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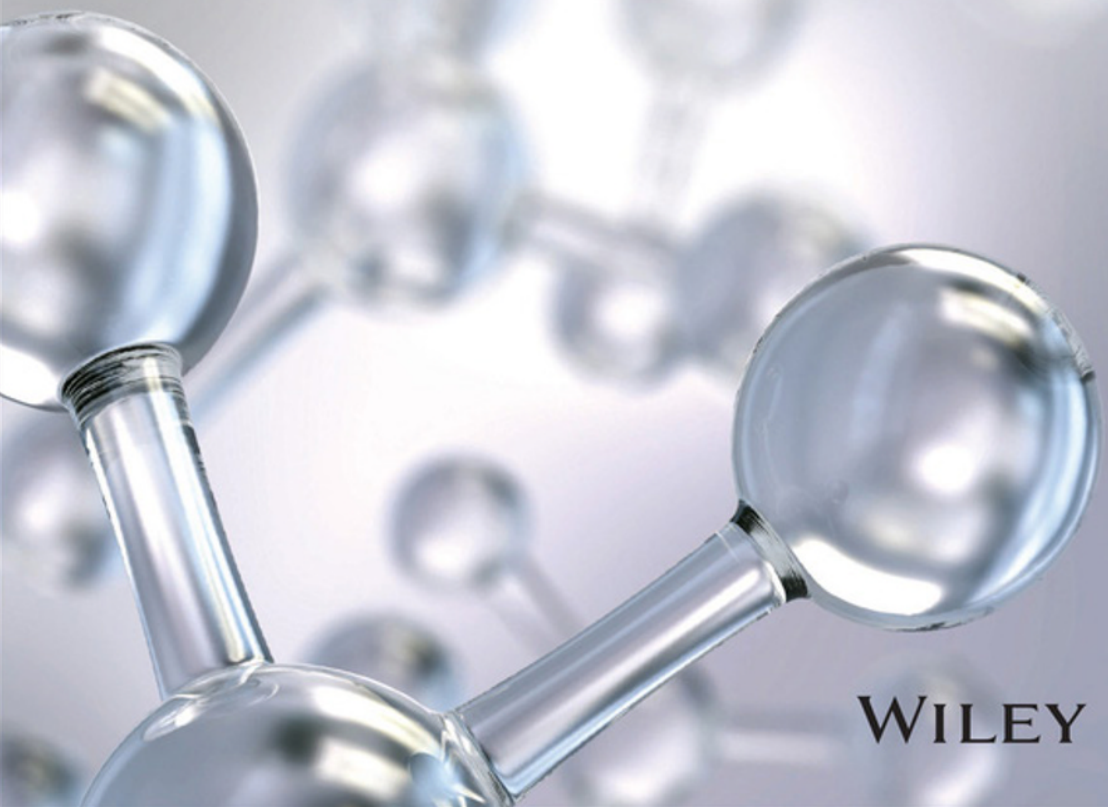
POISONOUS PLANTS AND PHYTOCHEMICALS IN DRUG DISCOVERY



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Edited by

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I dedicate this book to Thoko, Collins, Jed, and Michelle. You guys keep motivating.
Andrew

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Preface

Phytochemicals play a major role in the day-to-day management of diseases and health. There have been many reports of their effectiveness as community medicines and as alternatives to conventional drugs. However, there is one area that has been grossly underrepresented by researchers in phytochemistry. This is the area of poisonous plants and the role that phytochemicals play as toxins in society and how they could be harnessed for the betterment of mankind. There are many open-source, non-scholarly outlets and information that is not reviewed and that receives minimal scientific discussion.

Poisonous Plants and Phytochemicals in Drug Discovery seeks to address the roles that poisonous plants and phytotoxins play as friends and foes in society. It covers the mechanisms, benefits, risks, and management protocols of phytotoxins in scientific laboratories and their usefulness in drug discovery. This book contains insights that can help in the development of antidotes against some phytochemicals and other synthetic toxic chemical agents and raises awareness of which plants need to be categorized for protection and controls and those that can be helpful in assisting as emergency medicines. This book is carefully designed to show the contribution that phytochemicals play in safety and health management and how they could inform policies at national and international levels. Various industrial communities, researchers, and scholarly drug developers will be well guided on how best to create relevant measures to counter and/or manage toxins using phytochemicals or other means. The chapters in this book are presented in a clear and consistent manner to aid flow and continuity.

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Andrew is a member of the American Chemical Society, the Phytochemical Society of Europe and the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers and an associate member of the Royal Society of Chemistry. He studied Education (Chemistry) for a Bachelor's degree and Applied Chemistry (Natural Product Chemistry) for his MSc at Chancellor College, University of Malawi. For his PhD, he trained in Medicinal Chemistry, focusing on drug design, synthesis, and development at the University of Dundee, UK, under the Wellcome Centre for Anti-Infectives Research training program, and in Pharmbiotechnology (Medicinal Chemistry) at Mbarara University of Science and Technology in Uganda. His current interest and the focus of his research group is in the designing, synthesis, and hit expansion of potential drugs against diseases that cause a heavy burden on developing countries. He is also interested in encouraging developing countries to be much more involved in medicinal chemistry and all preclinical aspects of the drug development pipeline beyond just extracts. He is a lead editor of another book on phytochemistry with Elsevier and carries out reviews for some journals, including BMC's *Systematic Reviews and Elsevier's Tuberculosis*. Andrew teaches Chemistry at the Malawi University of Science and Technology.

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1

Historical Use of Toxic Plants

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CONTENTS

1.1 Introduction to Toxic Plants

Numerous poisonous plants exist in our environment and have been the subject of great speculation, study, and concern. Poisonous plants have been used as food, medicines, agents for crime, means of dispensing justice, capital punishment, suicide, bioterrorism, fishing poisons, and for recreational and spiritual purposes as hallucinogens or psychoactive agents. Such plants can cause a wide range of adverse effects when ingested by animals or people, depending on the organ system affected [1, 2].

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Plant toxicity is due to a wide diversity of chemical toxins [1–3]. Certain plant species have multiple toxins with the ability to affect different systems [2]. Other less studied forms of toxicity of plant compounds, such as those that manifest as subacute and chronic types in the form of carcinogens, teratogens, endocrine disruptors, and genotoxic compounds, are difficult to attribute to specific plants or their compounds [4]. The toxicity of different plant species is dependent on the susceptibility of the organism in question, the growth stage or part of the plant ingested, the amount consumed, and the species [2]. In the same vein, the observed effects of ingestion of toxic plants range from mild forms to those that result in death after ingestion of even very small quantities. Additionally, there are only a few plant toxins with antidotes, with most other poisoning cases managed symptomatically [1]. The good news is that antidotes are rarely necessary when managing poisonings by plant toxins in patients [5].

This chapter looks at some notable examples of widely studied toxic plants but is by no means exhaustive. Even among the widely studied examples, there is incomplete toxicity information [6–8]. The chapter introduces the reader to numerous general aspects of toxic plants. Toxic plants are of great economic importance in the livestock industry but also pose a threat to the health of humans and domestic animals, which are often exposed to the same toxic plants because of a shared environment. Various toxic compounds from plants such as those used in arrow and dart poisons could prove valuable for drug discovery and research [9].

1.2 Poisonous Plants as Sources of Traditional and Modern Medicines

Traditional medicine has been the source of several modern drugs. Many well-known poisonous plants are widely used in traditional medicine and are a source of life-saving drugs [3, 9]. Nature produces a variety of toxic compounds, many of which have found applications as anticancer drugs [10]. The same toxic compounds can serve as therapeutic compounds depending on the dose administered. This fact is well articulated in Paracelsus' dictum, which states that only the dose determines that a thing is not a poison [3]. Table 1.1 shows some common poisonous plants from which modern drugs have been obtained.

Several toxic compounds from plants belong to several classes of compounds, including alkaloids, glycosides, flavonoids, proteins, and saponins [1, 3, 25]. Most saponins can cause hemolysis of erythrocytes at doses of only a few milligrams per milliliter but may also have other toxic properties. Saponin-containing plants are extensively used as fishing poisons. Alkaloids have extraordinary structural diversity and a wide variety of pharmacological activities, such as muscle relaxants and cardiovascular and respiratory agents [2, 3]. They belong to the most powerful plant constituents and are responsible for the activity of a host of poison

Table 1.1 Some common drugs derived from toxic plants.

Name of plant	Toxic principles/ drug(s) derived	Use of drug
Strychnos spp.	Strychnine	Neuropharmacological science [11]
Strophanthus spp.	Ouabain	Acute cardiac insufficiency [12, 13]
Strophanthus spp.	k-Strophanthin	Acute cardiac insufficiency/ cardiotonic research [12, 13]
Physostigma venenosum	Physostigmine	Glaucoma and myasthenia gravis [14, 15]
Chondrodendron tomentosum	D-Tubocurarine	Muscle relaxant in anesthesia [14, 16]
Rauwolfia serpentina	Reserpine (Serpalan and Serpasil)	Antihypertensive and psychotropic [17, 18]
Rauwolfia vomitoria	Ajmaline	Cardiac arrhythmias [19]
Catharanthus roseus	Ajmaline	Cardiac arrhythmias [19]
	Vincristine	Pediatric malignancies, acute lymphocytic leukemia, lymphoid blast crisis of chronic myeloid leukemia, and both Hodgkin's and non-Hodgkin's lymphomas [14]
Digitalis purpurea	Digitoxin	Tachyarrhythmia [14]
Taxus brevifolia	Taxol (docetaxel/ Taxotere)	Ovarian, breast, and colon cancers and Kaposi's sarcoma [20]
Colchicum autumnale	Colchicine	Gout [21]; familial Mediterranean fever [22]
Melilotus officinalis/*Melilotus albus*	Dicoumarol (warfarin)	Thrombotic conditions [23]
Camptotheca acuminata	Camptothecin	Anticancer [20]
Podophyllum emodi/ *Podophyllum peltatum*	Podophyllotoxin	Anticancer [5, 24]
Papaver somniferum	Morphine	Analgesic [14]
Erythroxylum coca	Cocaine (benzoylmethylecgonine)	Local anesthetic [14]
Atropa belladonna *Datura stramonium* *Hyoscyamus niger* *Hyoscyamus muticus*	Scopolamine	Prevention of motion sickness and postoperative nausea and vomiting; parkinsonism; ophthalmic treatment [14]

ingredients. Flavonoids, which have a wide structural variety, show a broad range of activities but are rarely used as a primary source of toxicity for mammals, except for several compounds of special structure such as rotenoids, which are used for fish poisoning [3].

Almost all the active ingredients of African arrow poison come from plants. At least 80% of the poisons are based on cardioactive components, mostly cardiac glycosides from the genera *Acokanthera*, *Parquetina*, and *Strophanthus*. Other genera with numerous poisonous species are *Adenium, Mansonia, Calotropis, Pergularia, Corchorus, Erythrophleum, Euphorbia, Gnidia*, and *Jatropha* [3]. Many other plant toxins used have a variety of activities, but their toxic effects are more long term than acute, e.g. sesquiterpene lactones, iridoids, pyrrolizidine alkaloids, and tannins. Some alkaloid-bearing plants that are used as base poisons include *Strychnos* spp., *Boophone disticha*, *Crinum* spp., *Triclisia dictyophylla*, *Nicotiana* spp., *Physostigma venenosum*, *Sarcocephalus latifolius*, and *Erythrophleum* spp. [3, 26].

Some medicinal plant species used in African traditional medicine have potential or well-known toxicities. However, some of the toxic effects of plants are not easy to discern because they are either subacute or chronic. This is compounded by the fact that very few African plants have been tested for toxicity. Tamokou and Kuete [27] evaluated 120 African medicinal plants that had been previously screened for their toxic effects and found that about 40% of them were potentially toxic, with symptoms affecting neurological, hepatic, renal, gastrointestinal, and cardiovascular systems.

1.3 Toxic Plants and Justice

1.3.1 Toxic Plants in Capital Punishment

In 399 BCE, Socrates was sentenced to death for corrupting the youth of Athens and failing to recognize the city's traditional gods. Socrates is perhaps the most prominent victim of poison from hemlock (*Conium maculatum*), which was the standard form of capital punishment during his time [28, 29]. *C. maculatum* contains coniine, a polyketide-derived alkaloid that is poisonous to humans and animals [30].

1.3.2 Trial by Ordeal

Trial by ordeal is a judicial practice by which the guilt or innocence of the accused is determined by subjecting them to an unpleasant – usually dangerous – experience. In some cases, the accused were considered innocent only if they survived the test, or if their injuries healed [9, 31]. Ordeal by poison is peculiar to Africa [31, 32].

There was a substantial variation in the way ordeal poisons were prepared and dispensed across Africa but the basic procedures were similar. The procedure

involved a suspect being given some of the poison to eat or drink, depending on the form in which it was prepared or presented. If the suspect vomited the poison, an indication that the subject's stomach had rejected it, they would be ruled innocent. However, if their system retained the poison, they would be judged guilty and left to die from the effects of the poison [31, 33].

Most of the ordeal poisons were from the Loganiaceae, Apocynaceae, Leguminosae, and Solanaceae families. Other families with one or fewer representatives of ordeal poisons are Combretaceae, Sapotaceae, Euphorbiaceae, Polygalaceae, and Asclepiadaceae. In most cases, poisons were referred to by a local tribal name, which was mainly a general reference to the ordeal. This rendered the classification of ordeal poisons by tribe practically impossible [33]. The medicine men highly guarded their trade secrets, often making it impossible to identify the plant or formula used [3, 33]. Two of the well-documented cases are of ordeal poisons with *Physostigma venenosum* and *Tanghinia venenifera*.

The Efik-speaking chiefs of Nigeria used the toxic Calabar bean (*P. venenosum* Balf.; family Leguminosae). *P. venenosum* was used in the trial of people accused of witchcraft, sorcery, or murder. Medicine men were used to determine the effective dose of portions administered to the accused. It was believed that the poison would kill only the guilty and spare the innocent. This trial by ordeal was effective because of the toxic nature of the alkaloids contained in *P. venenosum*. When ingested hesitantly or slowly, as expected of a guilty person, the alkaloids had more time to be absorbed into the bloodstream, leading to death shortly after ingestion. When given to innocent people, they would quickly gulp it, resulting in emesis. In other words, the drug would quickly be vomited before exerting any lethal effects [31, 34, 35].

In Madagascar, a prominent example was the widely used ordeal plant tangena (*T. venenifera* Poir, synonym of *Cerbera manghas* (L). from the family Apocynaceae). All parts of *T. venenifera* are toxic, but the nuts that were used against those accused of various crimes, especially witchcraft – are the most toxic and contain the cardiac glycoside tanghin [32, 33]. *T. venenifera* use often resulted in a very high number of fatalities, with as many as 6000 people reported dead in one incident [33]. The suspect was made to eat a little rice and swallow three small pieces of fowl's skin followed by the tangena emulsion. After a few minutes, large quantities of tepid water were given, resulting in long and continued violent vomiting. If the three pieces of skin were expelled, the victim was exonerated, as a rule, and left to be nursed by their friends. *T. venenifera* has not been pursued for the possible development of any modern drug.

Other plant species used as ordeal poisons across Africa include *Strychnos icaja* Baillon, whose roots are widely used in ordeal poisons in West and Central Africa [36].

Menabea venenata Baill. (family Asclepiadaceae) was used in a similar manner to *T. venenifera*, but was dwarfed by its power. It was used exclusively as an ordeal poison by the Sakalave tribe in the arid regions of the west and northwest of Madagascar. *M. venenata* also contains a powerful cardiac glycoside [33].

Erythrophleum couminga Baill. (family Leguminosae) was also used as an ordeal plant poison. The bark of *E. couminga* is a highly toxic bark and was used as an accessory poison in ordeal trials throughout Madagascar and the Seychelles [33].

1.4 Toxic Plants in Poisoned Weapons

The use of poisoned weapons has been part and parcel of man's existence since time immemorial in virtually all parts of the world. Poisoned weapons have been used as a means of obtaining food through hunting game, protecting self from enemies and wild animals, and tribal warfare. Even today, the use of poisoned weapons such as arrows for similar purposes continues, especially in Africa, albeit less frequently [3]. Arrow and ordeal poisons are still considered to be conventional natural sources for future drug discovery [37]. Some of the earliest evidence of the use of arrow poisons is from around 218–2050 BCE in the tombs of ancient Egypt. One arrow had a mainly water-soluble poison, whose aqueous extract was cardioactive in mice [3].

The bow and arrow is the weapon most used by local tribesmen. Generally, forest dwellers have small bows and mostly wooden-tipped arrows; savannah people have large bows and arrows with iron tips and mostly complicated barbs. The barbs are wrapped behind with plant material for better adhesion of the poison. Another efficient weapon used in many parts of Africa, particularly Central Africa, is the crossbow [3].

1.4.1 Arrow Poisons

Arrow poisons can be roughly classified into African, South American, and Asian types. Arrow poisons from Africa are predominantly cardiac poisons containing cardenolides, whereas those from South America are almost exclusively muscle-paralyzing or curarizing poisons and contain alkaloids. Arrow poisons from Asia are mainly cardiac poisons with tetanizing poisons and thus contain cardenolides and alkaloids. With few exceptions, African and most Asian arrow poisons are extremely deadly with no antidote. South American curare poisons, on the contrary, can usually be survived by true antidote or artificial respiration [1, 3]. There are concerns about the rapidly disappearing use of arrow poisons [3, 9].

1.5 Plant Fishing Poisons/Piscicides/Ichthyotoxins

Piscicidal/ichthyotoxic plants are widely distributed throughout the world [38]. The use of plant toxins in fishing was widespread in tropical Africa but is now restricted to remote parts of the continent because it is largely banned [39].

In tropical Africa alone, Neuwinger [39] documented 258 fishing poisons/piscicidal/ichthyotoxic plants from 25 years of field research and concluded that 10–20% of fishing poisons are yet to be discovered. Ten years later, Neuwinger [40] documented an additional 325 fish-poisoning plants.

Using plant extracts or toxins for fishing involves pounding the plant material and throwing it into shallow pools or sections of small rivers that have been dammed to give relatively still water. The fish are stupefied and, after a short while, float to the surface of the water. They are easily picked up by hand and then eaten without any untoward effects on the health of the consumers. Women have traditionally carried out this activity in Africa [39–41].

The main active compounds are saponins, rotenoids, and diterpene esters. They represent the most important and most common constituents in ichthyotoxic plants and are of great interest to scientists [39, 40]. These biologically active compounds have potential as insecticides and many are widely used in traditional medicine and in preparation of arrow poisons [39]. For example, *Cissus quadrangularis* is used in Nigeria both as a fish poison and as an arrow poison to kill small birds. Many piscicidal plants from Africa are predominantly from the Leguminosae family, followed by the Euphorbiaceae family [39, 40]. Ichthyotoxic compounds have several other biological activities, such as the anticancer and potent antiviral activities of compounds isolated from *Dryopteris fragrans* [42] and the antibacterial activity of rotenoids against the ulcer-causing *Helicobacter pylori* [43].

The most commonly used piscicidal plants in Africa are *Tephrosia vogelii*, *Mundulea sericea*, *Euphorbia tirucalli*, *Gnidia kraussiana*, *Adenia lobata*, *Balanites aegyptiaca*, *Swartzia madagascariensis*, *Neoratanenia mitis*, *Tetrapleura tetraptera*, and *Strychnos aculeata* [40]. *C. quadrangularis* is often used in conjunction with other local plant poisons such as *B. aegyptiaca* and *T. vogelii*. The mixture of the three toxic plants is more toxic than any one of the poisons used alone [39].

1.6 Poisonous Plants as Food

Oftentimes, there is no clear line between food plants and toxic and medicinal plants [44]. In many cases, one plant may have particular parts that are poisonous, whereas the other parts are edible or medicinal. A case in point is *Abrus precatorius*, the seeds of which are highly toxic; however, the leaves are edible and are used in traditional medicine [45]. The seeds of *Malus* spp. (apple) are also poisonous and contain the toxin amygdalin, which is a cyanogenic glycoside [5]. In some cases, the toxic parts have to be detoxified first through various elaborate and careful processes to render them edible. Although several plant species that are potentially toxic are consumed as food and also used as medicine, this section will focus on a few examples of plant species that are widely used as food and are also known to be toxic.

One of the most prominent examples of such toxic and edible plants is the cycad. Cycads have been widely researched as poisonous food plants. Cycads are indigenous to the tropics and subtropics, where they have been used both as a staple and as emergency food and medicine for various ailments [46, 47]. Natives of particular areas have long been aware of their toxicity, which especially manifests as gastrointestinal and neurological effects. In cattle, for instance, continued ingestion leads to irreversible paralysis of the extremities [44].

The nutritional value of cycads principally lies in an edible starch extracted from the roots, stems, and nuts [44]. Cycads produce flour with a high nutritional value [48]. Several precautions are taken when preparing it as a food. A high-quality food starch is extracted from the fibrous pulp of cycads through alternate processes of cutting, drying, and soaking [44]. In some parts of Uganda, for example, the hard seed of the cycad *Encephalartos hildebrandtii* can be boiled and ground into flour in times of famine. The starchy center of the stem is also edible [49].

There have been repeated accounts of poisoning from cycad ingestion during periods of famine. This has been attributed to inadequate preparation of cycad products, possibly because of a lack of knowledge of the toxicity of the plants or because of their unpredictable variations in toxicity [44].

Compounds from cycads are carcinogenic in various laboratory animals [50]. Hirono et al. [51] showed a high death rate from liver cirrhosis in the Miyako Islands of Japan that may be correlated to the consumption of cycads during periods of crop loss. Cycad flour contains the neurotoxin beta-methylamino-L-alanine (BMAA) [52] as well as other neurotoxins, as reviewed by Rivadeneyra-Domínguez and Rodríguez-Landa [48].

Duncan et al. [52] showed that 87% of the total BMAA content of *Cycas circinalis* seeds collected on Guam island was removed during traditional processing. They concluded that processed cycad flour as prepared on Guam contains extremely low levels of BMAA (0.005% by weight), making it unlikely to cause the delayed and widespread neurofibrillary degeneration of nerve cells observed in amyotrophic lateral sclerosis and the parkinsonism–dementia complex of Guam [52].

Another widely used toxic plant species is cassava (*Manihot* spp.), which is consumed after detoxification. Cassava contains potentially toxic levels of cyanogenic glucosides, made up of linamarin (95% of total cyanogen content) and lotaustralin (5%) [53]. Sun-drying and crushing cassava roots to make flour removes 96–99% of total cyanogens [54]. Cyanide intake from a cassava-dominated diet has been put forward as a contributing factor in two forms of nutritional neuropathies in Africa: tropical ataxic neuropathy and epidemic spastic paraparesis. Therefore, proper processing of the cassava root is required

to detoxify it for safe consumption [5, 55]. It is thus important to beware of the potential toxicity of various plant species while trying to meet the food security needs of vulnerable populations [48].

1.7 Poisonous Plants as Biopesticides

Botanical pesticides have a wide range of biological activities such as repellents, insecticides, fungicides, bactericides, molluscicides, nematicides, and rodenticides [21, 56]. Some of the plant species used as fishing poisons also have proven insecticidal properties include *Derris* sp. (containing rotenone) and *Nicotiana* sp. (containing nicotine) [57].

Rotenones are extremely toxic isoflavones from the roots or rhizomes of several tropical legumes. They act by suppressing the appetite of insects, leading to death within hours or a few days. There are more than 67 species of legumes that synthesize a broad spectrum of non-systemic insecticides [58]. The roots of many species of *Derris* and *Lonchocarpus* (family Leguminosae) have insecticidal properties, which are mainly attributed to the presence of rotenone (3–10%), although other insecticidal compounds are usually present. Other genera with rotenoid-producing species are *Millettia*, *Neorautanenia*, and *Tephrosia* [21]. Strychnine from *Strychnos* spp. has also been historically used as a pesticide [21]. Such compounds of botanical origin can be highly effective with low levels of toxicity toward non-target organisms and multiple mechanisms of action [59, 60]. However, poor stability and other technological issues limit the large-scale application of natural compounds for pest control [21, 61].

1.8 Toxic Psychoactive Plants for Recreational and Religious Purposes

All cultures around the world have some kind of drug culture that relies on psychoactive compounds for medicinal, recreational, or ritual purposes [62]. Psychoactive substances are compounds that have the ability to change consciousness, mood, and thoughts [63]. Psychoactive plant species contain compounds that work as hallucinogenics, sedatives, or stimulants [64, 65].

Alrashedy and Molina [64] conducted a phylogenetic analysis of 126 traditionally used psychoactive plants that indicates multiple ethnobotanical origins. The plant species documented were also used for several medicinal purposes. Rätsch [66] presented a detailed account of psychoactive plants. Some of the well-known psychoactive plant species with medicinal, recreational, and other purposes

include *Cannabis* spp. (marijuana), which has hallucinogenic, stimulant, antianxiety, antidepressant, sedative, analgesic, and aphrodisiac properties; *Atropa belladonna* (belladonna), which has hallucinogenic, stimulant, sedative, and aphrodisiac properties; and *Papaver somniferum* (opium poppy), *Datura* spp., and *Mandragora* spp. (mandrake), which all have hallucinogenic, sedative, analgesic, and aphrodisiac properties. In addition, *Catha edulis* (khat) has stimulant, antidepressant, and aphrodisiac properties.

Some plants containing psychoactive substances should be classified as harmful drugs since chronic administration has been linked to addiction and cognitive impairment [65]. Not much is known about the toxicity of many of the psychoactive plant species, mainly because of the limited number of studies conducted on their toxicity. A case in point is *Datura stramonium*, in which all parts are toxic. The plant contains a mixture of anticholinergic alkaloids such as atropine, hyoscyamine, and scopolamine, which are mainly responsible for its neurotoxic and hallucinogenic effects [67]. *Datura* has a narrow therapeutic window, implying a small difference between the active and lethal dose. It has been widely documented as a cause of accidental poisoning, particularly in contaminated food [68]. Another example is *C. edulis*, the consumption of which has been associated with several cases of acute liver failure and autoimmune hepatitis [69].

1.9 Poisonous Plants in Warfare and Bioterrorism

Bioterrorism refers to the use of biological agents to inflict disease and/or death on humans, animals, or plants by a political or religious group or cult to achieve a political or ideological objective [70, 71]. Such agents include some bacteria such as *Bacillus anthracis* (anthrax), viruses such as variola virus (smallpox), rickettsiae, fungi, or biological toxins such as ricin. For the agent to be used successfully, it must first be "weaponized," or produced in sufficient quantities in relatively stable and easily disseminated forms [70]. Therefore, human populations, crops, and livestock are considered possible bioterrorist targets [70]. Concerns over the use of biological as well as chemical weapons have increased recently. In fact, bioterrorist incidents have increased markedly since 1985 [71], with attempted uses of ricin by various groups, especially in the USA [72]. According to Balali-Mood et al. [73] there was a 10-fold increase in the number of published articles following the terrorist attacks in the USA on 11 September 2001.

Ricin is a toxalbumin derived from the seeds of the poisonous plant *Ricinus communis* (castor bean; family Euphorbiaceae). Ricin acts by inhibiting protein synthesis and is one of the most toxic biological agents known. Ricin is classified as a category B bioterrorism agent and a schedule 1 chemical warfare agent according to the US Centers for Disease Control and Prevention [74]. It can be

extracted from castor beans and purified. It is stable under ambient conditions and is readily accessible and relatively easy to extract. The fact that ricin has been used previously in high-profile assassination cases – such as that of Georgi Markov, a leading communist dissident exiled in London, in 1978 [75, 76] and similar assassination attempts with ricin elsewhere [71] – has contributed to its publicity. On a larger scale, Iraq developed a weapons of mass destruction program between 1985 and 1991, in which approximately 10 L of concentrated ricin solution was produced for field testing [77]. Schep et al. [78] have, however, argued that although ricin is deadly it is not suitable as an agent of bioterrorism for a large population since a substantial mass of powder needs to be extracted, formulated, and produced in the right particle size to target the relevant parts of the lung to be fatal. Additionally, the facilities for the large-scale manufacture of ricin are not available to terrorists. Ricin has also been used for suicide [8].

In 1975, the Biological and Toxin Weapons Convention was ratified by several countries. In spite of the agreement, bioweapon threats from groups such as terrorist organizations and rogue states continue to worry public health authorities [71].

R. communis is widely used for medicinal purposes in traditional medicine [45], despite the highly toxic nature of ricin. Tyagi et al. [79] conducted an extensive review of the recent advances in ricin research and its potential therapeutic applications, especially as an anticancer agent.

1.10 Poisonous Plants as Carcinogens and Teratogens

Some plant species contain teratogenic substances, which can cause deformities or abnormalities in the developing fetus in animals when ingested by the mother. However, no species have been shown to be specifically responsible for malformations in humans. The teratogenicity of plants has been demonstrated when they are ingested by some animals as part of their fodder. Teratogens usually act early in the gestation period of an animal, making it hard to pinpoint the exact causative agent when the malformations manifest [80]. Some plant species and their respective or suspected teratogenic compounds have been demonstrated in laboratory animals at large doses not normally consumed by humans. These include alkaloids from *Senecio* spp., which are responsible for possible teratogenic effects in rats and *in utero* deaths of calves, and *Nicotiana* spp. and *Lobelia* spp., which are responsible for some skeletal deformations in pigs [21].

Some compounds have been shown to promote liver cancer in rats, although they occur naturally in very low concentrations, which are typically far below the toxic concentrations in products consumed by humans. Such carcinogenic

compounds include alkenylbenzene and its derivatives, which are found in products such oils from *Sassafras*, star anise, and nutmeg [21].

Other carcinogens or co-carcinogens are the betel quid and tigliane and daphnane derivatives and related diterpenes. Bracken (*Pteridium aquilinum*) is also carcinogenic and has been implicated in bovine poisoning. Aristolochic acid from *Aristolochia* spp. is both nephrotoxic and carcinogenic and has thus been banned in many countries.

1.11 Conclusion

Poisonous plants offer an exciting window of opportunity for the discovery of various biologically active substances that could find applications as novel drugs, biopesticides, or research tools. However, more research is needed to determine the actual toxicity of several medicinal plants used in traditional medicine, especially in Africa.

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2

Classification of Phytotoxins and their Mechanisms of Action

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CONTENTS

Poisonous Plants and Phytochemicals in Drug Discovery, First Edition.
Edited by Andrew G. Mtewa, Chukwuebuka Egbuna, and G.M. Narasimha Rao.

2.1 Introduction

Phytotoxins are basically toxic secondary metabolites that are found in plants. They largely have low molecular weight and are capable of deranging vital plant physiology and plant cell activities and/or causing the death of a plant at less than 10 mM concentrations [1]. There are hundreds of such substances in a single plant in addition to non-toxic chemicals, which demonstrates the complexity of phytochemical systems. Phytotoxins can be synthesized by the plants themselves [2], by endophytes (bacteria, fungi, etc.) in the plants [3], and also by phytophagous insects [1].

Phytotoxins can be categorized into two broad groups: those that have an effect on plant systems and physiology and those that have an effect on animals and humans. Those that affect plant systems and physiology are the ones that enjoy the most scientific limelight; they are most commonly discussed in the field of phytotoxins with a focus on the quality of plant life. There is a relatively narrower discussion on phytotoxins that the plant produces with a focus on elements foreign to and outside the plants themselves, such as predators.

Some of the existing phytotoxins are produced by plants for defense purposes against predators, such as insects, microorganisms, and higher animals. At other times, they are produced in response to infestation by environmental and physical stress, such as extreme drought, humidity, and confinement, e.g. growing under a huge rock.

Although phytotoxins are largely known to show adverse effects on plants such as necroses, chloroses, general suppression of growth, wilting, and spotting of aerial portions [1], they may also produce toxins and other metabolites that are beneficial to drug discovery.

Apart from plant health, phytotoxins have been established as playing a significant role in human health. It is therefore important to categorically detail their important identity characteristics to make it easy to understand how they can be used or controlled when it comes to drug development. In this way, their molecular clustering can more efficiently and conveniently lead to the identification of potential drug scaffolds. This chapter discusses the categorization of phytotoxins within the two broad distinctions stated earlier and relates the same to the risks and benefits that these toxins have for disease management and prevention.

2.1.1 Endophytic Phytotoxins

Endophytes in plants are known to produce compounds that are different than those that the plant produces. One of the earliest studies on phytotoxin categorization was carried out successfully in 1980 by Yoder [4], who used the terms ‘pathogenicity’ and ‘virulence’ of toxins as major descriptors, among others.

By pathogenicity, Yoder meant the ability to induce disease(s) or disorder, whereas he used the term virulence to describe the severity of the disorder induced.

Phytotoxins that affect plants are usually further categorized by the target range, from the host's range to a wider radius outside the host plant. These are technically called host selective toxins (HSTs) and non-host selective toxins (NHSTs) [5], where HSTs affect the plant producing the phytotoxin as it hosts the endophyte while NHSTs have no effects on the host plant but affect other plants around it. The mechanisms of action of most HSTs have elements of pathogenicity, where the endophyte liable for a particular phytotoxin invades host tissues, causing some diseases in the plant. In contrast, it is not clear about the roles of NHSTs in pathogenicity [5–7]. Some authors have suggested that NHSTs are not the only pathogenicity determinants in the plants that they affect and they largely contribute to the virulence of the pathogens that produce the toxins [8]. There is a hypothesis that the compounds that a plant synthesizes resemble those that are formed from metabolic processes of endophytes within its cells [9].

2.1.2 Secondary Metabolites

Plants are known to produce various compounds, some of which are directly beneficial to human and animal health. These compounds are secondary metabolites, synthesized by plants and sometimes associated by endophytic activities. Some secondary metabolites are reported to have allelopathic activities [10] and adversely act on the host plant itself or other surrounding plants.

2.2 Possible Categorization

Depending on convenience and the details available about the phytotoxins under consideration, there are a number of ways of systematically categorizing them into meaningful classes. These could be any from: the biological characteristics, chemical characteristics, occurrence, pharmacological characteristics, taxonomic details, response to growth conditions, or habitat. However, some of these aspects can be carefully combined to better qualify other descriptions. Below are brief discussions on the possible categories that can be used to classify phytotoxins.

2.2.1 Biological Characteristics

Phytotoxins differ in terms of their biological characteristics. These characteristics can emanate from the biology of the responsible endophyte or from the bioactivities that the toxins are reported to exhibit, if already studied. Categorization of phytotoxins based on biological characteristics has been a traditional method of

phytotoxin classification [8]. For instance, mycotoxins, secondary metabolites from fungi that adversely affect plants and humans alike, often fatally [11], present a good example of classification based on biological properties. The categorization of mycotoxins and other phytotoxins into two different groups had to be revised. They were made into one group of endophytic phytotoxins after similar biological activities of the toxins, among other characteristics, were noticed [11].

Some wild mushrooms, for example, have phytotoxins such as muscarine and muscimol. When ingested, these toxins cause nausea, confusion, diarrhea, visual challenges, hallucinations, and salivation. Symptoms are key to determining the effects of poisoning and, when their onset is delayed, there is only a very small chance of survival because of delayed intervention.

In the same way, some taxonomic details of the host plant can also be of great significance in categorizing toxins from their respective plants of origin. Chemotaxonomy has already proven to be reliable when markers, such as race, genera, species, and pathotypes, among others, are used to determine the phylogenetic relationships among endophytes responsible for the production of some toxic metabolites with similar traits [1].

2.2.2 Chemical Characteristics

Chemical characteristics are one of the easiest and most convenient ways to classify phytotoxins. This is usually done by examining the chemical structures of the phytotoxins, among other chemical properties. Since chemical structures are easily classified by their biosynthetic pathways, those for phytotoxins can also be done in a similar fashion. For example, phytotoxins from *Alternaria* and *Fusarium* pathogens share similar chemical structures [11]. Usually, chemical structures are categorized as polyketides, alkaloids, ribosomal and non-ribosomal, terpenes producing peptides, and metabolites of mixed biosynthetic origin. Other examples include *Stagonospora nodorum* and *Pyrenophora tritici-repentis*; these are both fungi and are known to produce phytotoxic metabolites that are ribosome-produced peptides [12].

2.2.2.1 Cyanogenic Glycosides

Cyanogenic glycosides are plant-based phytotoxins occurring in more 2000 plant species, and some of these species are used as food. Plant foods that contain these phytotoxins include sorghum, cassava, bamboo sticks, almonds, and summer fruits. Their toxicity to humans emanates from the cyanide that they contain; the clinical signs of contamination in humans include elevated breathing rates, dizziness, diarrhea, a reduction in blood pressure, headache, stomachache, nausea, confusion, convulsions, and cyanosis. If the body fails to detoxify these cyanides, it can end up in fatality.

2.2.2.2 Furocoumarins

Furocoumarins are found in many plant species, such as celery, citrus plants, and parsnips. In plants, they are released in the presence of stressful events, such as drastic climatic changes and physical damage. They may cause skin reactions and, in susceptible people, gastrointestinal problems may follow.

2.2.2.3 Lectins

Lectins are common phytotoxins in beans, with the highest concentration in red kidney beans. This is the reason why even a few beans eaten raw can cause nausea, severe stomachache, and sometimes diarrhea. At high temperatures, lectins are destroyed. In local settings, boiling beans for over 10 minutes helps to eliminate these phytotoxins.

2.2.2.4 Solanines and Chalcones

Solanines and chalcones are glycoalkaloid phytotoxins present mostly in the Solanaceae family. This is a family that includes potatoes, tomatoes, and eggplants. Generally, the concentration of these toxins is low but they are found to be high in some plants depending on the stage of growth and the environment. Unripe tomatoes and potato sprouts have relatively high levels of solanines and chalcones. Stressful conditions such as ultraviolet light, high temperatures, biochemical attack from microorganisms, and physical damage such as bruising provide a good environment for the release of these toxins.

2.2.2.5 Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids are a group of toxins produced by about 600 plant species. They are most commonly found in the Asteraceae, Boraginaceae, and Fabaceae families. They generally grow as weeds that, in the course of growth, tend to contaminate food plants. Pyrrolizidine alkaloids have high stability during processing and they have been detected in honey, herbs and spices, herbal teas, and wheat products. The overall risk to health is yet to be explored [13]. This demonstrates the convenience and importance associated with the chemical characteristic classification of phytotoxins.

2.2.2.6 Pharmacological Characteristics

These are related to biological characterization, but with the addition of relevance in therapeutic, pharmacokinetic, and pharmacodynamics activities. Although endophytes usually cause diseases to the host plants, they sometimes have beneficial effects to the plants as well as the animal kingdom that consume the attacked plants. Endophytic fungi has been known to have damaging properties on the leaves of c. Papaya for example but some toxic compounds isolated from these endophytes possess very good cytotoxic properties which can be used

to develop drug compounds to control, manage and possibly cure cancers in its various specific stages. In the same understanding, these toxins can be instrumental in the pharmaceutical industry focusing on what they do as a means of their interaction with biological targets. Some can act better on fungi, others on bacteria, viruses and protozoa. Their interactions could span from cytotoxicity, metabolic modulation, imunal modulation, cell growth arrest or enhancement, transportation efficiency and excretion among other pharmacokinetic and physiological proccesses. All phytotoxins that have similar properties as described in the sections above can be categorised based on their pharmacological relevance and studied together.

2.3 Currently Available Classification Tools

Apart from some textbooks [14, 15], there is also software available to researchers to help them to make collections and classifications of phytotoxins that are uploaded in their respective libraries. These include the Aggregate Computational Toxicology Online Resource database [16], which is managed by the US Environmental Protection Agency; the Clinical Toxicology (CliniTox) database [17, 18]; the Toxic Plants–PhytoToxins (TPPT) database [19]; the SuperToxic database [20]; the compendium of the European Food Safety Authority [21]; the Super Natural II database [22]; and KNApSAcK-3D [23–25]. Despite the existence of these databases, there are still gaps in systematically clustering phytotoxins because of the limitations of the tools. For example, the ACToR database fails to link toxins and toxin metabolites to some of their effects on, for example, environmental management [16] and the CliniTox database falls short in providing details of chemical characterization [19].

The TPPT database has made efforts to be better than most; however, it is primarily focused on European plant phytotoxins, in particular Swiss plants, apart from a few of the most commonly known toxins from elsewhere. The justification for this is that Swiss vegetation is a good representation of central Europe, with several altitude zones, giving it the advantage of having a wide range of plant species [26]. This being the case, there remains a need for a more improved version of a tool or a non-computer-based standard that can incorporate regional databases and standards into one that can give a wider picture in one resource. The databases that are available can be useful in modeling a standard that can be used in the classification of phytotoxins. In this way, phytotoxins can be optimized using the databases and/or the standards developed thereof to suit the needs of researchers, government agencies, and industries.

2.4 Role of Phytotoxin Classification

The classification of phytotoxins plays a role mainly in human survival and economic development [1]. This is evident in agricultural management, studies of natural medicines, the discovery of novel drugs and their pro-drug metabolites, the preservation and protection of the quality of water in various water bodies, the security of societies, and the proper management of the environment to ensure people's safety. The sections below briefly detail how important the classification of phytotoxins is to society.

2.4.1 Drug Discovery

The classification of phytotoxins by their biological activities is helpful in drug discovery as researchers can then easily target a metabolite and study it just because it has similar bioactivities to some already known standard drugs. Ascochytin, a phytotoxin from *Ascochyta pisi* that induces spotting disease on the leaves of peas, is structurally similar to citrinin, a mycotoxin with antifungal activities. As such, ascochytin was regarded as a potential hit for an antifungal [1, 27]. The same is true for griseofulvin, an antibiotic produced by *Penicillium griseofulvum* as one of its natural products, which is also a known phytotoxin [28].

2.4.2 Environmental Monitoring

Understanding the type and nature of phytotoxins in an environment is essential to the safety of the community. Among many other potentially harmful and toxic effects of phytotoxins, some can be fatal if ingested in very small amounts; others are irritants to the skin, eyes, and the respiratory tract and can cause more significant physiological harm with time; and yet other phytotoxins are carcinogenic. Understanding these phytotoxins can help in devising ways through which the community can avoid or effectively minimize the risks associated with each one of them or, at least, know which category they may fall in. It is imperative for environmental monitors to include phytotoxins in their various assessments, including environmental impact assessments for community projects. The incorporation of phytotoxins in environmental monitoring is said to be hampered by the high diversity of phytotoxin structures and the unavailability of reliable and well-validated standard analytical methods [19]. This challenge can be overcome by devising standard protocols that are only feasible with the development of a sound systematic categorization of the toxins.

2.4.3 Phytotoxins, Aquatic Life, and Water Quality

Phytotoxins that are produced by algae in bodies of water, both fresh and oceans, are known as algal toxins [13]. They contaminate fish and other aquatic animals to different extents; they also contaminate drinking water and cannot be eliminated by freezing or cooking [13].

Phytotoxins can also potentially contaminate bodies of water, particularly in situations where leaching of chemicals from plants into the water is possible. This happens particularly for phytochemicals that are polar and are easily soluble in water owing to the molecular nature of their structures being similar to that of water. In this regard, it is imperative to always be alert and vigilant to find ways that are useful in both detecting the presence of phytochemicals in bodies of water and improving water quality following phytochemical contamination.

2.4.4 Air Contamination

In the same regard, air can also be susceptible to phytotoxin contamination. This is particularly the case for volatile phytochemicals or very lightweight microparticles that can be used as vehicles to ferry phytotoxins to various areas. Most of these phytotoxins are irritants to the eyes, respiratory tract, and skin. It is generally difficult to protect the environment from airborne phytotoxins because they are mostly distributed naturally by the wind. Non-toxic materials with a higher density than that of the toxins or their carriers can be employed in limited areas to weigh down the contaminants to the ground. However, such interventions should only be done after thorough environmental and health impact assessments have been conducted and approved.

2.4.5 Food Contamination

Phytotoxins can potentially be found in foodstuffs, particularly those of marine origins. However, other terrestrial food sources can still contain phytotoxins from ground-plant contamination through root uptake or through leaf and stem openings into the metabolic system of plants. Herbivorous meat sources can be easily contaminated by such phytotoxins or their metabolites from plants. Phytotoxin metabolites can be non-toxic or as toxic as, or more toxic than, the original phytotoxin obtained from the ground or the air. In Malawian history, hunters and fishermen were known to use plant-based phytotoxins, locally known as *mwabvi*, to make lethal material that could make their fish or animal targets drowsy.

2.4.6 Security and Safety Services

Phytotoxins should be considered a group of chemicals potentially endangering the security of communities. Their use in various historical conflicts should serve as a lesson from which security agents need to do all they can to prevent the recurrence of abuse and/or misuse of plant based materials as weapons during conflicts. Weaponisation of phytotoxins remain a worrisome thought that threaten water bodies and the general environment in which people live. Chatters and organizations that checks and controls the use of chemicals such as the Organisation for the prohibition of chemical weapons (OPCW) exist to make sure no chemicals are weaponized. However, there is need to extend the reach to phytotoxins which currently aren't been exhaustively studied. Security services need to be made aware of the potential dangers that they can pose and the challenges in their remediation. In this way, it will be much easier for scientists, working alongside security service guidelines and policy, to develop appropriate remedial interventions.

2.4.7 Agricultural

The accumulation of NHST phytotoxins in some plants may be dangerous to other plants [1]. This can be useful in selectively and strategically growing these plants to protect intended plants from invasive weeds. However, this can only be done if the intended plant–weed relationship has been studied and is well understood. If need be, biotechnology approaches to customize the relationship can be introduced. Some phytotoxins have already been reported to be significant in the management of weeds in farming [29].

2.5 Brief Mechanisms of Action

Phytotoxins have various mechanistic activities on the physiological processes of the organisms they are affecting. These include, but are not limited to, lipid biosynthesis (cyperine: *Ascochyta cypericola*), energy production (tentoxin: *Alternaria* spp.), the polymerization of actin (cytochalasins: many fungal species), and reactive oxygen species production (cercosporin: *Cercospora* spp.) [1]. Generally, their mechanism of action is to interact with body cells and possibly cause slow degeneration or other long-lasting complications. Interventions against such adverse mechanisms involve blocking the mechanistic processes or introducing entirely new agents, such as known drugs, to undo or redirect the mechanisms.

2.6 Conclusion

Phytotoxins are a very significant group of compounds in all sectors of society, including health, security, agriculture, and drug discovery, among others. Although many studies have been carried out, it is imperative to consider the categorization of these compounds in terms of their sources, mechanisms of action, hazards, and their benefits. In this way, there will be a very important multidisciplinary contribution to science in a way that is more meaningful to the general public.

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3

Poisonous Plants as Sources of Anticancer and Other Drugs

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CONTENTS

3.1 Introduction

Cancer is one of the leading causes of morbidity and mortality in both developing and developed countries. It is the second leading cause of death, after cardiovascular disease, among non-communicable diseases [1]. In spite of the use of modern drugs, different procedures (surgery, chemotherapy, and radiotherapy), and technological developments in the management of cancer, its burden has increased worldwide. The World Health Organization (WHO) has estimated that the number of new cancer cases will probably rise by 70% by 2038 [2].

According to WHO [3], the incidence of cancer is generally marked by three of the five most deadly cancers: lung, female breast, and colorectum. Smoking, some

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infections, exposure to ionizing radiation and environmental pollution, age, and lifestyle – especially poor diet and lack of physical activity (with the appearance of obesity) – are the risk factors for the occurrence of cancer. These factors can interact directly with the existing cellular genetic components, resulting in the onset of disease. However, the causes of the increasing incidence of cancer are not very well known [4].

Poisons produced by plants have been discovered by observing nature and by experimentation [5]. Moreover, a high number of medicinal plants possess a degree of toxicity. For example, it was reported that about one-third of medicinal plants used in the treatment of diabetes are considered to be toxic [6]. Natural products are being used to meet the urgent need to develop effective drugs, and they will play a leading role in the discovery of drugs for treating human diseases, especially critical diseases [7].

Extracted or isolated primary and/or secondary metabolites from natural remedies play a crucial role in the management of diseases and contribute significantly to the improvement of cancer conditions. These metabolites are reported to have pharmacological potentials by different mechanisms involved in the genesis, occurrence, and development of cancer and other diseases. Among their anticancer properties, these metabolites can act by suppressing the stimulating enzymes produced by tumors, repairing DNA, stimulating the production of antitumor enzymes in cells, increasing body immunity, and inducing antioxidant effects [8]. A certain number these metabolites produced by "poisonous plants" are known to possess properties that enable them to interact with DNA or RNA by either intercalation or alkylation. Among these primary and/or secondary metabolites there are alkaloids, enzyme inhibitors, aristolochic acids, ptaquiloside, antivitamins, cycasin, furanocoumarins, epoxide or aldehyde groups, phytoestrogens, terpenes, saponins, proteins, oxalates, volatile etheric layers, photosensitizing substances, etc. [9–11]. It is possible to use that potential against cancer cells. Therefore, it is very important for scientists to explore the potential of poisonous plants for the development of novel agents in the management of cancer and other diseases as future therapeutic strategies.

Poison is a substance that causes damage or injury to the body and endangers one's life [12]. Many in vivo and in vitro studies of a number of poisonous plants have been carried out, and this chapter aims to document their potential in the management of diseases, and especially in the treatment of the cancer.

3.2 Poisonous Plants in the Treatment of Cancer and Other Diseases

Poisonous plants have been used against cancers and are also used as the sources of molecular scaffolds for drug development in cancer therapy. These plants include *Abelmoschus esculentus* (L.), *Abelmoschus esculentus* (L.) Moench

(Malvaceae), *Abrus precatorius* (L.) (Leguminosae), *Achillea millefolium* (L.) (Compositae), *Actaea* spp., *Ananas comosus, Annona squamosa* (L.)*, Anacardium occidentale, Bidens pilosa, Capsicum frutescens, Carica papaya, Catharanthus roseus, Centella asiatica, Elaeis guineensis, Ginkgo biloba, and Kigelia africana* [6, 8, 9].

Also, some phytochemical constituents have shown profound anticancer activities. These constituents include coumarins, iridoids, lignans, naphthoquinones, meroterpenoid naphthoquinones, sterols, and flavonoids [9], which have been isolated from plants such as *Macadamia* spp., *Mangifera indica, Melia azedarach, Phyllanthus amarus* Schumach. & Thonn., *Plumbago zeylanica, Silybum marianum, Vernonia amygdalina,* and *Withania somnifera* [9]. Table 3.1 presents poisonous plants with their common areas of growth.

3.3 Poisonous Plant-Based Anticancer Drugs that are on the Market

Having presented a list of poisonous plants that are known to have anticancer components in them, it is also important to note that there already exist conventional drugs that have been synthesized or semisynthesized from plants. Table 3.2 shows some of the plant-based anticancer drugs currently on the market together with their mechanisms of action.

3.4 Poisonous Plant-Based Drugs Against Other Diseases that are on the Market

Besides anticancer drugs, there are plant-based drugs on the market that were derived from known poisonous plants. Some of the diseases managed by these toxin-based drugs can be fatal, such as heart conditions, or chronic, such as type 2 diabetes and pain. Table 3.3 presents some of the drugs on the market, their natural plant sources, and the diseases they are used to manage.

3.5 Conclusion

For a long time, poisonous plants have been ignored in drug development or traditional medicines, particularly because of fear of death or other complications from the toxins in the plant. Most research into natural product-related therapy focuses on edible plants and their parts. This chapter has demonstrated that poisonous plants should also be a focus of research when considering drug development.

Table 3.1 Selected poisonous plants from around the world.

Plant species	Family	Part concerned	Country	References
Abelmoschus esculentus (L.) Moench	Malvaceae	Fruit and roots	Nigeria	[13]
Abrus precatorius (L.)	Leguminosae	Seeds (mainly), roots, and stem leaves	India, Nigeria	[14, 15]
Achillea millefolium (L.)	Compositae	Entire plant	Turkey	[16]
Achillea wilsoniana (Heimerl) Hand.-Mazz.	Compositae	Entire plant	China	[17]
Acokanthera oppositifolia (Lam.) Codd.	Apocynaceae	Sap	South Africa	[18]
Acokanthera schimperi (A.DC.) Schweinf.	Apocynaceae	All parts	Ethiopia	[19]
Acokanthera spp.	Apocynaceae	Entire plant	USA	[20]
Aconitum carmichaelii Debeaux	Ranunculaceae	Root tuber	China	[17]
Aconitum chasmanthum Stapf ex Holmes	Ranunculaceae	Seeds	Himalaya	[21]
Aconitum napellus (L.)	Ranunculaceae	All parts, especially dried tuberous roots	India, Europe, Pakistan	[14, 22, 23]
Aconitum sinomontanum Nakai	Ranunculaceae	Roots		
Aconitum spp.	Ranunculaceae	All parts, especially roots and seeds	Canada, USA, China, Tibet	[20, 24–26]
Aconitum vulparia Rchb.	Ranunculaceae	Roots	Morocco	[6]

Actaea spp.	Ranunculaceae	All parts, especially roots and berries	Canada	[24]
Adenanthera microsperma Teijsm. & Binn.	Leguminosae	Seeds, roots, barks, and leaves	China	[17]
Adenium obesum (Forssk.) Roem. & Schult.	Apocynaceae	Juice	Ethiopia	[19]
Adenium somalense Balf.				
Adenopus breviflorus Benth.	Cucurbitaceae	Fruit	Nigeria	[13]
Adonis aestivalis (L.)	Ranunculaceae	Leaves and stems	Morocco	[6]
Adonis spp.	Ranunculaceae	All parts, especially leaves and stems	Canada, USA	[20, 24]
Aerva lanata (L.) Juss.	Amaranthaceae	Leaves	Nigeria	[13]
Aesculus glabra Willd.	Sapindaceae	All parts, especially mature fruit	Canada	[24]
Aesculus hippocastanum (L.)	Sapindaceae	All parts, especially seeds	India	[14]
Aesculus spp.	Sapindaceae	Leaves, seeds, and flowers	USA	[27]
Agapanthus spp.	Amaryllidaceae	—	New Zealand	[28]
Agave americana (L.)	Asparagaceae	Century plant, roots, and leaf juice	Pakistan, USA	[20, 23, 29]
Agelanthus brunneus (Engl.)	Loranthaceae	Leaves	Nigeria	[13]
Aglaonema spp.	Araceae	Aboveground parts	USA	[20]
Agrostemma githago (L.)	Caryophyllaceae	Seeds	Turkey	[16]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Ailanthus altissima (Mill.) Swingle	Simaroubaceae	Leaves and flowers	USA	[20]
Akebia spp.	Lardizabalaceae	—	China	[25]
Alangium chinense (Lour.) Harms.	Cornaceae	Roots and root bark	New Zealand	[28]
Alkanna orientalis (L.) Boiss.	Boraginaceae	Entire plant	Turkey	[16]
Alocasia acuminata Schott	Araceae	All parts	India	[14]
Alocasia macrorrhizos (L.) G.Don	Araceae	Stems	China	[17]
Aloe andra (L.) Burm.f.	Leguminosae	Entire plant	Turkey	[16]
Aloe barbadensis Mill. *Aloe vera* (L.) Burm.f.	Xanthorrhoeaceae	Leaves, stems, and latex	Pakistan	[23]
Alstonia scholaris (L.) R.Br.	Apocynaceae	Bark, leaves, and pollen	India, China	[15]
Alternanthera repens J.F.Gmel.	Amaranthaceae	Leaves	Nigeria	[13]
Amaranthus retroflexus (L.) *Amaranthus hybridus* (L.)	Amaranthaceae	Entire plant especially foliage	Pakistan, Turkey	[16, 23]
Amaranthus spinosus (L.)	Amaranthaceae	Leaves and stems	Nigeria	[13]
Amaryllis belladonna (L.)	Amaryllidaceae	Underground parts	USA	[20]
Amianthium muscitoxicum (Walter) A.Gray	Melanthiaceae	Bulbs, fruit, and leaves	Europe	[22]
Amorphophallus rivieri Durieu ex Carrière	Araceae	Tuber	China	[17]

Ampelopsis japonica (Thunb.) Makino	Vitaceae	Root tuber	China	[17]
Amydrium sinense (Engl.) H.Li	Araceae	Entire plant	China	[17]
Anacardium occidentale (L.)	Anacardiaceae	Seeds	Nigeria, India	[13, 15]
Anagallis arvensis (L.)	Primulaceae	Entire plant	Pakistan, Cyprus	[16, 23, 29]
Anagyris foetida (L.)	Leguminosae	Seeds	Morocco, Turkey	[6, 16]
Ananas comosus (L.) Merr.	Bromeliaceae	Peels	Nigeria	[13]
Anemone hupehensis (Lemoine) Lemoine	Ranunculaceae	All parts, especially roots	China	[17]
Anemone rivularis Buch.-Ham. ex DC.	Ranunculaceae	All parts, especially roots	China	[17]
Anisodus tanguticus (Maxim.) Pascher	Solanaceae	Roots	Tibet	[26]
Anemone tuberosa Rydb.	Ranunculaceae	Entire plant, especially flowers	USA	[20]
Annona squamosa (L.)	Annonaceae	Seeds	India	[15]
Anthurium spp.	Araceae	Entire plant	USA	[20]
Antiaris toxicaria Lesch.	Moraceae	Leaves, bark, and sap/latex	India, Nigeria, China	[13, 14, 17]
Apium graveolens (L.)	Apiaceae	Sap and aboveground parts	USA	[20]
Arceuthobium spp.	Santalaceae	Entire plant	USA	[20]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Argemone mexicana (L.)	Papaveraceae	All parts, especially seeds, fruits, and leaves	India, Nigeria	[13–15]
Arisarum vulgare Targ.-Tozz.	Araceae	—	Cyprus	[16]
Arisaema consanguineum Schott	Araceae	Corm	China	[17]
Arisaema erubescens (Wall.) Schott	Araceae	Tuber	China	[17]
Arisaema fargesii Buchet	Araceae	Tuber	China	[17]
Arisaema rhizomatum C.E.C.Fisch.	Araceae	Tuber	China	[17]
Arisaema triphyllum (L.) Schott	Araceae	Entire plant	USA	[20]
Aristolochia bottae Jaub. & Spach	Aristolochiaceae	Entire plant	Turkey	[16]
Aristolochia debilis Siebold & Zucc.	Aristolochiaceae	Roots	China	[17]
Aristolochia griffithii Hook.f. & Thomson ex Duch.	Aristolochiaceae	Roots	China	[17]
Aristolochia longa (L.)	Aristolochiaceae	Roots	Morocco	[6]
Aristolochia tubiflora Dunn	Aristolochiaceae	Entire plant, especially roots	Brazil	[30]
Arum detruncatum C.A.Mey. ex Schott	Araceae	Leaves, fruit, and tubers	Turkey	[16]

Arum dioscoridis Sm.	Araceae	—	Cyprus	[16]
Arum hydrophilum Boiss.	Araceae	—	Cyprus	[16]
Arum italicum Mill.	Araceae	Entire plant, especially leaves	Turkey	[16]
Arum maculatum (L.)	Araceae	Entire plant	USA	[20]
Artemisia absinthium (L.)	Compositae	All parts	Pakistan, Turkey	[16, 23]
Artemisia nova A.Nelson	Compositae	—	USA	[31]
Artemisia spp.	Compositae	Leaves	USA	[20]
Asarum sieboldii Miq.	Aristolochiaceae	Entire plant, especially roots	China	[17]
Asarum wulingense C.F.Liang	Aristolochiaceae	Entire plant, especially roots	China	[17]
Astragalus spp.	Leguminosae	Seeds	USA	[31]
Arisaema triphyllum (L.) Schott	Araceae	Seeds	Himalaya	[21]
Asclepias curassavica (L.)	Apocynaceae	Entire plant, especially juice and roots	Pakistan	[29]
Asclepias spp.	Apocynaceae	Entire plant	USA	[20, 31]
Asparagus africanus Lam.	Asparagaceae	Berries	Nigeria	[13]
Asparagus densiflorus (Kunth) Jessop	Asparagaceae	Aboveground parts	USA	[20]
Asparagus officinalis (L.)	Asparagaceae	Aboveground parts	USA, Pakistan, India	[20, 23, 32]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Atractylis gummifera Salzm. ex (L.)	Compositae	Roots	Morocco	[6]
Atropa belladonna (L.)	Solanaceae	All parts	India, Spain, Morocco	[6, 14, 33]
Baileya multiradiata Harv. & A.Gray ex Torr.	Compositae	Entire plant	USA	[20]
Bambusa vulgaris Schrad.	Poaceae	Leaves	Nigeria	[13]
Begonia semperflorens Link & Otto	Begoniaceae	Rhizomes, tubers, and roots	Pakistan	[23]
Bellis perennis (L.)	Compositae	Entire plant	Turkey	[16]
Bidens pilosa (L.)	Compositae	Spikes	Nigeria	[13]
Blighia sapida K.D.Koenig	Sapindaceae	Leaves	Nigeria	[13]
Bowiea volubilis Harv.	Asparagaceae	—	South Africa	[34]
Brassica nigra (L.) K.Koch	Brassicaceae	Seeds and underground parts	USA	[20]
Brassica oleracea (L.)	Brassicaceae	Flowers, roots, and leaves	Pakistan	[23]
Bryonia alba (L.)	Cucurbitaceae	Leaves	Turkey	[16]
Bryonia dioica Jacq.	Cucurbitaceae	Fruit and leaves	Morocco	[6]
Butea monosperma (Lam.) Taub.	Leguminosae	All parts, particularly seeds	India, Pakistan	[15, 29]
Buxus microphylla Siebold & Zucc.	Buxaceae	Flowers and stems	USA	[20]

Buxus sempervirens (L.)	Buxaceae	Flowers, roots, leaves, and stems	USA, Turkey	[16, 17, 20]
Caesalpinia minax Hance	Leguminosae	Seeds	China	[17]
Caesalpinia spp.	Leguminosae	Entire plant	USA	[20]
Caladium bicolor (Aiton) Vent.	Araceae	Entire plant	USA, India	[20, 32]
Calla palustris (L.)	Araceae	All parts, particularly rhizomes	Canada	[24]
Calotropis procera (Aiton) Dryand.	Apocynaceae	Latex and leaves	India, Nigeria, Pakistan	[10, 13–15, 29]
Caltha palustris (L.)	Ranunculaceae	All parts, except young plants	Canada	[24]
Camellia oleifera Abel	Theaceae	Seeds	China	[17]
Cannabis indica (Lam.) Cannabis sativa (L.)	Cannabaceae	Leaves, fruits, flowers, top of female plant, resin of leaves, seeds, and stems	India, Europe, Nigeria, Pakistan, Himalaya, Turkey, Morocco	[10, 13–16, 21–23, 29, 35]
Cannabis spp.	Cannabaceae	Leaves	Spain	[33]
Canarium schweinfurthii Engl.	Burseraceae	Leaves	Nigeria	[13]
Capsicum annuum (L.) *Capsicum frutescens* (L.)	Solanaceae	Roots, fruit, and seeds	Nigeria, USA, Pakistan	[13, 27, 29]
Caragana arborescens (Lam.)	Leguminosae	Seeds	Canada	[24]
Carica papaya (L.)	Caricaceae	Latex	India	[15]
Carpesium abrotanoides (L.)	Compositae	Entire plant	China	[17]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Cassia spp.	Leguminosae	—	Ethiopia	[19]
Cassytha filiformis (L.)	Lauraceae	Entire plant	China	[17]
Catharanthus roseus (L.) G.Don	Apocynaceae	Roots and leaves	India, Pakistan	[15, 29]
Ceiba pentandra (L.) Gaertn.	Malvaceae	Stems	Nigeria	[13]
Centaurea spp.	Compositae	—	USA	[31]
Centaurea urvillei DC.	Compositae	Entire plant	Turkey	[16]
Centella asiatica (L.) Urb.	Apiaceae	Entire plant	Pakistan	[29]
Celastrus scandens (L.)	Celastraceae	Leaves, seeds, and roots	Canada	[24]
Cephalanthus occidentalis (L.)	Rubiaceae	Leaves	USA	[20]
Cerbera odollam Gaertn.	Apocynaceae	Fruit, sap, and seed	India, Nigeria	[13, 14]
Cerbera thevetia (L.)	Apocynaceae	All parts, especially leaves and fruits	India	[14]
Cestrum nocturnum (L.)	Solanaceae	All parts	Pakistan	[29]
Cestrum spp.	Solanaceae	Leaves and stems	USA	[20]
Chamaedorea spp.	Arecaceae	Fruit	USA	[20]
Chelidonium majus (L.)	Papaveraceae	Entire plant	China	[17]
Chenopodium ambrosioides (L.)	Amaranthaceae	Oil	Pakistan, Brazil	[23, 30]
Chenopodium album (L.)	Amaranthaceae	Entire plant, especially roots	USA, Morocco	[20]

Chimaphila umbellata (L.) Nutt.	Ericaceae	Leaves and stems	Canada	[24]
Chloranthus erectus (Buch.-Ham.) Verdc.	Chloranthaceae	Entire plant	China	[17]
Chloranthus henryi Hemsl.	Chloranthaceae	Roots	China	[17]
Chloranthus holostegius (Hand.-Mazz.) S.J.Pei & Shan	Chloranthaceae	Leaves and roots	China	[17]
Chlorophytum spp.	Asparagaceae	—	USA	[27]
Chromolaena odorata (L.) R.M.King & H.Rob.	Compositae	Leaves	Nigeria	[13]
Chrysanthemum spp.	Compositae	Aboveground parts	USA	[20, 27]
Chrysophyllum albidum G.Don	Sapotaceae	Unripe fruits	Nigeria	[13]
Cicuta virosa (L.)	Apiaceae	—	China	[25]
Cicuta spp.	Apiaceae	Leaves and roots	Canada, USA	[24, 31]
Cionura erecta (L.) Griseb.	Apocynaceae	Entire plant	Turkey	[16]
Cistus laurifolius (L.)	Cistaceae	Entire plant	Turkey	[16]
Citrullus colocynthis (L.) Schrad.	Cucurbitaceae	Fruit, roots, and dried pulps	India, Nigeria, Pakistan, Morocco	[6, 13, 14, 29]
Cleistanthus collinus (Roxb.) Benth. ex Hook.f.	Phyllanthaceae	Leaves and bark	India, Turkey	[14, 16]
Clematis spp.	Ranunculaceae	All parts	Canada, USA	[20, 24]
Clematis vitalba (L.)	Ranunculaceae	Leaves	USA	[20]
Cnidoscolus aconitifolius (Mill.) I.M.Johnst.	Euphorbiaceae	Leaves and sap	Nigeria	[13]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Colchicum autumnale (L.)	Colchicaceae	All parts, especially bulbs, seeds, and capsules	Europe, Canada, USA, Morocco, Turkey	[6, 16, 20, 22, 24]
Colchicum atticum Spruner ex Tommas.	Colchicaceae	Seeds	New Zealand	[28]
Colchicum luteum Baker	Colchicaceae	All parts, especially corms and seeds	Pakistan	[29]
Colocasia antiquorum Schott	Araceae	Entire plant	USA	[20]
Colocasia esculenta (L.) Schott	Araceae	Leaves and rhizomes	Nigeria, China, New Zealand	[13, 17, 28]
Conium maculatum (L.)	Apiaceae	All parts	India, Europe, USA, Morocco, New Zealand	[6, 14, 20, 22, 28, 31]
Consolida ambigua (L.) P.W.Ball & Heywood	Ranunculaceae	All parts, especially seeds	Pakistan	[29]
Conyza canadensis (L.) Cronquist	Compositae	Leaves	Pakistan	[29]
Convallaria majalis (L.)	Asparagaceae	All parts, especially seeds	Canada, USA, Himalaya, Turkey	[16, 20, 21, 24]
Convolvulus arvensis (L.)	Convolvulaceae	Roots	Pakistan	[10]
Corchorus olitorius (L.)	Malvaceae	Roots	Nigeria	[13]

Coriaria myrtifolia (L.)	Coriariaceae	Stems, branches, and fruits	Europe	[22]
Coriaria nepalensis Wall.	Coriariaceae	—	China	[25]
Coriaria sinica Maxim.	Coriariaceae	—	China	[17]
Coronilla coronata (L.)	Leguminosae	Leaves and seeds	Turkey	[16]
Coronilla emerus Boiss (L.)	Leguminosae	Leaves and seeds	Turkey	[16]
Coronilla scorpioides (L.) Koch	Leguminosae	Leaves and seeds	Turkey	[16]
Coronilla varia (L.)	Leguminosae	Leaves and seeds	Turkey	[16]
Corynocarpus laevigatus	Corynocarpaceae	Seeds	USA, New Zealand	[20, 28]
Costus speciosus (J.König) Sm.	Costaceae	Rhizomes	China	[17]
Cotyledon spp.	Crassulaceae	—	South Africa	[34]
Crassula spp.	Crassulaceae	—	USA	[27]
Cremastra appendiculata (D.Don) Makino	Orchidaceae	Bulbs	China	[17]
Crinum asiaticum (L.)	Amaryllidaceae	Underground parts	USA	[20]
Crotalaria retusa (L.)	Leguminosae	—	Ethiopia	[19]
Crotalaria spectabilis Roth	Leguminosae	—	India	[14]
Crotalaria spp.	Leguminosae	Entire plant	USA	[20]
Croton caudatus Geiseler	Euphorbiaceae	Entire plant	China	[17]
Croton macrostachyus Hochst. ex Delile.	Euphorbiaceae	Seeds and resins	Ethiopia	[19]
Croton penduliflorus Hutch.	Euphorbiaceae	Seeds and leaves	Nigeria	[13]
Croton tiglium (L.)	Euphorbiaceae	Seeds and leaves	China	[17]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Croton zambesicus Müll.Arg.	Euphorbiaceae	Seeds and leaves	Nigeria	[13]
Cucumis melo (L.)	Cucurbitaceae	Fruit and sap	Nigeria	[13]
Cucumis prophetarum (L.)	Cucurbitaceae	Fruit	Ethiopia	[19]
Cucumis trigonus Roxb.	Cucurbitaceae	Roots	India	[15]
Curculigo orchioides Gaertn.	Hypoxidaceae	Rhizomes	China	[17]
Cuscuta reflexa Roxb.	Convolvulaceae	Entire plant	India	[15]
Cyathula prostrata (L.) Blume	Amaranthaceae	Leaves	Nigeria	[13]
Cycas revoluta Thunb.	Cycadaceae	All parts, especially seeds or "nuts"	Pakistan	[23]
Cyclamen purpurascens Mill.	Primulaceae	Entire plant	USA	[20]
Cynanchum acutum (L.)	Apocynaceae	Entire plant	Turkey	[16]
Cynoglossum montanum (L.)	Boraginaceae	Entire plant	Turkey	[16]
Cypripedium calceolus (L.)	Orchidaceae	Leaves and stems	Canada	[24]
Cypripedium spp.	Orchidaceae	Leaves and stems	USA	[20]
Dacryodes edulis (G.Don) H.J.Lam	Burseraceae	Bark	Nigeria	[13]
Daphne gnidium (L.)	Thymelaeaceae	Leaves	Morocco	[6]
Daphne laureola (L.)	Thymelaeaceae	Leaves	Morocco	[6]
Daphne spp.	Thymelaeaceae	All parts, especially stems	Canada, USA	[20, 24]
Datura alba F.Muell.	Solanaceae	Seeds	India	[15]

Datura arborea (L.)	Solanaceae	All parts	Europe	[22]
Datura fastuosa (L.)	Solanaceae	All parts, especially seeds and fruits	India, Pakistan	[14, 29]
Datura innoxia Mill.	Solanaceae	All parts, especially seeds and juice	Pakistan, Cyprus	[16, 29]
Datura meteloides DC. ex Dunal	Solanaceae	All parts	Europe	[22]
Datura stramonium (L.)	Solanaceae	All parts, especially leaves, roots, and fruit	Europe, Pakistan, USA, Spain, Morocco, India, China, Turkey, Cyprus, Tibet	[6, 16, 17, 20, 22, 23, 25, 26, 29, 32, 33, 35]
Datura spp.	Solanaceae	—	China	[25]
Daucus carota (L.)	Apiaceae	Sap	USA	[20]
Delphinium brunonianum Royle	Ranunculaceae	Seeds	Himalaya	[21]
Delphinium spp.	Ranunculaceae	All parts, young plants, seeds, and aboveground parts	USA, South Africa	[20, 31, 34]
Delphinium virescens Nutt.	Ranunculaceae	Entire plant	USA	[20]
Dianthus calocephalus Boiss.	Caryophyllaceae	Entire plant	Turkey	[16]
Dianthus spp.	Caryophyllaceae	Leaves and underground parts	USA	[20]
Dicentra spp.	Papaveraceae	All parts	Canada	[24]
Dictamnus albus (L.)	Rutaceae	All parts, especially seed pods and plant juices	Canada, USA	[20, 24]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Dieffenbachia spp.	Araceae	All parts, especially leaves and underground parts	India, USA, Spain	[14, 20, 27, 33]
Dieffenbachia picta Schott *Dieffenbachia seguine* (Jacq.) Schott	Araceae	All parts, especially plant juice	Pakistan, India	[29, 32]
Digitalis purpurea (L.)	Plantaginaceae	All parts, especially seed, leaves, and twigs	India, Canada, USA, Pakistan, Morocco, South Africa, China, New Zealand	[14, 18, 20, 23–25, 28]
Dioclea reflexa Hook.f.	Leguminosae	Leaves	Nigeria	[13]
Dioscorea bulbifera (L.)	Dioscoreaceae	Fruit and tuber	Nigeria, China	[13, 17]
Dioscorea cirrhosa Lour.	Dioscoreaceae	Tuber	China	[17]
Dioscorea dumetorum (Kunth) Pax	Dioscoreaceae	Tuber	Nigeria	[13]
Dioscorea hispida Dennst.	Dioscoreaceae	Tuber	China	[17]
Dioscorea praehensilis Benth.	Dioscoreaceae	Tuber	Nigeria	[13]
Dipterocarpus turbinatus C.F.Gaertn	Dipterocarpaceae	Leaves	Brazil	[30]
Dracunculus vulgaris Schott	Araceae	Entire plant	Turkey	[16]
Drimia sanguinea (Schinz) Jessop	Asparagaceae	—	South Africa	[18, 34]

Dryopteris filix-mas (L.) Schott	Dryopteridaceae	Entire plant, especially juice	Pakistan, Turkey	[23]
Duchesnea indica (Andrews) Focke	Rosaceae	Entire plant	New Zealand	[28]
Duranta repens (L.)	Verbenaceae	Fruit and green parts	Pakistan, USA	[20, 29]
Dysosma difformis (Hemsl. & E.H.Wilson) T.H.Wang	Berberidaceae	Rhizomes and roots	China	[17]
Dysosma majorensis (Gagnep.) T.S.Ying	Berberidaceae	Rhizomes and roots	China	[17]
Dysosma veitchii (Hemsl. & E.H.Wilson) Fu ex Ying	Berberidaceae	Rhizomes and roots	China	[17]
Dysosma versipellis (Hance) M. Cheng ex T.S.Ying	Berberidaceae	Rhizomes and roots	China	[17]
Ecballium elaterium (L.) A.Rich.	Cucurbitaceae	Fruit	Turkey	[16]
Echium vulgare (L.)	Boraginaceae	Leaves and stems	USA	[20, 31]
Echium italicum (L.)	Boraginaceae	Entire plant	Turkey	[16]
Ehretia cymosa Thonn.	Boraginaceae	Fruit	Nigeria	[13]
Elaeis guineensis Jacq.	Arecaceae	Seeds	Nigeria	[13]
Entada gigas (L.) Fawc. & Rendle	Leguminosae	Leaves and fruit	Nigeria	[13]
Eomecon chionantha Hance	Papaveraceae	Entire plant	Brazil	[30]
Epipremnum aureum (Linden & André) G.S.Bunting	Araceae	Leaves	USA, Spain	[20, 27, 33]
Epipremnum pinnatum (L.) Engl. Pinellia	Araceae	Entire plant	China	[17]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Eriobotrya japonica (Thunb.) Lindl.	Rosaceae	Leaves and seeds	USA	[20]
Erythrophleum suaveolens (Guill. & Perr.) Brenan	Leguminosae	Roots and bark	Nigeria	[13]
Erythrina arborescens Roxb.	Leguminosae	Bark	China	[17]
Erythroxylum coca Lam.	Erythroxylaceae	Leaves	India	[14]
Eschscholzia californica Cham.	Papaveraceae	Entire plant	USA	[20]
Euadenia trifoliolata (Schumach. & Thonn.) Oliv.	Capparaceae	Leaves	Nigeria	[13]
Eucalyptus globulus Labill.	Myrtaceae	Oil of the plant	Pakistan, USA	[27, 29]
Euonymus europaeus (L.)	Celastraceae	All parts, especially leaves and fruit	USA, Turkey	[16, 20]
Euonymus latifolius	Celastraceae	Entire plant	Turkey	[16]
Euonymus spp.	Celastraceae	Leaves, barks, and fruit	Canada	[24]
Eupatorium chinense (L.)	Compositae	Entire plant	China	[17]
Eupatorium rugosum DC.	Compositae	Leaves and seeds	USA	[20]
Euphorbia amygdalina (L.)	Euphorbiaceae	Leaves, seeds, and latex	Turkey	[16]
Euphorbia antiquorum (L.)	Euphorbiaceae	Leaves and stems	China	[17]
Euphorbia cyparissias (L.)	Euphorbiaceae	Entire plant	Turkey	[16]
Euphorbia deightonii Croizat	Euphorbiaceae	Leaves and sap	Nigeria, Pakistan	[13, 29]

Euphorbia falcata (L.)	Euphorbiaceae	All parts, especially resins	Turkey	[16]
Euphorbia grandicornis Goebel ex N.E.Br.	Euphorbiaceae	Flowers, stems, and sap	USA	[20]
Euphorbia helioscopia (L.)	Euphorbiaceae	Milky latex, leaves, and seeds	India, Pakistan, Cyprus	[10, 14, 16, 23]
Euphorbia hirta (L.)	Euphorbiaceae	Sap	Nigeria	[13]
Euphorbia kamerunica Pax	Euphorbiaceae	Latex	Nigeria	[13]
Euphorbia lactea Haw.	Euphorbiaceae	Flowers, stems, and sap	USA	[20]
Euphorbia lateriflora Schumach.	Euphorbiaceae	Roots and sap	Nigeria	[13]
Euphorbia lathyris (L.)	Euphorbiaceae	Entire plant, especially seeds	China	[17]
Euphorbia marginata Pursh	Euphorbiaceae	Latex	Morocco	[6]
Euphorbia milii Des Moul.	Euphorbiaceae	Entire plant	USA, Pakistan	[20]
Euphorbia peplus (L.)	Euphorbiaceae	Entire plant	Turkey	[16]
Euphorbia pulcherrima Willd. ex Klotzsch	Euphorbiaceae	Leaves and latex	Pakistan, USA	[20, 27, 29]
Euphorbia seguieriana Neck	Euphorbiaceae	Entire plant	Turkey	[16]
Euphorbia spp.	Euphorbiaceae	Sap	USA, Ethiopia, New Zealand	[20, 28]
Euphorbia tirucalli (L.)	Euphorbiaceae	All parts	Ethiopia	[19]
Euphorbia unispina N.E.Br.	Euphorbiaceae	Sap	Nigeria	[13]
Equisetum arvense (L.)	Equisetaceae	Young shoots	Turkey	[16]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Equisetum spp.	Equisetaceae	—	Europe	[22]
Epilobium hirsutum (L.)	Onagraceae	Entire plant	Pakistan	[29]
Fadogia homblei De Wild.	Rubiaceae	—	South Africa	[34]
Ferula communis (L.)	Apiaceae	Resin	Morocco, Cyprus	[6, 16]
Ficus exasperata Vahl	Moraceae	Leaves, sap, and roots	Nigeria	[13]
Ficus spp.	Moraceae	Sap	USA	[20, 27]
Flacourtia indica (Burm.f.) Merr.	Salicaceae	Leaves	Nigeria	[13]
Funtumia elastica (Preuss) Stapf	Apocynaceae	Latex	Nigeria	[13]
Galega officinalis (L.)	Leguminosae	Hay	Europe	[22]
Gaultheria leucocarpa Bl. var. *crenulata* (Kurz) T.Z.Hsu	Ericaceae	Entire plant, especially roots	China	[17]
Gelsemium elegans (Gardner & Chapm.) Benth.	Gelsemiaceae	Entire plant, especially roots	China	[17, 25]
Gelsemium sempervirens (L.) J.St.-Hil.	Gelsemiaceae	Entire plant	USA	[20]
Genista tinctoria (L.)	Leguminosae	Entire plant	Turkey	[16]
Gentiana gelida Bied	Gentianaceae	Entire plant	Turkey	[16]
Ginkgo biloba (L.) (female plants)	Ginkgoaceae	Fruit	USA, China	[17, 20, 25]
Glochidion puberum (L.) Hutch.	Euphorbiaceae	Roots and fruit	China	[17]
Gloriosa spp.	Colchicaceae	Entire plant	USA	[20]

Gloriosa superba (L.)	Colchicaceae	Tubers, roots, berries/ leaves	India, Nigeria, China	[13–15, 17]
Glycine max (L.) Merr.	Leguminosae	—	China	[25]
Glyryrrhiza glabra (L.)	Leguminosae	Entire plant	Turkey	[16]
Gouania leptostachya DC.	Rhamnaceae	Stems, leaves, and roots	China	[17]
Gutierrezia spp.	Compositae	Entire plant	USA	[31]
Gynura procumbens (Lour.) Merr.	Compositae	Entire plant	China	[17]
Halogeton glomeratus (M.Bieb.) Ledeb.	Amaranthaceae	Entire plant	USA	[31]
Haplopappus heterophyllus (A.Gray) S.F.Blake	Compositae	Roots	USA	[31]
Hedera helix (L.)	Araliaceae	Leaves and fruit	USA	[20, 27]
Helenium autumnale (L.)	Compositae	All parts	Canada	[24]
Helenium ssp.	Compositae	Entire plant	USA	[20]
Helianthus annuus (L.)	Compositae	Hair, and leaves	Pakistan	[23]
Heliotropium spp.	Boraginaceae	Leaves	USA	[31]
Helleborus spp.	Melanthiaceae	Entire plant	USA	[20]
Hemerocallis spp.	Xanthorrhoeaceae	—	China	[25]
Heracleum canescens Lindl.	Apiaceae	Seeds	Himalaya	[21]
Heracleum lanatum Michx.	Apiaceae	Leaves	Canada	[24]
Heteromeles arbutifolia Greene	Rosaceae	Leaves	USA	[20]
Hippeastrum spp.	Amaryllidaceae	Underground parts	USA	[20]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Hosta plantaginea (Lam.) Asch.	Asparagaceae	Flowers	China	[17]
Huperzia serrata (Thunb.) Trevis.	Lycopodiaceae	Entire plant	China	[17]
Hyacinthus orientalis (L.)	Asparagaceae	All parts, especially bulbs and underground parts	Canada, USA	[20, 24]
Hybanthus enneaspermus (L.) F.Muell.	Violaceae	Leaves	Nigeria	[13]
Hydrangea spp.	Hydrangeaceae	All parts	Canada, USA	[20, 24]
Hymenocardia acida Tul.	Phyllanthaceae	Leaves	Nigeria	[13]
Hymenocallis aviaricana (L.)	Amaryllidaceae	Underground parts	USA	[20]
Hypericum perforatum (L.)	Hypericaceae	All parts, especially leaves, stems, and petals	Europe, USA, Turkey	[16, 20, 22]
Hypericum triquetrifolium Turra	Hypericaceae	—	Cyprus	[16]
Hyoscyamus albus (L.)	Solanaceae	—	Cyprus	[16]
Hyoscyamus aureus (L.)	Solanaceae	—	Cyprus	[16]
Hyoscyamus niger (L.)	Solanaceae	Resin and seeds	Morocco, Himalaya	[6]
Ilex aquifolium (L.)	Aquifoliaceae	Fruit and leaves	USA, Turkey	[16, 20]
Ilex spp.	Aquifoliaceae	Fruit	USA	[20, 27]
Illicium spp.	Schisandraceae	—	China	[25]
Impatiens uliginosa Franch.	Balsaminaceae	Entire plant	China	[17]

Impatiens spp.	Balsaminaceae	Entire plant	USA	[20]
Ipomoea alba (L.)	Convolvulaceae	Seeds	USA	[20]
Ipomoea tricolor Cav.	Convolvulaceae	Seeds, leaves, and stems	USA, Pakistan	[20, 23]
Ipomoea purpurea (L.) Roth	Convolvulaceae	Seeds	Pakistan	[29]
Ipomoea spp.	Convolvulaceae	Seeds	Canada	[24]
Iris pseudacorus (L.)	Iridaceae	Rhizomes	Morocco	[6]
Iris spp.	Iridaceae	Leaves, rhizomes, underground parts, and plant juices	Canada, USA	[20, 24]
Iris foetidissima (L.)	Iridaceae	—	New Zealand	[28]
Iris tectorum Maxim.	Iridaceae	Rhizomes	China	[17]
Jasminum officinale (L.)	Oleaceae	Berries	Pakistan	[23]
Jatropha gossypiifolia (L.)	Euphorbiaceae	Seeds	India	[15]
Jatropha curcas (L.)	Euphorbiaceae	Leaves, bark, and latex	Nigeria, Pakistan, China	[13, 17, 23]
Jatropha multifida (L.)	Euphorbiaceae	Seeds and sap	Pakistan	[23]
Juglans spp.	Juglandaceae	Sap	USA	[20]
Juniperus communis (L.)	Cupressaceae	Fruit	Turkey	[16]
Juniperus excelsa M.Bieb.	Cupressaceae	Entire plant	Turkey	[16]
Juniperus oxycedrus (L.)	Cupressaceae	Essential oil	Morocco	[35]
Juniperus spp.	Cupressaceae	Berries	Canada	[24]
Justicia adhatoda (L.)	Acanthaceae	All parts	Pakistan	[29]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Justicia pectoralis Jacq.	Acanthaceae	—	Brazil	[30]
Kalanchoe blossfeldiana Poelln.	Crassulaceae	Flowers	Brazil	[30]
Kalanchoe spp.	Crassulaceae	—	South Africa	[34]
Kalmia latifolia (L.)	Ericaceae	Leaves	USA	[20]
Kalmia polifolia Wangenh.	Ericaceae	All parts	Canada	[24]
Kigelia africana (Lam.) Benth.	Bignoniaceae	Stems	Nigeria	[13]
Kochia prostrata (L.) Schrad.	Amaranthaceae	Entire plant	Turkey	[16]
Kopsia officinalis Tsiang & P.T.Li	Apocynaceae	Fruit	China	[17]
Laburnum anagyroides Medik.	Leguminosae	Leaves	USA	[20]
Lagenaria siceraria (Molina) Standl.	Cucurbitaceae	—	China	[25]
Lantana camara (L.)	Verbenaceae	Entire plant, especially the berries	India, Nigeria, South Africa, Pakistan, USA	[13–15, 20, 34]
Lantana spp.	Verbenaceae	Entire plant	USA	[20]
Lannea welwitschii (Hiern) Engl.	Anacardiaceae	Leaves	Nigeria	[13]
Laportea aestuans (L.) Chew	Urticaceae	Fresh leaves	Turkey	[16]
Laportea interrupta (L.) Chew	Urticaceae	Leaves and fruit	India	[32]
Lathyrus aphaca (L.)	Leguminosae	Seeds	Turkey	[16]
Lathyrus latifolius (L.)	Leguminosae	Seeds	Canada	[24]
Lathyrus odoratus (L.)	Leguminosae	Entire plant, especially seeds	Canada, USA, Pakistan	[20, 23, 24]

Lathyrus sativus (L.)	Leguminosae	Seeds	India, Pakistan, Turkey	[15, 16, 23]
Lathyrus spp.	Leguminosae	Pea-like seeds and foliage	Pakistan	[23]
Laurus nobilis (L.)	Lauraceae	Leaves, fruit, and stems	USA	[20]
Lawsonia inermis (L.)	Lythraceae	—	Morocco	[35]
Leontice leontopetalum (L.)	Berberidaceae	Entire plant	Cyprus	[16]
Ligustrum lucidum W.T.Aiton	Oleaceae	All parts, especially berries	Pakistan	[29]
Ligustrum spp.	Oleaceae	Leaves and fruit	USA	[20]
Linum usitatissimum (L.)	Linaceae	Entire plant, especially seeds	USA	[20]
Litsea glutinosa (Lour.) C.B.Rob.	Lauraceae	Root bark, bark, and leaves	China	[17]
Lobelia clavata E.Wimm.	Campanulaceae	Leaves and roots	China	[17]
Lobelia colorata subsp. *taliensis* (Diels) T.J.Zhang & D.Y.Hong	Campanulaceae	Roots	China	[17]
Lobelia spp.	Campanulaceae	All parts	Canada, USA	[20, 24]
Lolium temulentum (L.)	Poaceae	Seeds	Pakistan, Turkey	[16, 23, 29]
Luffa cylindrica (L.) M.Roem.	Cucurbitaceae	Fruit	India	[15]
Lupinus spp.	Leguminosae	All parts, especially seeds	Europe, Canada, USA	[22, 24, 31]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Lycopersicon esculentum Mill.	Solanaceae	Green parts, leaves, and stems	Canada, Pakistan, USA	[20, 24, 29]
Macadamia ternifolia F.Muell.	Proteaceae	Leaves	USA	[20]
Macleaya cordata (Willd.) R.Br.	Papaveraceae	Entire plant	China	[17]
Maclura pomifera (Raf.) C.K.Schneid.	Moraceae	Sap	USA	[20]
Malus domestica Borkh.	Rosaceae	Leaves and seeds	USA, Pakistan	[20, 23]
Malus spp.	Rosaceae	Foliage and seeds	Canada, USA	[24, 27]
Mandragora autumnalis Bertol.	Solanaceae	Roots and leaves	Morocco	[6]
Mangifera indica (L.)	Anacardiaceae	Leaves, stems, mango peel, and sap	Pakistan	[23]
Manihot esculenta Crantz (uncooked)	Euphorbiaceae	Underground parts	USA	[20]
Manihot utilissima Pohl	Euphorbiaceae	Tubers and leaves	Nigeria	[13]
Marrubium parviflorum Fisch. & C.A.Mey.	Lamiaceae	Entire plant	Turkey	[16]
Medicago sativa (L.)	Leguminosae	Leaves and stems	Pakistan	[23]
Melia azedarach (L.)	Meliaceae	Fruit, bark, and seeds	Pakistan, USA, South Africa, Himalaya, China	[17, 21, 29, 36]
Melianthus major (L.)	Melianthaceae	—	South Africa	[34]
Melilotus alba Desr.	Leguminosae	Entire plant	Turkey	[16]
Millettia pachycarpa Benth.	Leguminosae	Leaves, roots, and seeds	China	[17]

Menispermum dauricum DC.	Menispermaceae	—	China	[25]
Mercurialis annua (L.)	Euphorbiaceae	—	Turkey	[16]
Mercurialis perennis (L.)	Euphorbiaceae	Roots, seeds, and shoots	Turkey	[16]
Mimosa pudica (L.)	Leguminosae	Leaves	Nigeria	[13]
Mirabilis jalapa (L.)	Nyctaginaceae	Entire plant, especially seeds	Pakistan, USA	[20, 23, 29]
Momordica charantia (L.)	Cucurbitaceae	Seeds and roots	Nigeria, Brazil	[13, 30]
Monstera deliciosa Liebm.	Araceae	Fruit and leaves	USA	[20]
Moraea pallida (Baker) Goldblatt	Iridaceae	Leaves and roots	South Africa	[34]
Morus spp.	Moraceae	Fruit (unripe), sap	USA	[20]
Mucuna pruriens (L.) DC.	Leguminosae	Fruits	Nigeria	[13]
Mucuna sloanei Fawc. & Rendle	Leguminosae	Sap	Nigeria	[13]
Myoporum laetum G.Forst.	Scrophulariaceae	Leaves	USA	[20]
Myristica fragrans Houtt.	Myristicaceae	Seeds	India, Europe, Morocco	[14, 22, 35]
Nandina domestica Thunb.	Berberidaceae	Fruit and roots	China	[17]
Narcissus spp.	Amaryllidaceae	All parts, especially bulbs and underground parts	Canada, USA, New Zealand	[20, 24, 28]
Narcissus tazetta (L.)	Amaryllidaceae	All parts, especially bulbs and underground parts	Pakistan, USA	[20, 23, 29]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Nerium indicum Mill. *Nerium oleander* (L.) (Synonym)	Apocynaceae	All parts, especially leaves and seeds	India, Pakistan, USA, Spain, Morocco, South Africa, Turkey, China, Cyprus, New Zealand	[6, 14–18, 27–29, 33, 35]
Newbouldia laevis (P.Beauv.) Seem.	Bignoniaceae	Leaves and roots	Nigeria	[13, 23]
Nicotiana alata Link & Otto	Solanaceae	All parts	Canada	[24]
Nicotiana glauca Graham	Solanaceae	Leaves	Cyprus	[16]
Nicotiana spp.	Solanaceae	Entire plant	USA	[20]
Nicotiana tabacum (L.)	Solanaceae	All parts, except ripe seeds and leaves	India, Pakistan, China	[14, 17, 23]
Nolletia gariepina (DC.) Mattf.	Compositae	—	South Africa	[34]
Oplopanax horridus (Sm.) Miq.	Araliaceae	Spines along leaf stalks and leaf veins	Canada	[24]
Opuntia stricta (Haw.) Haw.	Cactaceae	Roots and stems	China	[17]
Ormosia hosiei Hemsl. & E.H.Wilson	Leguminosae	Seeds	China	[17]
Ormosia nuda (F.C.How) R.H.Chang & Q.W.Yao	Leguminosae	Fruit	China	[17]

Ornithogalum spp.	Asparagaceae	Entire plant	USA	[20]
Ornithogalum umbellatum (L.)	Asparagaceae	All parts	Canada	[24]
Oxalis corniculata (L.)	Oxalidaceae	All parts	Pakistan, Cyprus	[16, 29]
Oxalis pes-carpae (L.)	Oxalidaceae	All parts	Cyprus	[16]
Oxalis pretoensis Lourteig	Oxalidaceae	All parts	Pakistan	[29]
Oxalis spp.	Oxalidaceae	All parts	USA	[20]
Oxytropis spp.	Leguminosae	Seeds	Himalaya	[21]
Pachyrhizus erosus (L.) Urb.	Leguminosae	Seeds and root tubers	China	[17]
Pachystigma latifolium Sond.	Rubiaceae	—	South Africa	[34]
Pachystigma thamnus Robyns	Rubiaceae	—	South Africa	[34]
Papaver somniferum (L.)	Papaveraceae	Petals, stems, fruit, seeds, and ripe dried capsules	India, Pakistan, USA, Morocco	[14, 15, 20, 23, 29, 35]
Papaver spp.	Papaveraceae	All parts, especially raw, green seeds; ripe poppy seeds	Canada, China	[24, 25],
Paris polyphylla Sm.	Melanthiaceae	Rhizomes	China	[17]
Parthenium hysterophorus (L.)	Compositae	All parts, especially leaves and seeds	India	[14, 15, 29]
Parthenocissus quinquefolia (L.) Planch.	Vitaceae	Berries, fruit, and leaves	Canada, USA	[20, 24]
Pavetta harborii S.Moore	Rubiaceae	—	South Africa	[34]
Pavetta schumanniana F.Hoffm. ex K.Schum.	Rubiaceae	—	South Africa	[34]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Peganum harmala (L.)	Nitrariaceae	All parts	India, Morocco	[14, 35]
Periploca forrestii Schltr.	Apocynaceae	Entire plant, especially roots	China	[17]
Periploca graeca (L.)	Apocynaceae	Entire plant	Turkey	[16]
Petiveria alliacea (L.)	Phytolaccaceae	—	Turkey	[16]
Pinellia ternata (Thunb.) Makino	Araceae	Entire plant	China	[17, 25]
Pinus spp.	Pinaceae	—	USA	[31]
Piper guineense Schumach. & Thonn.	Piperaceae	Roots	Nigeria	[13]
Pittosporum spp.	Pittosporaceae	All parts	USA	[20]
Pharbitis purpurea (L.) Voigt	Convolvulaceae	Seeds	China	[17]
Phaseolus vulgaris (L.)	Leguminosae	Entire plant, especially fruit	Spain, Pakistan, China	[25, 29, 33]
Philodendron spp.	Araceae	All parts	USA	[20, 27]
Phlomis armeniaca Willd.	Lamiaceae	Entire plant	Turkey	[16]
Phlomis pungens Willd.	Lamiaceae	Entire plant	Turkey	[16]
Phoradendron spp.	Santalaceae	All parts, especially fruit	USA	[20]
Phyllanthus amarus Schumach. & Thonn.	Phyllanthaceae	Leaves and stems	Nigeria	[13]

Physalis longifolia Nutt.	Solanaceae	Raw fruit	Canada	[24]
Physalis peruviana (L.)	Solanaceae	Raw/immature fruit	Canada, Pakistan	[23, 24]
Physostigma venenosum Balf.	Leguminosae	Bark and seeds	Nigeria	[13]
Phytolacca acinosa Roxb.	Phytolaccaceae	Roots	China	[17]
Phytolacca americana (L.)	Phytolaccaceae	—	USA	[27]
Phytolacca dodecandra L'Hér.	Phytolaccaceae	Roots	Ethiopia	[19]
Plumbago indica (L.)	Plumbaginaceae	Entire plant	China	[17]
Plumbago rosea (L.)	Plumbaginaceae	Root	India	[14]
Plumbago zeylanica (L.)	Plumbaginaceae	All parts, especially roots and leaves	Nigeria, India, China	[13, 15, 17]
Pleiocarpa pycnantha (K.Schum.) Stapf	Apocynaceae	Flowers	Nigeria	[13]
Polygonum hydropiper (L.)	Polygonaceae	Entire plant	China	[17]
Primula spp.	Primulaceae	All parts	USA	[20]
Prosopis africana (Guill. & Perr.) Taub.	Leguminosae	Seeds	Nigeria	[13]
Prunus americana Marshall	Rosaceae	Fruit	USA	[20]
Prunus amygdalus Batsch	Rosaceae	Almond	India	[14]
Prunus spp.	Rosaceae	All parts, especially fruit, leaves, and stems	Canada, USA, Pakistan	[20, 23, 24]
Psammosilene tunicoides	Caryophyllaceae	Roots	China	[17]
Pteridium aquilinum (L.) Kuhn	Dennstaedtiaceae	All parts, green or dry	Europe, Canada, USA	[20, 22, 24, 31]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Pyracantha spp.	Rosaceae	Fruit	USA	[20, 27]
Pyrus spp.	Rosaceae	Seeds	USA	[20]
Quercus prinoides Willd.	Fagaceae	Leaves and acorns	Pakistan	[23]
Quercus spp.	Fagaceae	Leaves, fruit, acorns, and oak leaves	Europe, Canada, USA	[20, 22, 24]
Quisqualis indica (L.)	Combretaceae	Fruit	China	[17]
Ranunculus grandis Honda	Ranunculaceae	Entire plant	China	[17]
Ranunculus sceleratus (L.)	Ranunculaceae	All parts	Pakistan	[29]
Ranunculcus spp.	Ranunculaceae	Fresh leaves and inflorescence	Pakistan	[23]
Raphanus sativus (L.)	Brassicaceae	Leaves and seeds	Pakistan	[23]
Rauvolfia serpentina (L.) Benth. ex Kurz	Apocynaceae	Roots	India	[15]
Rauvolfia verticillata (Lour.) Baill.	Apocynaceae	Roots	China	[17]
Rauvolfia vomitoria Afzel.	Apocynaceae	Seeds and roots	Nigeria	[13]
Remusatia vivipara (Roxb.) Schott	Araceae	Entire plant, especially tuber	China	[17]
Rhamnus spp.	Rhamnaceae	Fruit and bark	Canada, USA	[20, 24]
Rheum rhabarbarum (L.)	Polygonaceae	Leaves	Canada, New Zealand	[24, 28]
Rhus radicans (L.)	Anacardiaceae	All parts	Pakistan	[29]

Rhododendron caucasicum Pall.	Ericaceae	Flowers and leaves	Turkey	[16]
Rhododendron luteum Sweet	Ericaceae	—	Turkey	[16]
Rhododendron ponticum (L.)	Ericaceae	Flowers and leaves	Turkey	[16]
Rhododendron spp.	Ericaceae	All parts	USA	[20, 27]
Ricinus communis (L.)	Euphorbiaceae	Entire plant, especially seeds, foliage, and young seedlings and leaves	India, Europe, Turkey, Nigeria, Pakistan, Canada, USA, Spain, Morocco, China, Cyprus	[6, 10, 13–17, 20, 22–25, 29, 33]
Robinia pseudoacacia (L.)	Leguminosae	Leaves, young shoots, pods, seeds, and inner bark	Europe, Pakistan, Turkey	[16, 20, 22]
Rohdea japonica (Thunb.) Roth	Asparagaceae	Roots and rhizomes	China	[17]
Rotheca serrata (L.) Steane & Mabb.	Verbenaceae	Entire plant	China	[17]
Rudbeckia spp.	Compositae	All parts	Canada	[24]
Rumex spp.	Polygonaceae	Leaves	USA	[20]
Rumex abyssinicus Jacq.	Asparagaceae	Berries	Spain	[33]
Salvia candidissima Vahl	Lamiaceae	Entire plant	Turkey	[16]
Salvia multicaulis Vahl	Lamiaceae	Entire plant	Turkey	[16]
Salvia trichoclada Benth.	Lamiaceae	Entire plant	Turkey	[16]
Sambucus ebulus (L.)	Adoxaceae	Leaves, fruit, and cork of stem	Turkey	[16]
Sambucus nigra (L.)	Adoxaceae	Leaves, fruit, and cork of stem	Turkey	[16]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Sambucus spp.	Adoxaceae	All parts, roots and especially berries; harmless when cooked	Canada, USA	[20, 24]
Saintpaulia spp.	Gesneriaceae	Leaves and fruits	USA	[27]
Sansevieria trifasciata Prain	Asparagaceae	Leaves	China	[17]
Sapium sebiferum (L.) Roxb.	Euphorbiaceae	Latex	Pakistan	[29]
Saponaria vaccaria (L.)	Caryophyllaceae	Seeds	USA	[20]
Sarcocephalus latifolius (Sm.) E.A.Bruce	Rubiaceae	Leaves	Nigeria	[13]
Saruma henryi Oliv.	Aristolochiaceae	Roots and rhizomes	China	[17]
Saxifraga stolonifera Curtis	Saxifragaceae	Entire plant	China	[17]
Schefflera spp.	Araliaceae	Aboveground	USA	[20]
Schlumbergera bridgesii (Lem.) Loefgr.	Cactaceae	Roots	USA	[27]
Schwenkia americana Kunth	Solanaceae	Leaves	Nigeria	[13]
Scilla spp.	Asparagaceae	All parts, especially the bulbs	Canada	[24]
Schinus terebinthifolia Raddi	Anacardiaceae	—	Brazil	[30]
Scleria depressa (C.B.Clarke) Nelmes	Cyperaceae	Fruit	Nigeria	[13]
Scuttelaria orientalis (L.)	Lamiaceae	Entire plant	Turkey	[16]

Securidaca longipedunculata Fresen.	Polygalaceae	Leaves, fruits, and seeds	Ethiopia	[19]
Semecarpus anacardium (L.)	Anacardiaceae	Juice	India	[14]
Senecio jacobaea (L.)	Compositae	All parts	USA, Turkey	[16, 20]
Senecio mikanioides Otto ex Walp.	Compositae	Leaves and stems	USA	[20]
Senecio paludosus (L.)	Compositae	Entire plant	Turkey	[16]
Senecio vulgaris (L.)	Compositae	Entire plant	Turkey	[16]
Senecio spp.	Compositae	Seeds, flowers, and leaves	Europe, USA	[22, 31]
Senna hirsuta (L.) H.S.Irwin & Barneby	Leguminosae	Leaves	Nigeria	[13]
Sesbania punicea (Cav.) Benth.	Leguminosae	Seeds	USA	[20]
Sida acuta Burm.f.	Malvaceae	Leaves	Nigeria	[13]
Siegesbeckia pubescens (Makino) Makino	Compositae	Entire plant	China	[17]
Silene laxa Boiss. & Kotschy	Caryophyllaceae	Entire plant	Turkey	[16]
Silybum marianum (L.) Gaertn.	Compositae	All parts, especially seeds	Pakistan, Himalaya	[21, 29]
Sinapis arvensis (L.)	Brassicaceae	—	Turkey	[16]
Sisymbrium officinale (L.) Scop.	Brassicaceae	—	Turkey	[16]
Solandra spp.	Solanaceae	Leaves and fruit	USA	[20]
Solanum americanum Mill.	Solanaceae	Entire plant	China	[17]
Solanum dasyphyllum Schumach. & Thonn.	Solanaceae	Roots	Nigeria	[13]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Solanum dulcamara (L.)	Solanaceae	Leaves and fruit	USA	[20, 27]
Solanum elaeagnifolium Cav.	Solanaceae	—	Cyprus	[16]
Solanum hazenii Britton	Solanaceae	Roots, leaves, and fruit	China	[17]
Solanum lycopersicum (L.)	Solanaceae	Leaves and unripe fruit	India	[15]
Solanum melongena (L.)	Solanaceae	Leaves and stems	USA	[20]
Solanum nigrum (L.)	Solanaceae	All parts, including leaves, immature fruit, and unripe berries	Canada, Pakistan, Morocco, Cyprus, New Zealand	[6, 16, 23, 24, 28]
Solanum pseudocapsicum (L.)	Solanaceae	Berries	Pakistan, USA, Spain	[20, 29, 33]
Solanum sodomaeum (L.)	Solanaceae	Leaves	Morocco	[6]
Solanum spp.	Solanaceae	All parts, especially leaves and fruit	USA	[20]
Solanum torvum Sw.	Solanaceae	Roots	China	[17]
Solanum triflorum Nutt.	Solanaceae	Leaves and berries	Canada	[24]
Solanum tuberosum (L.)	Solanaceae	Spoiled potatoes	Canada, USA, Pakistan, China	[20, 23–25]
Solanum villosum Mill.	Solanaceae	—	Cyprus	[16]
Solanum xanthocarpum Schrad. & H.Wendl.	Solanaceae	Seeds	Himalaya	[21]

Solenanthus stamineus (Desf.) Wettst.	Boraginaceae	Entire plant	Turkey	[16]
Solidago decurrens Lour.	Compositae	Entire plant	China	[17]
Solidago spp.	Compositae	All parts	Canada	[24]
Sophora alopecuroides (L.)	Leguminosae	Entire plant	Turkey	[16]
Sophora japonica (L.)	Leguminosae	All parts, especially seeds	Pakistan	[29]
Sophora spp.	Leguminosae	—	New Zealand	[28]
Sorghum halepense (L.) Pers.	Leguminosae	—	Cyprus	[16]
Spartium junceum (L.)	Leguminosae	Flowers	Spain	[33]
Spathiphyllum spp.	Araceae	All parts	USA, New Zealand	[20, 28]
Spilanthes callimorpha A.H.Moore	Compositae	Entire plant	China	[17]
Spilanthes paniculata Wall. ex DC.	Compositae	Entire plant	China	[17]
Spiraea japonica L.f.	Rosaceae	Roots	China	[17]
Stachys lavandulifolia Vahl	Lamiaceae	Entire plant	Turkey	[16]
Stemona tuberosa Lour.	Stenonaceae	Root tuber	China	[17]
Strophanthus sarmentosus DC.	Apocynaceae	Leaves	Nigeria	[13]
Stephania cephalantha Hayata	Menispermaceae	Root tuber	China	[17]
Stephania epigaea H.S.Lo	Menispermaceae	Root tuber	China	[17]
Strychnos nux-vomica (L.)	Loganiaceae	All parts, especially seeds of ripe fruits and leaves	India, Nigeria, China, Tibet	[13–15, 25, 26]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Symphytum officinale (L.)	Boraginaceae	—	Brazil	[30]
Synedrella nodiflora (L.) Gaertn.	Compositae	Tuber	Nigeria	[13]
Syngonium podophyllum Schott	Araceae	All parts	USA	[20]
Tabernaemontana divaricata (L.) R.Br. ex Roem. & Schult.	Apocynaceae	Fruit and seeds	India	[15]
Talinum triangulare (Jacq.) Willd.	Talinaceae	Roots	Nigeria	[13]
Tamus communis (L.)	Dioscoreaceae	Leaves and stems	Morocco	[6]
Tanacetum balsamita (L.)	Compositae	Entire plant	Cyprus	[16]
Tanacetum vulgare (L.)	Compositae	All parts, especially leaves	Canada, USA, Turkey	[16, 20, 24]
Taraxacum officinale (L.) Weber ex F.H.Wigg.	Compositae	All parts	USA	[27]
Taxus baccata (L.)	Taxaceae	Seeds and leaves	Europe, Pakistan, Morocco	[6, 22, 23]
Taxus canadensis Marshall	Taxaceae	Entire plant	Pakistan	[23]
Taxus spp.	Taxaceae	All parts, especially seeds	Canada, USA	[20, 24, 27]
Tetracarpidium conophorum (Müll.Arg.) Hutch. & Dalziel	Euphorbiaceae	Seeds	Nigeria	[13]
Tetradymia spp.	Compositae	—	USA	[31]
Teucrium chamaedrys (L.)	Lamiaceae	Entire plant	Turkey	[16]

Teucrium pollium (L.)	Lamiaceae	Entire plant	Turkey	[16]
Thapsia garganica (L.)	Apiaceae	Roots	Morocco	[6]
Thevetia peruviana (Pers.) K. Schum.	Apocynaceae	All parts	India, Pakistan, USA, South Africa	[15, 18, 20, 29]
Thuja occidentalis (L.)	Cupressaceae	Branches	Europe	[22]
Toddalia asiatica (L.) Lam.	Rutaceae	Roots and bark	China	[17]
Toxicodendron diversilobum (Torr. & A.Gray) Greene	Anacardiaceae	All parts	USA	[20]
Tragia benthamii Baker	Euphorbiaceae	Leaves	Nigeria	[13]
Toxicodendron radicans (L.) Kuntze	Anacardiaceae	—	USA	[27]
Toxicodendron rydbergii (Small ex Rydb.) Greene	Anacardiaceae	All parts	USA	[20]
Tragia involucrata (L.)	Euphorbiaceae	Leaves	India	[15]
Tephrosia vogelii Hook.f.	Leguminosae	Seeds, leaves, and roots	Ethiopia	[19]
Tribulus terrestris (L.)	Zygophyllaceae	—	Pakistan	[10]
Trichosanthes cucumeroides (Ser.) Maxim.	Cucurbitaceae	Fruit	China	[17]
Tridax procumbens (L.) L.	Compositae	Leaves	Nigeria	[13]
Trillium spp.	Melanthiaceae	Rootstocks	Canada	[24]
Tripterygium hypoglaucum (H.Lév.) Hutch.	Celastraceae	—	China	[25]
Tulipa spp.	Liliaceae	Underground parts	USA	[20]

(*Continued*)

Table 3.1 (Continued)

Plant species	Family	Part concerned	Country	References
Tylecodon ventricosus (Burm.f.) Toelken	Crassulaceae	—	South Africa	[34]
Tylecodon wallichii (Harv.) Toelken	Crassulaceae	Leaves	South Africa	[34]
Typhonium roxburghii Schott	Araceae	Entire plant, especially tuber	China	[17]
Typhonium trilobatum (L.) Schott	Araceae	Leaves and tuber	China	[17]
Uapaca togoensis Pax	Phyllanthaceae	Leaves	Nigeria	[13]
Uraria picta (Jacq.) DC.	Leguminosae	Leaves	Nigeria	[13]
Urera obovata Benth.	Urticaceae	Leaves	Nigeria	[13]
Urginea maritima (L.) Baker	Asparagaceae	Bulbs	South Africa	[18]
Urtica dioica (L.)	Urticaceae	Leaves and stems	Nigeria, USA	[13]
Urtica ferox G.Forst.	Urticaceae	—	New Zealand	[28]
Urtica fissa E.Pritz.	Urticaceae	Entire plant	China	[17]
Valeriana jatamansi Jones	Valerianaceae	Roots and rhizomes	China	[17]
Veratrum californicum Durand	Melanthiaceae	All parts	USA	[20]
Veratrum mengtzeanum O.Loes.	Melanthiaceae	Rhizomes	China	[17]
Veratrum spp.	Melanthiaceae	Leaves and flowers	USA	[31]
Vernicia fordii (Hemsl.) Airy Shaw	Euphorbiaceae	—	China	[25]
Vernonia amygdalina Delile	Compositae	Roots	Nigeria	[13]
Vernonia condensata Baker	Compositae	—	Brazil	[30]
Veronica virginica (L.)	Compositae	Underground parts	USA	[20]
Viburnum lantana (L.)	Adoxaceae	—	Turkey	[16]

Vicia faba (L.)	Leguminosae	Seeds, raw or cooked, pollen	Canada, Turkey	[16, 24]
Vicia sativum (L.)	Leguminosae	Seeds	Turkey	[16]
Viscum album (L.)	Santalaceae	All parts, especially berries	USA, Spain	[20, 33]
Wisteria spp.	Leguminosae	All parts, especially seeds	USA	[20]
Withania somnifera (L.) Dunal	Solanaceae	Seeds and roots	Morocco	[6]
Xanthium strumarium (L.)	Compositae	Entire plant when fresh and seeds	Pakistan, Turkey	[16, 29]
Xanthosoma sagittifolium (L.) Schott	Araceae	Fresh leaves	Brazil	[30]
Zantedeschia aethiopica (L.) Spreng.	Araceae	—	New Zealand	[28]
Zantedeschia ssp.	Araceae	—	USA	[20]
Zea mays (L.)	Poaceae	Roots and stems	Nigeria	[13]
Zanthoxylum nitidum (Roxb.) DC.	Rutaceae	Roots, stems, and leaves	China	[17]
Zanthoxylum planispinum Siebold & Zucc.	Rutaceae	Roots and fruit	China	[17]
Zehneria capillacea (Schumach.) C.Jeffrey	Cucurbitaceae	Fruits	Nigeria	[13]
Zephyranthes spp.	Amaryllidaceae	Leaves and underground parts	USA	[20]
Zigadenus spp.	Melanthiaceae	Leaves	USA	[31]
Zygadenus spp.	Liliaceae	All parts, especially leaves and bulbs	Canada	[24]

Table 3.2 Drugs from poisonous plants used as anticancer agents.

Drug	Mechanism of action	Poisonous plant	References
Vinblastine, vincristine		*Catharanthus roseus*	
Paclitaxel, docetaxel	Arrest multiplication of cancerous cells by cross-linking the microtubules	*Taxus* spp.	[4]
Cannabinoid (Sativex®)	Inhibition of cell proliferation of colorectal carcinoma, neuroblastoma, gliomas, lymphomas, thyroid epithelioma, and breast cancer	*Cannabis sativa*	[1]
Curcumin	Inhibition of cell growth in many types of cancerous cells	*Curcuma longa*	[2]

Table 3.3 Some examples of drugs developed from poisonous plants.

Drug	Disease treated	Poisonous plant
Atropine	Hypertension and other diseases	*Atropa belladonna*
		Hyoscyamus niger
		Mandragora autumnalis
Scopolamine	Nausea, motion sickness, and vomiting	*Datura stramonium*
		Hyoscyamus niger
		Mandragora autumnalis
Hyoscyamine	Gastrointestinal disorders	*Hyoscyamus niger*
		Datura stramonium
		Mandragora autumnalis
		Atropa belladonna
Reserpine	Hypertension	*Rauwolfia serpentina*
Codeine	Analgesic, antitussive	*Papaver somniferum*
Morphine	Analgesic	
Papaverine	Antispasmodic	
Digoxin	Heart diseases	*Digitalis purpurea*

Table 3.3 (Continued)

Drug	Disease treated	Poisonous plant
Colchicine	Gout	*Colchicum autumnale*
Ginkgolide-B	Cerebral infarction	*Ginkgo biloba*
Ternatolide	Antituberculosis	*Ranunculus ternatus*
Curcumin	Hypolipidemic	*Curcuma longa*
Apomorphine hydrochloride	Parkinson's disease	*Papaver somniferum*
Tiotropium bromide	Chronic obstructive pulmonary disease	*Atropa belladonna*
Metformin	Type 2 diabetes	*Galega officinalis* (L.)

It is important to note, however, that these plants or their parts cannot just be taken as they are but rather need to be extracted into pure compounds that should be thoroughly tested, optimized, and studied for their safety in their individual molecular forms. These may then be studied further in combination with other compounds, if need be.

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4

Drugs in Clinical Practice from Toxic Plants and Phytochemicals

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CONTENTS

Poisonous Plants and Phytochemicals in Drug Discovery, First Edition.
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4.1 Introduction

The use of natural products as medicines is assumed to have presented a great challenge to early humans. Despite the potential adverse outcomes, perhaps early humans often used poisonous plants on themselves to ultimately discover natural medicines [1]. Traditionally, a single herb or formula may contain many phytochemical constituents, such as alkaloids, terpenoids, or flavonoids. Generally, these chemicals function alone or in conjunction to effect the desired medicinal outcome [2]. However, with advances in the theoretical background, therapeutic principles, associated technologies, and understanding of the life sciences, a deeper understanding of the active compounds of traditional and complementary medicine has been reached [3].

Shortly after the beginning of the era of "modern" drugs in the nineteenth century, a German pharmacist, Friedrich Sertürner, isolated the first pharmacologically active compound, morphine, from the opium plant in 1805. Subsequently, numerous active compounds have been separated from natural products. Despite the fact that the development of synthetic techniques has led to a significant reduction in their importance, natural products still continue to be crucial for the development of new drugs. To this effect, categories of medicines, such as anticancer, antihypertensive, and antimigraine medication, have benefited greatly from natural products [4–6]. The acceptability, convenience, and accessibility of traditional medicine has been, and will continue to be, helpful for new drug research [7].

The toxicity of medicinal plants derives primarily from the fact that they contain diester diterpene alkaloids such as aconitine, mesaconitine, and hypaconitine [8]. Poisoning as a result of taking homemade medicated liquor containing aconite and traditional medicine containing *Aconitum carmichaelii* was reported in China between 1999 and 2008 [9]. Severe cases of cardiac toxicity from the consumption of aconitine-containing herbal preparations manifesting as ventricular tachycardia and fibrillation and eventually leading to death have also been reported [10, 11].

Venoms have been included in numerous systems of traditional healing since prehistoric times, but the modern translation of toxins into medicines began only in the 1940s with the introduction of tubocurarine, a vegetal compound, into anesthetic practice as a selective muscle relaxant. Generally speaking, plant toxins are representative of a large group of structurally diverse small molecules that result from plant secondary metabolism, whereas most animal toxins are peptides and proteins that are often resistant to protease owing to their disulfide-rich architectures [12, 13]. In this chapter, the drugs in clinical practice obtained from toxic plants and their chemistry will be discussed.

4.2 Drugs in Clinical Practice from Toxic Plants

4.2.1 Curare

Curare is the common name for various plant alkaloid toxins originating from Central and South America. Originally, it was familiar as an "arrow poison" because the indigenes used it for hunting; it was traditionally produced by boiling diverse plants (e.g. *Chondrodendron tomentosum*, *Menispermaceae*, or *Strychnos*). In the 1860s, the scientists Thomas Richard Fraser and Alexander Crum Brown, working on the relationship between chemical structure and biological activity, discovered that when alkaloids such as atropine, brucine, codeine, morphine, and nicotine had their nitrogen atoms changed from the tertiary to the quaternary form they acquired curare-like activity. Harold King isolated D-tubocurarine from a museum sample of curare, and in 1942 Oscar Wintersteiner and James Dutcher isolated the alkaloid D-tubocurarine from the plant *C. tomentosum*. Its first clinical use as a muscle relaxant during an operation was in the same year [14, 15].

In addition, the molecular mechanism of curare as a competitive antagonist of nicotinergic neuromuscular synaptic junctions was finally elucidated in the twentieth century. This non-depolarizing muscle relaxant acts by paralyzing skeletal musculature, including respiratory muscles. Tubocurarine is a long-acting benzylisoquinoline that is eliminated by both renal excretion and hepatic metabolism. It also causes the release of histamine, which is associated with a significant reduction in blood pressure following rapid infusions of large doses [16, 17]. Histamine-associated hypotension can be minimized by slow injection, incremental dose increases, and co-administration of histamine-1 and histamine-2 receptor blockers [18].

With the ongoing rapid development of medical science, new derivatives, such as atracurium, succinylcholine, gallamine, pancuronium, rocuronium, vecuronium, and mivacurium, have been synthesized with an excellent effectiveness to safety ratio and thus the original tubocurarine is no longer used clinically in most countries around the world. The most successful of the new muscle relaxants is atracurium [15].

4.2.2 Drugs Acting on the Central Nervous System

4.2.2.1 Morphine

Morphine's history begins with the use of opium poppy plants (*Papaver somniferum*), which are native to Eurasia and have been cultivated for more than 5000 years. Opium was used for its analgesic and sedative effects, whereas its poisonous effects were characterized by lethal respiratory depression at high doses. The molecule was discovered in 1805 by Friedrich Wilhelm Adam Sertürner

(1783–1841), a pharmacy pupil in Germany who was working on active opium compound isolation. Morphine is a morphinan isoquinoline alkaloid. Later, a number of other alkaloids, such as codeine and papaverine, were isolated from opium. Currently, codeine is obtained from morphine and is used as an analgesic and antitussive drug. Papaverine formed the basis for developing verapamil, a calcium channel blocker that is used to treat hypertension [19, 20].

Currently, morphine is approved by the US Food and Drug Administration (FDA) in sulfate form and is still accepted as the gold standard treatment for severe pain. Morphine was also the prototype for several opioid receptor agonists in clinical use, such as fentanyl, oxycodone, and methadone [20]. Nowadays, codeine is used in the management of mild to moderately severe pain and short-term relief of cough in select patients. Recently, the FDA has issued a drug safety communication after reviewing reports of children who developed serious adverse effects, including death, after receiving codeine in the usual dosage range for pain relief following tonsillectomy and/or adenoidectomy for obstructive sleep apnea syndrome [21, 22].

Papaverine is currently indicated in various vascular spasms associated with smooth muscle spasms, such as myocardial infarction, angina, peripheral and pulmonary embolism, peripheral vascular disease, cerebral angiospastic states, and visceral spasms (ureteral, biliary, and gastrointestinal colic). However, because of the availability of safer and more effective alternative medications, these uses of papaverine have recently declined. Papaverine is also occasionally used in the prevention of vasospasm during harvesting of mammary arteries for coronary artery bypass graft surgery [23, 24].

4.2.2.2 Cocaine

Cocaine (benzoylmethylecgonine), supposed to be the most potent stimulant of natural origin, is extracted from the leaves of the coca plant (*Erythroxylum coca*), which is indigenous to the Andean highlands of South America. Natives in this region chew or brew coca leaves into a tea for refreshment and to relieve fatigue, similar to the customs of chewing tobacco and drinking tea or coffee in other cultures. Cocaine acts mainly by increasing dopamine levels by binding to the dopamine transporter and blocking the reuptake of dopamine into presynaptic cells. The therapeutic use of cocaine dates back to 1884, when Carl Koller used it in ophthalmic surgery. Despite legislative attempts dating from the early twentieth century to eradicate its use, cocaine remains a common and dangerous drug of abuse. The alkaloidal form of cocaine is extracted from the coca leaf by mechanical degradation in the presence of a hydrocarbon solvent. The resultant product is converted into a hydrochloride salt and extracted into an aqueous phase, from which water is subsequently evaporated to yield a white powder (cocaine hydrochloride) [25, 26].

Currently, its toxicity and potential for addiction have stringently limited cocaine's therapeutic purposes to topical anesthesia in ophthalmological and nasal surgery. However, the identification of the benzoyl moiety of cocaine enabled the synthesis of different molecules, such as: in 1890 benzocaine, which is the cocaine benzoic acid ester; in 1905 procaine, which is the cocaine para-aminobenzoic acid; and, finally, in 1943 lidocaine, which is the diethyl-aminoacetic acid derivative of cocaine that started the amide-type local anesthetic age. These drugs are now most commonly used for local anesthesia. Lidocaine is also often used in cardiac arrhythmias [26–28].

4.2.2.3 Ergot Alkaloids

Being primarily responsible for ergotism, or St. Anthony's fire disease, ergot alkaloids are currently used in the management of various medical conditions, particularly through their psychoactive and vasoconstrictive effects. This class of molecules belongs to the indole alkaloid group and can be classified according to their structures; namely, clavines, lysergic acid amides (ergoamides), and peptides (ergopeptines). Ergot alkaloids lead to the formation of the tetracyclic ergoline ring system, except the simplest one, the tricyclic compound. Convolvulaceae, Poaceae, and Polygalaceae are the three families of higher plants in which these metabolites are found, but their production is often dependent on the presence of plant-associated fungi. Moreover, fungi from the phylum Ascomycota, such as *Claviceps*, *Epichloë*, *Penicillium*, and *Aspergillus* spp., are the main producers of ergot alkaloids. *Claviceps purpurea* is the most studied species related to ergotism [29].

The ergot alkaloids have a strong affinity for the 5-hydroxytryptamine, dopamine, and adrenergic receptors in the central nervous system and also the adrenergic receptors in blood vessels. Owing to its specific uterotonic action, ergometrine started to be used for the prevention and treatment of postpartum hemorrhages. At the beginning of the nineteenth century, because of repeated cases of associated intrapartum ergometrine use and tetanic uterine contractions that led to fetal asphyxia, stillbirth, and uterine rupture, the role of ergometrine changed from *pulvis ad partum* (the powder of birth) to *pulvis ad mortem* (the powder of death), and its use was restricted to the management of postpartum hemorrhage. Additionally, ergot alkaloids were the first antimigraine drug available [29, 30].

Currently, ergotamine is indicated for the prevention and treatment of vascular headaches, such as migraine, migraine variants, or so-called "histaminic cephalalgia." Natural and semisynthetic ergot alkaloids are used as a second-line intervention if uterine atony persists after oxytocin administration during cesarean delivery. These are used as blood pressure modulators and pituitary hormone regulators for migraine prevention and as dopaminergic agents.

The cardiovascular adverse reactions of the class include: absence of pulse, bradycardia, cardiac valvular fibrosis, cyanosis, edema, electrocardiograph changes, gangrene, hypertension, ischemia, precordial distress and pain, tachycardia, and vasospasm. The contraindications of the class include hypersensitivity to ergotamine or any component of the formulation; peripheral vascular disease; hepatic or renal disease; coronary artery disease; hypertension; sepsis; coadministration with CYP3A4 (includes protease inhibitors, azole antifungals, and some macrolide antibiotics); and pregnancy [30, 31].

4.2.3 Atropine, Scopolamine, and Hyoscyamine

Atropine, scopolamine, and hyoscyamine are alkaloids found in plants of the Solanaceae botanical family, such as *Atropa belladonna*, *Datura stramonium*, *Hyoscyamus niger*, and *Hyoscyamus muticus*. Atropine and scopolamine are esters derived from the reaction of an aromatic acid (tropic acid) with tropine (tropanol) or scopine. Scopine differs from tropine only in having an oxygen bridge between C6 and C7. On the other hand, hyoscyamine is the tropine ester of tropic acid. It is an asymmetric molecule and forms atropine when (−)-hyoscyamine is racemized into the (±)-compound. During the Roman Empire and in the Middle Ages, *A. belladonna* (also called deadly nightshade) was frequently used to poison people by ingestion. To date, it is the major cause of poisoning [32].

Atropine and scopolamine work by blocking the action of acetylcholine at parasympathetic sites in smooth muscle, secretory glands, and the central nervous system, increasing cardiac output and drying secretions. Atropine reverses the muscarinic effects of poisoning by acetylcholinesterase inhibitors by acting as a competitive antagonist. Owing to their mechanisms of action, atropine and scopolamine are used as preoperative medications to inhibit salivation and secretions; in the treatment of symptomatic sinus bradycardia; as an atrioventricular block (nodal level); as an antidote for anticholinesterase poisoning (carbamate insecticides, nerve agents, organophosphate insecticides); and as an adjuvant to anticholinesterases (e.g. edrophonium, neostigmine) to decrease their side effects during reversal of neuromuscular blockade [33].

Significant adverse reactions of these drugs include cardiovascular (arrhythmia, flushing, hypotension, palpitation, tachycardia), central nervous system (ataxia, coma, delirium, disorientation, dizziness, drowsiness, excitement, fever, hallucinations, headache, insomnia, nervousness), dermatological, gastrointestinal, genitourinary, neuromuscular and skeletal weakness, ocular, and respiratory effects as well as anaphylaxis [34].

4.2.4 Physostigmine and Other Acetylcholinesterase Inhibitors

Physostigmine is a tertiary amine belonging to the indole alkaloid class. It is a highly unstable white powder that becomes red upon exposure to light, air, and heat. Physostigmine is present in the ripe seeds of *Physostigma venenosum* from Western Africa [35].

The first therapeutic use of the drug dates from 1877, when Ludwig Laqueur used it in the treatment of glaucoma. Further studies in 1929 led Edgar Stedman to identify the mechanism of its parasympathomimetic effect through acetylcholinesterase inhibition, thus acting as a substrate and facilitating carbamylation of the enzyme. Currently, physostigmine is indicated to reverse toxic, life-threatening delirium caused by atropine, diphenhydramine, dimenhydrinate, *A. belladonna* (deadly nightshade), or jimson weed (*Datura* spp.). Significant adverse reactions include cardiovascular (asystole, bradycardia, palpitation), central nervous system (hallucinations, nervousness, restlessness, seizure), gastrointestinal (diarrhea, nausea, salivation, stomach pain), genitourinary (urinary frequency), neuromuscular and skeletal (twitching), ocular (lacrimation, miosis), and respiratory (bronchospasm, dyspnea, pulmonary edema, respiratory paralysis) effects as well as diaphoresis [36–39].

The chemical structure of physostigmine has provided a template for the development of other molecules with highly significant anticholinesterase activity, such as rivastigmine, galantamine, and huperzine, all of which have demonstrated efficacy in Alzheimer's disease. Currently, rivastigmine is used for the treatment of mild to moderate dementia associated with Alzheimer's disease or Parkinson's disease as well as for the relief of severe dementia associated with Alzheimer's disease, including Lewy body dementia [35, 38].

4.2.5 Antitumor Agents

4.2.5.1 Podophyllotoxin and Etoposide

Podophyllotoxin is an aryltetralin-type lignan isolated from podophyllin, a resin produced by species belonging to the *Podophyllum* genus, such as *Podophyllum emodi* and *Podophyllum peltatum*. Because of its tremendous cytotoxicity, podophyllin is nowadays indicated topically in the treatment of genital warts and condylomata. Among the numerous compounds of the resin, podophyllotoxin is the main cause of the associated cytotoxic and neurotoxic effects. The manifestations of podophyllotoxin intoxication include vomiting, diarrhea, abdominal pain, and abnormal hepatic functions, in addition to neurological disturbance. Podophyllotoxin exerts its pharmacological actions by irreversibly binding to tubulin; therefore, inhibiting its polymerization and inducing cell cycle arrest at the G2/M phase. Podophyllotoxin (podofilox) is included in many pharmacopoeias and is

used as an antiviral agent against the human papillomavirus, cytomegalovirus, Sindbis virus, molluscum contagiosum, and venereal warts. Additionally, the antitumor activity of podophyllotoxin has been demonstrated: it is effective in the treatment of some types of genital tumors and in non-Hodgkin's and other lymphomas, as well as in lung cancer. The limiting factors of the clinical uses of podophyllotoxin include its non-selectivity against tumor cells and its narrow therapeutic window. Consequently, derivatives of podophyllotoxin, such as the semisynthetic derivative etoposides synthesized in 1963, were developed. These compounds present good clinical effects against several types of neoplasms [40, 41].

Etoposide has been shown to delay the transit of cells through S phase and arrest cells in late S or early G2 phase. The drug may inhibit mitochondrial transport at the protonated nicotinamide adenine dinucleotide dehydrogenase level or inhibit the uptake of nucleosides into HeLa cells. It is a topoisomerase II inhibitor and appears to cause DNA strand breaks. Etoposide is used in combination with other chemotherapeutic agents for the treatment of non-Hodgkin's lymphomas, refractory testicular tumors, small cell/non-small cell lung cancer (NSCLC), lymphoma, non-lymphocytic leukemia, and glioblastoma multiforme as well as many other cancers. The most common adverse reactions of etoposide include dermatological (alopecia), gastrointestinal (nausea, vomiting, anorexia, diarrhea), and hematologic (leukopenia, thrombocytopenia, anemia) effects [42–45].

4.2.5.2 Taxanes

Taxanes are modified diterpenes, which are also known as non-heterocyclic pseudo-alkaloids. These chemicals are synthesized by the yew tree, which belongs to the *Taxus* spp. The noxious nature of yew has been quoted since the second century BCE, when the "juice" was used for poisoning and in ritual suicides and as emmenagogues. Taxanes gained popularity in the 1980s and 1990s as an innovation against cancer; at the time, they were considered to be the most promising new chemotherapeutic agents developed for cancer treatment, particularly paclitaxel and docetaxel. To date, several taxanes have been isolated and their structural analogs described. Chemical features of this class of compounds include a taxane ring with a four-member oxetane ring attached at positions C4 and C5 and a bulky ester side chain at C13. The configuration of this ester chain is essential for the antitumor activity through a special mechanism of action. The prototype of taxanes, paclitaxel, was discovered as part of a National Cancer Institute program in which extracts of thousands of plants were screened for anticancer activity. Paclitaxel was initially supplied from the bark of the Pacific yew, *Taxus brevifolia*, which is not a sustainable source because of plant scarcity. Further investigations led to an approved semisynthetic molecule, 10-deacetylbaccatin III, being derived from the needles of a readily available precursor, *Taxus baccata*,

which is the European yew species; the European species is more abundant than the Pacific one and is able to meet commercial demands [46, 47].

Taxanes act by microtubule stabilization, interfering with the normal mitotic process as a result of induced resistance to cell division. Both paclitaxel and docetaxel bind to the β-subunit of tubulin, but higher activity for tubulin has been observed with docetaxel, which results in a longer intracellular period than with paclitaxel. This may explain why docetaxel appears to be two to four times more potent than paclitaxel. The transition between microtubule stabilization and cell death that is effected by taxanes is not well understood [48].

Currently, the common indications of taxanes include breast cancer (locally advanced/metastatic), NSCLC, metastatic prostate cancer, advanced gastric adenocarcinoma, locally advanced squamous cell head and neck cancer, and metastatic ovarian cancer. Paclitaxel is also used for the treatment of AIDS-related Kaposi's sarcoma. Significant adverse reactions include fluid retention, neurosensory events including neuropathy, fever, neuromotor events, alopecia, cutaneous events, nail disorders, stomatitis, diarrhea, nausea, vomiting, neutropenia, leukopenia, anemia, thrombocytopenia, febrile neutropenia, muscle weakness, pulmonary events, and hypersensitivity [49–51].

4.2.5.3 Vincristine and Vinblastine

The vinca alkaloids are indole alkaloid molecules primarily encountered in the pink periwinkle (*Catharanthus roseus*). This is known as the vinca plant; it is native and endemic to Madagascar and also encountered in Europe, Northwest Africa, Southwest Asia, and Southern USA. These have dimeric chemical structures containing an indole (catharanthine) and a dihydroindole nucleus (vindoline) joined together with other complexes. The earlier therapeutic use of vinca is related to diabetes treatment in the population of Madagascar. Further evaluation of the hypoglycemic activity of its extracts evidenced a granulocytopenia produced as a result of bone marrow suppression in animals, directing studies to model leukemia and lymphoma treatment. Identification of their bone marrow suppression activity led to the isolation of vinblastine and vincristine alkaloids, which today are widely used in the treatment of Hodgkin's and non-Hodgkin's lymphoma, testicular cancer, breast cancer, mycosis fungoides, Kaposi's sarcoma, histiocytosis (Letterer–Siwe disease), and choriocarcinoma. Vincristine is also used for the treatment of Wilms' tumor, neuroblastoma, and rhabdomyosarcoma. Though wide in range, the frequency of adverse reactions is not well established for these drugs. The toxicity of vincristine and vinblastine is mainly characterized by peripheral neuropathy and neutropenia. Despite being less neurotoxic, vinblastine presents similar side effects to vincristine, particularly when it is combined with or follows other neurotoxic agents such as taxanes [52–59].

Structurally, vinblastine and vincristine are identical except for the substituent found on the indoline nitrogen in the lower vindoline portion of the molecule. This single structural difference distinguishes both the clinical activities and toxicity profiles of these molecules. This modification, however, does not affect their mechanism of action, which is through binding to tubulin and inhibiting microtubule formation, thus arresting the cell at metaphase by disrupting the formation of the mitotic spindle; this is specific for the M and S phases [53]. The vinca alkaloids vincristine and vinblastine are structurally identical apart from the substituent attached in the indoline nitrogen in the lower vindoline portion of the molecules; these substituents are an aldehyde and a methyl group, respectively.

Knowledge of the structure and functional groups as well as the toxicity of vinca alkaloids guides studies in a natural direction: the search for new analogs that are more active, less toxic, and exhibit a broader spectrum of anticancer efficacy. To this effect, there are two other major vinca alkaloids in clinical use based on vincristine and vinblastine: vinorelbine and vindesine.

4.2.6 Other Drugs

4.2.6.1 Cardiac Glycosides

Cardiac glycosides are perfectly individualized chemical groups with excellent structural homogeneity. They possess a β-lactone unsaturated ring at C17 and are divided into cardenolides, such as ouabain and digoxin, and bufadienolides, such as bufalin. Although historical records indicate that extracts of the common foxglove *Digitalis purpurea* were used (mainly as poisonous preparations) as early as Egyptian and Roman times, the first scientific reports on the medical application of cardiac glycosides date back to 1785. In the nineteenth century, the cardiac glycosides began to be used in the control of tachyarrhythmia, despite being considered tremendously toxic [60, 61].

The fundamental cardiac glycoside digoxin is well known to have a complex mechanism of action. It remains the only positive ionotropic drug for chronic heart failure. Digoxin acts by inhibiting the sodium pump, which indirectly promotes calcium influx by sodium–calcium exchange. One of the major concerns related to the medical use of cardiac glycosides originates from their rather narrow therapeutic index, with the most prominent adverse effects including anorexia, nausea, vomiting, diarrhea, and life-threatening alterations of cardiac rhythm. Nevertheless, the prototypical cardiac glycosides digoxin and digitoxin were approved by the FDA for the treatment of atrial fibrillation, atrial flutter, and paroxysmal atrial tachycardia prior to 1982. In 1998, the FDA extended the indications of digoxin to congestive heart failure. Currently, digoxin is indicated for the treatment of congestive heart failure, atrial fibrillation, and

atrial flutter with rapid ventricular response, whereas the use of digitoxin has been discontinued in several Western countries. The significant adverse reactions of digoxin include cardiovascular, central nervous system, and dermatological reactions [61–64].

4.2.6.2 Colchicine

Colchicine is an alkaloid originally extracted from the meadow saffron *Colchicum autumnale* (L.). Because of its feared toxicity, preparation of the plant was not recommended for pain treatment until the sixth century CE [65]. Colchicine was approved in 2009 by the FDA as a monotherapy drug to treat familial Mediterranean fever and acute gout flares.

Colchicine disrupts cytoskeletal functions by inhibiting β-tubulin polymerization into microtubules and preventing the activation, degranulation, and migration of neutrophils associated with mediating some gout symptoms. The uses of colchicine include the prevention and treatment of acute gout flares and the treatment of familial Mediterranean fever. Unlabeled uses for primary biliary cirrhosis and pericarditis are also significant. In addition, colchicine is being investigated as an anticancer drug. However, the therapeutic value of colchicine against cancer is restrained by its low therapeutic index. Its toxicity includes dose-dependent gastrointestinal toxicity, neutropenia, bone marrow damage, and anemia [66–69].

4.2.6.3 Coumarins

Previously unseen hemorrhagic disease in cattle was associated with the sweet clover recently imported from Europe as cattle feed in the early twentieth century. When fresh, the plant appeared to be harmless. The problem arose when sweet clover was fed to cattle in the form of hay or silage, which when spoiled as a result of mold caused spontaneous and uncontrollable bleeding. It took many years for the active compound in spoiled sweet clover to be identified as the toxin dicoumarol, which had been converted from coumarin in the plants during the spoiling process. The most potent compound was patented in 1948 under the name of warfarin; it was initially marketed and used as a rat poison, the belief being that it was too poisonous to be used in humans. Warfarin later became the standard treatment for long-term thrombotic conditions. Currently, warfarin is used in the prophylaxis and treatment of thromboembolic disorders (e.g. venous, pulmonary) and embolic complications arising from atrial fibrillation or cardiac valve replacement and as an adjunct to reduce the risk of systemic embolism (e.g. stroke) after myocardial infarction. Bleeding is the major adverse effect of warfarin. Hemorrhage may occur at virtually any site. The risk is dependent on multiple variables, including the intensity of anticoagulation and patient susceptibility [70–73].

4.2.6.4 Nicotine and the Neonicotinoids

Nicotine is an alkaloid found in the Solanaceae family (nightshade) of plants, predominantly in tobacco and in lower quantities in some vegetables and the coca plant. Nicotine, which is well known as the addictive compound in cigarettes, has been used both as a pesticide and as the model for a series of synthetic insecticides called neonicotinoids. Because of their preferential binding to receptors in the nervous systems of insects and not to those in mammals, the neonicotinoids are considered to be safer options than nicotine itself. Currently, the clinical uses of nicotine are to aid smoking cessation by relieving nicotine withdrawal symptoms and the management of ulcerative colitis. The most significant adverse reactions of nicotine include headache, mouth/throat irritation, dyspepsia, cough, and rhinitis [74–77].

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5

Toxicology and Health Benefits of Plant Alkaloids

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CONTENTS

5.1 Introduction

Alkaloids are a class of natural products containing carbon (C), hydrogen (H), nitrogen (N), and usually oxygen (O). These compounds have been categorized into different classes based on their sources, pharmacokinetics, and chemical structure. They include isoquinoline alkaloids, indole alkaloids, pyrroloindole alkaloids, piperidine alkaloids, aporphine alkaloids, pyridine alkaloids, methylxanthine derivatives, vinca alkaloids, lycopodium alkaloids, indole beta-carboline, and erythrine by-products [1, 2]. Alkaloids are derived from plants (especially in

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certain flowering plants) [1, 2], microorganisms, and animals and are widely found in products such as herbal preparations, beverages, well-cooked foods, and tobacco smoke. Some alkaloids, such as beta-carbolines, are naturally present in body fluids and tissues [3, 4]. Most alkaloids are available in many plant species that are used for human and animal nutrition [5]. Most of the plant species contain a limited number of alkaloids, but others in families such as Solanaceae (nightshades), Papaveraceae (poppies), Ranunculaceae (buttercups), and Amaryllidaceae (amaryllis) contain several kinds of alkaloids [1, 2]. Alkaloids have a variety of psychopharmacological, biochemical, and behavioral effects in both humans and animals [3, 4]. Plant alkaloids exist as the salts of organic acids such as malate, acetate, and citrate or in combination with other molecules such as tannins. Alkaloids are mostly basic and lipophilic, which makes them soluble in polar organic solvents.

Some alkaloids, such as pyrrolizidines, are derived from ornithine as well as insects that consume alkaloids; these insects then use the alkaloids in their fight against predators [6]. Alkaloids mostly occur as monoesters, diesters, or macrocyclic diesters and are formed by an amino alcohol (necine base) and (a) necic acid(s) (mono- or dicarboxylic aliphatic acids), which then give them their structural diversity. Alkaloids rarely exist as a free form of the pyrrolizidine base; usually, they exist as tertiary bases or pyrrolizidine alkaloid *N*-oxides (PANO) [6, 7].

Pyrrolizidines are an important source of amino alcohols. Pyrrolizidines have a core composed of at least two five-membered rings attached by a nitrogen. Sometimes, they acquire a double bond at positions 1 and 2, and this may be responsible for the frequently reported enhanced toxicity in pyrrolizidine alkaloids (PAs) [8]. In addition, a single alcohol can be formed at C1, while a second and less often third alcohol can be formed at positions C7 (di-hydroxylated) and C2 or C6 (tri-hydroxylated), respectively [9–11]. Furthermore, an esterification reaction can occur at C7 and/or C9 [11]. Several approaches have been made to partially or fully synthesizing many naturally occurring PAs and related non-natural analogs [12, 13].

Most PAs can be ingested orally and absorbed from the gastrointestinal tract; they are excreted in urine, feces, and milk. Passage to the placenta is limited because they have high lipophilicity [14]. The majority of the compounds are bioactivated or biotransformed in the liver, and this makes the liver the organ most susceptible to the toxicity of PAs [15]. The other most susceptible organ is the lung, followed by the kidney, as the PAs pass through these organs on their way to their targets, metabolic processes, excretion through blood circulation. Excretion and toxicity of the PAs occur only after biotransformation [16].

The activation of the PAs occurs metabolically through three principal pathways. These include hydrolysis, *N*-oxidation, and oxidation, which lead to the formation

of necic acids and necines, PANO, and dehydropyrrolizidine alkaloids (DHPAs) or pyrrolic esters, respectively. Hydrolysis is also responsible for detoxification through the promotion of clearance of the compounds [17, 18]. *N*-oxidation also facilitates detoxification by facilitating excretion of the compounds through the formation of easily conjugated PANO, which is then easily excreted [16].

Several alkaloids exhibit both toxicity and potent pharmacological activities. Many alkaloids have been used both lawfully and illicitly as pharmaceuticals, stimulants, and narcotics. Concentrations or amounts of these alkaloids in foodstuffs have not been a cause for concern in acute poisoning. However, the concentrations have been sufficient enough to cause chronic toxicity because the alkaloids are either consumed from different sources of food in a day or consumed from one foodstuff that is frequently ingested in a day, such that they exceed the maximum daily intake suggested by authorities, which poses a risk of development of chronic disease [5].

5.2 Pharmacological Properties of Alkaloids

Alkaloids have demonstrated several health benefits in humans and animals. Notable ones in humans include antimicrobial activity, anti-inflammatory activity, anticancer activity, anti-human immunodeficiency virus (HIV) activity, and acetylcholinesterase (AChE)-inhibiting activity, as described below.

Alkaloids show significant antimicrobial activity, the defensive role that is expected of this class of secondary metabolites in plants [19]. Monocrotaline, usaramine, and azido-retronecine are examples of PAs that have shown antimicrobial activity against some bacteria species [20]. Analysis of usaramine activity against the effective formation of biofilms in *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* has shown that it reduced the formation of biofilms by *S. epidermis* by about 50% at 1 mg/ml, but had no activity against the formation of biofilms by *P. aeruginosa*. Monocrotaline and azido-retronecine had activity against *Trichomonas vaginalis* (concentrations up to 1 mg/ml), being lethal to 70% and 85% of bacterial cells, respectively, but had no toxicity toward *T. vaginalis*. They also exhibited desirable selective toxicity but did not interfere with vaginal epithelial cells, which is an important trait for compounds under consideration for development into drugs such as topical antimicrobial agents [5].

Anti-inflammatory activity has also been elucidated for alkaloids. The inflammatory process is a physiological response of the body aimed at eliminating, neutralizing, and/or destroying stimuli from infection or tissue damage [21, 22]. Also, the inflammatory process involves the upregulation of inducible synthase of nitric oxide (NO) as a consequence of proinflammatory mediators, such as cytokines, that lead to increased levels of NO, which is important for mediation of

the inflammatory response [6, 23]. Therefore, the regulation of its production in tissues may be important for the treatment of inflammation [5]. Alkaloids nervosine I–VI and the PAs lindelofidine and labumine have been found to exhibit inhibitory capacity toward NO production by lipopolysaccharide-challenged macrophages from the RAW 264.7 cell line. The results indicated that the molecules that were effective in this model had half-maximal inhibitory concentration (IC_{50}) values ranging from 2.16 to 38.25 μM [24].

Anticancer activities have also been reported for many types of cancers. For example, alkaloids have been used in the treatment of pediatric cancers such as acute lymphoblastic leukemia with indicine-*N*-oxide at two dose levels (2000 and 2500 mg/m^2/day) for five consecutive days. However, the therapeutic index was very narrow and the dose–response curve was very steep and registered mild acute hepatotoxicity at the doses tested. Treatment with indicine-*N*-oxide in some patients has led to liver failure. Indicine-*N*-oxide that was obtained from *Heliotropium indicum* (L.) inhibited the proliferation of cancers in various human cancer cell lines (breast, cervical, prostate, and cervical squamous) with IC_{50} values ranging from 46 to 100 μM. At these concentrations, cell cycle arrest at mitosis was detected without noticeable changes in the organization of the spindle or interphase microtubules [5, 25, 26].

Anti-HIV activity of polyhydroxylated PAs has also been tested and the results have shown that these PAs are able to affect the progression of HIV. For example, 10 mM alexine in conjunction with 0.1 mM australine, obtained from *Castanospermum australe* A.Cunn. and *Alexa Leiopetela* Sandwith, respectively, inhibit glycosidase activity, especially for the HIV glycosylation process that is linked with nitrogen. The activity is attributed to reduced fusion of cells with virions, which leads to restricted syncytium formation. Another alkaloid in the class of PAs and alexine from *C. austral* and *A. leiopetala*, respectively, also had inhibitory activity against HIV-1. Activity was obtained with 7,7a-diepialexine and an IC_{50} of 0.38 mM was found. The anti-HIV activity results correlated positively with the inhibition of pig kidney α-glucosidase 1 and the diminished cleavage of the precursor HIV-1 glycoprotein gp160 [5].

Inhibition of AChE has also been reported for alkaloids. AChE is involved in the catalysis of acetylcholine (ACh) and other esters that act as neurotransmitters. AChE is also important for neural function. It is mainly present in the synaptic gaps of the peripheral and central nervous systems and is responsible for terminating nerve impulses. When ACh is overstimulated, this can lead to disorders such as depression, while lower levels of ACh can lead to other diseases such as Alzheimer's disease and myasthenia gravis [27, 28]. Therefore, because of these factors, inhibitors of AChE are exploited as therapeutic targets [27]. Four PAs from *Solenanthus lanatus*, including 7-*O*-angeloylechinatine-*N*-oxide, 3′-*O*-acetylheliosupine-*N*-oxide, heliosupine-*N*-oxide, and heliosupine, had AChE inhibitory activity, with IC_{50} values ranging between 0.53 and 0.60 mM.

Another study of 7-*O*-angeloyllycopsamine-*N*-oxide, echimidine-*N*-oxide, and 7-*O*-angeloylretronecine, obtained from *Echium confusum*, against AChE showed IC_{50} values of 0.275–0.769 mM [3, 17].

The effects of an alkaloid extract of *Senecio brasiliensis* (Spreng) leaves on rats and mice showed the potential of using PAs in the treatment of stomach pain and ulcerogenic disease. Results showed that lesions significantly decreased by 32.9%, 42.5%, and 66.8%, respectively, with concentrations of PA extract of 12.5, 25, and 50 mg/kg (containing integerrimine, senecionine, retrorsine, seneciphylline, and usaramine). Similarly, a dose of 12.5 mg/kg of the same PA extract in the same study was shown to ameliorate non-steroidal anti-inflammatory drug-induced gastric ulcers [29, 30].

Alkaloids have also shown promising antidepressant effects [31, 32]. For example, strictosidinic acid isolated from *Psychotria myriantha* showed antidepressant-like effects on 5-hydroxytryptamine (5-HT) in a rat hippocampal system [9, 33]. Another alkaloid, berberine, resulted in a significant increase in mobility and climbing behavior in the forced swim test in rats [34, 35]. Additionally, alkaloids isolated from *Annona cherimola*, which included anonaine, 1,2-dimethoxy-5,6,6a,7-tetrahydro-4H-dibenzoquinoline-3,8,9,10-tetraol, nornuciferine, and liriodenine, also showed antidepressant-like activity in mouse tests [36, 37]. Beta-carboline alkaloids such harmine, harmane, and norharmane also increased the mobility time in a mouse forced swim test, thus producing the effects of an antidepressant [38, 39]. Tetrahydrosecamine, akuammidine, and rhaziminine from *Rhazya stricta* also showed a significant antidepressant effect in experimental animals on administration of a lyophilized extract. Mitragynine, a bioactive agent from *Mitragyna spicosa*, which grows in Malaysia, administered as an injection, also significantly reduced the immobility time of mice in both the tail suspension test and the forced swim test without any significant effect on locomotor activity. Mauritine A, an active compound from *Ziziphus apetala* collected in the People's Republic of China, showed strong activity against 11-β-hydroxysteroid dehydrogenase inhibition in an in vitro assay. The diterpene alkaloids songorine, napelline, mesaconitine, and hypaconitine from *Aconitum baicalens* have also demonstrated antidepressant effects in a depression animal model. The alkaloid punarnavine, isolated from *Boerhaavia diffusa* (L.), showed significant antidepressant activity in unstressed and stressed mice. Evodiamine, isolated from *Evodia fructus*, favored the increases in sucrose preference, 5-HT, and sodium level as well as increasing immobility time [40, 41]. Several other alkaloids such as mesembrine from *Sceletium tortuosum*, which grows in the USA; piperine from *Piper nigrum*, which grows in Thailand; leatispicine, an amide alkaloid from *Piper laetispicum*, which grows in China; *Dactylicapnos scandens* Hutch.; and the non-ergoline alkaloid pramipexole showed significant clinical efficacy in an antidepressant activity model [32].

5.3 Toxicological Properties of Alkaloids

Different species are affected by alkaloid compounds differently. This is attributed to the balance between the formation of DHPA and the formation of detoxification compounds such as necines, necic acids, and PANO. DHPA is formed through necine base hydroxylation at the C3 and C8 positions in the case of heliotridine and retronecine types. For otonecine, oxidative *N*-demethylation is required. After the formation of the highly reactive metabolites, they are bound to glutathione (GSH) to form GSH conjugates and are eliminated in the process. This is why conjugation to GSH is considered to be a route of detoxification. Similarly, pyrrole esters can bind to DNA and other proteins, later forming adducts. These metabolites can also be hydrolyzed and transform to dehydronecines, which are also toxic metabolites but are less reactive than the previously mentioned forms [5].

DHPA has been recognized as the main toxic mechanism of PAs, and is concerned particularly with binding the groups containing sulfur, nitrogen, and oxygen present in proteins to form adducts, such as 2,3-dihydro-1H-pyrrolizine-protein [5], mainly at the site of formation. Pyrroles are also capable of penetrating the nucleus and reacting with DNA, consequently causing DNA cross-links and DNA-to-protein linkages with poor functions, which cause damage, mainly in hepatocytes. They can pass into the sinusoidal lumen and attack sinusoidal cells. The injury caused by the toxic metabolites in hepatocytes and in hepatic vein walls leads to veno-occlusive disease (VOD), which is also known as hepatic sinusoidal obstruction syndrome (HSOS) [16]. This proposed mode of activity has been supported by other subsequent studies [42–44].

5.4 Acute and Chronic Toxicities

As already stated, the liver is the main target of toxicity. HSOS is the clinical manifestation most frequently found, being considered a marker for PA intoxication, and causing symptoms such as vomiting, enlargement of the liver, and bleeding diarrhea [17, 18]. PA intoxication can be acute, subacute, or chronic. Each type of intoxication exhibits its own symptoms. Acute intoxication is recognized by symptoms such as hemorrhagic necrosis, hepatomegaly, and ascites. Subacute intoxication is characterized by the blockage of hepatic veins, which causes HSOS (primary sinusoidal damage and parenchymal cell dysfunction) [1, 15, 45]. Additionally, chronic PA exposure manifests in the form of necrosis, fibrosis, cirrhosis, and proliferation of the bile duct epithelium [21, 25], while liver failure and death occur at the highest level of toxicity [1].

5.4.1 Genotoxicity and Tumorigenicity

Some alkaloids have been shown to be capable of affecting genes and inducing tumors. For example, retrorsine was reported to be capable of inducing lung, pancreatic, skin, liver, bladder, spinal cord, brain, and gastrointestinal tract tumors in vivo [14]. The mechanism of formation of the tumor is believed to be as a result of DNA adduct formation in the form of DHPA [46, 47], and high levels of DHPA-induced DNA adducts were associated with the appearance of tumors. Hence, these can be used as tumorigenicity biomarkers caused by PAs [29]. Furthermore, studies have shown that the tumors can also be caused by reactions between the compounds and proteins that cause chromatid exchange, DNA cross-linkages, and the aberration of chromosomes [11, 17, 18].

Alkaloids in the PA family are also linked to skin cancer owing to their ability to undergo photosensitization in animals after consumption and metabolism [48, 49]. It is believed that, normally, a porphyrin (phylloerythrin) produced as a result of chlorophyll damage by microorganisms in the gastrointestinal tract travels via the blood circulation through the liver to the bile. However, when the liver is compromised by a PA, it fails to eliminate the phylloerythrin, which accumulates in the skin and blood. When subjects are then exposed to sunlight, the metabolites produced can trigger oxidative stress and lipid peroxidation in skin tissues, which may facilitate the development of tumors [49, 50].

Although there are no reports of cancer in humans as a direct consequence of PA consumption, it is reasonable to conclude that such PAs may be tumorigenic and genotoxic to humans. This is because riddelliine metabolism in rodent liver microsomes is similar to that in humans, which includes DNA adduct formation; also, PAs induce liver tumors in rodents through the formation of DNA adducts [51, 52]. Similarly, the US National Toxicology Program asserted that riddelliine has the potential to cause cancer [5].

Some studies have already been done to assess the potential of PAs in causing, for example, cancer, liver diseases, congenital anomalies, and pulmonary hypertension [34]. These alkaloids are genotoxic and initiate such diseases slowly. This may be difficult for clinicians to identify since they cannot know about patients' dietary exposure to the alkaloids. Researchers have therefore attempted to define two pointers that can indicate a dietary dehydrogenase plant alkaloid etiology: cirrhosis, mainly one associated with HSOS and/or copper accumulation in the liver, and congenital anomalies and/or cancers where evidence of asymptomatic or overt HSOS, bone deformities, pulmonary arterial hypertension or immunological deficiencies is available. The presence of several of these indicators affirms that it is possible that dietary exposure to PAs is involved in disease etiology [5].

5.4.2 Lung Toxicity, Neurotoxicity, and Teratogenicity

The lungs are one of the target organs for alkaloid toxicity because DHPA can move from the liver into pulmonary arterioles to cause damage similar to HSOS. When the alkaloid reaches the organ, occlusion and inflammation may occur as a result of thrombi forming in the vessels and thickening in their walls. Overall, the combined effects of these phenomena consequently stimulate pulmonary hypertension and subsequent congestive heart failure. This was confirmed in a hooded Wistar rat study which showed that PAs can cause lung lesions (intravascular accumulation of mononuclear cells) as a result of low-level (0.025 mmol/kg body weight) and long-term exposure to PAs. This may lead to venous occlusion and extravascular alteration, whereby alveolar septa are thickened and the number of cells increased. The study results also led to the conclusion that rats developing lung lesions always also presented with chronic liver lesions [5].

Neurotoxicity has also been reported for alkaloids. For example, tricodesmine causes encephalitis, which is associated with headaches and vertigo, which can cause derilium and consciousness loss. Teratogenicity has also been reported as one of the consequences of consuming alkaloids. This is based on the finding that some alkaloids are capable of crossing a placenta from a mother to an unborn baby (fetus). For example, a hepatic HSOS case has been reported in the newborn of a mother who had taken herbal tea extracted from *Tussilago farfara* and the consumption of *Senecio madagascariensis* Poir. by a mare in Australia was reported to have led to hepatic failure [5]. Furthermore, clivorine from *Ligularia hodgsonii* Hook., in concentrations between 10 and 100 μM, showed that this PA can induce DNA fragmentation, which is compatible with apoptosis, in a human fetal hepatocyte cell line and mouse hepatocytes with an IC_{50} value of 40.8 μM [51, 53].

5.5 Factors that Influence the Toxicological Profile of Alkaloids

Both chemical and biological factors influence the toxicological effects of PAs. For example, the presence of a 1,2 double bond and/or one or two hydroxyl groups attached to the pyrrole ring can play a significant role in the toxicity of alkaloids; the presence of this functional group in retronecine, heliotridine, and otonecine types has been associated with their toxic effects. Furthermore, the presence of a methyl group at C1, two esterified groups, and branching in at least one of the carboxylic acids have also been implicated in the toxicity of alkaloids. Based on the reasons above, it is concluded that cyclic diesters, open chain diesters, and monoesters exhibit the highest, intermediary, and lowest levels of toxicity, respectively. To illustrate the relationship between the esterification level and toxicity, macrocyclic DHPA was found to be more toxic than open chain diesters [14–16].

Among the important factors that affect the toxicity of alkaloids are age, sex and differences activating metabolism within and between species. It is reported that men are at higher risk of toxicity than women, and children and fetuses are even more vulnerable to the toxicity of alkaloids than men [1]. There are also variations in toxicological vulnerability between distinct PAs within a species and of the same PA in different species [31, 54].

Finally, the presence of bacteria and metals influences the toxicity of alkaloids. This was demonstrated when exposure to low doses of the alkaloid monocrotaline, which has no toxicity risk normally, elicited hepatotoxicity in the presence of bacteria [42, 55]. In this case, centrilobular and midzonal liver lesions were registered. In addition, coadministration of retrorsine and copper led to more serious liver damage than retrorsine alone; a result that was confirmed by Moreira et al. [5].

Therefore, with the increasing consumption of herbal medicines and the toxicities widely reported in the literature, alkaloid poisoning has been increasingly considered as a public health problem. Some countries such as the USA, the Netherlands, and Austria are now establishing regulations around alkaloids in foodstuffs [36, 38, 40, 46].

5.6 Conclusion

Alkaloids are a very important class of compounds, particularly in drug discovery. They are mostly found as bases in nature because of the presence of a nitrogen group. Nitrogen significantly contributes to toxicity and other important pharmacological properties in drug design. Alkaloid forms of phytotoxins are not widely exploited in research. There is a need for more studies into the individual properties of common alkaloids as well as their characteristics in terms of toxicity.

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6

Chemical and Pharmacological Mechanisms of Plant-Derived Neurotoxins

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Poisonous Plants and Phytochemicals in Drug Discovery, First Edition.
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6.1 Introduction

The nervous system (NS) comprises the central and peripheral divisions. While the central nervous system (CNS) consists mainly of the brain and spinal cord, the peripheral nervous system (PNS) is subdivided into the somatic nervous system and autonomic nervous system (ANS). The ANS is further subdivided into the sympathetic and parasympathetic systems. In every component of the NS, information passes from one cell to another or from the NS to the muscular system by means of neurotransmitter molecules that are secreted by neurons. All neurotransmitters have a general mode of action, involving binding to a receptor and altering the target cell's response to a particular signal (the stimulus). The molecular interaction between a neurotransmitter and its receptor induces a conformational change, which, in turn, changes the electrochemical properties of the nerve cell, promoting entry and/or exit of some electrolytes and generation of an action potential, which enables communication between cells. A number of neurotransmitters are known and well understood in terms of their molecular and biological activities [1]. It is also known that exogenous molecules can enter the body, traverse all the way to the NS, and bind to either receptors or their counter-ligands. The binding of exogenous molecules by either means could promote undesirable effects, some of which exert toxic metabolic responses. In a unique mechanism, nerve agents bind to the enzyme acetylcholinesterase (AChE) and inhibit its ability to recycle the neurotransmitter acetylcholine (ACh) across nerve junctions. There are two types of ACh receptor (AChR), namely nicotinic (N) and muscarinic (M) receptors. Among other organisms, plants are known to be sources of an enormous number of neurotoxic molecules [1]. We highlight the most potent neurotoxins related to well-established modes of neurotransmission.

6.2 Nerve Agents

Under normal nerve impulse transmission, in the CNS, a presynaptic neuron secretes the neurotransmitter ACh into the synaptic cleft, enabling the neurotransmitter to interact with its cholinergic receptors on the postsynaptic membrane surface. This interaction leads to opening of the sodium channels on the postsynaptic neuron, followed by generation of an action potential and propagation of the impulse along the axon of the postsynaptic neuron. In so doing, the message spreads from one nerve cell to the next. In the PNS, when the action potential reaches a junction between a motor neuron and a muscle (a neuromuscular junction), a similar mechanism occurs via the same neurotransmitter across the neuromuscular junction and the impulse results in a muscular response related to movement depending on the nature and goal of the stimulus. After the

action potential passes, the enzyme AChE cleaves ACh into acetyl and choline, thereby stopping its interaction with its receptor in order to block the sodium channel and the subsequent effects of the action potential. When the enzyme is bound to nerve agents, its ability to break the neurotransmitter is blocked, promoting continued ACh–AChR interaction and a progressive action potential, leading to undesirable nerve and muscular effects.

The history of nerve agents dates back to the early 1930s with the discovery of tabun by Gerhard Schrader, who was working to discover novel insecticides. Other prominent nerve agents discovered during these earliest stages include sarin, cyclosarin, and soman, all of which are organophosphates (OPs) and were deployed as biological weapons during World War II [2]. Another well-known OP is diisopropyl fluorophosphate, which is used in the treatment of glaucoma and as an inhibitor of AChE in the parasympathetic division of the PNS.

The physical and chemical properties of OP nerve agents are similar. As a general property, all OP nerve agents are volatile liquids with varying faint odors ranging from fruit juice to camphor [3].

6.3 Chemical Mechanisms of Neurotoxicity Induced by Organophosphate Nerve Agents

By binding to the enzyme AChE, poisonous agents such as OP compounds prevent its ability to hydrolyze its substrate, ACh, at the synapse. ACh then remains in higher than required concentrations and promotes progressive nerve action potentials that result in nerve hyperactivity and fatigue [4]. Sarin (propan-2-yl methylphosphonofluoridate) has been used as chemical warfare because of its irreversible binding activity to AChE, which causes permanent enzyme inactivation. Sarin inhibition starts by phosphorylation of the hydroxyl group of the serine residue on the active site of the enzyme, increasing its half-life from hours to days. In addition, as a result of increased phosphorylation, the enzyme undergoes a process known as aging, which is characterized by the loss of alkyl groups with subsequent resistance to ACh-mediated cleavage [2, 5]. The clinical manifestations of sarin-induced neurotoxicity vary depending on the type of cholinergic receptor involved, but they generally include pinpoint pupils; blurred and dimmed vision; salivary, sweat, bronchial, and lacrimal gland hypersecretions; cardiovascular upset; seizures; ataxia; musculoskeletal distortion; and even paralysis [6]. These signs are indicative of acute and chronic neurotoxicity and are caused by overaccumulation of ACh in the synaptic junctions, leading to hyperstimulation of the individual's NS [5, 7]. Exposure to sarin can cause death within a few minutes or hours, making it historically one of the most potent chemical warfare agents.

On the other hand, soman (*O*-pinacolyl methylphosphonofluoridate) is a synthetic nerve agent whose mechanism of action involves binding AChE as well

as butyl cholinesterase, which is a plasma cholinesterase [2]. On binding to AChE, soman phosphorylates the serine residues and loses is phosphoryl group, forming methylphosphonic acid. The latter can be decomposed via hydrolytic activity or the action of oximes, regenerating the enzyme.

Unlike sarin and soman, which contain a fluorine substituent, tabun contains a cyanide substituent. Other names for tabun include *N*′-*N*-phosphoramidophosphate or ethyl *N*′-*N*-dimethylaminocyanophosphate, among others. The acute toxicity induced by tabun is characterized by seizures, salivation, miosis, muscle fasciculation, and paralysis, all of which result from overstimulation of both the muscarinic and nicotinic AChRs in the entire body [8]. Exposure to lethal doses of tabun through the skin, eyes, and gastrointestinal tract can cause death in less than 10 minutes [3]. After the nerve agent accumulates in the NS it can undergo spontaneous or enzymatic hydrolysis via cleavage of the P–CN bond [5].

6.4 Mustards

Mustards are vesicant agents that not only cause blistering of the skin and mucous membranes but also damage the eyes and respiratory tract at high doses [9]. Sulfur mustard (2,2′-dichloroethyl sulfide, HD) and nitrogen mustard (*N*-methyl-2,2′-dichlorodiethylamine) both exert toxic effects, but the former is more potent than the latter [10]. Mustards are colorless to light yellow viscous liquids that smell like garlic or fish in high concentrations [11–14]. Figure 6.1 shows the absorption and distribution pattern of sulfur mustard on contact with the skin.

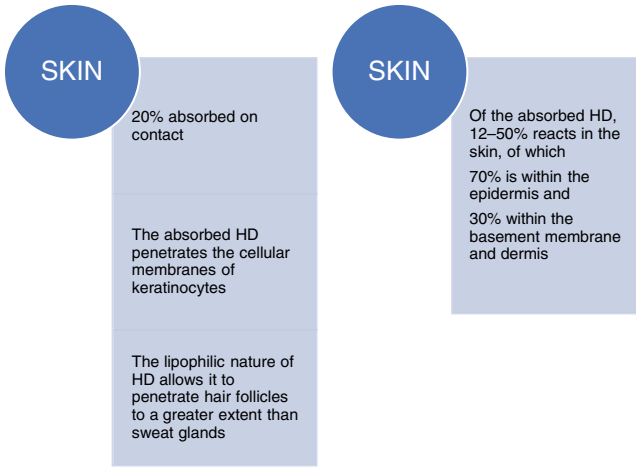

Figure 6.1 Penetration of sulfur mustard (HD) into skin through hair follicles.

The greasy nature of HD makes it more attractive to the lipophilic parts of the body, such as hair follicles [15–17]. After exposure to HD, DNA adducts, as measured by monoclonal antibodies, were easily seen within keratinocytes, yet no adducts were seen within the dermis [16]. Systemic absorption of HD is rare because it is hydrolyzed within organs such as the eyes, skin, and lungs [16]. The vaporized form of HD causes more major injuries than the liquid form. Human contact with the liquid form of HD is rare since most chemical warfare agents are usually packaged in and used with explosives.

6.4.1 Effect of HD on Skin

Figure 6.2 shows how HD affects the skin.

6.4.2 Effect of HD on Other Organs

According to the literature, HD has a profound adverse effect on various organs of the body, including the eyes, skin, and soft organs, such as the lungs, liver, spleen, and kidneys [15–17]. It is important to note that the human body is weakened when the liver is compromised [18]. Both physical weakness and biological weakness may be experienced in the course of HD acting on soft organs [19]. Figure 6.3 summarizes the effects of exposure to HD on various organs.

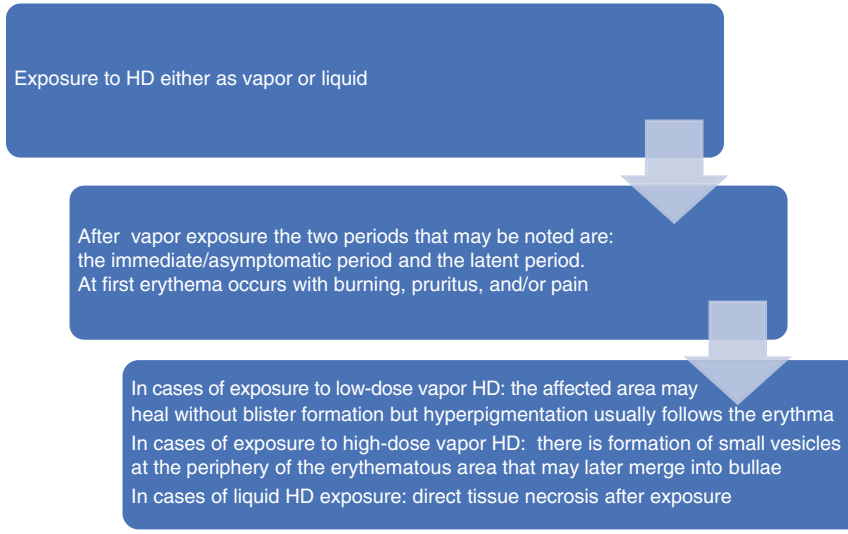

Figure 6.2 Skin exposure to sulfur mustard (HD).

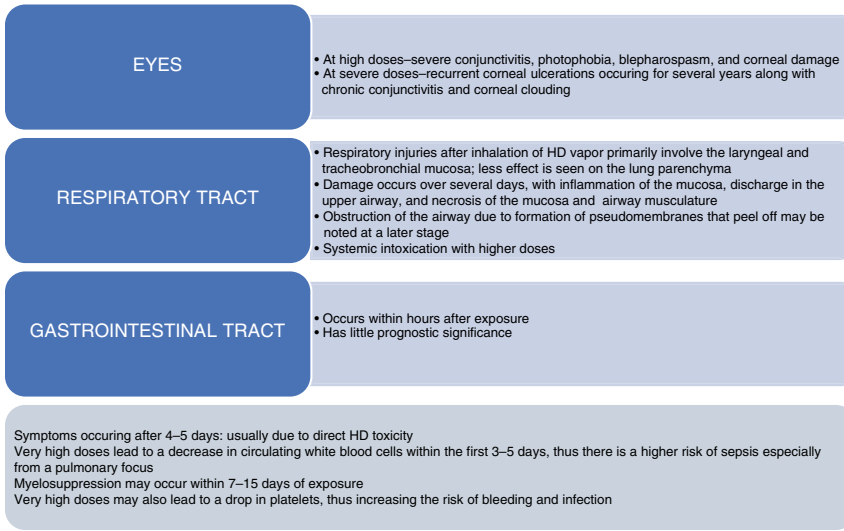

Figure 6.3 Adverse effects of sulfur mustard (HD) on various organs.

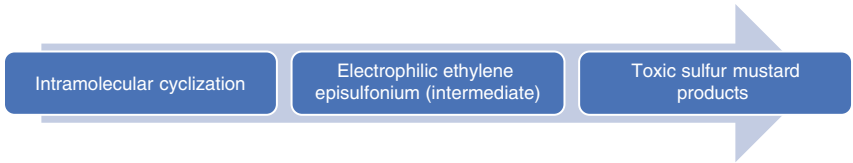

Figure 6.4 The activation process of sulfur mustard (HD).

6.4.3 The Activation of HD

Despite being lipophilic, HD requires reaction with an aqueous medium for biotransformation [14] (Figure 6.4).

The avidity of compounds for the electrophilic products produced by hydrolysis of HD is determined by the availability of electrons within the molecules as well as the functional groups in the molecules that increase electron availability and decrease reactivity. There is similarity between the rate of alkylation and the rate of hydrolysis by HD, but the products of alkylation exhibit greater stability than those of hydrolysis [14, 15, 17, 20, 21].

6.4.4 Mechanism of Action

There are several mechanisms proposed in the literature for the action of HD.

It is known that thiols are one of the body's main defense mechanisms against electrophilic stress (ES) and reactive oxygen species (ROS). Researchers have also proposed that a significant proportion of HD toxicity is secondary to ES or ROS with depletion of cellular detoxifying thiols, including glutathione. Note that microfilamentous proteins, which maintain the cytoskeletal and structural integrity of the cell, can induce apoptosis or necrosis through activation of endonucleases, proteases, and/or phospholipases, and thus can induce DNA and/or membrane damage.

HD can cross-link DNA and produce single-stranded DNA breaks since they are both bifunctional alkylating agents [16, 17, 22, 23].

The molecular mechanism involved in HD-induced epidermal cell injury and death is not completely understood yet. Following DNA alkylation, with induction of DNA repair of strand breaks/apoptosis, the enzyme poly(adenosine diphosphate-ribose) polymerase (PADPRP) is activated [15, 17]. In enzymatic reactions with a number of nuclear proteins, PADPRP utilizes nicotinamide adenine dinucleotide (NAD+) as a substrate in these reactions, as shown in Figures 6.5 and 6.6.

Increased levels of proteases are proposed to play a role in HD-induced blister formation and cellular damage.

In the absence of steric effects, most of the neutrophilic sites are susceptible to HD alkylation [16]. Runs of guanines are present in only 1.3% of the human genome and act as preferential sites for HD alkylation; on the other hand, a significant number of oncogenes and viral oncogenes are rich in guanine, thus explaining why some tumors – particularly virus-induced tumors – respond to this class of drugs [23, 24].

Since there is a preference for a specific site in the DNA binding, this also means that some structural proteins, adhesion molecules, cytokines, and/or enzymes are affected whereas others are not. The interstrand cross-links are major inhibitors of DNA synthesis, with comparatively minor effects on total protein and RNA synthesis, which are the primary targets in rapidly proliferating cells. There is a variation in the sensitivity of different cells to such agents. For example, cells in

Figure 6.5 A simple process illustrating nicotinamide adenine dinucleotide (NAD+) depletion.

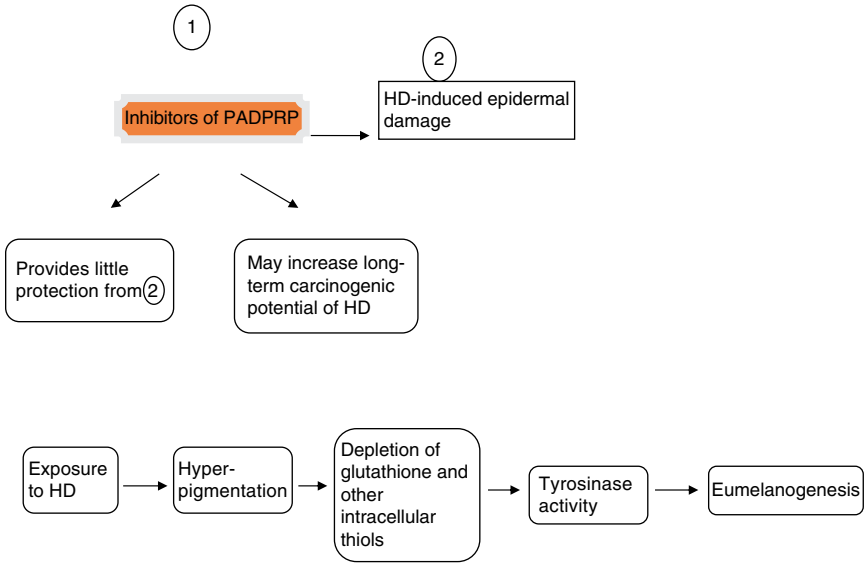

Figure 6.6 Sulfur mustard (HD) blister formation as a result of increased levels of protease. PADPRP, poly(adenosine diphosphate-ribose) polymerase.

the basal cell layer of the epidermis that are transiently amplifying are expected to be more sensitive to these agents; this is also the case for stem cells, which need to maintain a low cytoplasmic/nuclear ratio. The sensitivity of stem cells to these agents can be explained by unbalanced growth along with an increase in the cytoplasmic/nuclear ratio as a result of marked inhibition of nuclear DNA synthesis, cytoplasmic proteins, and RNA [24]. In addition, melanogenesis may be enhanced by DNA repair enzymes that are upgraded by enzymes that damage DNA [16].

6.5 Plant Natural Neurotoxins

A variety of plants used for pharmaceutical, nutritional, and other industrial purposes contain a wide range of toxic compounds from various plant parts at different levels. A range of groups of compounds from various plants have been implicated in a number of neurotoxic pathologies.

Non-proteinogenic amino acids and glycosides are examples of groups of compounds that are notorious for their neurotoxicity [1].

Plants synthesize a significant range of non-proteinogenic amino acids for ecological purposes. Although a good number of these secondary amino acids are

utilized by some plants for physiological development, a wide range are deployed as weapons in the fight against ecological competitors. The majority of these amino acids resemble the endogenous amino acids of the human body and therefore are comparatively active toward their de novo receptors in vivo.

The genera *Lathyrus* and *Panax* constitute some of the plant groups known to possess neurotoxic amino acids [25–27]. An unusual non-protein amino acid, β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), is a strong neurotoxic molecule produced by these plants and has been found to mediate its activity via molecular interaction and agonistic effects with non-*N*-methyl-D-aspartate (NMD) receptors. As it binds to these receptors, it induces excitotoxicity by inhibiting exchange of L-cystine and L-glutamate via the system $x_c(-)$ transporter, an amino acid transporter of the glycoprotein-associated amino acid transporter (gpAT) family [26, 28]. Accumulating amounts of β-ODAP in the CNS cause the slowly spreading disease neurolathyrism; in addition to its structural similarity to L-glutamate, the compatibility of β-ODAP at multiple sites prompts its multitarget-mediated neuroaccumulation and toxicity [29].

L-Glutamate is a major excitatory neurotransmitter and precursor of another neurotransmitter, γ-aminobutyric acid (GABA), the principal inhibitory neurotransmitter. Glutamate receptors are both synaptic and non-synaptic, residing in the membranes of neurons as well as in neuroglia [30]. Glutamate interaction with its receptors mediates postsynaptic neuronal excitation and plays a major role in neural communication, memory formation, and regulatory processes. Chronic overactivation of glutamate receptors is implicated in a number of neuropathological conditions as a result of excitotoxicity. The accumulation of glutamate or its analogs such as β-ODAP induces prolonged activation of *N*-methyl-D-aspartate receptor (NMDARs), leading to high levels of calcium ions (Ca^{2+}) following their massive influx into the postsynaptic cell. The accumulation of calcium ions induces a number of biochemical cascades that promote neuronal damage and subsequent death [31]. Excessive intracellular Ca^{2+} promotes neuronal death by activating several forms of hydrolytic enzymes, including proteases (such as calpains), caspases, nucleases, and lipases [32]. In addition, high levels of calcium promote activation of nitric oxide synthase (NOS) and generation of free radicals, with consequent overwhelming oxidative stress leading to neuronal death [32]. The clinical syndromes associated with β-ODAP accumulation and its excitotoxic effects include convulsions, trauma, ischemia, lower limb paralysis, gluteal muscle emaciation, and long-lasting seizures [29].

In the early 1960s, *Lathyrus cicero* and *Lathyrus sativus* were found to produce the amino acid homoarginine, also known as L-2-amino-6-guanidinohexanoic acid [33]. The neurotoxic effect of this compound is linked to its propensity to increase ammonia levels while inhibiting uptake of ornithine and lysine in the brain [34].

Studies have shown that homoarginine binds neural nitric oxide synthase (nNOS), the enzyme that catalyzes the synthesis of the signaling molecule nitric oxide (NO), normally from L-arginine [31]. NO is a signaling molecule in the CNS and PNS that acts by increasing the activity of soluble guanylate cyclase, which in turn synthesizes cyclic guanosine monophosphate (cGMP) [35, 36]. Homoarginine activates the G-protein-coupled receptor GPRC6A in a similar manner to L-arginine. In the nervous tissue, this activation is associated with increased levels of calcium ions [36], which may account for Ca^{2+}-induced neurotoxicity and mortality.

Caramboxin (CBX) is another NMDAR agonist, with a similar mechanism of action to that of β-ODAP. The compound is produced by plants of the genus *Averrhoa* (commonly known as starfruit, *Averrhoa carambola*). The earliest neurotoxic effect was described in Malaysia following intraperitoneal administration of crude extracts; preliminary findings showed convulsions, unconsciousness, and immediate death, suggesting that the starfruit plant produces a depressant metabolite [37]. CBX is known to act on the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) ionotropic receptor, mediating convulsant, excitatory, and neurodegenerative effects [38].

Structurally, CBX resembles the amino acid phenylalanine, with slight modifications on the aromatic ring, which contains hydroxyl, carboxyl, and methoxy groups. CBX is both a neurotoxin and a nephrotoxin owing to its ability to interact with oxalic acid in the fruit, suggesting that consuming star fruits poses neurotoxic and nephrotoxic risks not only to renally impaired individuals but also to normal individuals [39].

Very well described neurotoxic amino acids from the genus *Polygonatum* include L-α,γ-diaminobutyric acid, L-γ-aminobutyrine, and L-2,4-diaminobutanoic acid (DAB) [34]. Exposure to this amino acid is associated with mass accumulations in the liver, followed by chronic ammonia toxicity to the CNS [40]. The mechanism of action of DAB occurs by competitive inhibition of ornithine carbamoyltransferase, an enzyme catalyzing the formation of citrulline from ornithine and carbamoylphosphate in the urea cycle. This reaction is essential in the detoxification of ammonia, thus inhibition by DAB promotes the increase of ammonia in the blood and the brain, resulting in neurolathyrogenic symptoms such as tremors, hyperirritability, and convulsions, as consequences of liver and brain damage [40].

6.6 Plant Glycosides

Glycosides are a group of abundant natural products in plants as well as in microorganisms. Fabaceae, Rosaceae, Leguminosae, Linaceae, and Compositae are among the well-known families producing cyanogenic glycosides. Cyanogenic

glycosides have been used as a chemotaxonomic group of compounds and they are found extensively in many edible plants such as peaches, cherries, bamboo, cassava, coco yam, beans, and cashew. As toxins, glycosides are implicated in a wide range of pathological conditions, including goiter spastic paraparesis and tropical and ataxic neuropathy [41]. The potential risk of cyanogenic glycosides is posed by their ability to produce hydrocyanic acid or hydrogen cyanide (HCN) [42]. Well-known examples of cyanogenic glycosides include lotaustralin, amygdalin, linamarin, taxiphyllin, and dhurrin, among many others; these are produced in variable amounts among plant species [41].

Cyanogenic glycosides are biosynthesized as defensive secondary metabolites from one of the five proteinogenic amino acids – L-valine, L-isoleucine, L-leucine, L-phenylalanine, and L-tyrosine – as well as from one non-proteinogenic amino acid, cyclopentenyl-glycine [42].

One of the common neurological diseases caused by cyanogenic intoxication is an upper motor neuron disease known as *konzo*, which is most widely known to be due to consumption of cassava [43]. The disease afflicts mostly children and women of child-bearing age and is marked by irreversible non-progressive symmetric spastic paraparesis [43]. The cyanide produced by cyanogenic glycoside mediates toxicity by halting cellular oxidative respiration via cytochrome oxidase a_3 inhibition of the terminal enzyme in the respiratory chain [42].

6.7 Conclusion

Plants are well known to contain medicinally important phytocompounds, but little is known about their synthesis of metabolites that lead to neurotoxins and other agents affecting the NS. It is important to note that the plant kingdom is capable of enhancing adverse effects on the NS; at the same time, it can produce antidotes. If no direct antidotes can be produced, synthetic ones from the plant kingdom can also be made.

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7

Phytosedatives for Drug Discovery

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CONTENTS

7.1 Introduction

The central nervous system (CNS) consists mainly of the brain and spinal cord. The brain receives information and responds by coordinating and performing an action; hence, controlling movements, thoughts, awareness, and memory [1]. Anxiety, nervous tension, agitation, insomnia, depression, epilepsy, dementia, and severe chronic pain represent the major neuropsychological disturbances [2]. Most of these disorders have common symptoms such as fatigue, restlessness, sleep disorders, and fears. The pathophysiology of major CNS disorders involves an imbalance in the level of the major neurotransmitters, such as acetylcholine,

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dopamine, 5-hydroxytryptamine (5-HT), and γ-aminobutyric acid (GABA). Such an imbalance leads to changes not only in the emotional state but also in the cognitive functions.

Anxiety, for instance, is a psychological and physiological state of emotional and behavioral components. Anxiety is a normal response that may happen to anyone as a result of uncertainty. The problem becomes serious when the symptoms exceed the events triggering them. World Health Organization (WHO) statistical estimates revealed that around 264 million people worldwide have anxiety disorders [3]. Insomnia is a persistent disorder in which the person has difficulty in falling asleep or experiences non-restorative sleep along with repeated awakenings. Depression is a common hidden burden that affects more than 350 million people worldwide [3]. Epilepsy is a chronic CNS disorder that leads to recurrent seizures resulting from excessive cerebral neuronal discharges. Around 50 million people have epilepsy, with nearly 80% of them in less developed countries [4].

7.2 Treatment of Neuropsychological Disorders: The Current Scenario

The current treatment for major neuropsychological disorders includes sleep hygiene measures, behavioral therapies, and pharmacological treatment [5]. Sleep hygiene involves some practices and habits that are aimed at obtaining good quality sleep [6, 7]. Behavioral therapies, particularly cognitive behavioral therapy and mindfulness-based interventions, have been used extensively for the management of several CNS disorders, including headache, pain, tension stress, and anxiety [8–10].

Pharmacological treatments include benzodiazepines (such as clonazepam, diazepam, and lorazepam) that induce the neurotransmitter GABA [11]. Benzodiazepines have been used beneficially in the treatment of anxiety, sleep disorders, and panic attacks as well as in seizures, depression, and alcohol withdrawal [12]. A second major class of sedative hypnotics are the barbiturates (e.g. benzylbutylbarbiturate, pentobarbital, phenobarbital, and sodium thiopental). Like benzodiazepines but more potent, barbiturates, which act through binding to the GABA type A ($GABA_A$) receptor, have also been used extensively for the management of several CNS disorders, such as headache, insomnia, and seizures [13]. The binding sites of benzodiazepines and barbiturates are at different locations on the GABA receptor; hence, they exert different pharmacological actions. Azapirones (e.g. buspirone) also exert anxiolytic, antidepressant, and antipsychotic action by binding to 5-hydroxytryptamine 1A ($5\text{-}HT_{1A}$) receptors [14]. Opioids (such as tramadol, tapentadol, and morphine) act on opioid receptors to exert powerful analgesic activity. Selective serotonin reuptake inhibitors (SSRIs), the most commonly prescribed

antidepressants, block the reuptake of 5-HT into neurons and thus increases its levels [15].

7.3 Phytosedatives: Desirable Alternatives to Synthesized Drugs

The overall disease burden is expressed as disability-adjusted life years (DALYs), as per WHO guidelines [16]. This scale represents the lifetime lost due to an illness or a major disability. For instance, schizophrenia and episodes of moderate depression, among CNS disorders, have an increase in DALYs in years from 2004 to 2010 [17]. Pharmacological treatment is the most common and effective method of management of several CNS disorders. However, most anxiolytics and antidepressant drugs have severe adverse effects, ranging from dizziness, nausea, and vomiting to dependence, amnesia, sexual disorders, and restlessness [18, 19].

Several plants, such as valerian and hops, have been evaluated extensively for promising secondary metabolites that can be used as phytosedatives. Additionally, several essential oil components have been reported in aromatherapy as good alternatives to classic anxiolytics and antidepressant drugs. The major essential oils of several plants, such as *Lavender officinalis*, *Citrus aurantium*, *Citrus sinensis*, *Schisandra sphenanthera*, and *Achillea wilhelmsii*, have been shown to exert promising anxiolytic-like activity [20]. Inhalation of the fragrant constituents within aromatherapy oils is believed to relieve tension, anxiety, and depression.

In the following, we provide details about the current knowledge of plants that have been reported to manage CNS disorders. Additionally, details about the phytosedative chemical constituents are discussed as lead compounds.

7.4 Different Classes of Phytosedatives

Following the great advances and achievements in the fields of chromatographic and spectroscopic techniques, especially in recent decades, such as high-performance liquid chromatography, Fourier transform infrared spectroscopy, mass spectrometry (MS), nuclear magnetic resonance (NMR), and their different tandem applications [21, 22], a number of compounds have been isolated and identified from traditional herbal medicines using a bioassay-guided approach [23–25]. These compounds have shown promising and remarkable bioactivities and have been considered as leads for the discovery of novel medicaments, such as the anticancer drugs taxol and vinblastine, which were isolated from *Taxus brevifolia* and *Catharanthus roseus*, respectively; the antimalarial agent quinine, which was isolated from *Cinchona* spp.; and artemisinin, which was isolated from *Artemisia annua* [25, 26]; all of these compounds were isolated in the twentieth century.

Specifically, isolation and characterization of phytosedatives has drawn great attention from phytochemists and natural products professionals, especially those interested in CNS-active herbal drugs that affect behavior. These crude drugs were known to possess sedative, hypnotic, tranquilizer, and anxiolytic activities and have been used for the treatment of insomnia and depression in folk medicine, such as the traditional Chinese medicine [27]. Interestingly, their major ingredients were shown to have potential activity on the storage, reuptake, or release of the inhibitory neurotransmitter GABA and its receptors [28].

Chemically, these compounds revealed diverse structural features and could be grouped into different chemical classes. They include flavonoids, bioflavonoids, essential oils, alkaloids, coumarins, lignans, terpenoids, sterols, and others. The following sections discuss these classes and their chemical structures and possible mechanisms of action.

7.4.1 Flavonoids

A wide spectrum of available flavonoids have proven CNS activity [29]. Their reaction and affinity to the benzodiazepine binding site (BDZ-bs) of $GABA_A$ receptors ($GABA_A$/BDZ) and allosteric modification of chloride ion flux were explored through the ion channel complex [30]. These flavonoids include hesperidin, linarin, baicalein, vitexin-2″-*O*-xyloside, quercitrin, isoquercitrin, and rhusflavone (Figure 7.1).

7.4.1.1 Hesperidin

Hesperidin, or hesperetin-7-*O*-rutinoside (hesperetin-7-rhamnoglucoside), is a flavonoid glycoside of a flavanone nucleus. Historically, it was found that the flowers of several species of *Citrus* were used as a sedative to treat insomnia in Mexican traditional medicine [31]. Moreover, Loscalzo et al. [32] showed that the psychoactivity of hesperidin was mediated through its action on 5-HT_2 receptors and α_1-adrenoceptors in addition to $GABA_A$ receptors.

However, Martínez et al. [33] proposed that this interaction with the different involved receptors is not direct, as other changes in different areas of the brain were observed, especially in intracellular signaling cascades. These changes included a marked reduction in the phosphorylation state of the extracellular signal-regulated kinases 1/2 (ERK1/2) in the cerebral cortex, cerebellum, and hippocampus of the experimental animals [33].

7.4.1.2 Linarin

Linarin, or acacetin-β-rutinoside, is a flavone glycoside that is found in extracts from the roots and rhizomes of *Valeriana* spp. It was identified in *Valeriana wallichii* as the isovaleryl ester initially [34]. Nevertheless, *via* a sleep-inducing assay,

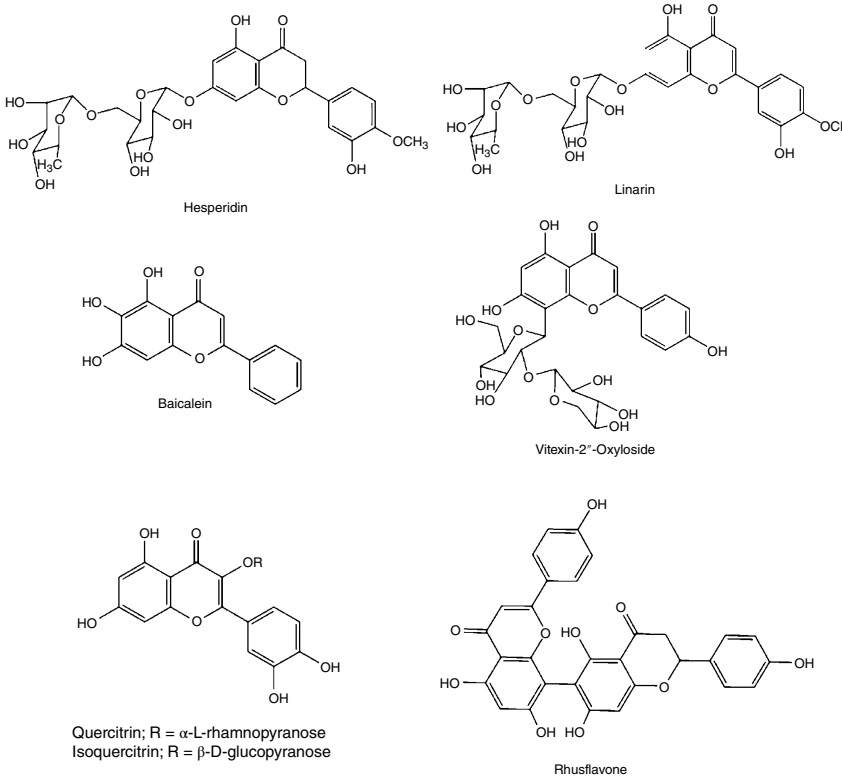


Figure 7.1 Chemical structures of flavonoids reported to have sedative–hypnotic activity.

Fernández et al. [35] demonstrated its activity and identified the molecular structure through ultraviolet absorption, ^{1}H-NMR, and MS. The exact mechanism of action is not yet understood, as linarin did not show any affinity for the BDZ-bs of the $GABA_A$ receptor complex in brain. However, linarin demonstrated a synergistic effect with the other anxiolytic ingredients in a *Valeriana* extract, such as the flavonoids hesperidin and 6-methylapigenin and the sesquiterpenoid valerenic acid [35].

7.4.1.3 Baicalein

Baicalein (5,6,7-trihydroxyflavone) is a flavone isolated mainly from the roots of *Scutellaria baicalensis*. Beside its reported multiple pharmacological activities, such as antioxidant and cytoprotective effects against cytotoxicity induced by oxidative stress [36], baicalein exerted an anxiolytic-like and sedative action through GABAergic non-benzodiazepine sites, when directly injected into the CNS [37].

Moreover, its glycoside baicalin was reported to also have sedative diurnal effects on the sleep–wake rhythm, in addition to inhibition of interleukin 1 action and induction of $GABA_A$ receptors during the light and dark phases, respectively [38].

7.4.1.4 Vitexin-2″-*O*-xyloside

Vitexin-2″-*O*-xyloside, a C-glycoflavonoid, is the major flavonoid in the leaves of *Passiflora quadrangularis*, which is used in traditional medicine as a sedative and mild tranquilizer [39]. It showed sedative activity after oral administration in mice. One study reported that the GABAergic pathway is principally involved, which was confirmed with the benzodiazepine antagonist flumazenil [40].

7.4.1.5 Quercitrin and Isoquercitrin

Quercitrin (quercetin-3-*O*-α-L-rhamnopyranoside) and isoquercitrin (quercetin-3-*O*-β-D-glucopyranoside) are flavonoid glycosides with a flavonol aglycon. Both glycosides were reported to be isolated from the flowers of *Albizia julibrissin* Durazz and their traditional uses for the treatment of sleeping disorders have been described [41]. The mechanism of action was revealed when a similar flavonoid pattern was isolated from the inflorescences of *Tilia americana* var. *mexicana*; the sedative effect was explained by their effect on GABA/BDZ and 5-HT_{1A} serotonergic receptors [42].

7.4.1.6 Rhusflavone

Rhusflavone is a biflavonoid found in *Rhus* sp., which is traditionally involved in the treatment of neurological diseases such as anxiety, insomnia, and epilepsy. Its sedative mechanism involves specific allosteric modulation of $GABA_A$/BZD receptors [43].

7.4.2 Alkaloids

7.4.2.1 Matrine and Oxymatrine

Matrine and oxymatrine are quinazoline alkaloids (Figure 7.2) isolated from the family Fabaceae, such as the aerial parts of *Sophora* sp. [44]. Their sedative effect was investigated and it was shown that it is mediated through multiple targets, such as alleviating caffeine-induced hyperactivity, increasing the non-rapid eye movement sleep, activation of the sleep-promoting center, and modulating 5-HT-related transmission [45]. In addition, oxymatrine induces the release of the inhibitory neurotransmitter GABA [27].

7.4.2.2 Theacrine

Theacrine, or 1,3,7,9-tetramethyluric acid, is a purine alkaloid found in *Camellia* sp. that can be extracted in variable concentrations. Unlike other CNS-stimulating

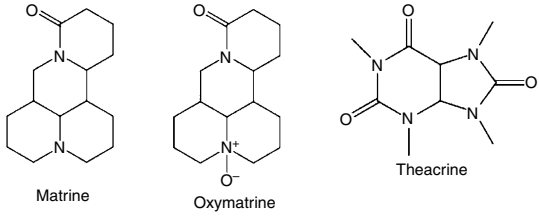


Figure 7.2 Chemical structures of some sedative–hypnotic alkaloids.

purine alkaloids, such as caffeine, theacrine can potentiate the sleeping effect of phenobarbital [46].

7.4.2.3 Total Alkaloid Extracts

Total alkaloids isolated from *Eschscholzia californica*, male *Eucommia* sp., and *Lotus* leaves are reported to have sedative–hypnotic effects. It is likely that most of these alkaloids act through modulation of the GABA level in the brain or its receptors, activating monoaminergic neurotransmitters [47–49].

7.4.3 Essential Oils

The term essential oils is originated in the sixteenth century and takes its name from the herb *Quinta essentia*. Volatile or essential oils, or "essences," were named because of their volatility [50]. Chemically, essential oils are complex mixtures of diverse chemical classes that give plants their characteristic aroma. Typically, essential oils are composed of terpenes, including monoterpenes and sesquiterpenes, phenolics, and some hydrophobic ingredients [51]. These pleasant constituents are widely used in unconventional medicine and aromatherapy [52].

Previous reports have stated that the essential oil of *Annona vepretorum* had sedative and anxiolytic effects. Chemical analysis of *A. vepretorum* essential oil showed the presence of 16 different compounds. (*E*)-β-ocimene (42.59%), bicyclogermacrene (18.81%), germacrene D (12.19%), and limonene (10.02%) were identified as its major constituents (Figure 7.3). Pharmacologically, GABAergic and serotonergic systems were related to sedative activity [53].

In addition, the essential oil from the leaves of *Myrtus communis*, or myrtle, showed hypnotic activity. Myrtenol, myrtenol acetate, limonene (23%), linalool (20%), pinene (14%), and cineol (11%) were the most important constituents. The oil did not itself have any hypnotic effect. Nevertheless, it worked by potentiating the hypnotic effect when taken with significant CNS depressant drugs [54].

Interestingly, linalool, also a constituent of lavender and rose oils, showed $GABA_A$ modulatory activity and confirmed its calming, anxiolytic, and sedative effects [55–57]. Moreover, linalool and 1,8-cineol were reported as major

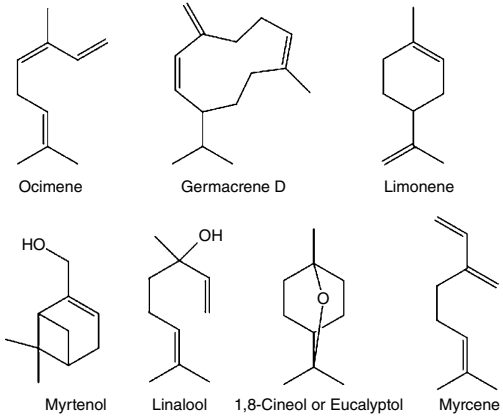


Figure 7.3 Chemical structures of some sedative–hypnotic volatile constituents.

monoterpenes in the essential oil of *Lippia alba*. The oil has sedative effect and prevents the stress response caused by using the anesthetic 2-phenoxyethanol for the purposes of fish anesthesia [58, 59]. It is likely that myrcene can induce sedation and fish anesthesia [60].

7.4.4 Other Classes of Phytosedatives

The other chemical classes of phytosedatives involve coumarins, resin glycosides, naphthoquinones, adenine ribosides, etc. All these classes are summarized in Table 7.1, which gives a clear overview, and their chemical structures are shown in Figure 7.4.

7.5 Plants with Reported Sedative Actions

Many plants are reported to possess sedative effects, the most famous of which are opium, henbane, and mandrake, which were known in medieval times as the "great rest" (requies magna). The three plants were used in combination in premodern science as a sedative and/or analgesic. Recent research has precisely determined the therapeutic dose of the great rest to be 3.1 ± 0.1–5.3 ± 0.76 g and has revealed that its lethal dose is double the therapeutic dose, where all three alkaloid compounds are biologically active [74]. In addition, many other plants have been shown to possess sedative and anxiolytic activity as a result of being tested in different animal models. Table 7.2 shows a list of such pants, their effective doses, the model used for their testing, and their mechanism of action.

Table 7.1 Some other classes of phytochemicals possessing sedative–hypnotic activity.

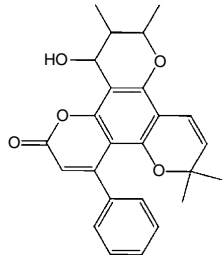
Chemical class	Example	Effects	Molecular mechanism	Reference
Coumarins	Soulattrolide	Mild sedative and anxiolytic properties; demonstrated experimentally by showing significant results in pentobarbital-induced sleeping time and the rotarod assay	Unclear	[61]
Resin glycosides	Convolvulin	Sedative and vasodilator. It increased the pentobarbital-induced hypnotic effect	Enhancement of GABA release in the brain cortex	[62]
Sesquiterpenoids	Ricinusoids A and ricinusoids B	Sedative and analgesic effects in the open field test and acetic acid-induced writhing test, respectively	Unclear, but reduction in the locomotive activity was observed in a dose-dependent manner	[63]
	Guaiane-type sesquiterpene lactone; lactucin and lactucopicrin	Sedative effect in the spontaneous locomotor activity test. It also possesses analgesic activity	Unclear	[64]
Diterpenoids	Unnamed tricyclic pimarane nucleus-containing diterpenoids	Sedative and skeletal muscle relaxant	$GABA_A$ receptor is involved	[65]
Phenyl propanoids	a) Salidroside (tyrosol glucoside)	Anxiolytic and sedative through shortening of sleep latency and prolonging sleeping time	Unclear	[66]
	b) Betonyoside F and verbascoside	Strong sedative effect after oral administration	Unclear, but indirect effect on GABA/BDZ receptor may be involved	[67]
Lignans	Schizandrin	Sedative through augmentation of pentobarbital-induced sleep effect	Serotonergic system is involved	[68]

(*Continued*)

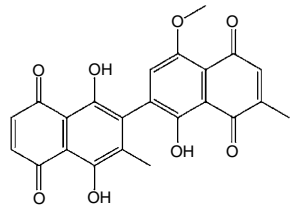
Table 7.1 (Continued)

Chemical class	Example	Effects	Molecular mechanism	Reference
Iridoids	Paederosidic acid	Significant anticonvulsant and sedative effects	Increase of GABA and decrease of glutamic acid release in brain	[69]
Triterpenoids and phytosterols	Lupeol and betulinic acid (triterpenoids); stigmasterol and β-sitosterol (sterols)	Mild sedative in open field animal models	Unclear, but a reduction in locomotor activities was observed	[70]
Miscellaneous	a) **Benzoquinones** (e.g. jacaranone)	Sedative, anxiolytic, and mild tranquilizer activities	Direct activation or modulation of $GABA_A$ receptors may be involved	[71]
	b) **Adenine riboside** (e.g. N^6-(4-hydroxybenzyl) adenine riboside)	Potentiating the sedative–hypnotic effect of sodium pentobarbital and significant decreasing of wakefulness time and increasing of non-raid eye movement sleep times	A functional ligand for the adenosine A_1 and A_{2A} receptors, decreasing spontaneous locomotor activity	[72]
	c) **Naphthoquinones** • Di-naphthodiospyrol	Significant sedative-hypnotic-like effect	$GABA_A$ receptor agonist	[73]
	• Diospyrin and 8-hydroxyisodiospyrin	Mild to moderate sedative effect in open field animal models, in addition to analgesic and anti-inflammatory effects	Exact mechanism is unknown, but a reduction in locomotor activities, including frequency and amplitude of motion, was observed	[70]

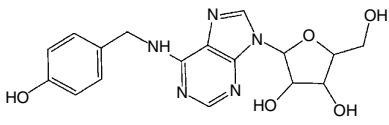
BDZ, benzodiazepine; GABA, γ-aminobutyric acid.



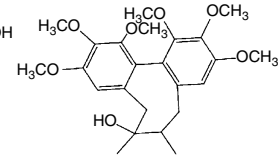
Soulattrolide



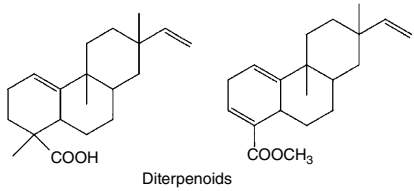
di-Naphthodiospyrol



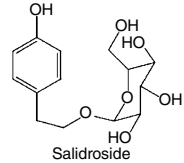
N^6-(4-hydroxybenzyl) adenine riboside



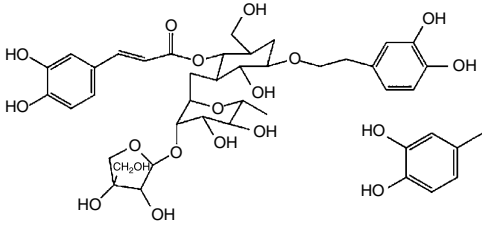
Schizandrin



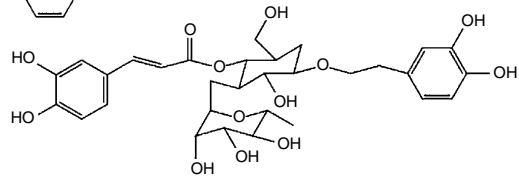
Diterpenoids



Salidroside

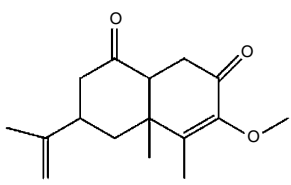


Betonyoside F

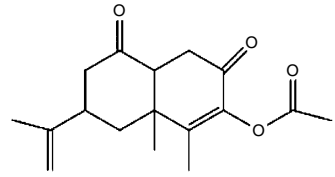


Verbascoside

Figure 7.4 Chemical structures of some natural bioactive phytosedatives.



Ricinusoids A



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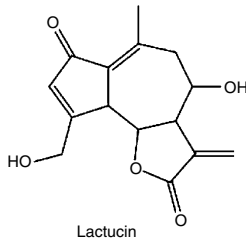
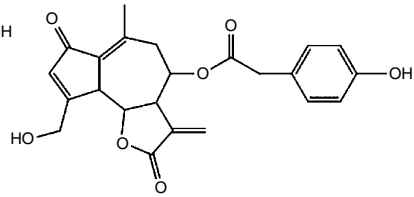
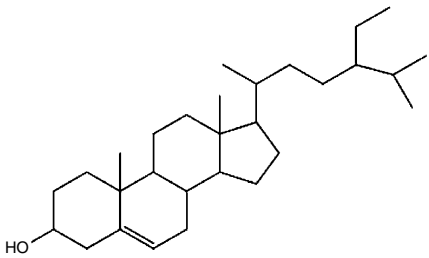


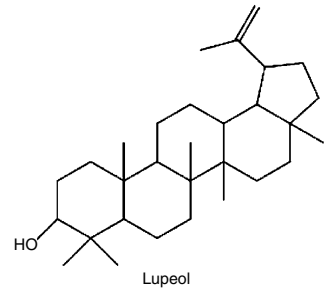
Lactucin



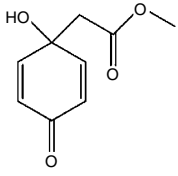
Lactucopicrin



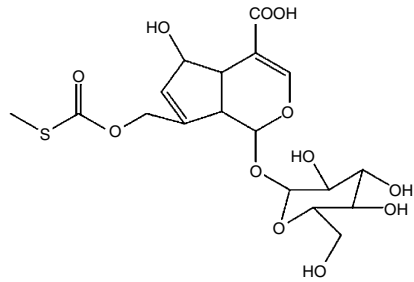
β-Sitosterol



Lupeol



Jacaranone



Paederosidic acid

Figure 7.4 (Continued)

Table 7.2 Plants with reported sedative actions.

Plant	Type of extract	Doses	Model	Results	Mechanism of action	Reference
Euphorbia hirta (L.)	Lyophilized aqueous extract of the whole dried plant	12.5, 25, 50, 100, and 200 mg/kg i.p. in mice	Behavioral effects in mice (activity test and staircase test)	100 and 200 mg/kg showed decrease of behavioral parameters	Sedative and anxiolytic properties	[75]
Telfairia occidentalis Hook.f.	Hydroethanolic extract of the leaves	50–400 mg/kg, p.o. in mice	The hole-board, elevated plus-maze test, open-field, light–dark, and social interaction tests	At 50 mg/kg there was an increased number of sectional crossings and number of central squares crossed. At 100 mg/kg there was an increased duration of head dips and number of entries into open arms. Both the doses increased the number of social interactions. At doses of 200 and 400 mg/kg, there was an increased latency of entry into and time spent in a dark box and a reduced number of social interactions	Anxiolytic property at doses of 50 and 100 mg/kg and sedative activity at doses of 200 and 400 mg/kg	[76]
Cyperus articulatus (L.)	Decoction of the rhizomes	20 ml/kg i.p. in mice	Effect on sodium thiopental- and diazepam-induced sleep	Facilitates sleep induction and increases the total sleep time	Pharmacological properties similar to those of sedatives	[77]

(*Continued*)

Table 7.2 (Continued)

Plant	Type of extract	Doses	Model	Results	Mechanism of action	Reference
Equisetum arvense (L.)	50% EtOH–water of the aerial stems	50, 100, 200, or 400 mg/kg i.p. in rats	Behavioral tests	200 and 400 mg/kg showed a significant activity on the open-field, enhanced the number of falls in the rotarod, reduced the time of permanence in the bar, and increased the sleeping time (46% and 74%) in the barbiturate-induced sleeping time	Sedative effect	[78]
Vateria copallifera (Retz.)	Aqueous extract of the bark	250, 1000, 2000, and 2500 mg/kg p.o. in rats	Rat hole-board model	Dose-dependent sedative activity		[79]
Monotheca buxifolia and *Bosea amherstiana*	Methanolic extract	50, 100, and 150 mg/kg i.p. in mice	White wood apparatus divided by black lines in different squares	Both plants exhibit mild to moderate sedative effects in the tested doses		[80]
Fruits of *Fructus schisandrae*	EtOH fraction	25, 50, and 100 mg/kg	Open-field test	Significantly inhibited the motor activity of mice compared with the normal. Results also showed SY3 potentiated pentobarbital-induced sleep by not only increasing the frequency of falling asleep and prolonging sleeping time but also reducing sleep latency	Hypnotic and sedative	[81]

Securidaca longepedunculata Fresen.	Aqueous extract of the roots	100–400 mg/kg p.o. to albino mice	Hexobarbitone-induced sleep and the hole-board models	The extract (100–400 mg/kg) produced significant reduction of onset of sleep induced by hexobarbitone. The prolongation of hexobarbitone sleeping time by the extract (200 mg/kg) was comparable to that produced by diazepam (3 mg/kg). At doses of 100–400 mg/kg, the extract produced a dose-dependent decrease in exploratory activity of the mice	CNS depressant	[82]
Noni (*Morinda citrifolia*)	MeOH extract, and its BuOH and H_2O partitions of the fruits	*In vitro*	$GABA_A$ binding assay	The MeOH extract and its BuOH and H_2O partitions exhibited IC_{50} values of 22.8, 27.2, and 17.1 mg/ml, respectively		[83]
Schisandra chinensis (Turcz.) Baill.	Supercritical carbon dioxide fluid extraction of the fruits	50 mg/kg, 100 mg/kg, and 200 mg/kg, intragastrically in mice	Locomotor activity and pentobarbital-induced sleep test	Strong hypnotic effect in synergy with pentobarbital in mouse sleep, and reversal of insomnia induced by caffeine, *p*-chlorophenylalanine, and flumazenil by decreasing sleep latency and sleep recovery and increasing sleeping time. It produced a synergistic effect with 5-hydroxytryptophan	Relevant to the serotonergic and GABA-ergic system	[84]

(*Continued*)

Table 7.2 (Continued)

Plant	Type of extract	Doses	Model	Results	Mechanism of action	Reference
Nauclea latifolia Sm.	Decoction of the root bark	16, 40, 80, and 160 mg/kg i.p. in mice	MES-, PTZ-, and STR-induced convulsions; NMDA-induced turning behavior; elevated plus-maze test; stress-induced hyperthermia; open field; and diazepam-induced sleep	The decoction increased the diazepam-induced total sleep time. Protected against MES-, PTZ-, and STR-induced seizures. Inhibited the turning behavior induced by NMDA. In the elevated plus-maze test, it increased the number of entries into, percentage of entries into, and percentage of time in open arms, and reduced rearing, head dipping, and percentage of time in closed arms. In the open-field test, it increased crossing and reduced rearing and defecation	Sedative, anticonvulsant, anxiolytic	[85]

Spondias mombin (L.)	Aqueous, MeOH and EtOH solvents of the leaves	12.5–100 mg/kg i.p. in mice and rats	Hexobarbital-induced sleeping time and NIR behaviors	The methanolic and ethanolic extracts (12.5–100 mg/kg i.p.) prolonged the hexobarbital-induced sleeping time and reduced the NIR in both mice and rats in a dose-dependent manner. The aqueous extract prolonged the hexobarbital-induced sleeping time and reduced NIR at doses of 50 and 100 mg/kg. The three extracts blocked PIC-induced convulsions	Sedative and antidopaminergic effects. The effect is not mediated *via* muscarinic, α_2-adrenergic, and μ-opioid receptors, whereas, the extracts appear to facilitate GABAergic transmission	[86]
Cecropia pachystachya Mart. from neotropical rainforest and from temperate region	Aqueous extract of the leaves of each variety	180 and 600 mg/kg i.p. in mice	Open-field test	Both plants decreased the spontaneous locomotion and exploratory behavior of mice at doses between 180 and 600 mg/kg. The plant from the neotropical rainforest potentiated the effect of 3 mg/kg diazepam to one similar to 10 mg/kg diazepam, but was not antagonized by 0.5 mg/kg flumazenil. Amphetamine at 5 mg/kg prevented its sedative effect	Exert a sedative effect additive to benzodiazepines but do not bind to the same site on the $GABA_A$ receptor. Prevented by the dopamine release produced by amphetamine	[87]

(*Continued*)

Table 7.2 (Continued)

Plant	Type of extract	Doses	Model	Results	Mechanism of action	Reference
Tilia americana (L.) var. *mexicana* (Schltdl.) Hardin	Inflorescence hexane and MeOH extracts	Hexane (10–1000 mg/kg) and MeOH (10–300 mg/kg) i.p. in mice	SP-induced hypnosis potentiation	The MeOH extract was more active in showing a dose-dependent lengthening in the hypnosis time induced by SP. Also produced a significant and dose-dependent attenuation in the anxiety response in the plus-maze test and exploratory cylinder activity, but also a diminution in the ambulatory activity and in the head dipping response were observed, resembling the response to diazepam	Depressant activity on the CNS	[88]
Tilia americana (L.) var. *mexicana* (Schltdl.) Hardin	Aqueous extract of the inflorescences	10, 30, and/or 100 and 300 mg/kg p.o.	SP-induced hypnosis potentiation, ambulatory activity	All doses caused lengthening in the hypnosis time. A significant attenuation in the anxiety response in the plus-maze test and a diminution in both head dipping response and ambulatory activity were comparable to diazepam at a dose of 0.3 mg/kg i.p.	Sedative and anxiolytic-like	[89]

Sansevieria liberica	Aqueous extract of the roots	100–400 mg/kg p.o. in mice	Pentobarbitone sleeping time and hole-board exploratory behavior for sedation tests, and STR, PIC, bicuculline, and PTZ-induced convulsions in mice	A dose-dependent prolongation of pentobarbitone sleeping time and suppression of exploratory behavior. 100 and 200 mg/kg produced dose-dependent and significant increases in onset to clonic and tonic convulsions, and 400 mg/kg showed complete protection against seizures induced by STR, PIC, and bicuculline, but not with PTZ	Sedative and anticonvulsant activities	[90]
Kaempferia galanga (L.)	Hexane extract	Inhalation of the hexane extract at doses of 1.5 and 10 mg		Significant reduction in locomotor activity, indicating considerable sedative and relaxant effects		[91]

(*Continued*)

Table 7.2 (Continued)

Plant	Type of extract	Doses	Model	Results	Mechanism of action	Reference
Passiflora edulis f. *flavicarpa*	Ethanolic extract of the aerial part and its fractions	100 mg/kg, 200 mg/kg, 300 mg/kg, and 400 mg/kg of the EtOH extract p.o. in mice. Isoorientin was used in 20 mg/kg, 40 mg/kg, 75 mg/kg, 80 mg/kg, 125 mg/kg, 200 mg/kg, and 300 mg/kg of fractions	Elevated plus-maze test and spontaneous activity	EtOH extract (300 mg/kg and 400 mg/kg), *n*-BuOH fraction (125 mg/kg and 200 mg/kg), aqueous fractions (200 mg/kg and 300 mg/kg), BEF-I (200 mg/kg), BEF-II (200 mg/kg), BEF-III (100 mg/kg), or isoorientin (20 mg/kg) resulted in anxiolytic-like effects, but sedative-like activity was produced at higher doses, such as 300 mg/kg of the *n*-BuOH fraction or 40 mg/kg and 80 mg/kg of isoorientin. The results of the spontaneous activity test manifested that treatment with 400 mg/kg of EtOH extract, 300 mg/kg of *n*-BuOH fraction, or 40 mg/kg and 80 mg/kg of isoorientin compromised motor activity in mice	Anxiolytic at low doses. Sedative at high doses	[92]

Dorstenia arifolia	MeOH extract of the rhizomes	10 and 50 mg/kg i.p. in mice	Locomotor activity evaluation, pentobarbital-induced sleeping time, and PTZ-induced convulsion	Significantly decreased locomotor activity and increased the duration of pentobarbital-induced sleeping. Also promoted a significant protection of PTZ-induced seizures	Sedative and anticonvulsant activities related to a facilitation of the GABAergic transmission	[93]
Pinelliae praeparatum	The EtOH fraction of the rhizomes	0.2 ml/10 g, volume/body weight was administered intragastrically	Locomotion activity, pentobarbital-induced sleeping, and NKTM-induced convulsion tests	Dose of 12 g/kg significantly inhibited the locomotion activity of mice. The effect of the extract on pentobarbital-induced sleeping was inhibited by L-malic acid and flumazenil	Sedative, hypnotic, and anticonvulsant activities, which may be related to the GABAergic system	[94]
Viscum album (L.)	The aqueous leaf extract	50 and 150 mg/kg, p.o. in rats and mice	Measurement of locomotor activity and pentobarbital sleeping time	Prolonged the pentobarbital-induced sleeping time and reduced the locomotor activity in an actophotometer. Reduced maximum electric shock-induced seizures, isoniazid-induced convulsions, and PTZ-induced convulsions. Decreased the apomorphine-induced stereotyped behavior and potentiated the haloperidol-induced cataleptic score	Facilitates GABAergic transmission and possesses antidopaminergic activity	[95]

(*Continued*)

Table 7.2 (Continued)

Plant	Type of extract	Doses	Model	Results	Mechanism of action	Reference
Dracocephalum moldavica (L.)	Aqueous extract of aerial parts	1, 10, 100, or 200 mg/kg i.p. in mice	Pentobarbital-induced sleeping time observed using the hole-board and the avoidance exploratory behavior tests and on the forced swimming test	Prolonged the pentobarbital-induced sleeping time, induced sedation in the hole-board test, decreased spontaneous activity, and produced motor coordination impairment in mice	Sedative actions and a general inhibition of CNS activity	[96]
Crassocephalum bauchiense (Hutch.) Milne-Redh.	Aqueous extract of the leaves and its alkaloid fraction	20, 40, 80, and 160 mg/kg p.o. for the aqueous extract; 5, 10, 20 and 40 mg/kg, p.o. for the alkaloid fraction	Novelty-induced rearing behavior	Caused dose-dependent inhibition of novelty-induced rearing behavior; decreased the apomorphine-induced stereotypy and fighting. The aqueous extract prolonged the SP sleeping time. The concentration of the inhibitory amino acid GABA was significantly increased	Antipsychotic and sedative effect which is mediated through the blockade of dopamine D_2 receptors and GABAergic activation	[97]

Paris polyphylla Smith var. *yunnanensis* (Franch.) Hand.-Mazz.	Ethanolic extract of roots	250 and 500 mg/kg (i.p.) in mice	Rotarod test and SP-induced hypnosis	The extract acted in synergy with SP at doses of 250 and 500 mg/kg while motor coordination was not influenced	Sedative–hypnotic activity	[98]
Carica papaya (L.)	80% ethanolic extract of the pulps	50, 100, 200, and 400 mg/kg in mice	Elevated plus-maze test, staircase and open-field tests, and ketamine-induced sleeping time test for sedation	At 100 mg/kg significantly increased the percentage of open-arm time and entry and reduced the percentage of entry and time spent in the closed arm in the elevated plus-maze test; reduced the amount of rearing in the staircase test and increased the time spent and entries in the central squares while the total number of entries into the open field were not significantly affected	A synergistic reduction in the amount of rearing and inverted U-shaped dose–response curves were obtained with important parameters of anxiety	[99]

(*Continued*)

Table 7.2 (Continued)

Plant	Type of extract	Doses	Model	Results	Mechanism of action	Reference
Dichrocephala integrifolia	Decoction of the leaves	4, 22, and 40 mg/kg p.o. in mice	Elevated plus-maze and open-field tests while the sedative effect was evaluated by the diazepam-induced sleep test	The extract induced an increase in the percentage of entries into open arms and a decrease in the percentage of entries into closed arms at a dose of 22 mg/kg. The extract also induced a decrease in rearing and head dipping at 22 and 40 mg/kg. In addition, the extract induced a significant increase in crossing and time spent at the center of the experimental set at a dose of 40 mg/kg during the open-field test. It also caused a significant reduction in the latency to sleep and an increase in total sleep time at doses of 22 and 40 mg/kg in the diazepam-induced sleep test	Anxiolytic and sedative properties	[100]

Pearl in oysters, *Pteria martensii* (Dunker), or mussels, *Hyriopsis cumingii* (Lea) or *Cristaria plicata* (Leach), and nacre (mother of pearl) ii the conch of these mollusks	The aqueous extract	p.o. in mice	Locomotor activity test	Pearl original powder (1.1 g/kg), pearl water-soluble protein (0.2 g/kg), pearl acid-soluble protein (0.275 g/kg), pearl conchiolin protein (1.1 g/kg), nacre original powder (1.1 g/kg), nacre water-soluble protein (0.2 g/kg), nacre acid-soluble protein (0.7 g/kg), and nacre conchiolin protein (1.1 g/kg) could downregulate the expression of 5-HT_3 and upregulate the level of $GABA_B$ to varying degrees compared with the control group. In addition, drug administration also reduced the locomotor activity and increased convulsion latency with a certain mortality	[101]
Kava kava and *Passiflora incarnata*	EtOH extract	100 mg kava soft extract, 250 mg passiflora extract, and a combination of both (350 mg/kg) p.o. by stomach tube in mice	Amphetamine-induced hypermotility test	Caused a significant decrease in amphetamine-induced hypermotility and significant prolongation of the sleeping phase induced by subcutaneous injection of barbiturates	[102]

(*Continued*)

Table 7.2 (Continued)

Plant	Type of extract	Doses	Model	Results	Mechanism of action	Reference
Semen *Ziziphi spinosae* (Suanzaoren) and Radix et Rhizoma *Salviae miltiorrhizae* (Danshen)	Water extract of Suanzaoren and the ether extract of Danshen	400 and 800 mg/kg of Suanzaoren; 300 and 600 mg/kg of Danshen	Pentobarbital-induced sleep test	Both extracts could shorten sleep latency significantly, increased sleeping time, and prolonged movement convalescence time induced by SP (55 mg/kg body wt.) administration in mice. The combination of the extracts showed a significant synergistic effect in decreasing sleep latency and increasing sleeping time	Sedative–hypnotic activity	[103]
Acanthus montanus,	Dried leaves	Mice	Animal models (MES, NMDA, PTZ, isonicotinic hydrazide acid, PIC, and STR-induced convulsions, turning dried leaves behavior and diazepam-induced sleep)	*A. montanus* protected 66.6% of mice against MES-, PIC-, and STR-induced convulsions and 83.3% of mice from PTZ-induced convulsions. *A. laxiflora* protected 75% and 87.5% of mice in the STR and NMDA tests, respectively, at a dose of 120 mg/kg. *H. spicigera* protected 100% and 87.5% of mice against STR- and	Sedative activity through increasing the total duration of sleep induced by diazepam	[104]
Alchornea laxiflora,	Fresh leaves					
Hyptis spicigera,	Fresh leaves					
Microglossa pyrifolia,	Fresh leaves					
Piliostigma reticulatum,	Fresh leaves					
Voacanga africana	Dried bark					

				PTZ-induced convulsions, respectively, at a dose of 160 mg/kg. *M. pyrifolia* protected 50–100% of mice against convulsions. *P. reticulatum* protected 62.5–100% of mice against convulsions and turning behavior. *V. africana* protected 62.5–87.5% of mice against convulsions and turning behavior		
Bridelia micrantha and *Croton macrostachyus*	Decoction of each bark	The doses are 152, 76, and 30 mg/kg for *B. micrantha* and 135, 67, 34, and 13 mg/kg for *C. macrostachyus*	Diazepam-induced sleep was used for the evaluation of the sedative properties	The coadministration of the subeffective dose of the decoction of *B. micrantha* or *C. macrostachyus* with the subeffective dose of diazepam or clonazepam resulted in a synergistic effect. The decoctions of *B. micrantha* and *C. macrostachyus* also exerted sedative activity by increasing the total duration of sleep induced by diazepam and by reducing the latency time to sleep	Sedative	[105]

(*Continued*)

Table 7.2 (Continued)

Plant	Type of extract	Doses	Model	Results	Mechanism of action	Reference
Gladiolus dalenii Van Geel	Macerate of corms, aqueous extract, and lyophilized extract	Macerate: 7.5, 15, 30, 75, and 150 mg/kg. Aqueous extract: 100, 200, 500, and 1000 mg/kg. Lyophilized extract: 100, 200, 500, and 1000 mg/kg. p.o. administration	MES- and PTZ-induced convulsions were used to evaluate the anticonvulsant activities of the plant extracts. Diazepam-induced sleep was used for the evaluation of the sedative properties	The macerated extract of *G. dalenii* protected 100% and 83.3% of mice against PTZ- and MES-induced seizures, respectively. The aqueous extract of *G. dalenii* protected 100% and 83.3% of mice against PTZ- and MES-induced seizures, respectively. The lyophilized extract of *G. dalenii* also protected 100% and 83.3% of mice against PTZ- and MES-induced seizures, respectively. The coadministration of *G. dalenii* with diazepam resulted in an additive effect, while the coadministration of *G. dalenii* with flumazenil or FG7142 resulted in antagonistic effects. The macerate of *G. dalenii* also exerted sedative activity by reducing the latency time to sleep and increasing the total duration of sleep induced by diazepam	Anticonvulsant and sedative activities that might show efficacy against secondary generalized tonic–clonic seizures and primary generalized seizures and insomnia in humans	[106]

Diospyros lotus (L.)	MeOH root extract and its fraction *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH	50 and 100 mg/kg i.p. in mice	The open-field and rotarod tests	Exhibited significant sedative effect in mice (45.98%) at 100 mg/kg i.p. When the extract was partitioned with different solvents, the *n*-hexane fraction was inactive whereas the chloroform fraction was the most active with 82.67% sedative effect at 50 and 100 mg/kg i.p. On the other hand, the EtOAc and *n*-butanol fractions displayed significant sedative effects (55.65% and 40.87%, respectively) at 100 mg/kg i.p. Among the tested extracts/ fractions, only chloroform and EtOAc fractions showed significant ($P < 0.05$) muscle relaxant activity in the rotarod test	Sedative	[107]

5-HT, 5-hydroxytryptamine; BEF, *n*-butanol extract fraction; BuOH, butanol; CNS, central nervous system; EtOAc, ethyl acetate; EtOH, ethanol; IC_{50}, half-maximal inhibitory concentration; i.p., intraperitoneally; MeOH, methanol; MES, maximal electroshock; NIR, novelty-induced rearing; NKTM, nikethamide; NMDA, *N*-methyl-D-aspartate; PIC, picrotoxin; p.o., per os (orally); PTZ, pentylenetetrazol; SP, sodium pentobarbital; STR, strychnine.

7.6 Conclusion

Phytocompounds play an important role as sedatives both in times of emergency and in regular operations and in local analgesia. Some of these phytocompounds are toxic in nature, and it may be the toxic chemicals that affect CNS cells and induce sedative effects. It is therefore important to study this group of compounds, particularly when phytotoxins and poisonous plants are regarded as not so useful to the community.

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8

Mushroom Species and Classification

Bioactives in Poisonous and Edible Mushrooms

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CONTENTS

8.1 Introduction

The term mushroom is generally used to indicate a stem or stalk, a cap (pileus), and the gills (lamellae). The part of the fungus that is usually visible is the sporophore, which is an umbrella-shaped structure that has fertile surfaces on its

Poisonous Plants and Phytochemicals in Drug Discovery, First Edition.
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lamellae. The lamellate fungi are commonly called mushrooms or toadstools, according to whether they are edible or non-edible, respectively [1]. Mushrooms are ancient species and have had different names; for example, Theophrastus used "truffles" for mushroom fruiting bodies. The Romans viewed mushrooms as "ambrosia," which means heavenly food or the food of Gods and only used mushrooms for special celebratory events, whereas the Chinese view mushrooms as a "health food." Since the Early Middle Ages, mushrooms have been cultivated according to their importance [2]. The genus *Amanita* is known as the death cap or destroying angel and has killed several mushroom hunters [2]. In this chapter, we will discuss edible and non-edible mushrooms, their bioactivities, and their uses.

8.2 Classification of Mushrooms

According to modern classification, which is based on rDNA sequences, the kingdom Fungi is divided into four phyla: Zygomycota, Chytridiomycota, Basidiomycota, and Ascomycota. The phyla Ascomycota and Basidiomycota are the main phyla with fruiting bodies and are considered to be mushrooms. The phylum Ascomycota has approximately 40 000 different types of species around the world. These are also known as sac fungi and ascomycetes. Their spores, called ascospores, are found in the ascus, which is a sac-like structure. Ascospores divide by meiosis and haploid spores are produced; this type of asexual reproduction is observed only in mushrooms, single-celled yeasts, and truffles [2, 3].

The members of the phylum Basidiomycota are also known as basidiomycetes or club fungi. The spores of Basidiomycota are known as basidia. Smut and rust are dominating members that cause numerous diseases in grains. Order Agaricales (genus *Agaricus*; species *Agaricus campestris* [field mushroom]) of the phylum Basidiomycota is the most commonly found and has gills on the fruiting body; these are called gilled mushrooms. Globally, *Agaricus bisporus* is known as a button mushroom, and is the most widely cultivated mushroom. *Marasmius oreades* is a fairy ring mushroom belonging to the order Agaricales, and is also commonly found. *Hypomyces lactifluorum* (lobster mushroom) belongs to the phylum Ascomycota. It parasitizes other mushrooms such as *Russula* and *Lactarius*, forming on the outer side of the organism with the white flesh of the parasitized basidiomycete mushroom remaining on the inner side [2, 4].

Mushrooms (Basidiomycota) produce spores (basidiospore) on the basidium. The spores are haploid and become binucleate at maturity or remain uninucleate. After ripening of the spores, they are released from the fruiting body and start to germinate and form hyphae, which develop into the mycelium [5]. In some cases, two compatible mycelia fuse and a secondary mycelium develops that is binucleate and develops into fruiting bodies.

8.2.1 Edible Mushrooms

It is estimated that a total of 140 000 species of mushrooms have been discovered, out of which around 14 000 species have been described [6]. Less than 10% of the described species are edible mushrooms and 700 species are used in pharmacology [7]. However, data for edible mushrooms have been collected for culinary purposes around the world, the number of which varies greatly from 200 to 3000 mushroom species. Many wild and cultivated mushroom species are consumed globally, in approximately equal amounts. Both fresh and preserved mushrooms are also consumed because of their particular texture and aroma and also because of their lower fiber content and energy. The demand for mushrooms becomes higher when there is a shortage of staple foods, such as during wars [8]. About 100 species are cultivated commercially; of these, 10–20 species are cultivated on an industrial scale [6]. The mushroom industry can be divided into three parts: wild growing, cultivated medicinal, and edible mushrooms [8].

8.2.2 Non-Edible Mushrooms

Numerous mushroom species have psychoactive toxins. They are poisonous and a serious threat to health [9]. Some mushrooms are very toxic and are dangerous even in very small quantities. Many toxins are well explained in the literature, such as amatoxin, which causes damage to the kidneys and liver, and the toxin orellanine, which is nephrotoxic in nature [9, 10]. Some species are consumed for food and medicine because they contain pharmacologically active compounds. Also, mushrooms have been used in traditional medicine since ancient times because of the pharmacological compounds they contain [11, 12]. However, some of the pharmacological compounds were found to be toxic. In fact, only 70–80 mushroom species are fatal out of all the poisonous mushroom species; however, unfortunately, several poisonous mushroom species are morphologically similar to edible mushrooms [13].

8.3 Bioactive Agents in Mushroom Species

The demand for mushrooms is increasing very rapidly because of the presence of the biologically active compounds that are very beneficial for human health, either directly or indirectly [14]. The biologically active compounds that mushrooms contain are also present in cell walls, such as proteins, β-glucans (polysaccharides), and other secondary metabolites like terpenoids, steroids, and phenolics. Bioactive compounds vary greatly from species to species or even within a single mushroom, depending upon the concentration of the bioactive compounds, developmental stage, conditions of the fruiting body, the mushroom's

age, and the storage conditions [15]. Previous studies have described the diversity of biologically active compounds and their action in the medicinal field as, for example, antioxidant, anti-inflammatory, antitumor, antiviral, antifungal, antibacterial, anti-diabetic, immunomodulatory agents [16–19]. Bioactive compounds that are found in mushrooms are described in Tables 8.1 and 8.2.

8.4 Bioactive Agents in Non-Edible Mushroom Species

Both poisonous and non-poisonous mushrooms contain various phytocompounds, some of which are toxins, particularly in wild mushrooms. The following sections describe various components of bioactive agents in both poisonous and edible mushrooms.

8.4.1 Polysaccharides

Edible mushrooms contain polysaccharides. The polysaccharides can be used as immunomodulator, anticancer, anti-inflammatory, antimicrobial, antidiabetic, and antioxidant agents [18, 34, 126].

8.4.2 Glucans

Glucan polysaccharides vary in molecular weight, primary structure, degree of branching, solubility, type of linkage, etc. The fungal glucans can be insoluble or soluble in alkali but are soluble in water. Glucans help to preserve material and are intracellular; other glucans are released in the medium; and some exist in the cell wall [127]. For those glucans that are insoluble, they classically constitute the cell wall's structural components and make cross-linkages to other polysaccharides (chitin, proteins). Soluble and insoluble glucans represent 20–50% and 50–80% of total glucans, respectively [128]. Two glucose units can connect in a minimum of eight different ways in a variety of glucans. As a result of condensation reactions, α or β bonds are formed. On cyclic sugar structures, different branches of chains and length further increase the variety of glucans [129]. In the cell wall of fungi α- and β-glucans are present. *Pleurotus pulmonarius* fruit extract displayed a mixture of α- and β-anomeric carbon links, while mycelial extracts had mostly α-glucan connections in polysaccharides [130, 131]. In numerous basidiomycetes 9–46% of α-1,3-glucan is present in the cell wall. It can exist in the cell wall of various mushrooms such as in the fruiting bodies of *A. bisporus* [132]. The main constituent of the cell wall in numerous ascomycetes and basidiomycetes is β-glucan. This is a long-chain polysaccharide with β-D-glucose as the basic

Table 8.1 Bioactive compounds of edible mushroom species.

Scientific name	Phylum	Bioactive compounds	Action of bioactive compounds	References
Agaricus bisporus	Basidiomycota	Pyrogallolhydroxyl benzoic acid-derived flavonoids	Anti-inflammatory activity	[20, 21]
Agaricus macrosporus	Basidiomycota	Agaricoglycerides	Anti-inflammatory activity	[22]
Agaricus subrufescens	Basidiomycota	Glucan, glycoprotein, polysaccharide, and protein segments	Modulate the immune response	[23–25]
Agrocybe cylindracea	Basidiomycota	Agrocybin, β-glucans	Hypoglycemic, antioxidant, and antifungal activity	[14, 26]
Albatrellus ovinus	Basidiomycota	Grifolin and its derivatives	Anti-inflammatory and antioxidant activity	[27]
Albatrellus caeruleo porus	Basidiomycota	Phenolic compounds, grifolinones A, B	Anti-inflammatory activity	[27, 28]
Auricularia auricula	Basidiomycota	Glucans	Immunomodulatory, anti-inflammatory, hyperglycemic, and anticancer activity	[29]
Boletus edulis	Basidiomycota	Polysaccharides	Anti-inflammatory activity	[20]
Boletus spp.	Basidiomycota	Glutamyl tryptophan, lithocholic, 2,4,6-trimethyl acetophenone imine, glycine conjugate, and azatadine	Antioxidant activity	[30]
Cantharellus cibarius	Basidiomycota	Flavonoids, polysaccharides, pyrogallol, caffeic acid, catechin	Anti-inflammatory, antimicrobial, and antioxidant activity	[20, 31, 32]

(*Continued*)

Table 8.1 (Continued)

Scientific name	Phylum	Bioactive compounds	Action of bioactive compounds	References
Calvatia gigantea	Basidiomycota	Calvacin mucoprotein	Anticancer activity	[14]
Caripia montagnei	Basidiomycota	Glucans, polysaccharides	Anti-inflammatory activity	[33]
Coprinus comatus	Basidiomycota	Polysaccharide and protein fractions, β-1,3-glucan	Modulate the immune response	[34]
Cordyceps militaris	Ascomycota	Cordycepin, cordymin	Antiangiogenic, anti-inflammatory, and antitumor activity	[35–37]
Cordyceps sinensis	Ascomycota	ciclosporin, cordycepin, cordymin (peptide)	Antioxidant, immunosuppressive, and anti-inflammatory activity	[38–41]
Craterellus cornucopioides	Basidiomycota	Myricetin (phenolic compound)	Antioxidant activity	[32]
Craterellus tubaeformis	Basidiomycota	Polysaccharides (starch, glycogen, cellulose)	Anti-inflammatory activity	[42]
Dictyophora indusiata	Basidiomycota	Dictyoquinazol, dictyophorines A and B	Antineurodegenerative and neuroprotective activity	[43]
Flammulina velutipes	Basidiomycota	Peptidoglycan, polysaccharides, and flammulin (protein)	Anti-inflammatory, antiviral, and antitumor activity	[44–47]
Ganoderma tsugae	Ascomycota	Fungal immunomodulatory proteins (FIP)	Modulate the immune response	[48]
Grifola frondosa	Basidiomycota	Grifolan, glucoxylan, proteoglycan, galactomannan, mannogalactofucan, heteroglycan, agricoglycerides, and fucomannogalactan	Immunomodulatory, antiviral, anticancer, anti-inflammatory, and hepato-protective activity	[22, 49, 50]

Hericium erinaceus	Basidiomycota	Phenol-analogous compounds, hericenones, erinacines, hericerins, dilinoleoyl phosphatidylethanolamine, resorcinols, heteroglycan peptide, steroids, terpenoids, lectin (glycoprotein)	Antioxidant, anticancer, antifatigue, antiviral, antihyperglycemic, antidiabetic, antihypertensive, antisenescence, immunomodulatory, neuroprotective, cardioprotective, and antineurodegenerative activity	[19, 51–55]
Hypsizygus marmoreus	Basidiomycota	Ergosterol, mannitol, trehalose, methionine, marmorin, phenolic compounds, flavonoids	Antifungal, anti-inflammatory, antitumor, antioxidant, antibacterial, and antiallergic activity	[56–58]
Inonotus obliquus	Basidiomycota	Mannogalactoglucan, sterols, β-D-glucans, triterpenes	Anti-inflammatory, anticancer, antioxidant, and gastrointestinal disease activity	[14, 20, 59–62]
Lactarius deliciosus	Basidiomycota	Polysaccharides, pyrogallol, flavonoids	Anti-inflammatory activity	[63]
Lactarius rufus	Basidiomycota	1,3- and 6-β-D-glucans	Anti-inflammatory activity	[64]
Lentinula edodes	Basidiomycota	Lentinan, glucan, mannoglucan, fucomannogalactan, lentin (protein), catechin (phenolic compound), phenolic compounds, flavonoids	Immunomodulatory, antitumor, anti-inflammatory, antifungal, antibacterial, antifungal, and antioxidant activity	[58, 65–67]
Lyophyllum decastes	Basidiomycota	1,3- and 6-β-D-glucans	Anti-inflammatory activity	[68]
Morchella esculenta	Ascomycota	Heteroglycan, galactomannan, β-1,3-D-glucan	Anticancer and hyperglycemic activity	[69]
Pholiota adiposa	Basidiomycota	Lectin	Antiviral and anticancer activity	[70]

(*Continued*)

Table 8.1 (Continued)

Scientific name	Phylum	Bioactive compounds	Action of bioactive compounds	References
Pholiota nameko	Basidiomycota	Polysaccharides	Anti-inflammatory activity	[71]
Pleurotus citrinopileatus	Basidiomycota	Glycoprotein	Anticancer activity	[72]
Pleurotus eryngii	Basidiomycota	Laccase	Antiviral activity	[73]
Pleurotus florida	Basidiomycota	β-Glucan	Antioxidant activity	[74]
Pleurotus ostreatus	Basidiomycota	Pleuran (β-1,3-glucan with galactose and mannose), proteoglycan, pleurostrin, and laccase	Immunomodulatory, antifungal, antioxidant, antiviral, hyperglycemic, and anticancer activity	[75–78]
Pleurotus pulmonarius	Basidiomycota	β-(1,3)-glucopyranosyl and 1,3- and 6-β-D-glucans	Anti-inflammatory activity	[79, 80]
Sparassis crispa	Basidiomycota	β-Glucan	Immunomodulatory activity	[81, 82]
Termitomyces albuminosus	Basidiomycota	Termitomycesphin, termitomycamides (fatty acid amides)	Antineurodegenerative activity	[83, 84]
Trametes versicolor	Basidiomycota	Krestin, coriolan (β-glucan protein complex)	Antimetastatic hypoglycemic effect	[12, 14]
Tremella aurantia alba	Basidiomycota	Heteroglycan (heteropolysaccharides)	Modulate the immune response	[85]
Tremella mesenterica	Basidiomycota	Glucuronoxylomannan	Hypoglycemic and immunomodulatory activity	[26]
Tricholoma giganteum	Basidiomycota	Trichogin	Antifungal activity	[86]
Tricholoma mongolicum	Basidiomycota	Laccase	Anticancer and antiviral activity	[87]
Volvariella volvacea	Basidiomycota	FIP-*Volvariella volvacea*	Immunomodulatory activity	[88]

Table 8.2 Bioactive compounds of non-edible mushroom species.

Scientific name	Phylum	Bioactive compounds	Bioactivity	References
Antrodia camphorata	Basidiomycota	Glycoprotein ACA, diterpenes	Immunomodulatory and neuroprotective activity	[89, 90]
Clitocybe maxima	Basidiomycota	Laccase	Antitumor activity	[91]
Cortinarius infractus	Basidiomycota	6-hydroxyinfractine and infractopicrine	Antineurodegenerative activity	[92, 93]
Cyathus africanus	Basidiomycota	Diterpenoid (cyathatriol, 11-O-acetylcyathatriol, and neosarcodonin)	Anti-inflammatory activity	[94]
Daldinia concentrica	Ascomycota	1-(3,4,5-trimethoxyphenyl) ethanol, caruilignan C	Neuroprotective activity	[95]
Elaphomyces granulatus	Ascomycota	Syringaldehyde, syringic acid	Anti-inflammatory activity	[96]
Fomitopsis pinicola	Basidiomycota	Polysaccharides	Anti-inflammatory activity	[97]
Ganoderma lucidum	Basidiomycota	Ganoderic acids, ganoderiol, ganodermanontriol, ganoderan A and B, ganopoly, triterpenes, lucidenic acids, ganoderic acids, lanostane-type triterpenic acids; lingzhi-8 (protein), ganodermin (protein), Se-containing protein	Antimetastatic, hypoglycemic, anti-HIV, hepatoprotective, anti-inflammatory, antiviral, immunomodulatory, and antitumor activity	[14, 98–108]
Ganoderma microsporum	Basidiomycota	Protein GMI	Immunomodulatory activity	[109]
Ganoderma pfeifferi	Basidiomycota	Sesquiterpenoid hydroquinones (lucialdehyde D, ganoderone A, ganoderone C)	Antiviral, antibacterial, and antifungal activity	[110]

(*Continued*)

Table 8.2 (Continued)

Scientific name	Phylum	Bioactive compounds	Bioactivity	References
Geastrum saccatum	Basidiomycota	β-glucans, polysaccharides	Anti-inflammatory activity	[111]
Ganoderma tsugae	Order: Polyporales, Class: Agaricomycetes	FIP-gts protein	Immunomodulatory activity	[48]
Lentinus polychrous	Basidiomycota	Catechin (flavan-3-ol)	Antioxidant activity	[66]
Lentinus squarrosulus	Basidiomycota	Catechin (flavan-3-ol)	Antioxidant activity	[66]
Lenzites betulina	Basidiomycota	Betulinan A	Antioxidant activity	[14]
Lignosus rhinocerus	Basidiomycota	Polysaccharides–protein	Anticancer activity	[26]
Phellinus linteus	Basidiomycota	Glucans, acidic polysaccharides, hispidin (polyphenol)	Antitumor, immunomodulatory, and antioxidant activity	[39, 112–114]
Psilocybe cubensis	Basidiomycota	Psilocybin	Antidepressant activity	[115–119]
Psilocybe samuiensis	Basidiomycota	Psilocybin	Sedative	[115–119]
Psilocybe mexicana	Basidiomycota	Psilocybin	Sedative	[115–119]
Russula lepida	Basidiomycota	Lectin (proteins)	Antitumor activity	[120]
Schizophyllum commune	Basidiomycota	Schizophyllan, 1,6-monoglucosyl-branched β-1,3-D-glucan	Immunomodulatory and antitumor activity	[121, 122]
Wolfiporia cocos	Basidiomycota	Dehydrotrametenolic acid, lanostane	Hypoglycemic and anti-inflammatory activity	[14, 123, 124]
Xylaria hypoxylon	Ascomycota	Lectin (glycoprotein)	Antitumor and antimitogenic activity	[125]

ACA, anti-cardio0lipin antibodies; GMI-protein, *Ganoderma microsporum* immunomodulatory protein; HIV, human immunodeficiency virus; *-gts*, from *Ganoderma tsugae*.

subunit in β-glucan. These β-glucans have shown an antimicrobial immune response on numerous immune receptors such as dectin-1, which is the main β-glucan receptor, innate immune receptor, and complement receptor [34]. This is why β-glucans have the ability to boost the immune system, prevent various common diseases, and promote health [133]. β-Glucan is attached to macrophages in the innate immune system, which is responsible for distinguishing invaders and managing the defense system of the body. When they leave the bloodstream, monocytes become macrophages, which are then activated by β-glucan; this boosts their ability to recognize and destroy invaders by the process of phagocytosis. The thymus gland produces T lymphocytes, which contain T-cell receptors for antigens and have specialized cells whose aim is to kill pathogens. B lymphocytes play a vital role in humoral immunity and make antibodies. T lymphocytes are natural killer cells that destroy bacteria, infected cells, tumor cells, and viruses. Thus, these types of white blood cells help the immune system to defend the body against destructive pathogens [34, 134]. Several biologically active fungal β-glucans have been found in the fruiting bodies of mushrooms and have been isolated [33, 64, 71, 111, 135]. For example, pleuran has been isolated from *Pleurotus ostreatus* and consists of β-(1,4)- or β-(1,6)-branches for every fourth β-(1,3)-glucan backbone [136]. Lentinan is derived from *Lentinula edodes*; it has a molecular weight of 400–1000 kDa and has shown antitumor and immunomodulatory activity [23]. The active β-glucan schizophyllan from *Schizophyllum commune* has a molecular weight of 450 kDa [121]. In *Grifola frondosa* maitake the D-fraction was found to consist of a combination of a main chain of β-1,6-glucan with branched β-1,4-glucan and a main chain of β-1,3-glucan with branched β-1,6-glucan [49]. Extract of *Agaricus subrufescens*, which contains β-1,3-, β-1,4-, and β-1,6-glucans, induces proinflammatory cytokine release in monocytes and veins of human endothelial cells [137]. Anti-inflammatory properties are also seen in *P. pulmonarius,* which contains (1,3)-glucopyranosyl that is responsible for this action [80]. The glucans ganoderan A and B from *Ganoderma lucidum* show hypoglycemic properties, as reported by Rathee et al. [14]. Moreover, ganopoly of *G. lucidum* revealed hepatoprotective actions in patients with chronic hepatitis B [102]. Immunomodulating properties have also been demonstrated for *G. lucidum* glucans. They enhance the proliferation of lymphocytes and production of antibodies. Antigenotoxic and antitumor activities are also displayed by these polysaccharides [12, 138]. These studies showed that *G. lucidum* has antioxidative and scavenging effects [14]. Antitumor activity against HeLa tumor cells has also been confirmed in *P. ostreatus* fruiting bodies, which contain a β-glucan that is responsible for this action [76, 139]. β-Glucan has two mechanisms that are responsible for the anticancer properties: (i) directly through cytotoxic activity and (ii) indirectly via immune modulation [34]. The anti-inflammatory action of *L. edodes* has also been reported. Its active fraction was made by chain reaction of α-D-galactopyranosyl-1,6-linked

units with fucomannogalactan, incompletely substituted at O-2 [140]. Moreover, it is reported that 1,3-D-glucopyranosyl glucans of *P. pulmonarius* exhibit anti-inflammatory activity [80]. Wu et al. [46] reported that *Flammulina velutipes* polysaccharides consist of three monosaccharides, namely xylose, glucose, and mannose in a 3.5 : 0.8 : 1.4 molar ratio, that have anti-inflammatory properties. This study reported that mushrooms contain polysaccharides that have anti-inflammatory activity.

8.4.3 Polysaccharide–Protein Complexes

Polysaccharide–protein complexes have also been recognized as polysaccharides that possess immune modulatory and antitumor properties. For example, polysaccharide-K protein, also known as krestin, was obtained from a few mushroom species that exhibited antimetastatic action [12, 141]. Hypoglycemic effects have been shown for *Trametes versicolor*, a type of mold that affects waterlogged trees [14]. Calvacin, which is a mucoprotein that has shown antitumor activity, is obtained from *Calvatia gigantea* [142], whereas *Phellinus linteus* contains a proteoglycan and its ethanolic extracts show anti-inflammatory activities [143, 144].

8.4.4 Terpenes

The terpenes present in mushrooms constitute the major group of anti-inflammatory compounds. The numerous terpenes obtained from mushrooms are non-polar metabolites, including mono- and sesquiterpenes, volatile oils, carotenoid pigments, involatile triterpenoids and sterols, and less volatile diterpenes. Four ganoderic acids and nine lucidenic acids have been isolated from mushroom fruiting bodies by Akihisa et al. [106] and Iwatsuki et al. [105], whereas numerous triterpenic acids (lanostane-type) and terpenoids were isolated from Reishi mushrooms [103, 106]. Anti-inflammatory properties are shown by all of these terpenes. In addition, biosynthesis of cholesterol is inhibited by some triterpenes [145]. The studies reported that mushrooms contain other triterpenes, which give protection against atherosclerosis and provide antiviral and antioxidative protection [14, 146]. Some sterol compounds have been isolated that show antiviral, antifungal, antibacterial, and potent anti-inflammatory properties [10, 59, 60, 110]. Numerous terpenes with anticancer and anti-inflammatory properties have been isolated from the sclerotia of *Inonotus obliquus* [62]. Also, three diterpenes have been isolated from *Cyathus africans*, that is, neosarcodonin, 11-O-acetylcyathatriol, and cyathatriol, and five novel cyathane diterpenes have also been isolated that

have potent anti-inflammatory properties [94]. One study [90] reported that numerous triterpenes that showed neuroprotective activity were isolated from *Antrodia campho*.

8.4.5 Phenolic Compounds

The phenolic complexes are aromatic hydroxylated composites that have one or more hydroxyl groups and aromatic rings. These phenolic compounds contain hydroxybenzoic acids, phenolic acids, flavonoids, lignans, hydroxycinnamic acids, tannins, oxidized polyphenols, and stilbenes [147, 148]. These studies have demonstrated that these phenolic compounds have antioxidant properties: they are inhibitors of free radicals, decomposers of peroxide, oxygen scavengers, and metal inactivators. Thus, antioxidants play a vital role in the response to free radicals, which possess one or more unpaired electrons and are chemical compounds. Reactive oxygen species (ROS) can be formed either by external sources or by metal-catalyzed oxidation and oxidoreductase enzymes, or by aerobic respiration during mitochondrial electron transport as a by-product. Radicals find ways to pair with their electrons because of their reactive properties, which is why radicals repeatedly attack adjacent chemical complexes. Apoptosis and cell function are lost if radicals change the chemical structure of the cells. Numerous non-enzymatic small molecules perform a role as antioxidants. The most vital intracellular defense against the harmful properties of ROS is glutathione. This is a tripeptide which presents a sulfhydryl group as a goal for attack. Polyphenol, vitamins C and E, and lycopene have the ability to reduce ROS [149]. The antioxidant properties of phenolic complexes have been studied by Palacios et al. [32] in numerous species of mushroom, as shown in Table 8.1. The maximum amount of myricetin is present in *Craterellus cornucopioides* and large amounts of catechin and caffeic acid are present in *Cantharellus cibarius*. Anti-inflammatory properties have also been demonstrated for the phenolic molecule pyrogallol from *Lactarius deliciosus, A. bisporus,* and *Cantharellus cibaromius* [31, 139, 150]. Catechin, a major phenolic compound that is extracted from *Lentinula squarrosulus*, *Lentinula polychrous*, and *L. edodes*, has antioxidant properties. Chowdhury et al. [58] isolated flavonoids and phenolic compounds from fungi and demonstrated that they had antibacterial, antioxidant, and antifungal properties. Mushrooms reduce the risk of neurodegenerative diseases [19]. The synthesis of nerve growth factor has been reported from hericenones and erinacines produced by the fruiting body and the mycelium of *Hericium erinaceus* [54, 151]. It has been reported that hispidin, an important medicinal metabolite that belongs to a class of polyphenols that show ROS scavenger properties, was isolated from *Phellinus* spp. [114, 152].

8.4.6 Peptides and Proteins

Different bioactive peptides and proteins are produced by mushrooms. These essentially include lectins, in which enzymatic activity is absent, and some proteins, such as fungal immune modulatory proteins, laccases, and ribosome-inactivating proteins that possess enzymatic activity. Chu et al. [75] demonstrated antifungal activity in *P. ostreatus* and in *Agrocybe cylindracea* [153]. Peptide from *Russule paludosa* exhibited antiviral properties [154]. Cordymin, which has inflammatory activity and a low molecular weight, has been isolated from *Cordyceps sinensis* [40, 41] and *Cordyceps militaries* [106, 155]. Some enzymes deactivate ribosomes, such as ribosome-inactivating proteins, which eliminate adenosine remnants from rRNA. It has been stated that these ribosome-inactivating proteins exhibited antitumor activity [56]. Ascomycete and basidiomycete fungi use laccases, which are phenol oxidases, to reduce lignocellulosic substrates.

8.5 Other Bioactive Compounds of Mushroom Species

Agaric glycerides, which contain glycerol and an ester of 4-hydroxybenzoic acid (chlorinated), are fungal secondary metabolites that show strong anti-inflammatory properties [22]. Dilinoleoyl phosphatidylethanolamine is obtained from *H. erinaceum* fruiting bodies; this reduces oxidative stress and has a strong effect on neurodegenerative diseases, as reported by Nagai et al. [51]. It has been reported that termitomycesphins and termitomycamides were extracted from *Termitomyces albuminosus* dried fruiting bodies [83]. These biologically active compounds have antineurodegenerative properties [156].

8.6 Conclusion

Mushrooms exist in two broad categories: poisonous and edible. Poisonous mushrooms are mostly found in the wild and their toxicity is usually fatal. Within both of the groups are phytocomponents with bioactive properties. Eating mushrooms remains a risk, particularly to people who are not familiar with the properties of species that are available to them. It is therefore imperative for food scientists and other allied researchers to identify these groups of mushrooms and present the information to the communities that use them. In terms of drug discovery, most alkaloid toxins tend to make good drugs or drug scaffolds. It is also important for researchers to explore this area for the development of drugs that can be used reliably against various ailments.

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9

Toxicity Protocols for Natural Products in the Drug Development Process

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CONTENTS

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9.1 Introduction

Natural products are less toxic than synthetic compounds because of the adaption of humans and animals through exposure over time, which has enabled them to develop detoxification mechanisms [1]. Phytochemicals are extracted from plants and contain many interrelated chemical compounds that may have different pharmacological effects, when the whole plant preparation is used. Although occurring rarely compared with synthetic compounds, adverse effects of natural products have been reported in the literature [2]. Therefore, toxicity studies are necessary for medicinal products derived from natural products before humans are exposed to them.

In vitro studies, which are conducted outside the intact organism, provide important tools to develop our understanding of the hazardous effects of natural products and to enable us to predict these effects in humans. They are widely used for screening purposes [3]. Before testing the toxicity of a herbal product, the complexity of the herbal material should be considered. The product naming system (botanical, common, pharmaceutical name, or herbal drug name), botanical identity, and the relevant part of the herb to be tested should also be considered before testing [4].

9.2 In Vitro Toxicity Testing for Natural Products

Concerns for the welfare of animals have resulted in alternative methods being used for toxicological testing. In addition, the limited predictive capacity of in vivo testing for acute toxicity and the requirement for large quantities of test substance have encouraged the use of in vitro toxicity testing [5]. The three Rs method was designed to reduce unnecessary exposure of animals to experimental products. These are reduction (use the least number of animals for toxicological tests that provide full results), refinement (to improve animal research to reduce or eliminate pain and discomfort), and replacement (use of alternative toxicological tests

that do not involve the intact animal). This approach calls for alternative approaches to reduce the use of animals and replace them with in vitro toxicological testing [6]. In vitro studies can also be used to determine the mechanisms of toxicity at the cellular level, thereby allowing possible interventions with therapeutic or antidotal treatment [6]. The procedure of liver perfusion to isolate viable rat hepatocytes is used widely in toxicology testing [7]. The liver is the main target organ that is responsible for detoxifying toxic substances, and the development of this procedure has in vitro studies of toxicity. In vitro studies are a cornerstone of drug discovery and are widely applied for natural products [4].

9.2.1 Cell Culture Method for Toxicity Testing

The cell culture method was developed many years ago. Early attempts at tissue culture were carried out in the USA when scientists removed tissue explants from animals and allowed them to adhere to glass coverslips or put them in capillary tubes in clots formed from lymph or plasma. Following this, synthetic media, such as those formulated by Earle, Parker, and Eagle, were developed with different serum additives to support the growth of cells [8]. These early studies had a drawback of contamination with bacteria and fungi, which outpaced the growth of mammalian cells because of their rapid rate of mitosis. This was also addressed by the addition of liquid antibiotics to the media as well as the development of better aseptic methods, such as the use of laminar airflow hoods, autoclaves, and sterile disposable glassware, which reduced the requirement for antibiotics [9].

In vitro studies have been further modified by the development of accepted protocols, such as chemically defined culture media, the introduction of porous membranes, and filter inserts, which allow the passage of low-molecular-weight soluble substances. The use of biological safety cabinets (class II) reduced contamination of cells by microorganisms. These cabinets have a unidirectional airflow that separates the working area from the environment by blowing sterile air over the surface of the working area. Most of the supplies and plastic currently used for handling cell cultures are sterile and disposable, which reduces microbiological contamination [9].

In order to grow, cells need a liquid culture medium with defined components. The medium is usually composed of a buffered solution with physiological ion concentrations containing soluble amino acids, carbohydrates, vitamins, minerals, fatty acids, and other cofactors. Optional ingredients include a pH indicator, separate buffering systems such as 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and some non-essential amino acids that are incorporated when required by a particular cell type [9, 10].

The most commonly used media for cell culture are modified Eagle's medium, basal medium Eagle, Dulbecco's modified Eagle's medium, and Ham's F12

medium. These media are designed to be used with serum or serum proteins [8]. Serum (5–20%) is added to the medium formulation to promote cellular proliferation, and a balanced salt solution is used for irrigation, transport, washing, diluting fluids, and maintaining intracellular and extracellular osmotic pressure. The salt solution can also be supplemented with glucose to provide energy for cell metabolism during the washing procedure. The most commonly used prepared salt solutions include Dulbecco's phosphate-buffered saline, Earle's balanced salt solution, and Hanks' balanced salt solution [9, 11].

Most mammalian cells proliferate and differentiate at 37 °C [11]. A temperature higher than 39 °C may stimulate heat shock, which can irreversibly inhibit biological function. However, cells can tolerate falls in temperature; for example, falls up to 4 °C can reduce proliferation and differentiation but do not irreversibly affect biological function [9]. Another factor that affects cell proliferation is pH. A pH of 7.2–7.4 supports optimum cell growth. Rapidly growing cells release more acidic metabolic waste products, decrease the pH of the medium very rapidly, and need frequent washing or the addition of a buffering agent [11].

Most culture media contain bicarbonate as the buffering agent to avoid large and rapid changes in pH. To maintain an equilibrium concentration between the bicarbonate and carbonic acid, CO_2 supply needs to be controlled. This is because soluble CO_2 evaporates from the solution, thereby disturbing the equilibrium between carbonic acid and bicarbonate at 37 °C. Maintaining an increased partial pressure of CO_2 in the gas phase above the liquid is necessary to maintain this equilibrium. However, at room temperature and standard incubator pressures, bicarbonate and carbonic acid are in equilibrium. Many laboratories incorporate organic buffers such as HEPES in medium formulations to prevent pH changes when cultures are removed from a CO_2 incubator [12]. Water is also a fundamental requirement in cell culture and the quality of water used in the preparation of media and salt solutions needs to be considered. The contaminants in water, such as trace metals, divalent cations such as magnesium and calcium, and metabolic products of microorganisms, can interfere with cell growth and functional processes [9].

9.2.2 Cell Culture for Acute Toxicology Testing

The measurement of viable and dead cells in culture has a long history in toxicology [13]. Some indicators of toxicity are used to detect the effects of different natural products by measuring the number of cells that have intact membranes per unit volume to demonstrate toxic endpoints. Some indicators, for instance neutral red uptake, detect the fraction of cells with intact membranes, whereas 3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is used to measure the metabolism of surviving cells [14].

The inhibition of cell proliferation is a sensitive indicator for the cellular response to the effects of a natural product, especially when coupled with measurements of metabolism. Simultaneous measurements of proliferation and viability are standard indicators of cell integrity. Together with the data from metabolic experiments, they contribute significantly to the ability of a cell culture system to predict or screen for toxicity. Altered cell proliferation, in which the effects of a natural product on the ability of cells to replicate are measured, serves as an indicator of toxicity. This is measured by the median inhibitory dose (IC_{50}), which is the concentration of the test substance at which 50% of the cells do not multiply. Cell proliferation is measured by cell counting, DNA content, protein content, and enzyme activity. Cell viability is another general index of toxicity. This endpoint is measured by using vital stains such as trypan, which enters only the compromised membranes of dead cells, and neutral red uptake, which is actively absorbed by living cells [9].

9.3 Methods Used for In Vitro Toxicity Studies

9.3.1 MTT Assay

Viable cells could be measured by using several staining methods. MTT is a water-soluble yellow dye that is absorbed by viable cells. The MTT assay is a colorimetric assay used for measuring only living cells. A tetrazolium ring is cleaved in mitochondria that are active, that is, this occurs only in living cells. MTT is absorbed into the cells and undergoes a reduction in a mitochondrion-dependent reaction to give a formazan metabolite. The formazan product accumulates within cells because it cannot pass through intact cell membranes. Dimethylsulfoxide, isopropanol, or another suitable solvent is used to solubilize the formazan product and release it from intracellular stores. The released product can be readily quantified calorimetrically. The quantity of reduced MTT is proportional to cell viability because MTT is only reduced by viable cells [9, 15].

9.3.2 Neutral Red Uptake Assay

This assay estimates the number of cells deemed viable in a culture. It is one of the most used cytotoxicity tests and has a wide range of environmental and biomedical applications based on the ability of living cells to bind to dyes such as neutral red in lysosomes. Neutral red uptake is dependent on the cell's ability to maintain a pH gradient. The dye is best absorbed at physiological pH because of the net charge of approximately zero. After uptake the lower pH inside the lysosome results in the dye becoming charged and, therefore, retained in the lysosome.

Most primary cells and cell lines from diverse origins may be successfully used in this assay. Cells are seeded in 96-well tissue culture plates and allowed to adhere for 24 hours. The plates are then incubated for 2 hours with a medium containing neutral red. The cells are washed, and then the dye is extracted in each of the reaction wells. Absorbance is determined directly by reading the specific wavelength of absorption on a spectrophotometer. This procedure is relatively more sensitive and cheaper than other cytotoxicity tests, such as those that involve enzyme leakage, tetrazolium salts, or protein content. This assay has a good throughput and can be completed in 3 hours [3, 16].

9.3.3 Lactate Dehydrogenase Assay

Lactate dehydrogenase (LDH) is an enzyme that is released into the cytoplasm during cell lysis. It is a colorimetric assay that is based on the conversion of lactate to pyruvate. The level of LDH is higher in damaged cells than in normal cells. The H^+ ions formed during the reduction of nicotinamide adenine dinucleotide (NAD) to reduced NAD (NADH) catalyze the reduction reaction of the tetrazolium salt (INT) to give the colored formazan compound. The amount of formazan compound formed is directly proportional to the activity of LDH in the sample [17].

9.4 In Vitro Models for Liver Toxicity

Cell lines are commonly used to study liver toxicity because of their similarities in genotypic and phenotypic characteristics to normal liver cells that have enzymes responsible for phase I and phase II metabolism of natural products. Liver toxicity is damage derived from chemicals that leads to acute and/or chronic liver disease. Liver cell lines are the best choice for investigation of the pharmacological and toxicological effects of natural product and their cellular mechanisms of action. Commonly used immortalized liver-derived cell lines are HepG2, Hep3B, HBG, and HepaRG [18].

9.5 In Vitro Models for Nephrotoxicity Studies

Cultured cells are also used to investigate renal cellular injury that results from natural products. A primary renal culture system of rat cortical epithelial cells is one of the models used to evaluate nephrotoxicity [19]. The cortical cells stemming from the renal cortex constitute the most metabolically active cells of the

kidney. Of these, proximal tubule and distal tubule cells are more frequently used for the assessment of in vitro renal toxicity. The methods of isolating and obtaining enriched populations of tubular cells include enzymatic methods, mechanical methods, and, historically, microdissection techniques [9].

9.6 In Vitro Model for Dermal Toxicity Testing

The Draize test has been used for many years to test skin corrosivity. Because of ethical issues around using animal models, alternatives to this test have been sought. Of these alternatives, the Corrositex, EpiDerm™, Episkin™, and transcutaneous electrical resistance (TER) assays have been validated for in vitro testing by different validating organizations.

The Corrositex assay is quantitative in vitro test for assessing the skin corrosion effects of a chemical. It is based on the time required for a test chemical to penetrate a barrier membrane. The membrane used for this test is composed of a reconstructed collagen matrix, developed to mimic the physicochemical properties of rat skin. The time needed to pass through the collagen matrix is recorded. As the solution of test product passes through the bio-barrier, the chemical detection system changes color.

EpiSkin is an in vitro model that uses a three-dimensional system consisting of reconstructed human epidermis with a functional stratum corneum. The model uses topical application of the test material to the surface of reconstructed skin and assesses the viability of cells. The cell viability is assessed from formazan production as measured by the MTT assay [20]. EpiDerm is another in vitro method designed to replace the Draize test. It uses a reconstituted human epidermal model to show the cytotoxicity effects of the test product. Cytotoxicity is expressed by a reduction in mitochondrial dehydrogenase activity, as measured by formazan production from MTT [20].

9.7 Mutagenicity Testing In Vitro

Mutations are changes that occur in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) sequences. They affect the normal cell proliferation, reproduction, and physiology. Mutations can have immediate or delayed consequences and may also cause stable inherited changes in gene sequences, resulting in phenotypic alterations. The effect and the type of mutations depend on the dose, frequency, and duration of exposure of the cell to the mutagens. Currently there are different methods that are more rapid, economical, and convenient than in vivo testing [9].

9.7.1 Bacterial Cell System

Bacterial testing systems use auxotrophic organisms. These organisms depend on the presence of rate-limiting nutrient compounds in the medium. Auxotrophic organisms are mutant bacteria with a highly specific defect in a gene locus, whereas normal or wild-type prototrophic organisms lack the mutation and are capable of growing in the absence of rate-limiting amino acids in the medium.

The Ames test is a bacterial mutagenicity assay that can identify a direct-acting mutagen. The test is conducted by mixing the bacterial strain with known concentrations of a test agent. A suspension of the bacterial strain along with the natural product being tested is incubated in agar solution containing the rate-limiting component for growth of auxotrophic bacteria. In this case, the bacteria can grow freely. The mixture is then spread over the surface of an agar plate without the essential rate-limiting component in the medium. On the agar plate, auxotrophic organisms stop growing, and only those affected by the natural product being tested back-mutate to prototrophic growth. The concentration of the natural product being tested is proportional to the proportion of organisms that change from auxotrophic to prototrophic, unless there is evidence of extensive genetic changes or lethal damage to the auxotrophic bacterial genome. The method, which was developed by Ames et al. [21], uses tester strains of *Salmonella* that require histidine. The test measures the effect of test product reversal of growth on histidine-free medium. Three tester strains – TA1531, TA1532, and TA1534 – are used to test frameshift mutagens and TA1530 is used to detect mutagens that cause base pair substitution [21]. The sensitivity of the assay was later improved by the addition of other mutations, but all strains had in common some type of mutation in the histidine operon. For example, the *RFA* mutation causes loss of lipopolysaccharide surface coatings of bacteria and this increases permeability to large molecules and polar compounds that do not normally penetrate cell walls. Another improvement was mutation of *uvrB*, which greatly increases the sensitivity of the bacteria to mutagens by deleting the gene coding for DNA excision repair [9, 22].

9.8 Reproductive and Teratogenicity Studies In Vitro

The in vivo tests used today are mostly time consuming and expensive. They also require expertise, skills, and a number of laboratory animals, which are eventually sacrificed; thus, in vivo testing is surrounded by several ethical issues [23]. Therefore, over the years, several in vitro methods have been used and documented. For example, one study has demonstrated that the in vitro follicle growth (IVFG) assay is a robust, organotypic, cheap model system that can be applied to rapidly assess potential adverse reproductive outcomes following chemical

exposure of female reproductive systems. Further research on this assay has enabled in-depth studies regarding reproductive toxicities to be established [24]. Other examples of in vitro assays follow.

9.8.1 H295R Steroidogenesis Assay

This test describes an in vitro screening for chemical effects on steroidogenesis, especially the production of 17β-estradiol and testosterone [25].

The human H295R adrenocarcinoma cell line is used in this assay. It is acclimatized for a period of 24 hours in multiwell plates and then cells are exposed for 48 hours to seven concentrations of the test chemical in at least triplicate. The solvent, a known inhibitor, and an inducer of hormone production are run at fixed concentrations as negative and positive controls. Cell viability is analyzed in each well at the end of the exposure period. The concentrations of the hormones in the medium can be measured using commercially available hormone measurement kits. Data are expressed as the fold change relative to the solvent control and as the lowest observed effect concentration. If the assay is negative, the highest concentration tested is reported as the no observed effect concentration.

9.8.2 Embryonic Stem Cell Test

This test uses two cell lines – mouse embryonic stem (ES) cells and mouse 3T3 fibroblast cells – and three endpoints to predict embryotoxic chemicals. The assay endpoints are indicated by the inhibition of differentiation of the ES cells, inhibition of ES and 3T3 cell viability, and inhibition of ES and 3T3 cell proliferation [26–28].

9.8.3 Whole Rat Embryo Cultures

This assay uses isolated and cultured early postimplantation rat embryos to study the embryotoxic effects of chemicals or any test substance. The morphology of 48 hour cultured embryos exposed to the test chemical are compared with controls to determine any delays in the development of certain organ systems or the development of any malformations [29].

In addition, according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) criteria for reproductive toxicity, three hazard categories exist, as shown in Table 9.1.

In conclusion, it is important for any new or initially marketed herbal formulation or medicine to be analyzed to determine any possible effects on the reproductive systems of both males and females prior to consumption in order to avoid any side effects that affect the normal functioning of organs or cause other disorders in the body.

Table 9.1 Globally Harmonized System of Classification and Labelling of Chemicals (GHS) criteria for reproductive toxicity.

Category	Criteria
1A	Known human reproductive toxicant that is based on evidence from humans
1B	Largely based on animal studies – presumed human reproductive toxicants
	Clear evidence of adverse effects on sexual function and fertility or on development in the absence of other effects
	In the case of other toxicity effects, the reproductive toxicity is not considered to be a second non-specific consequence of other toxic effects
	If there is information that raises doubt about the relevance of effects for humans, category 2 will be more appropriate
2	Evidence from human/animal studies is limited and there is a suspected human reproductive toxicant
Effects via lactation	Toxicants may be harmful to breast-fed children, may interfere with lactation, or may be present in breast milk

9.9 In Vivo Toxicity Testing of Natural Products

9.9.1 Acute Toxicity Testing

Acute toxicity is the noxious effect produced after a single dose of a chemical [30]. The data derived are used to determine the safety of or hazards produced by the natural product with regard to humans. The effect of the administration of a product to animals mimics the effect in humans [9]. The LD_{50} has been widely used for a long time to estimate acute toxicity in experimental animals. It is defined as the estimated dose that causes the death of 50% of the test population under specific conditions. For each LD_{50} test subjects need to be exposed to at least two routes of exposure; these are mostly the oral route and parenteral routes. Based on the nature of the natural product, the route can be modified to dermal, inhalational, or other route [9].

Guidelines produced by the Organisation for Economic Co-operation and Development (OECD) recommend having all available information about the test product prior to starting the test. Such information will help in the selection of the starting dose for a test. The information includes the chemical structure if identified, its physical and chemical properties, any other in vitro and in vivo tests conducted, and toxicological data on related products. When there is no information

to estimate a preliminary LD_{50}, the OECD suggests that the starting dose should be approximately 175 mg/kg with a dose progression factor of 3.2 [31].

Experimental animals need to be acclimatized at least for 5 days before starting the test to minimize the effects of a new environment [32]. According to OECD guidelines there are two tests: the main test and the limit test. In the main test, the animals are given a single dose at a minimum of 48 hour intervals. The first animal is given a dose one step below the level of the estimated LD_{50}. If the animal survives the next dose, which is 3.2 times the previous one, will be administered, and so on. If the first animal dies, the next animal will receive a dose decreased by a similar progression factor below the lethal dose in the first animal. Each animal should be followed for 48 hours before deciding on the dose of the next animal. The decision is made based on 48 hour animal survival patterns.

The limit test is a sequential test that needs a maximum of five animals and is used to identify chemicals that are likely to have low toxicity. Testing starts at 2000 mg/kg or 5000 mg/kg [31]. In another method, which has been described by Carpejane et al. [30], animals are divided into different groups, including a control group and a treatment group for each different concentration of the test product given; animals are followed for 14 days [33, 34]. To assess toxicological effects, the animals are closely observed for behavioral change, clinical signs of toxicity, body weight, and food intake. Hematological, biochemical, and histopathological analysis on the brain, heart, lungs, liver, stomach, small intestine (section), and left kidney also conducted [35].

Some regulatory agencies require that at least two species are used: one rodent species and one non-rodent species [36]. The preferred rodent for acute toxicity testing is the rat, although other rodents could also be used. Female rats are usually used because most of the literature shows a sensitivity difference and females are slightly more sensitive than males [37]. However, if the toxicokinetic properties of a structurally related product show higher sensitivity in males than in females, then males will be used. Healthy young animals are commonly used; also, females should be nulliparous and non-pregnant. Animals should be between 8 and 12 weeks old at the start of dosing. The temperature of the experimental room should be 20 °C ± 3 °C with a humidity of 30–70%. It is also recommended that animals should be housed individually with artificial light in a 12 hour light/12 hour dark cycle and fed a conventional rodent diet with an unlimited supply of drinking water [31].

Another method for acute toxicity testing according to the OECD guidelines is the acute toxic class method. According to this guideline, a stepwise procedure using a minimum number of animals per step (usually three) is used. Animals should be fasted prior to and after dosing for 3–4 hours for rats and 1–2 hours for mice. The three animals used for each step are given a starting dose from one of the following fixed dose levels: 5, 50, 300, and 2000 mg/kg body weight. The dose

is selected based on that most likely to result in death in some of the dosed animals. In this case, when the study conducted uses doses up to 2000 mg/kg, it is unlikely that the drug will result in death. When there is no information available for the natural product to be tested, 300 mg/kg is the recommended starting dose [38, 39].

9.9.2 Subchronic Toxicity Testing

Subchronic toxicity involves the period of time between acute and chronic effects, which ranges from 1 month [40, 41] to 3 months [42]. Subchronic toxicity testing is conducted to provide information on the hazard likely occurring as a result of repeated or continuous exposure to a natural product for a long period of time [9]. It also provides information on the major toxic effects, indicates the organs that are affected, and demonstrates the possibility of accumulation of natural products. Subchronic toxicity studies can help to provide an estimate of a no observed adverse effect level (NOAEL) of exposure. NOAEL exposure is the maximum exposure of an organism for which there is no biological or statistically significant increments in toxicity. The NOAEL ascertained through subchronic toxicity testing can be used to determine the dose levels for chronic toxicity studies and for establishing safety criteria for human exposure [42].

The preferred animal for subchronic toxicity testing is the rat. Other rodent species such as mice can also be used. It is recommended by the OECD to use both male and female healthy young adult animals; the females should be nulliparous and non-pregnant. In contrast, the World Health Organization recommends that males and females of two species – one rodent species and one non-rodent species – should be used [43]. At least 10 males and 10 females for each dose level should be used. The number of animals should be increased if interim killing is planned. At the beginning of the study the weight variation should be less than 20% from the mean. According to OECD guidelines published in 2019 at least three dose levels and concurrent controls should be used. The controls should be an untreated group or a vehicle control group if a vehicle is used for administering a natural product. A limit test can be used when a test dose level of 1000 mg/kg body weight produces no observed adverse effects or if toxicity would not be expected from structurally related compounds [42].

The natural product undergoing testing is usually given orally or by the route of administration which would be used clinically [43] on a daily basis in increasing doses to different groups of animals. For each group one dose level is given for at least 90 days [44]. The volume of the natural product administered depends on the animal used for a test and should not be greater than 1 ml/100 g of animal body

weight. But for aqueous solution up to 2 ml/100 g can be used. The animal should be observed for at least 90 days and general clinical observations should be carried out at least daily until the time when the peak period is reached [42].

During the period of administration, the animals are observed for signs of toxicity. Animals that die before 90 days or humanely killed during the test are necropsied. At the conclusion of the test, the remaining animals are also humanely killed and necropsied after the full dosing period. All signs of morbidity and mortality in the animals should be recorded twice daily, preferably at the beginning and the end of a day.

The animals being tested should be weighed at least once a week. Their food consumption should also be measured before the start of the experiment, and then at least weekly. Water intake should also be followed, depending on its usefulness [43]. At the end of a test period, blood samples should be collected prior to necropsy for rodents. For non-rodents blood samples should be collected before the start of administration of the product and at least once during administration of the product, and finally before necropsy. Hematological examination, such as hemoglobin, hematocrit, erythrocyte count, reticulocyte count, white blood cell count, platelet count, and a measure of blood clotting time, should be conducted [40, 45].

Biochemical examination to investigate the toxic effects of the natural product on the kidneys and liver should be performed. The plasma or serum levels of sodium, potassium, glucose, total cholesterol, high-density cholesterol, low-density cholesterol, urea, blood urea nitrogen, creatinine, total protein and albumin, and at least two enzymes that are indicative of hepatocellular effects (e.g. alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, and sorbitol dehydrogenase) should be determined. At the end of the study, the weight of the testes and epididymis of all male animal should be recorded [42]. For histopathological examination, the viscera, that is, heart, stomach, large and small intestine, kidneys, lungs, and liver, should be immersed in fixative solution [30]. At least one epididymis from each male should be reserved for histopathological examination. At necropsy, the estrus cycle of all females should be determined by taking vaginal smears [42].

9.9.3 Chronic Toxicity Testing

Chronic toxicity is a term used to describe products that need repeated or continuous exposure to cause the toxic effects [9]. Chronic toxicity testing provides data on the possible hazards over the life span of the animal species used as a result of repeated exposure; demonstrates target organ damage due to accumulation of the natural product over long-term usage; and identifies the level of exposure of the

experimental organisms at which there is no significant increase adverse effects (NOAEL) [46].

Before beginning the study, all the available information about the product should be considered to reduce the number of animals used and the study should be designed efficiently to test the chronic safety of a natural product. Chronic toxicity testing is only conducted after the initial information on acute and subchronic toxicity is obtained. The route of administration basically depends on the route that would be used clinically. The natural product is given daily at different dose levels to a few groups of animals. The duration of testing is usually 12 months [9, 46], but 180 days can be used. The duration is chosen to sufficiently determine long-term effects and cumulative toxicity without being affected by changes in the animal [47]. The rat is the preferred rodent even though other rodents such as mice can be used. Rats and mice are preferred since the effects of the test product can be investigated over their life span. Both male and female test animals should be used, and at least 10 animals for each sex are used per dose level. For non-rodents a minimum of three animals for each sex per group are used [43]. If there is no available information on the progression of toxicological changes from previous studies, the interim killing may be needed to gather enough information. When such information is available from previous studies, interim killing is not scientifically justified [34]. Satellite groups can also be included to investigate the reversibility of any toxicological changes induced by the natural product. An additional sentinel group may also be included to monitor disease status during the study. These animals should undergo the same observations and measurements as animal in the toxicity testing study [46, 48].

In a chronic toxicity study, a minimum of three dose levels with additional control groups are used. The selection of dose level depends on the results from acute or subchronic toxicity data or any existing toxicology data on the product or structurally similar compounds. The control groups are either untreated or given the vehicle [49]. For oral administration, the animals are given the dose daily for 12 months.

The body weight of the animals should be monitored, preferably at the start of the experiment and every week for the first 13 weeks, then every month. In addition to this, food consumption and, if the product is given with water, water consumption should also be measured [50].

At the end of the experiment the animal is sacrificed and blood is collected in tubes containing ethylenediaminetetraacetic acid (EDTA) for hematological tests and in tubes without EDTA for biochemical tests. Clinical biochemistry is basically used to investigate the toxicity effects of the natural product on the major organs, especially the kidneys and liver. All surviving animals are necropsied at the end of the study. The organs and tissues are harvested for morphological examination, and fragments of these organs are fixed for histopathological investigation [47].

9.9.4 Dermal and Ocular Toxicity

The Draize skin test method has been used since the mid-twentieth century to test the safety of cosmetic agents. Draize et al. [51] published a protocol for dermal toxicity testing that used the presence of edema and erythema to quantify skin irritation. The rabbit model is preferred for this test because of the sensitivity of its skin, because these animals are easy to handle, and because their skin has high permeability.

The test area is shaved 24 hours before application. The test areas mostly frequently used are the back or the abdomen [52]. Initially, a single rabbit is exposed to 0.5 ml (liquid) or 0.5 g (solid) of the natural product for 3 minutes. If the product shows any corrosive effect the test is stopped and the test product is classified as corrosive. If it is not corrosive, two additional tests for 1 hour and 4 hours are conducted and any irritation is scored according to the Draize irritation potential classification (Table 9.2) [51, 53].

The skin sensitization study is one of the tests for allergic dermatitis that could be caused by a natural product. It involves an immunological reaction, which is the result of activation of antigen-specific T cells. The response takes 24–48 hours to develop. For skin sensitization studies, guinea pigs are preferred because of their known sensitivity to different chemical sensitizers. The most common skin sensitization methods include the guinea pig maximization test, which needs 10–20 animals in the treatment group and 5–10 animals in the control group. The study starts with an intradermal injection; this is followed after 1 week by topical application; and then after 2 weeks a topical challenge is conducted. The Buehler guinea pig skin sensitization test is another protocol in which three topical applications 1 week apart for the induction phase and 2 weeks later for the topical challenge are applied. The appearance of edema or erythema after the challenge dose greater than that of the sensitizing dose is indicative of sensitization [9, 55].

Table 9.2 Draize irritation potential classification [51, 54].

Dermal irritation score (DIS)	Classification of dermal irritability
$0 < \text{DIS} < 0.4$	Not irritant
$0.4 \leq \text{DIS} < 2$	Slightly irritant
$2 \leq \text{DIS} < 5$	Moderately irritant
$5 \leq \text{DIS} \leq 8$	Severely irritating

The score is based on observations at 1, 24, 48, and 72 hours.

$$\text{DIS} = \frac{\text{Value (erythema + edema)}}{\text{Number of animals} \times \text{Number of observations}}$$

Dermal phototoxicity and photosensitivity studies are conducted in guinea pigs or rabbits. The natural products being tested are administered orally, parenterally, or topically for 10–14 days. The challenge phase starts 2–3 weeks later with another dose along with exposure to an ultraviolet lamp. The control group is exposed to positive and negative photoallergic agents [9].

Draize published the first protocol for an eye irritancy test in 1940 [56]. The study raised many ethical issues and was, therefore, revised many times. In this test, the compound undergoing testing is placed onto the eye of conscious restrained rabbits, which were then observed for several days to see the effects of the test compound. Because the cornea is the most sensitive part of the body and is rich in nerve endings, irritation or ulceration may produce pain. OECD guideline 405 recommends that before in vivo testing all available information about a product with regard to eye corrosivity/irritancy should be evaluated and a sequential testing strategy should be used. Performing tests sequentially on one animal at a time is recommended and allows reassessment of data and avoids duplication. Generally before any in vivo eye test studies are carried out, in vitro or in vivo tests on the skin corrosive effects of a substance should be conducted [57].

Albino rabbits are the preferred laboratory animal for in vivo eye tests. According to OECD guidelines, 6 minutes before administration of the natural product a subcutaneous (SC) injection of buprenorphine 0.01 mg/kg should be given; then 5 minutes before administration one or two drops of local anesthetic such as 0.5% proparacaine hydrochloride or 0.5% tetracaine hydrochloride should be applied to give a therapeutic range of systemic analgesia. Eight hours after application of the natural product, meloxicam 0.5 mg/kg SC and buprenorphine 0.01 mg/kg SC are administered to give a sustained therapeutic range of systemic analgesia. Sixteen hours after application buprenorphine 0.01 mg/kg SC should be administered 12 hourly, alongside meloxicam 0.5 mg/kg SC 24 hourly to the point of resolution of ocular lesions and absence of clinical distress and pain signs [58]. Animal eyes need not be washed at least for 24 hours after test substance instillation, unless the substance is a solid. In the case of a solid, if the substance is still in the animal's eyes at the 1 hour observation time point, saline or distilled water can be used to rinse the eye. If appropriate, a complete washout can be conducted at the 24 hour observation time point. Follow-up of up to 21 days should be made on the animal to determine any cases of possible reversibility of the effects of natural product material. Ocular lesions must always be graded and recorded at every examination in an appropriate good laboratory practice manner [57].

9.9.5 Toxicity Testing for Fertility and Reproduction

In vivo models are known to be more reliable than in vitro models for toxicity testing for fertility and reproduction, although there are drawbacks such as

differences in biokinetic parameters [59]. The tests below suit in vivo testing of different herbal formulations and help to establish the effects on different reproductive systems over time with their daily use.

9.9.5.1 The Uterotrophic Bioassay

This is a rapid screening test that depends on the uterotrophic response or an increase in uterine weight. Its sensitivity is dependent on a test system for animals in which the hypothalamic–ovarian–pituitary axis is dysfunctional. The two estrogen-sensitive states in female rodents meeting this requirement are: (i) females prior to puberty but after weaning and (ii) females at a young adult age with adequate time for uterine tissue regress but after ovariectomy [60].

The route of test material administration is dependent on the expected route in clinical use, but test substances are mostly administered orally or SC on a daily basis. Treatment and control groups should have a minimum of six animals each. Well-regulated test material doses are administered to a minimum of two treatment groups of animals with one dose level in each group over an administration period of three consecutive days for immature animals and a minimum of three consecutive days for ovariectomized adult animals. Approximately 24 hours after the last dose, animals should be necropsied. In cases of agonists of estrogen, a significant increase in the mean uterine weight of the treated animal groups as compared with the control groups indicates a positive response to this bioassay. Information on daily body weights, the status of the animal, the wet and blotted uterine weight, and food consumption should be recorded and reported.

9.9.5.2 Hershberger Bioassay in Rats

This is an in vivo short-term screening test and evaluates the ability of a chemical to elicit biological activity consistent with androgen agonists or antagonists or 5α-reductase inhibitors. The bioassay considers changes in weight of androgen-dependent tissues, such as prostate, the seminal vesicles, and the epididymis in castrated/peripubertal male rats.

To determine the androgenic or antiandrogenic action of a test substance, two (respectively three) dose groups of the test substance as well as positive and negative controls are sufficient for this test. The test substance is administered by gavage or SC injection daily for 10 consecutive days. A minimum of six animals should be included in each treatment or control group. The antiandrogen test involves administration of the test substance together with a reference androgen agonist. Animals are to be necropsied approximately 24 hours after the last administration of the test substance. Tissues are then excised and their fresh weights determined. Results showing a statistically significant increase in weight of the five tissues indicate androgenic activity, whereas a decrease means antiandrogenic activity of the test substance [61].

9.9.6 Combined Repeated Dose Toxicity Study with Reproduction/ Developmental Testing

9.9.6.1 Toxicity Screening Test

This test describes the impacts of a test substance on female and male reproductive functioning. Endocrine disruptor endpoints, particularly measurement of anogenital distance, thyroid examination, and male nipple retention in pups, are noted in this test. This test guideline is devised for use with rats.

The test substance is administered in regular doses to several groups of females and males. Males should be dosed for a minimum of 4 weeks, whereas females are dosed for the entire length of the study, approximately 63 days. Mating of one male to one female is recommended for this kind of study. A minimum of 10 animals of each sex per group is recommended. At least three test groups and a control group should be used. Dose levels can be predicted based on information from acute toxicity tests or on results from repeated dose studies. The test substance should be administered orally and daily for the period of the study. The findings of this toxicity study should be evaluated in terms of observed effects such as body weight, food/water consumption, and necropsy and microscopic findings. Because of the short period of treatment in males, histopathology of the testis and epididymis should be considered along with fertility data for assessment of male reproductive effects [62].

9.9.6.2 Extended One-Generation Reproductive Toxicity Study

This study allows for the evaluation of developmental and reproductive effects that may occur as a result of pre- as well as postnatal chemical exposure and an evaluation of systemic toxicity in lactating and pregnant females and in young toward adult offspring.

Sexually mature female and male rodents (P generation) are exposed to regular doses of test material beginning from 2 weeks before mating and continuing through to mating, gestation, and weaning of their pups (referred to here as the F1 generation). At the weaning stage, the pups are selected and assigned to various cohorts of animals for developmental/reproductive toxicity testing (cohort 1), testing for developmental neurotoxicity (cohort 2), and testing for developmental immunotoxicity (cohort 3). F1 offspring are further treated with the test material from weaning to adulthood. Clinical observations and pathological examinations are performed on all animals to check for signs of toxicity. The integrity and performance of male and female reproductive systems as well as the health, growth, development, and function of offspring should be carefully recorded. Part of cohort 1 may be extended to include an F2 generation(cohort 1B); in this case, the procedure for F1 animals will be similar to that for the P animals [63].

9.9.7 In Vivo Carcinogenicity Testing

Transgenic rodent models have been used for many years in carcinogenic testing. In vivo carcinogenicity testing uses two species to identify trans-species carcinogens. If the natural product produces a carcinogenic effect in these two species, it may have a significant carcinogenic effect in humans [9]. The carcinogenic study is usually performed for 18–24 months in mice and for 24–30 months in rats, or for the life span of an animal if the survival rate is high [64].

Carcinogenicity testing depends on the development of neoplasia as the single endpoint of this study. In addition, morphological examination of the organs and tissues is also used to investigate any carcinogenic response. The experiment is conducted in two phases: one is a preliminary study, aimed at determining the dose level for a full carcinogenicity study. If enough data are available, the preliminary study may be omitted. Testing consists of three stages: the first is a single-dose toxicity study that is conducted on a small number of animals to determine the highest dose to be used for the second stage. The second stage is another dose toxicity study that is used to determine the maximum dose to be used in the full-scale carcinogenicity study. At least 20 animals (10 males and 10 females) should be used with three dose groups and a control group for at least 90 days [43].

The maximum tolerated dose determined from the preliminary study is the dose that inhibits the weight gain of an animal by less than 10% compared with the control group. This dose should also not result in mortality or morbidity because of toxicity and should not significantly change the laboratory findings for the animal [43]. In full-scale carcinogenicity testing a minimum of 50 males and 50 females is used. The route of administration depends on the expected route of administration of the natural product, but it is usually given with water or prepared with food. A minimum of three dose levels and a control should be used.

Hematological and blood chemistry examinations should be conducted; for rodents, this should be done on blood collected before necropsy, and for non-rodents, it should be done on blood collected before the start of administration of the natural product and at least once during administration and before necropsy. Renal and liver function tests are also important because these are the main organs where metabolism and excretion of a drug take place. At the end of the experiment the survivors are necropsied and all animals should be examined macroscopically; also histopathological examination should be performed on all those in the highest dose group and in the control group. Histopathological examination of all animals should be conducted if the incidence of neoplastic lesions in the highest dose group and in the control group are different. A natural product is considered to be carcinogenic when any of the following responses are observed: (i) if a tumor develops in the experimental groups and none are seen in the control groups; (ii) development of tumors with a greater frequency in the test group than

in the control group; (iii) a greater variety of organs and tissues involved in tumor development in the test group than in the control group; and (iv) if a tumor develops earlier in the test group even though there is no significant difference in the incidence of tumors between the test groups and the control group.

9.10 Conclusion

Claims that natural products are all safe to use is not a scientific conclusion that should preclude any investigations. The toxicity of natural plant products emanates from a number of properties, and various tests should be undertaken to verify claims on a compound, plant, or product basis and not on the whole plant. These issues should always be included in standardization processes and approvals of any herbal product.

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10

Quality Control for the Safety of Natural Products

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CONTENTS

Poisonous Plants and Phytochemicals in Drug Discovery, First Edition.
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10.1 Introduction

The World Health Organization (WHO) has defined a medicinal plant as any plant in which one or more of its parts consists of substances that are used for the synthesis of beneficial drugs [1]. These plants need to be studied to better comprehend their characteristics, safety, and efficacy [2]. They comprise biologically active chemicals, including saponins, tannins, essential oil flavonoids, and alkaloids, among others [3], which all serve remedial purposes. It is estimated that 30 000–70 000 medicinal plants exist worldwide, most of which have not been systematically investigated [4]. Generally, herbal products are safer than conventional medications. While monitoring thousands of people who used ginkgo, St. John's wort, and kava in Europe, a study revealed that mild adverse effects were encountered in fewer than 3% of users [5]. Controlled studies of other common European herbal medicines, such as *Echinacea*, horse chestnut, saw palmetto, and valerian, have shown rare and mild side effects, generally similar to those seen in placebo groups [6–9]. The relative scarcity of severe adverse effects from herbal medicinal products most likely reflects a combination of factors that set them apart from conventional drugs, including weaker and less potent pharmacological activity, less consistent usage, and a poorly established mechanism for distinguishing and reporting adverse outcomes.

Accurate authentication of natural products including those from medicinal plants is crucial, as mistakenly identified species or varieties are either remedially less effective or not active at all, or may even contain poisonous ingredients [10]. The methods of classical botany for plant identification have latterly been accompanied by numerous DNA-based technologies, including random amplification of polymorphic DNA, restriction fragment length polymorphism, the amplification–refractory mutation system, cleaved amplified polymorphic region, amplified fragment length polymorphism, DNA amplification fingerprinting, inter-sample sequence repeat, simple sequence repeat, hybridization, and microarrays [11]. Apart from correct identification of plants, establishing their safety is another topic of interest. Although herbal products are often promoted to the public as "natural" and totally "safe" alternatives to conventional medicines, many are possibly toxic [12].

In 1993, the US Food and Drug Administration (FDA) proposed that those herbal medicines and other nutritional supplements that were not already well controlled as drugs should undergo stringent marketing regulations. Strong opposition by consumer groups and the supplement industry led to a concession passed by Congress in 1994, called the Dietary Supplement Health and Education Act (DSHEA), which classified herbal medicines (along with vitamins, minerals, amino acids, enzymes, etc.) as "dietary supplements." Moreover, herbal medicines and other dietary supplements do not have to be approved by the FDA prior to marketing [13]. In European countries, however, herbal medicines are more strictly regulated. In Germany, where most of the Western world's scientific

botanical research is conducted, herbal medicines are well regulated and available as over-the-counter and prescription drugs [14].

10.2 Quality Assurance of Herbal Products

Quality control and standardization of herbal medicines is carried out by means of several steps. However, the source and quality of raw materials contribute a key role in assuring the quality and stability of herbal medicinal products. Other factors related to growth conditions, collection method, and processing technique of the medicinal plants – such as the use of fresh plants; temperature; light exposure; water availability; nutrients; period and time of collection; method of collecting, drying, packing, storage, and transportation of the raw material; age and part of the plant collected, etc. – can significantly affect the quality and, consequently, the healing value of herbal medicines.

Some plant ingredients are heat labile; therefore, the plants containing these ingredients have to be dried at low temperatures. Moreover, other active chemicals may be destroyed by enzymatic processes that continue for some time after plant collection. This could explain why the composition of medicinal products from plants is quite variable. Thus, proper quality control and standardization of raw material and herbal preparations themselves should be carried out stringently. In addition, other issues such as the method of extraction and contamination with heavy metals, microorganisms, pesticides, etc. can interfere with the safety, quality, and efficacy of these products. Current technological advances in the purification, isolation, and structural elucidation of naturally occurring substances has enabled appropriate methods to be established for the analysis of the quality of herbal preparations as well as the process of standardization. This, in turn, affects the possible homogeneity and consistency of the plant extract and ultimately the herbal medicinal product. Among others, thin layer chromatography, gas chromatography, high-performance liquid chromatography, mass spectrometry, infrared spectrometry, ultraviolet/visible spectrometry, etc., used alone or in combination, can be effectively used to standardize and control the quality of both the raw material and the finished herbal medicine [15].

As herbs are increasingly packaged and promoted to compete with pharmaceutical drugs, consumers and healthcare providers are anticipating medicinal products that meet equivalent quality standards. In contrast to pure pharmaceutical preparations, most herbal medicinal products have few generic equivalents. Herbal products from different manufacturers vary considerably because it is virtually impossible to control all the variables that affect a plant's chemical composition. Natural conditions, such as sunlight and rainfall, as well as manufacturing procedures, such as selecting, drying, purifying, extracting, and storing herbs, can result in high inconsistencies in product quality and in the concentration of plant chemicals across products. This problem is not just imagined. In one analysis, the

concentration of the active agent in St. John's wort varied sevenfold among different products [16]. In another study, an active ingredient in different products of garlic tablets fluctuated more than 40-fold [17]. The concentration of important ingredients among 10 varieties of ginseng products fluctuated 10-fold in an analysis commissioned by *Consumer Reports*, and other analyses of ginseng products identified preparations that contained no ginseng at all [18, 19]. Accordingly, these products contrast substantially with standard aspirin tablets, which are mandated by law and are certified to contain 95–105% of labeled amounts of acetylsalicylic acid and which must undergo a series of purification tests. Similarly, regarding the safety of these products, concern over the lack of manufacturing standards and quality control has already been demonstrated by several reports of contaminated herbal products causing serious adverse effects, such as digitalis toxicity and lead poisoning [20, 21]. When original reports of a variety of herbal medicine poisonings were carefully scrutinized, many cases were found to be due to substitution or contamination of the declared ingredients [22]. Contamination with environmental pollutants (such as microorganisms, pesticides, and toxic metals), deliberate adulteration (with non-steroidal anti-inflammatory drugs [NSAIDs] or benzodiazepines), and misidentification or mislabeling of herbal products have all been described [22–24]. Such cases are likely tremendously underreported, since only serious adverse outcomes are usually investigated.

10.3 Methods of Quality Control for Herbal Products

10.3.1 DNA-Based Technologies

Barcode DNA is among the recent technological developments for the authentication of medicinal plants. This method is based on the detection of variable sites of the rDNA internal transcribed spacer. In systematic botany, polymerase chain reaction-based determination of barcode DNA is commonly employed for taxonomy studies. DNA barcoding provides a suitable tool for the authentication of plants and is well suited to quality control of medicinal plants [25, 26]. Current investigations in this field place emphasis on how many and which DNA fragments are required for the best discernment of different species. The largest database of DNA barcodes of medicinal plants, with more than 1000 species enumerated in the *American Herbal Pharmacopoeia* and the *Chinese Pharmacopoeia*, is the Medicinal Materials DNA Barcode Database (MMDBD) [27].

10.3.2 Good Practice Guidelines

Having been collected and correctly identified, medicinal plants must be subsequently handled in a standardized manner [28]. To this effect, prominent guidelines have been devised, including good sourcing practice, good agricultural

practice, good laboratory practice, good manufacturing practice, and good clinical trials practice [29].

10.3.3 Chemoprofiling

As stated above, the chemical composition of medicinal plants may substantially vary and must be standardized to effect comparable therapeutic effects. Several chromatographic fingerprinting analyses are known to identify ingredients and their concentration distribution [30, 31]. Standard analytical technologies available to this effect include thin layer chromatography, high-performance liquid chromatography, and capillary electrophoresis. Recently, new technological developments have become available for chemoprofiling, including metabolic fingerprinting, infrared spectroscopy, and quantitative determinations based on nuclear magnetic resonance spectroscopy.

10.3.4 Toxicology

Another facet of quality control, besides confirming the proper composition of herbal prescriptions, is to exclude possible contamination with pesticides, mycotoxins, heavy metals, or other chemical toxins or microbial toxins [32, 33]. Moreover, herbal medicinal products adulterated with conventional drugs (glucocorticoids and NSAIDs) must be prevented from reaching consumers.

10.3.5 Monographs and Pharmacopeias

Because of the aforementioned concerns about some traditional herbal products, there is a need for sound legal frameworks for the pharmaceutical use of herbal products. Pharmaceutically relevant knowledge of medicinal plants is systematically gathered and documented in monographs, which mostly form part of national or international pharmacopeias. Examples of these include the *International Pharmacopoeia*, the *European Pharmacopoeia*, the *American Herbal Pharmacopoeia*, the *German Pharmacopoeia*, and the *Chinese Pharmacopoeia*. Monographs contain technical definitions, analytical techniques for the identification of content and purity testing, as well as logistical and warehousing regulations for all kinds of drugs (herbal, chemical, and biological). Each pharmaceutically used drug has to meet the requirements of the monograph [34].

10.3.6 Preclinical Evidence of Safety and Efficacy

Traditionally, candidate compounds pass through a pipeline of preclinical investigations, using in vitro and in vivo test models. If the preclinical evaluation is promising, the candidate drug advances to clinical trial phases I–IV before it can

be recommended for clinical use. Phytotherapy has a different approach. Herbal medicines have been used for centuries, and it was only in recent years that inquiry and expectations about their mode of action, safety, and efficacy became a concern. In this sense, phytotherapeutic research may be understood as reverse pharmacology. On the other hand, research on the overall or partial bioactivity of medicinal plants or their parts is a part of quality control of herbal preparations that certifies whether they are effective and safe [34].

10.3.7 Systems Biology

Although classical pharmacological approaches are able to explain some of the mechanisms of medicinal plants (e.g. receptor–ligand interactions), the chemical composition of herbal mixtures is extremely complex and can only be understood incompletely by reductionistic approaches. The advent of systems biology and "-omics" technologies have been received with much curiosity among scientists in the areas of traditional medicine, because "-omics" technologies are all encompassing as they assess entire profiles of molecules at various levels of life such as in whole cells, organs, or organisms [4]. Liquid chromatography/mass spectrometry and microarray hybridization are basic technologies that are used to detect changes in the genomic makeup (genomics), proteome (proteomics), transcriptome (transcriptomics), or metabolome (metabolomics). Metabolomics is especially of interest in herbal medicine [35, 36] since plants synthesize abundant varieties of chemicals, much more numerous than those produced by most other organisms. Therefore, systems biology is appreciated as an innovative discipline to investigate holistic phytotherapeutic approaches. Systems biological research may also enable the determination of synergistic interactions of herbal mixtures.

10.3.8 Animal Experimentation

There are many investigations documenting the activity of plant extracts in in vitro test models. Such assays are normally easy to perform and are used for bioactivity-guided isolation of the active phytochemicals in extracts. Sophisticated methods can also be easily carried out in vitro, as in vivo conditions complicate experimental designs even more. Despite their crucial roles, in vitro bioactivity results do not necessarily translate into bioactivity in vivo [37, 38]. In vitro studies more often fail to appreciate the possible enzymatic activation of prodrug components and the role of presystemic metabolism of some chemicals in the body.

10.3.9 Clinical Evidence of Safety and Efficacy

In contrast to the general public, who widely use the products, Western academies are still reluctant to investigate herbal medicines. The major reasons for this include the complexity of the composition of the products and the fact that they

are usually sold over the counter. Their efficacy and safety are, therefore, doubted. On the other hand, given that traditional and herbal medicines have been successfully used for thousands of years, it is not always obvious to herbalists and traditional medical doctors that preclinical or clinical studies should be conducted in order to prove the efficacy of herbal medicines. However, the only practical way to integrate traditional medicines into conventional ones in a realistic time frame requires clinical trials to be conducted and to convince physicians with strong evidence-based data from herbal medicines. In recent years, an ever-increasing number of clinical trials, reports, and meta-analyses have focused on the efficacy of herbal medicines [39, 40]. Once evidence-based traditional medicines are on the market, appropriate pharmacovigilance studies are required to monitor any adverse effects [41].

10.4 WHO Guidelines for Quality Standardization of Herbal Formulations

Quality control and standardization parameters for herbal products are based on the following basic parameters: plant preparation; quality control of crude drug material and finished products; stability and shelf life assessments; safety assessment; documentation of safety based on toxicological studies or experience; and assessment of efficacy by biological activity evaluations and ethnomedical information. The following sections discuss some of these parameters.

10.4.1 Quality Control of Crude Material

There are a few challenges as far as standardization of a herbal product is concerned, such as deliberate adulteration of plant material, controversial identity of various plants, and problems with storage and transportation, and they need to be considered and seriously addressed in every practice [42]. One of the obstacles in the recognition of herbal products worldwide is the lack of standard quality control profiles. Most of the herbal formulations, particularly the classical formulations of traditional medicine, are polyherbal. A formulation may be found to contain 10–20 or, at times, 50–75 ingredients [43].

10.4.2 Identity of Plant Material

Authenticity, purity, and assay are important aspects of standardization and quality control. Authenticity refers to the state that proves the material is genuine and corresponds to the correct identification and profile. Quality control of botanicals starts with plant identification. According to the WHO general guidelines for methodologies on the research and evaluation of traditional medicines, the first step in assuring the safety, quality, and efficacy of traditional medicines is correct identification. If appropriate taxonomical names are not used, misidentification is

likely to occur. For example, two or more different plants often have the same name in Ayurveda. The challenges in medicinal plant authentication include limited knowledge about the medicinal plants produced by different traders or suppliers, the collection process by untrained people, and non-homogeneity of plant material because of unsystematic collection from wild sources and widely and differently controlled geographical locations [43, 44]. Thus, chemical analysis serves as the best method for identification, standardization, and detection of contamination.

10.4.3 Safety Assessment and Documentation

Adulteration of botanical preparations is another important issue that not only relates to the therapeutic efficacy of the product but also raises safety concerns. Because of overexploitation, habitat loss, and deforestation, many medicinal plants have been listed as endangered or rare species. The unavailability of the genuine drug, in turn, can result in the adulteration of plant materials by substitution with inferior commercial varieties, artificially manufactured substances, and cheaper plant materials or by another vegetative part [45]. Several reports have disclosed that herbal products quite often contain hidden pharmaceuticals and heavy metals. Agrochemicals used while growing the plants might also contaminate the crude plant material. Moreover, the mechanisms of action, stability, pharmacokinetics, compatibility, and drug–drug interactions of numerous herbs are still unknown. At the same time, an increasing number of reports about fatal adverse effects of herbal preparations demand the need for more stringent regulation and registration of herbal medicines and the establishment of proper safety monitoring [43, 46].

10.5 Concept of Validation in Herbal Products

In order to control the quality of herbal products effectively, an amalgamation of newer techniques is required. The FDA defines validation loosely as establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics. This has applied to manufacturers of synthetic conventional drugs for many years now, but it is not yet quite consistently or methodically studied and applied in the manufacturing of herbal products. All international regulations such as the Medicines Control Council (South Africa), FDA (USA), Therapeutic Goods Administration (Australia), and Medicines and Healthcare products Regulatory Agency (UK), show that it is possible to apply validation to pharmaceutical manufacturing, but only a few of the regulators together with WHO apply the validation concept to manufacturing of herbal drugs. Even WHO places very little emphasis on validation. Generally, one can

describe this model in a straightforward way as starting from the input and ending with the output. However, in the case of validation one must go in the reverse direction. The first step should be to identify and define what quality of the product is required [43].

Such a model is applicable for herbal drugs but there are some limitations, such as certification. Certification of herbal product manufacturers is a demanding procedure, but it is still possible. Problems with the standardization of the strength of the active moiety imply the requirement for validation. There are many parameters that must be considered when certifying the manufacturer:

- type of herbs
- environmental conditions
- time of collection.
- variation in composition, etc.

10.6 Challenges Related to Quality Control and Monitoring the Safety of Herbal Products

It is very obvious that research protocols as well as the requirements and standards needed for the evaluation of the efficacy and safety of herbal medicines are much more complex than those required for conventional synthetic drugs [47, 48]. A single herbal formulation may comprise hundreds of natural constituents, and a mixed herbal medicinal product may contain several times that number. Such a high number of components may be impossible to isolate and study singly from the formulations [47].

WHO continues to encourage the establishment of good manufacturing practices to ensure good quality and safety for herbal products [49, 50].

Adverse events arising from the use of herbal medicinal products are ascribed to many factors, such as the use of the wrong species of plant; adulteration of herbal products with other, undeclared medicines or with toxic or hazardous substances; overdosage; misuse of herbal medicines by either healthcare providers or consumers; and drug–drug interactions with other medicines. Although the assessment of the safety of herbal medicines has become an important issue, the analysis of adverse events related to the use of these products is much more complex than in the case of modern medicinal products [47, 48]. The evaluation of safety is even further complicated by factors such as the geographical origin of the plant material, different processing techniques, the route of administration, and uncertain compatibility with other medicines [51]. In addition, the lack of knowledge and/or poor emphasis of most manufacturers regarding the significance of taxonomic botany and the documentation of herbal medicines poses tremendous difficulties for the identification and collection of medicinal plants [52].

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11

Secondary Metabolites and Toxins of Microbial Origin for the Treatment of Diseases

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CONTENTS

Poisonous Plants and Phytochemicals in Drug Discovery, First Edition.
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11.1 Introduction

Microorganisms such as bacteria and fungi are rich sources of chemically diverse secondary metabolites, including toxic compounds that have potent biological activities. The specific features of these metabolites differ by their taxonomic, and biochemical diversity. Today, most of the antibiotics in clinical use are direct secondary metabolites or the semisynthetic derivatives of different species of bacteria, such as actinomycetes or fungi, which are toxic to other organisms. Drugs isolated from microbial sources include erythromycin and its derivatives, vancomycin and teicoplanin, cephalosporins, rifampicin, and tetracyclines, all of which were discovered through conventional antibacterial screening techniques. The latest approaches for the discovery of drugs include target-based discovery using bacterial genomics, combinatorial chemistry, and high-throughput screening techniques. However, the results of these techniques are unsatisfactory because not many antibiotic molecules have been approved for clinical use. So far, different drugs obtained from microbial sources have been found to possess anticancer, antimicrobial, hypocholesterolemic, antifungal, immunosuppressant, enzyme inhibitor, and antiparasitic activities [1].

About 23 000 active compounds have been identified from completely different microbial sources; of these, 42% of compounds have been isolated from fungi and 32% were isolated from actinomycetes [2]. From about 1 million naturally obtained compounds, approximately 25% have shown biological activity, i.e. they show positive activities or negative activities (toxicity). Among all of the biologically active compounds obtained from natural sources, 60% of them are of plant origin and the remainder are from microbes. To date the structures of more than 160 000 naturally obtained compounds are known. Most of the bioactive compounds have been isolated from terrestrial microorganisms, but about 129 compounds were obtained from marine microorganisms between 2000 and 2003 [3–5].

The journey of drug research from microorganisms began with the discovery of the antibiotic penicillin from the fungus *Penicillium notatum* by Alexander Fleming in 1929. After this revolutionary discovery, many antibiotics such as tetracyclines, cephalosporins, aminoglycosides, and macrolides were discovered later in the 1940s and early 1950s. The majority of antimicrobial agents discovered during this period were isolated from *Streptomyces* spp., which represent about 70–80% of all the isolated bioactive compounds and the discovery of antitumor, antiviral, and non-antibiotic enzyme inhibitory metabolites. During the 1970s to 1990s, the rate of antimicrobial drug discovery decreased, but the number of newly discovered compounds was still on the increase. The majority of all of the discovered bioactive compounds are derivatives of already existing drug compounds. From the 1990s onwards, research interest in the discovery of newer metabolites (mainly non-antibiotic compounds and analogous compounds) increased exponentially. However, the rate of discovery of new chemical entities has decreased. Because of urgent clinical needs, chemotherapy problems such as multidrug-resistant strains,

reappearing mycobacteria, human immunodeficiency virus, etc. are significantly increasing, bringing about new challenges in physiological disease therapy, improved classical screening methods, and new technologies [6].

11.2 Antimicrobial Agents from Microbial Sources

The selective action of microbial secondary metabolites was initiated in the antibiotic age, more than 50 years ago, against pathogenic bacteria and fungi. About 350 or more microbial compounds have reached the market as antibacterial agents. Most of them are (i) natural microorganism products, (ii) semisynthesized from natural products, or (iii) synthetically obtained on the basis of the structure of natural products [7].

The majority of antibacterial agents available on the market have been obtained from microbial sources such as penicillin, the β-lactam antibiotic. It was found that penicillin inhibits the growth of certain Gram-negative bacteria. The β-lactams are a broad class of antibiotics including its derivatives (called penams) and they contain a β-lactam ring in their structure. Furthermore, other β-lactam agents have been discovered. The agents that have been discovered so far are the cephalosporins, penem, monobactam, and carbapenem subclasses, and clavulanic acid, all of which have a β-lactam ring structure but do not have any significant antibacterial activity. For example, clavulanic acid is an inhibitor of β-lactamases, so it is generally used in combination with penicillins to reduce the incidence of resistance [8–11].

Aminoglycosides are the other class of antimicrobial agents derived from microbes; these were discovered in 1944. The nomenclature of the class differs on the basis of the microbial genus from which it was isolated. The suffix -mycin is used in the nomenclature for the group of aminoglycosides isolated from bacteria of the genus S*treptomyces* (e.g. streptomycin), and the suffix -micin is used for the group of drugs obtained from the genus *Micromonospora* (e.g. gentamicin) [12].

Furthermore, many other classes of antimicrobial agents have been isolated from microbial sources (Figure 11.1), such as chloramphenicol (isolated from the bacterium *Streptomyces venezuelae* in 1949), tetracyclines (derived from the bacterium *Streptomyces aureofaciens*), macrolides (1952), lincosamides (lincomycin was isolated from the bacterium *Streptomyces lincolnensis* in 1952), streptogramins (derived from the bacterium *Streptomyces virginiae* in 1952), rifamycins (derived from the bacterium *Amycolatopsis mediterranei* in 1957), and lipopeptides (2003) [12].

There are many antibiotic-producing bacterial species, but about 75% of all antimicrobial agents are produced by actinomycetes. Among all the actinomycetes, the genus *Streptomyces* has alone produced about 75% of all antimicrobial agents. One single bacterial strain can be the source of a number of antibiotics, e.g. almost 200 antibacterial compounds have been obtained from *Streptomyces*

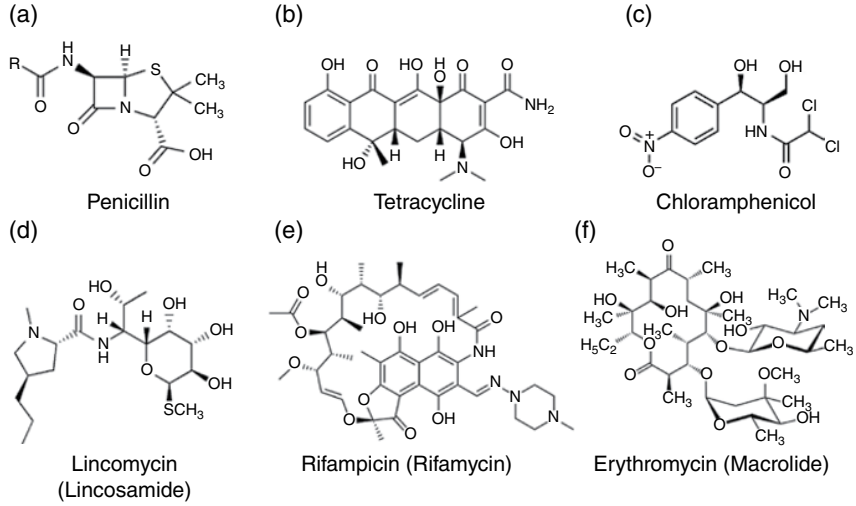


Figure 11.1 Structures of some antimicrobial agents obtained from microbes.

hygroscopicus, about 48 antibiotics have been obtained from a single strain of *Micromonospora*, and about 40 different antimicrobial compounds have been obtained from *Streptomyces griseus*.

The majority of antibiotics available on the market include cephalosporins (45%), penicillins (15%), quinolones (11%), tetracyclines (6%), and macrolides (5%); the aminoglycosides, glycopeptides, ansamycins, and polyenes constitute the remaining antibiotics available on the market. The synthetic agents include the sulfa drugs, azoles, oxazolidinones (linezolid), and fluoroquinolones [13, 14].

There are more than 40 β-lactam agents available on the market and these are prescribed on a regular basis. In spite of the fact that β-lactamases are the main reason for the development of antibiotic resistance, and there are more than 450 such enzymes, lactams are still extremely valuable because of the discovery of β-lactamase inhibitors. β-Lactamase inhibitors include clavulanic acid and the carbapenems as well as doripenem, tomopenem, ceftobiprole, ceftaroline, faropenem, and meropenem. They have a broad spectrum of activity, including β-lactamases from *Pseudomonas aeruginosa* [11].

The other class of most important antimicrobial agents is tetracyclines, which were discovered during the antibiotic age and which make a significant contribution in the treatment of bacterial infections. The first compound of this class, chlortetracycline, was discovered in 1948; this was followed by the discovery of oxytetracycline in 1950. These were the first broad-spectrum antibiotics known. The second-generation semisynthetic tetracyclines, i.e. minocycline and doxycycline, were more lipophilic and effective against resistant strains [15–17].

Another important class of antibiotics are the polyketide macrolides. According to R.B. Woodward, an American organic chemist, the term macrolide stands for

macro-lactone glycoside (it means a macro-lactone containing one or more deoxy sugars). The microorganisms that produce macrolide antibiotics are the actinomycetes, e.g. *Streptomyces*, *Actinoplanes*, *Micromonospora*, and *Saccharopolyspora*. The macrolide antibiotics of medical use are oleandomycin, amphotericin B, midecamycin, josamycin, and carbomycin.

The second-generation macrolides are semisynthetic in nature and include clarithromycin, azithromycin, dirithromycin, florithromycin, and roxithromycin. The advantages over first-generation compounds are that the second-generation macrolides are more acid stable, have a lower elimination rate, and only require dosing at one or two times per day. The third-generation macrolides appeared in the late 1980s, and were found to be more active against the microbial strains that were resistant against first- and second-generation macrolides. These agents are effective mainly against microbial strains causing respiratory diseases, sexually transmitted infections (*Chlamydia trachomatis*), Legionnaire disease (*Legionella pneumophila*), Lyme disease (*Borrelia burgdorferi*), peptic ulcers (*Helicobacter pylori*), gonorrhea (*Neisseria gonorrhoeae*), and *Mycobacterium avium* in patients with acquired immunodeficiency syndrome (AIDS) [18–26].

Microbial peptides have gained much more importance in the area of antibiotics. Many peptide compounds that show antibiotic activity were isolated; these include vancomycin, teicoplanin, the streptogramins, and the bacteriocins. Streptogramins, which are isolated from cultures of *S. virginiae*, include pristinamycin as well as virginiamycin M and virginiamycin S, which act together synergistically. Vancomycin has remained the drug of choice for many years to treat infections caused by antibiotic-resistant bacterial strains. More recently, other drugs belonging to these classes have been developed, e.g. the lipoglycopeptide teicoplanin (Targocid), daptomycin, linezolid, Synercid, and telavancin, and they are all effective against vancomycin-resistant bacterial strains. There are seven antimicrobials that could replace vancomycin and new carbapenems that are active against Gram-negative infections. They are the glycopeptides dalbavancin, televancin, and oritavancin; the lipopeptide daptomycin; the cephalosporins ceftobiprole and ceftaroline; and the diaminopyrimidine iclaprim [26–33].

11.3 Antifungal Agents from Microbial Sources

Mycosis is a fungal disorder that can cross the barriers to resistance, such as skin, and invade the tissues, leading to superficial, subcutaneous, or systemic disease in humans or animals. Generally, fungi are harmless; however, in some cases they can cause diseases. These infections are not life-threatening in most cases. However, when they are profoundly intrusive and spread, they lead to more serious infections, especially in critically ill patients, the elderly, and those with conditions affecting the immune system. Furthermore, the use of antibiotics, prosthetic devices, and grafts, as well as more aggressive surgery, has increased invasive fungal infections [34].

About 40% of deaths from nosocomial infections are caused by fungi, 80% of which are caused by *Candida* and *Aspergillus*, although the involvement of different species of *Cryptococcus*, *Fusarium*, *Scedosporium*, *Penicillium,* and *Zygomycetes* is now increasing. The need to discover newer antifungal agents is increasing day by day owing to the increase in the incidence of invasive fungal infections and the development of fungal resistance [35, 36].

The first class of antifungal agents obtained from a microbial source were the polyene antibiotics (polyketides obtained from *Streptomyces* spp.). These are broad-spectrum antifungal agents that act by causing alterations in membrane permeability. Polyene antibiotics consist of two different groups of antibiotics: partricin A (from *S. aureofaciens*) and partricin B (gedamycin). For the treatment of many systemic fungal infections, amphotericin B is the first-line agent because it has broad-spectrum and fungicidal activity. However, substantial side effects limit its clinical usefulness. Most of the current antifungal drugs have some connection to natural products, and especially to microorganisms; for example, the azoles, generally considered to be synthetic in origin, come from the metabolite azomycin of *Streptomyces*.

A newer group of compounds are the lipopeptide echinocandins, which are obtained from various fungi. They cause cell wall synthesis inhibition by blocking 1,3-D-glucan synthesis. Caspofungin (pneumocandin), micafungin, and anidulafungin (Figure 11.2) are echinocandins that are approved for the treatment of systemic fungal infections. In 2000, caspofungin was the very first compound among the echinocandin class approved as an injectable antifungal. It inhibits 1,3-β-D-glucan synthase irreversibly, prevents the formation of glucan polymers, and interferes with the integrity of the fungal cell walls. It has higher activity and fewer side effects than amphotericin B and is effective against many fungal strains of *Candida* (including fluconazole resistance), *Aspergillus*, *Histoplasma,* and *Pneumocystis carinii*. Micafungin is approved in Asian countries and in the USA for the clinical treatment of fungal infections. It was found that it is has highly potent antifungal activity against the fungus *Aspergillus* and azole-resistant strains of *Candida*. Animal studies have shown that micafungin is as effective as amphotericin B with respect to survival rate improvement. Anidulafungin is a semisynthetic derivative of echinocandin B, and was first obtained from *Aspergillus rugulovalvus*. At present, anidulafungin is approved in the USA [37–42].

11.4 Anticancer Agents from Microbial Sources

The fact that compounds with antibiotic activity also have other activities is an extremely important concept for the further development of natural products. Some of these activities have been used off-label in the past. Therefore, molecules

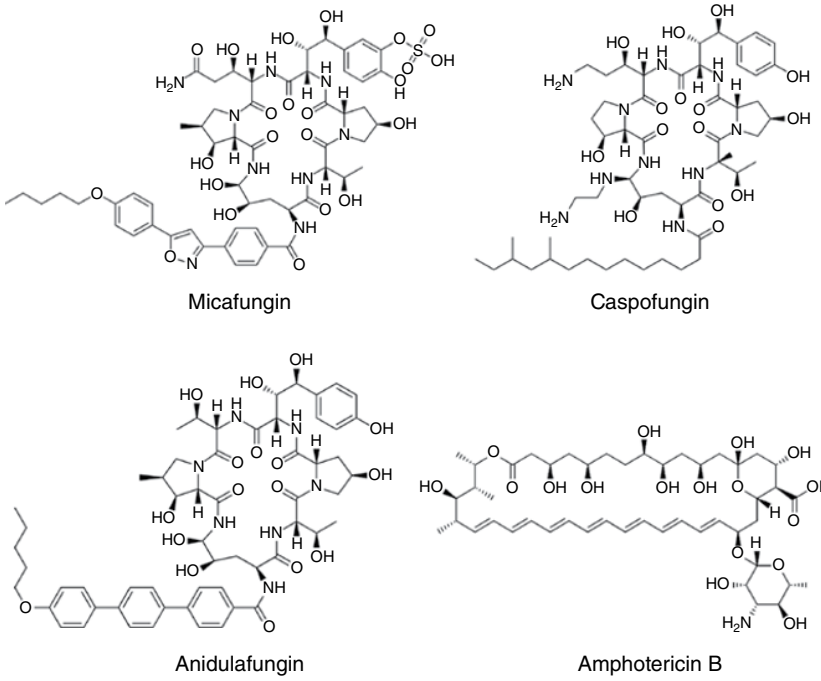
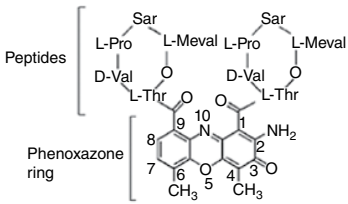


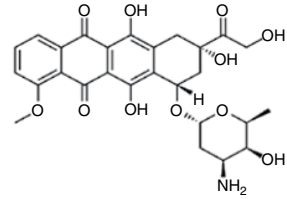
Figure 11.2 Antifungal agents from microbial sources.

with antibiotic activity were screened for antagonistic activity against organisms other than microorganisms and for activities that are useful for pharmacological or agricultural applications to provide new and beneficial uses for "failed antibiotics." As a result of this a large number of in vitro laboratory tests were developed for the detection, isolation, and purification of medicinally useful compounds. We have entered a new era in which microbial metabolites are used for synthetic compounds against diseases that were previously untreatable, i.e. especially for diseases caused by microorganisms other than bacteria and fungi, and great successes have been achieved.

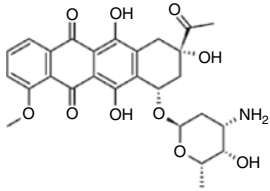
Microbial metabolites are among the most important chemotherapy agents for cancer. They began to appear around 1940 when actinomycin was discovered; since then many anticancer compounds have been isolated from natural sources. Among the 140 antitumor agents licensed since 1940 and used for treatment, more than 60% are obtained from different natural products. Nowadays the majority of available anticancer agents either are isolated from natural products or are derivatives of natural products. Some of the important compounds among all the products approved for marketing are actinomycin D, anthracyclines (daunorubicin, doxorubicin [Figure 11.3], epirubicin, pirarubicin, and valrubicin),



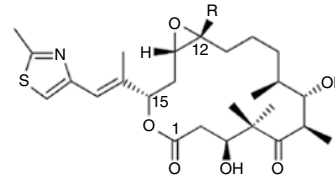
Actinomycin D



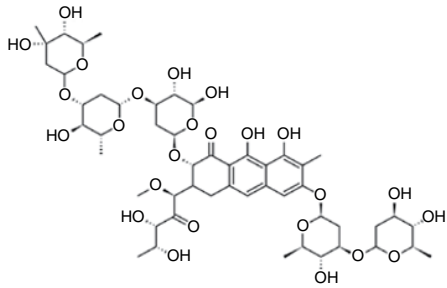
Doxorubicin



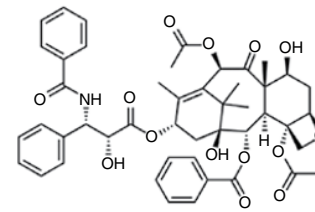
Daunorubicin



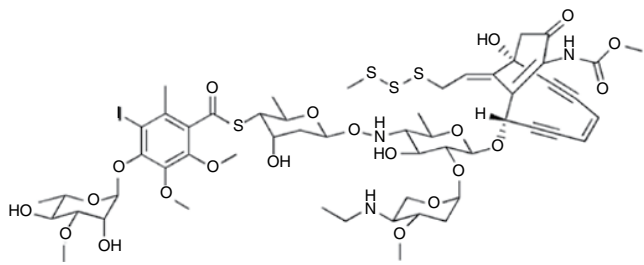
Epothilones A (R = H) and B (R = Me)



Mithramycin



Taxol



Calicheamicin gamma 1

Figure 11.3 Anticancer agents from microbial sources.

bleomycin, mitosanes (mitomycin C), anthracenones (mithramycin, streptozotocin, and pentostatin), enediynes (calicheamicin), taxol, and epothilones [43, 44].

Actinomycin D (Figure 11.3) is the oldest compound of microbial origin used in the treatment of cancer. A similar compound, actinomycin A, was the very first anticancer antibiotic obtained from the cultures of *Streptomyces parvulus*, *Streptomyces chrysomallus*, and *Streptomyces antibioticus*. They are chromopeptides. The chromophoric part is substituted 3-phenoxazone-1,9-dicarboxylic acid, which is known as "actinosin." Dactinomycin binds non-covalently to double-stranded DNA by partial intercalation between adjacent guanine–cytosine bases, resulting in inhibition of DNA function. This property leads to part of the toxic effect and was primarily used as an investigative tool [45].

The other most important class of antitumor antibiotics are the anthracyclines. They are effective against most types of cancer (uterine, ovarian, and lung cancers), which is not the case for other classes of anticancer agents. Anthracyclines cause DNA intercalation between adjacent nucleotides along the DNA, forming tight DNA–drug interactions. This leads to inhibition of DNA synthesis and transcription. It also causes inhibition of the enzyme topoisomerase II, a key enzyme involved in DNA synthesis and generation of free radicals; this assists the cytotoxic activity of the anthracyclines. Cardiotoxic effects of the drugs in this class limit their usefulness. Daunorubicin (known as daunomycin in 1966) was the very first anthracycline compound isolated naturally from *Streptomyces peucetius* (Figure 11.3). Later, in 1967, doxorubicin was developed. Another semisynthetic compound of this class is epirubicin (40-epidoxorubicin). It was approved in 1999 by the US Food and Drug Administration (FDA) and has fewer side effects than doxorubicin. The fewer side effects are due to its structural difference at C-4 of the sugar portion as compared with doxorubicin. It is effective in the treatment of breast and ovarian cancer, gastric cancer, lung cancer, and lymphomas. Valrubicin is a semisynthetic doxorubicin analog approved in 1999 as a chemotherapeutic agent and used to treat bladder cancer [46].

Bleomycin is a glycopeptide isolated from culture of the bacterium *Streptomyces verticillus*. It is a mixture of closely related compounds with bleomycin A2 and B2, which are available in nature as blue copper chelates. The cytotoxic properties of bleomycin are due to its ability to fragment DNA [47].

Mitosanes are another important group of anticancer compounds isolated from the cultivation of *Streptomyces caespitosus*. They have significant anticancer activities but their use was limited because of their toxicity. Mitomycin C, which was approved for use by FDA in 1974, was the first mitosane group compound to receive approval; it is effective against many types of cancer (lung, breast, bladder, anal, colorectal, head, and neck). Mitomycin dimers can be used to reduce toxicity but they also have reduced efficiency [48, 49].

The aromatic polyketide mithramycin is isolated from the culture of *Streptomyces argillaceous* and has antibacterial and anticancer activity. It binds with DNA and interferes with transcription. When used repeatedly, it shows kidney, liver, and blood toxicity that is not desirable [50].

A nitrosourea compound, streptozotocin, with anticancer activity is obtained from *Streptomyces achromogenes* microbial metabolites. It acts by alkylation of DNA strands like other nitrosourea compounds. It has a structural similarity to glucose, which helps to transport it via the glucose carrier into cells. As β cells contain a high level of glucose permease, so streptozotocin, a nitrosourea compound, is concentrated in pancreatic islet β cells and was therefore approved by FDA for the treatment of pancreatic islet cancer in 1982 [51].

Calicheamicin is an extremely potent microbial enediyne antitumor metabolite produced by *Micromonospora echinospora*. Its cytotoxic activity is caused by double-stranded DNA cleavage. These compounds are highly toxic [52].

Taxol, a non-bacterial compound, is isolated from the endophytic fungi *Taxomyces andreanae* and *Nodulisporium sylviforme*. But taxol was first isolated from the Pacific yew tree, *Taxus brevifolia*. It acts by inhibiting the rapid division of cells by promoting tubulin protein polymerization; the resulting microtubule–taxan complex is not able to disassemble. Taxol also has antifungal properties and is used to treat ovarian cancer, breast cancer, and Kaposi's sarcoma [44, 53].

The epothilones are 16-member ring polyketide macrolides originally isolated from the soil myxobacterium *Sorangium cellulosum* as a weak antifungal agent against rust fungi. Prior to 2005, more than 400 compounds were isolated from the microbial metabolites of *S. cellulosum*. Of these, only epothilones have promising anticancer activity. Their structure consists of an epoxide, thiazole, and ketone, and contains a methyl thiazole group attached by an olefinic bond; hence they are called epothilones. They act in a similar way to taxol by stabilizing microtubules in metaphase through the promotion of excessive tubulin protein polymerization. These compounds are 5–25 times more potent in inhibiting cancer cell growth than taxol. Among these compounds, five molecules are under investigation for their anticancer properties.

These molecules have a wide spectrum of activity and show activity against resistant cancer. The semisynthetic ixabepilone, derived from epothilone B, was approved by FDA in 2007 for the treatment of metastatic breast cancer that was resistant to recent chemotherapy. The *S. cellulosum* culture produces a blend of epothilone A and epothilone B, but only epothilone B is medically significant. Thus, sodium propionate is added to the culture to reduce the production of epothilone A. Epothilones have good water solubility compared with taxol [54–57].

Camptothecin is a monoterpene indole alkaloid mostly isolated from some plant species. It is also produced by *Entrophospora infrequens*, an endophytic fungus. It is used to treat recurrent colon cancer and is also effective against lung,

ovarian, and uterine cancers. Irinotecan and topotecan are the water-soluble camptothecin derivatives that are used clinically. Plant sources contain very low camptothecin concentrations, and the failure of the synthetic route to obtain a pure compound is the main limitation for industrial production. Fungal fermentation for the industrial production of camptothecin is promising in this regard. Camptothecin acts by inhibiting the enzyme topoisomerase I [58, 59].

For tumors to obtain oxygen and nutrients, angiogenesis (recruitment of new blood vessels) is necessary. For this purpose, tumor cells secrete endothelial growth factors that stimulate angiogenesis. Angiogenesis inhibitors can, therefore, be used as anticancer agents, i.e. inhibition of activated endothelial cells. Fumagillin, produced by *Aspergillus fumigatus*, was one of the first agents to act as an antiangiogenesis compound. It inhibits type 2 methionine aminopeptidase enzyme, which is an enzyme essential for proliferation.

11.5 Hypocholesterolemic Agents from Microbial Sources

Atherosclerosis is a disease in which fat, cholesterol, calcium, and other substances form plaques in the artery walls. The plaques harden, narrow the opening of the arteries, and limit the flow of blood over time. A variety of antiatherosclerotic drugs have been introduced over the last two decades. The diet accounts for only 30% of cholesterol in the human body. The remaining 70% is synthesized by the body, mainly in the liver. Statins inhibit the production of cholesterol in the liver, the principal source of cholesterol in the blood. High blood cholesterol leads to atherosclerosis, an important cause of human death in many types of coronary heart disease. The statins are among the important anti-hypercholesterolemic agents that act by inhibiting the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol biosynthesis mevalonate pathway. Inhibition of this enzyme in the liver stimulates low-density lipoprotein (LDL) receptors, leading to an increased clearance of LDL from the bloodstream and a decrease in blood cholesterol. Statins are successful because they reduce the total plasma cholesterol by 20–40%, while the fibrates previously used reduced it by only 10–15%. Some microbial sources provide statins (Figure 11.4). There are currently a number of statins in clinical use. Before becoming a generic pharmaceutical, the entire group of statins reached an annual market value of almost US$30 billion.

The discovery and evolution of statins is a fascinating story. Mevastatin (compactin) (Figure 11.4), the first member of the group, was isolated from *Penicillium brevicompactum* as an antibiotic product and later from *Penicillium citrinum*. Its derivatives have considerable medical and commercial importance.

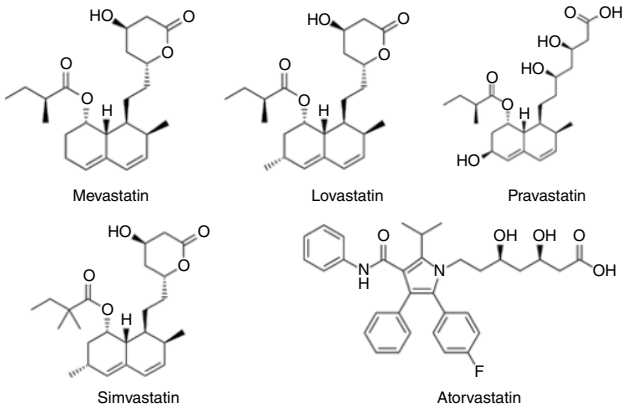


Figure 11.4 Hypocholesterolemic agents from microbial sources.

One of these important derivatives is lovastatin, which was isolated in the 1970s from cultures of *Monascus ruber* and *Aspergillus terreus*. In 1987, the FDA approved lovastatin as the first commercially marketed statin. Simvastatin, a semisynthetic derivative of lovastatin (in which the 2-methylbutanoate side chain of lovastatin is chemically modified to 2,2-dimethylbutanoate), was the top-selling hypocholesterolemic drug at that time. Another statin, pravastatin (obtained by biological hydroxylation using actinomycetes), is made from compactin by *Streptomyces carbophilus* and *Actinomadura* spp. through different biotransformation processes. Fungi from other genera, e.g. *Doratomyces*, *Eupenicillium*, *Gymnoascus*, *Hypomyces*, *Paecilomyces*, *Phoma*, *Trichoderma*, and *Pleurotus*, are involved in the production of statins. A synthetic compound, modeled from the natural statin structure, is atorvastatin, which has been the leading drug in the entire pharmaceutical industry for many years in terms of market share.

Statins reduce heart events such as myocardial infarction, stroke, and death. They are not only active against atherosclerosis, the most common cause of death in Western countries, but also have endothelial, anti-inflammatory, antiatherothrombotic, immunomodulatory, and antimigration effects. Their inflammatory effects are greater than their effects on cholesterol. Statins lower total cholesterol and LDL and increase high-density lipoprotein (HDL) cholesterol. They also lower the incidence of Alzheimer's disease. Statins reduce elevated levels of C-reactive protein (CRP), regardless of their cholesterol effect. This is important because half of all myocardial infarctions occur in patients with normal LDL levels. High CRP is associated with the inflammatory atherosclerosis response and is a predictor of future cardiovascular deaths. Statins may also prevent stroke and reduce peripheral vascular disease development. They have positive effects on multiple sclerosis and cancer as well. Experiments with oral statins demonstrated

efficacy in a multiple sclerosis mouse model. The effect seems to be independent of lowering cholesterol. Other activities under study include bone formation stimulation and antioxidation [60–62].

11.6 Immunosuppressants from Microbial Sources

Suppressor cells are of great importance in regulating the normal immune response. The immune system of an individual is capable of distinguishing between native and foreign antigens and mounting a response against only the latter. A major role for suppressor T lymphocytes in this phenomenon has been established. Suppressor cells also help to regulate the magnitude and duration of the antibody response to an antigenic challenge. The suppression of the immune response by drugs or radiation, to prevent graft or transplant rejection or to control autoimmune diseases, is called immunosuppression.

A number of microbial compounds have been discovered that can suppress the immune response (Figure 11.5). Ciclosporin A was originally manufactured by

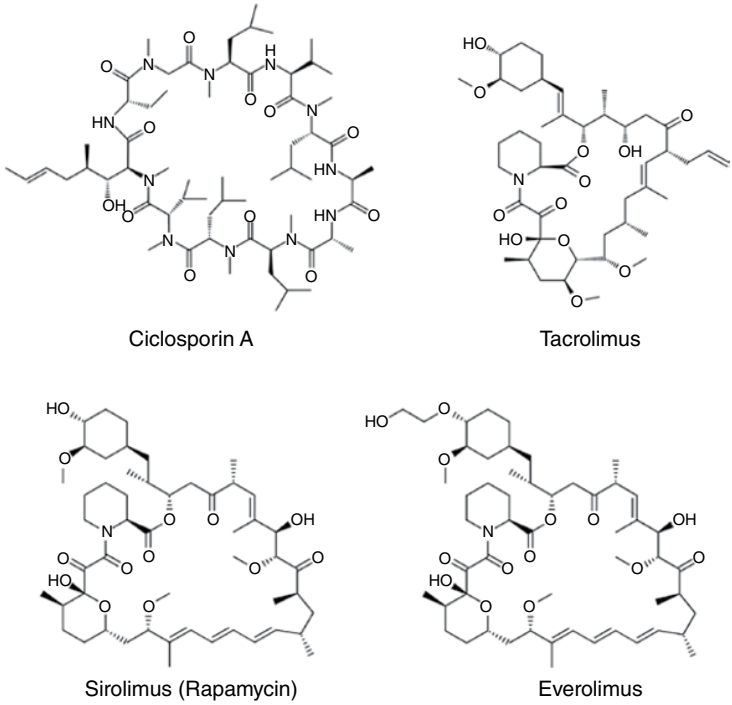


Figure 11.5 Immunosuppressants from microbial sources.

aerobic fermentation as a narrow-spectrum antifungal peptide produced by the mold *Tolypocladium niveum* (originally classified as *Trichoderma polysporum* and later *Tolypocladium inflatum*). Ciclosporins are a family of neutral, highly lipophilic, cyclic undecapeptides with certain unusual amino acids, synthesized with the non-ribosomal peptide enzyme cyclosporine synthetase. The discovery of immunosuppressive activity led to its use in heart, liver, and kidney transplants and its overwhelming success in the field of organ transplants. In 1983, ciclosporin was licensed for use. It binds immunocompetent lymphocytes, especially T lymphocytes, to the cytosolic protein cyclophilin (immunophilin). This complex of ciclosporin and cyclophilin inhibits calcineurin, which normally activates interleukin 2 (IL-2) transcription. It also inhibits the production of lymphokine and the release of interleukin, and thus reduces the function of T cells. ciclosporin A sales amount to US$1.5 billion per year [63, 64].

Ciclosporin A (Figure 11.5) is active against the malaria parasite *Plasmodium falciparum* because its genome contains cyclophilin- and calcineurin-encoding sequences. Ciclosporin analogs have been clinically tested against inflammatory asthma disease and have demonstrated promising results. They have reduced nephrotoxicity and differ in pharmacology and metabolism [63].

Tacrolimus was discovered in Japan in 1987. It comes from *Streptomyces tsukubaensis*. However, its use was almost terminated because of dose-related toxicity, which can be reduced by using lower doses. It was found to be about 100 times more active than ciclosporin A. It was introduced in Japan in 1993 and was approved by the FDA in 1994 for use as a liver transplant immunosuppressant. It has also been extended to include bone marrow, cornea, heart, intestines, kidney, lung, pancreas, trachea, small intestines, skin, and limb transplants and graft-versus-host disease prevention. It is also used topically against the common skin disease atopic dermatitis. Tacrolimus inhibits the mixed lymphocyte reaction, the development of IL-2 by T lymphocytes, and the formation of other soluble mediators. Recently, tacrolimus has been reported to inhibit tumor growth factor B-induced signaling and collagen synthesis in fibroblastic cells in the human lung. In tissue fibrosis, including pulmonary fibrosis, this factor plays a key role. Tacrolimus can therefore be useful in the treatment of pulmonary fibrosis, although its use in the acute inflammatory phase can exacerbate pulmonary injury [65, 66].

Another important immunosuppressant agent is sirolimus (rapamycin). Rapamycin is particularly useful in renal transplants because it lacks the nephrotoxicity demonstrated by ciclosporin A and tacrolimus. It is a macrolide that was first discovered in 1975 as a product of *S. hygroscopicus*, and an antifungal agent was originally proposed. This was abandoned, however, when it was found to have powerful immunosuppressive and antiproliferative properties. This compound binds to the immunophilin-binding protein; this binary complex then interacts

with the rapamycin-binding domain and inactivates a serine–threonine kinase known as rapamycin mammalian. The antiproliferative effect of rapamycin was also used in combination with coronary stents to prevent restenosis, which usually occurs after coronary artery disease has been treated with an angioplasty balloon. Rapamycin is not as nephrotoxic as either ciclosporin A or tacrolimus and is synergistic in immunosuppressive when in action with both compounds. Rapamycin is the basis for chemical modification in important products such as everolimus, temsirolimus, and ridaforolimus. Rapamycin has not only immunosuppressive properties, but also antifungal, neuroprotective, autoimmune, and antiaging properties. Rapamycin has antitumor activity, too. While ciclosporin A promotes tumor growth, resulting in the death of many transplant patients, rapamycin inhibits tumor growth by interfering with angiogenesis and is also an apoptosis inducer. Rapamycin can also reverse multidrug resistance in mammalian cells to antitumor agents. Ciclosporin and tacrolimus are also capable of this [67, 68].

11.7 Enzyme Inhibitors from Microbial Sources

Enzyme inhibitors (Figure 11.6) are now the focus of much attention, not only in the study of enzyme structures and reaction mechanisms but also for their possible use in medicine and agriculture. Many enzyme inhibitors are isolated from

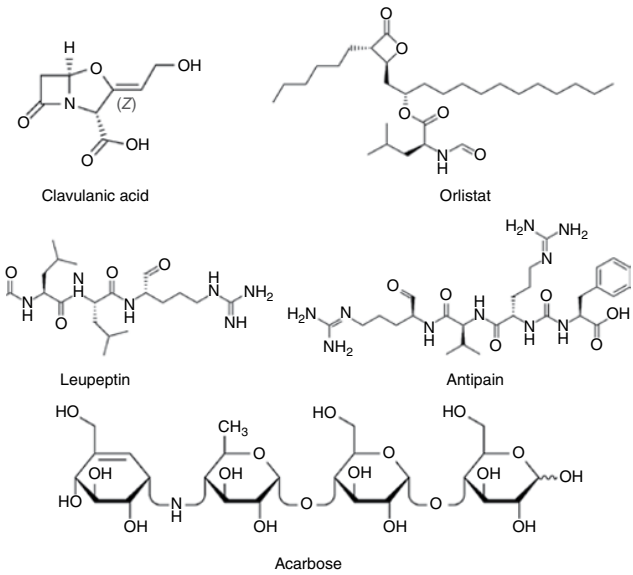


Figure 11.6 Enzyme inhibitors from microbial sources.

microbial sources, which are now used more than previously in industry. The best example of an enzyme inhibitor that is useful as a medical agent is clavulanic acid, the β-lactamase inhibitor. Some of the common targets for enzyme inhibitors are glucosidases, amylases, lipases, proteases, and xanthine oxidase (XO). In addition, the hypocholesterolemic drugs statins act by inhibiting the enzyme HMG-CoA, so they can also be included in this category [69].

Acarbose is a pseudotetrasaccharide isolated from cultures of *Actinoplanes utahensis*. Its chemical structure consists of an aminocyclitol moiety, valienamine, which is responsible for the inhibition of intestinal α-glucosidase and sucrase. As a result, the metabolism of starch decreases in the intestine, which is desirable in the fight against diabetes in humans.

Amylase inhibitors are important in the management of carbohydrate-dependent diseases such as diabetes, obesity, and hyperlipidemia. Amylase inhibitors prevent the absorption of dietary carbohydrates, so they can be used for weight loss. It was also reported that amylase inhibitors can be used to treat rumen acidosis. Examples of microbial α-amylase inhibitors are paim, which is obtained from *Streptomyces corchorushii* culture filtrates, and tau aggregation inhibitors (TAI-A and TAI-B), which are oligosaccharide compounds obtained from *Streptomyces calvus* TM-521. Lipstatin, produced by *Streptomyces toxytricini*, is a pancreatic lipase inhibitor used to fight obesity and diabetes. It interferes with fat absorption through the gastrointestinal system. Tetrahydrolipstatin, the saturated derivative of lipstatin, is also called orlistat [70, 71].

Protease inhibitors are potentially powerful tools for inactivating target proteases in the pathogenic processes of certain diseases, such as emphysema, arthritis, pancreatitis, cancer, and AIDS. Examples of microbial products include antipain, isolated from *Streptomyces yokosukaensis* cultures; leupeptin, isolated from *Streptomyces roseochromogenes*; and chymostatin, isolated from *S. hygroscopicus*. XO catalyzes uric acid oxidation of hypoxanthine through xanthine. Too much uric acid in the blood, known as hyperuricemia, causes gout. XO inhibitors reduce the uric acid level, leading to an antihyperuricemic effect. A powerful XO inhibitor, hydroxyakalone, was purified from the fermentation broth of the marine bacterial strain *Agrobacterium aurantiacum* [72].

11.8 Antiparasitic Agents from Microbial Sources

Antiparasitic agents are compounds that inhibit parasite growth or reproduction; some antiparasitic agents kill parasites directly. In general, parasites are much smaller than their hosts, exhibit a high degree of specialization in their lifestyle, and reproduce faster and in larger numbers. There are 3200 parasite varieties in four major categories: Protozoa, Trematoda, Cestoda, and Nematoda. Protozoans

(organisms with only one cell) and parasitic worms (helminths) are the main groups. Each of these can infect the digestive tract and occasionally cause two or more infections simultaneously. Classical examples of parasitism include the interactions between vertebrates and animals such as tapeworms, flukes, plasmodia, and fleas. Parasite infections can cause serious health problems and even kill the host. Parasites enter the body mainly through the mouth, usually by ingestion of contaminated food or drink.

Over the years, the predominant type of antiparasitic screening effort was the testing of synthetic compounds against nematodes, and some commercial products resulted. Some antibiotics have also been shown to have anthelmintic activity against nematodes or cestodes, but they failed to compete with synthetic compounds. Thiabendazole was developed by Merck as a synthetic, commercially useful anthelmintic agent and has been found to be toxic to humans.

The culture of *Streptomyces avermitilis*, isolated at the Kitasato Institute in Japan, produced a family of secondary metabolites (eight compounds) with anthelmintic and insecticidal activity. These compounds, known as "avermectins," are pentacyclic, 16-member macrocyclic lactones that contain the methylated disaccharide oleandrose, which has exceptional activity against parasites, in particular Nemathelminthes (nematodes) and arthropod parasites (10 times higher than any known synthetic anthelmintic). Avermectins are surprisingly lacking in activity against bacteria and fungi, do not inhibit protein synthesis, and are not ionophores. They interfere with neurotransmission in many invertebrates and cause paralysis and death through neuromuscular attacks. A semisynthetic derivative, 22,23-dihydroavermectin B1 (ivermectin), is 1000 times more active than thiabendazole and is a commercial veterinary product. Ivermectin's efficacy has made it a promising candidate for human onchocerciasis and strongyloidiasis control. Another avermectin, known as doramectin (or cyclohexyl avermectin B1), produced by mutational biosynthesis, was marketed for food animals. A doramectin derivative of a semisynthetic monosaccharide called selamectin is the most recently marketed avermectin and is active against heartworms (*Dirofilaria immitis*) and fleas in domestic animals. Although each of these molecules (ivermectin, doramectin, and selamectin) has the same macrocyclic backbone, different substitutions exist at pharmacologically relevant sites such as C-5, C-13, C-22, C-23, and C-25 [73, 74].

11.9 Recent Advances in Drug Discovery from Microbial Sources

The total number of microbial metabolites, including both bioactive and inactive compounds, that has been recognized to date is around 50 000, and the number of all known natural products is about 1 million. Regarding the possible number of

bioactive natural products, recall that chemists of natural products rarely investigated isolated compounds in the past, especially not for a wide range of bioactivity. Also, keep in mind that many of the compounds, including microbial products previously thought to be inactive, were proved to be active in later research or were rediscovered using various screening techniques. It is not possible to predict how many new bioactive microbial compounds will be discovered in this way.

The reinvestigation of known natural/microbial products and, in particular, of the whole microbial population (natural products and microbiological libraries) with a wide range of more selective, sensitive, and specific techniques, particularly in view of increased knowledge of microbial genetics and the acquired knowledge of different genomes, would be particularly fruitful. The main requirement is that every new bioactive compound can be used in human society. Nowadays, more food, new medicines, and other goods are essential for the benefit of mankind. The major problem is not only the discovery of new useful microbial metabolites but rather how to optimize and apply new discoveries quickly and efficiently.

The microbial resources have not yet been fully explored in terms of their dimensions and geographical and environmental aspects. There are certainly millions of microorganisms in the environment that have not yet been explored by science. DNA community analysis of microorganisms has shown that there are many more novel microorganisms in nature than originally thought; these microorganisms cannot be detected and cannot be isolated by conventional techniques. Many more drugs have been obtained from microbial sources than from other natural sources, such as fungi, viruses, and algae. Fungi include one of the largest groups of eukaryotes other than insects. The fungi appear to be one of the largest reservoirs from which to isolate further bioactive metabolites.

There is great potential in the isolation and cultivation of microbes that are difficult to isolate or cannot be isolated, that are less cultivable, that are almost uncultivable, or that grow slowly in both groups of well-known or less explored microbial types. Methods of DNA analysis have shown that the number of microbes in the ground was much higher than previously thought. It is estimated that approximately 1% of aquatic and 10% of terrestrial microorganisms (perhaps only 0.1% of soil microbes) can be easily grown or "cultivated" by conventional methods.

The most promising sources of microbials for exploration include the very diverse ecosystem, particularly from underrepresented sites (extreme circumstances, sea, etc.), and previously unexplored producers can lead to promising results. Generally speaking, the sea and, to a lesser extent, the rainforests are almost inexhaustible, untapped reservoirs for new compounds. In addition to aquatic microorganisms, some of the soft-bodied marine animals and endophytic fungi that grow alongside aquatic plants are probably the best targets for the discovery of new microbial sources.

Some unnatural precursors, which are the basis for the biosynthesis of important metabolites, cannot easily produce a mutant microorganism through biochemical means. Combinatorial biosynthesis is a further possibility for manipulating microbial physiology. Molecular biological approaches enable the identification and activation of genes operating the microbial biosynthetic machinery. Genetic engineering allows the production of hybrid enzymes that are capable of synthesizing a series of new analog molecules (e.g. erythromycin derivatives). There are encoded genes for numerous alternative biosynthetic pathways in the genomes of microbes (actinomycetes, fungi, myxobacteria) that allow new compounds to be generated. There are many silent genes responsible for the production of different, possibly new, actinomycetal metabolites, e.g. in *S. coelicolor*. The genome of *S. avermitilis* has been found to contain more than 30 metabolite-related gene clusters, but so far only a few such as avermectins, oligomycins, and pentalenolactones have been isolated.

Currently, microbial metabolites are under investigation for their usefulness in the treatment of viral diseases, neurological disorders, cardiovascular disorders, anemia, spasms, diabetes, irritable bowel syndrome, atopic dermatitis and Crohn disease.

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12

Development of Phyto-Antidotes Against Adverse Chemical Agents

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CONTENTS

12.1 Introduction

Modern chemical technologies are applied in almost all spheres of human activity and use a wide range of different chemicals. The high toxicity of these chemicals and their ability to pollute the environment and to penetrate the human body in different ways result in a high risk of acute and chronic mass poisoning [1].

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The impact of environmental pollution on human health is enormous. The World Health Organization estimates that 23% of all deaths worldwide are due to environmental factors, and at least 8.9 million deaths have been caused by non-communicable environmental causes. Low- and middle-income countries carry the main burden of pollution-related diseases [2].

Workers within and the populations surrounding industrial sites are exposed to toxic substances that enter the environment from the fuel/energy, chemical, metallurgical, military, and other sectors of modern industry. Mining and metallurgical enterprises emit compounds containing arsenic, phosphorus, antimony, lead, and mercury vapor into the atmosphere. The most dangerous pollution is caused by highly toxic polymetallic dust. The main sources of air pollution by lead are non-ferrous metallurgical enterprises. Metallic dust from zinc smelting contains 25–50% lead. Zinc emissions from zinc production can reach 62.5–77.5 kg of zinc emissions per tonne of zinc produced [1].

Global industry, especially within the fuel/energy sector, emits approximately 1–3 billion tonnes of chemicals every year in the form of particles of different chemical composition that are smaller than 1 μm in diameter. These particles contain a variety of chemicals, including toxic metals such as lead, mercury, and cadmium [3]. The release of toxic substances is particularly high in major cities and industrial centers.

As a result of the chemical interaction between two toxic substances, new, more toxic compounds can be formed. These chemicals disturb the normal chemical balance of the human body, might cause pathological changes in particular systems and organs, and negatively affect all physiological processes in the body. The word "toxic" is derived from the Greek word *toxikon pharmakon*, which originally meant the poison in which darts were dipped [4].

Among the European countries, Ukraine has the highest integral indicator of negative man-made environmental load over almost all its territory. Moreover, in two-thirds of the regions, the environmental situation and the quality of the environment are characterized as acute critical and unfavorable towards human health, respectively. More than 10% of the total territory of Ukraine is regarded as an "environmental disaster zone," which is an officially recognized international status. This status was applied to Ukraine after the accident at the Chernobyl Nuclear Power Plant, when radioactive contamination of a large part of its territory was added to all the other contaminants. As a result, the environmental impact of various chemical contaminants and ionizing radiation has increased significantly [5].

The need for detoxification, elimination, or neutralization of toxic substances in the human body has become a global concern that needs an immediate solution. One of the preventive measures aimed at detoxification of organisms under everyday conditions is the search for antitoxins of natural origin among native food plants. The use of plant-based foods for therapeutic purposes is a tradition in

all nations of the world. The main advantages of the use of biologically active substances from edible plants with detoxification properties are their low cost, sufficiently high efficiency, and absence of adverse effects.

This chapter discusses an information search in scientific publications and search databases on the detoxification properties of foods of plant origin according to their content and interaction of biologically active substances in the prevention of chronic heavy metal poisoning. Methods of analysis, systematization, comparison, and information generalization are discussed.

12.2 Heavy Metals and their Effects on the Body

Heavy metals belong to a wide-ranging group of pollutants. Recently, the meaning of this term has been interpreted differently in various scientific works, and therefore the number of elements belonging to the group of heavy metals varies widely. Numerous characteristics are used as inclusion criteria: atomic mass, density, toxicity, and distribution in the natural environment. In some cases, chemical elements such as bismuth or arsenic fall into the class of heavy metals because of their metallic properties and electrical conductivity [6].

In studies devoted to the problems of environmental pollution and ecological monitoring, more than 40 metals in the modern periodic table with an atomic mass higher than 50 atomic mass units are referred to as heavy metals: chromium, manganese, iron, cobalt, nickel, copper, zinc, molybdenum, cadmium, tin, mercury, lead, etc. [6]. In this case, high toxicity to living organisms at relatively low concentrations as well as the ability to bioaccumulate play an important role in inclusion of the chemical elements in the group of heavy metals. Some published works include the following heavy metals in the list of chemicals to be determined in biosphere reserves: lead, mercury, cadmium, arsenic. On the other hand, according to the Task Force on Emissions of Heavy Metals, which works under the auspices of the United Nations Economic Commission for Europe and collects and analyzes information on emissions of pollutants in European countries, only zinc, arsenic, selenium, and antimony should be classified as heavy metals [7].

Metal ions tend to accumulate in large quantities in the same tissues and organs where they are found under normal conditions as trace elements. Deposition of metal ions occurs in tissues and organs with a high rate of metabolism: the thyroid gland absorbs manganese, cobalt, nickel, chromium, arsenic, and rhenium; the pancreas absorbs manganese, cobalt, chromium, zinc, and nickel; the pituitary gland absorbs manganese, lead, and molybdenum; and the testes absorb cadmium and zinc. The deposition of most metal ions in the body is the result of their ability to form various organic complexes with proteins and amino acids. Ions of metals, such as zinc, cadmium, cobalt, nickel, thallium, copper, tin, ruthenium, chromium, and mercury, are distributed evenly throughout the body. They are detected

in cases of toxic poisoning in all tissues, but some selectivity in their accumulation is observed. Deposition of any form of mercury or cadmium occurs in the kidneys because of the specific affinity of these metals for kidney tissues. Several sparingly soluble rare earth metals are retained in tissues with high reticuloendothelial system activity: liver, spleen, and bone marrow. Metal ions tend to accumulate in bone tissue, forming strong bonds with phosphorus and calcium. Such metals include lead, beryllium, barium, strontium, gallium, yttrium, zirconium, uranium, and thorium. In addition, lead under steady-state conditions (i.e. stable inhalation) is also found in the liver, kidneys, spleen, and cardiac muscle in maximum quantities [8, 9].

Lead ranks first among heavy metals that pollute the environment. It is very toxic and, therefore, has been noted to be of special concern worldwide as a pollutant [10]. The ionic mechanism of lead toxicity arises mainly because of the ability of its ions to replace other divalent cations, such as Ca^{2+}, Mg^{2+}, and Fe^{2+}, and monovalent cations, such as Na^{+}, which ultimately disrupts the biological metabolism of the cell, causing significant changes in different biological processes, such as cell adhesion, intra- and intercellular signaling, protein folding, and apoptosis [11–13]. The biological half-life of lead in the blood is approximately 35 days, while in the brain this period is approximately 2 years, and in the bones it lasts for decades. Lead causes oxidative stress; affects calcium and zinc homeostasis, neurotransmission, and neurogenesis; damages the mitochondria and membrane integrity; and inhibits antioxidant enzymes, which ultimately leads to apoptosis, disorders of the blood–brain barrier, and neurodegeneration. Data concerning permissible limits of widely distributed heavy metals and their possible toxic effects on humans are summarized in Table 12.1.

In the case of heavy metal poisoning, ethylene diaminetetraacetic acid (EDTA), which belongs to a special class of chemical compounds, i.e. chelating (from the Greek word *chela*, meaning crab's claw) agents, is considered to be one of the main detoxifying compounds. These substances are capable of forming strong water-soluble complexes with heavy metals that are not biodegradable. Various EDTA salts (complexons), which do not hydrolyze in the body, are rapidly excreted by the kidneys; as a result, they are widely used as detoxifying agents for the prevention of heavy metal poisoning. However, complexons form coordination compounds with both heavy metals and other chemical elements. They accelerate the excretion of many metals that occur in the free state (Na, K, Ca, etc.) or as part of vital metalloproteins. The long-term use of chelating agents can lead to manifestations of toxic poisoning, changes in the blood coagulation system, binding of serum calcium, and impaired renal function because the kidneys eliminate large amounts of coordination compounds [14].

Complexons cause side effects; hence, preventive detoxifying agents are required that will not cause adverse events during prolonged daily use and at the same time exhibit a pronounced protective effect. Biologically active substances contained in food products of plant origins might be a source of such a group of active ingredients.

Table 12.1 Effects of heavy metals on the human body.

Heavy metal	Permissible limit (ppm)	Impact on the body
Lead, Pb	0.1	Mental retardation in children; damage to the liver, kidneys, gastrointestinal tract; infertility; anemia; muscle and joint pains; hypertension
Mercury, Hg	0.01	Skin rashes; eye irritation; muscle weakness; dermatitis; damage to kidneys and nerve trunks; anorexia; severe muscular pain
Arsenic, As	0.02	Bronchitis; carcinogenic dermatitis; liver tumors; damage to the gastrointestinal tract
Cadmium, Cd	0.06	Renal lesions; bronchitis; gastrointestinal disorders; bone marrow damage; cancer; weight loss
Chromium, Cr	0.01	Allergic dermatitis; lung tumors; a human carcinogen
Nickel, Ni	3.0	Chronic bronchitis; decreased lung function; diseases of the nasal cavity; lung cancer

12.3 Detoxification Properties of Biologically Active Substances of Plant-Based Foods

The detoxifying properties of foods of plant origin are the result of the content and interaction of biologically active components, which include dietary fiber, vitamins, antioxidants, organic acids, and others [8].

12.3.1 Pectins

Pectins are natural polymers that are found in vegetables, fruits, and berries [8]. Pectins have the ability to form complexes with metal ions because they contain polygalacturonic (pectic) acid (Figure 12.1). The complex-forming ability of pectins is based on the interaction of their molecules with heavy metal cations, which demonstrates the potential for application of these substances as detoxifying compounds [15–17]. After ingestion pectin increases in volume and stimulates intestinal peristalsis. The food manufacturing industry produces numerous specialized edible products based on vegetables and fruits containing pectins in significant quantities. The advantage of these products to the human body is that they simultaneously provide a

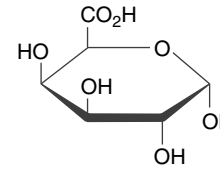

Figure 12.1 The structure of galacturonic acid.

constant supply of biologically active substrates from the product (vitamins, trace elements, and other natural compounds) and remove toxins [8].

Pectins have been widely used in the food and medical industries for many decades, but neither the macromolecular structure of pectic acids nor the molecular weight range of these compounds has been established to date [18]. It was believed that pectic acid is partially esterified with methyl alcohol to form polygalacturonic acid, or homogalacturonan. The substance is currently considered to be a heterogalacturonan, namely rhamnogalacturonan. Pectic acid and pectinic acid are thought to contain different amounts of neutral monosaccharides (10–25%), with only L-rhamnose residues in the main chain and the residues of other monosaccharides attached in the form of side chains. Using pectic acids isolated from soybeans as an example, it has been demonstrated that the side chains contain residues of D-xylose [19]. Available data show that pectinic acids from different plants have various degrees of esterification with methyl alcohol, but none of the pectinic acid is completely esterified [20].

It was found that the detoxification ability of pectins is determined by the presence of carboxyl groups, which are able to attach cations of many metals to form pectinates. The effect can be observed when absorption of lead, introduced into the body, is significantly inhibited under the influence of pectins. It has been established that only carboxyl groups of polygalacturonic acid residues participate in the formation of complexes with hydrated mercury ions, referred to as mercury galacturonate [21, 22]. It was demonstrated using in vitro studies that aqueous extracts of pectin preparations (beet, carrot, apple) possess a protective action against the cytotoxic effects of mercury chloride. Such a positive effect can be explained by the interaction of mercury ions with the carboxyl groups of polygalacturonic acid units of pectins in aqueous solution before mercury crosses the cell membrane [22, 23].

12.3.2 Phytin

Phytin, comprising a mixture of calcium and magnesium salts of inositol phosphoric (phytic) acids, is contained in plants. When these plants are consumed as part of the daily diet in large enough quantities, phytin has detoxifying properties. Experimental studies have shown that phytin completely protects animals that were poisoned by lethal doses of lead. In this case, the excretion of lead is carried out mainly through the gastrointestinal tract, rather than through the kidneys. Phytin may be recommended for detoxification of poisoning with other heavy metals and their salts [24].

Phytin is found in potatoes; nuts; the seeds of wheat, oats, and corn; peas; beans; and other cereals and legumes. The cyclic structure of phytin consists of myoinosit (or myoinositol), a component of cell membranes involved in the metabolism of carbohydrates, calcium, and phosphorus in plants.

An ester consisting of a cyclic polyalcohol of the six-carbon-atom myoinosit and six residues of phosphoric acid is a so-called phytic acid, or myoinositol hexaphosphoric acid (Figure 12.2) [25]. Phosphoric acid residues associated with myoinositol can either accept or donate hydrogen ions, owing to multistage dissociation, of which myoinositol hexaphosphoric acid exhibits properties of both strong and very weak acids, depending on temperature and other factors. In a neutral medium, the phosphate groups of myoinositol hexaphosphoric acid partially dissociate, and thereby acquire negative charges; this is because positively charged heavy metal ions can be strongly chelated by two or more phosphoric acid residues or form weaker ionic bonds with one phosphate group. Therefore, myoinositol hexaphosphoric acid, or phytic acid, exerts its detoxifying properties by the chelation of heavy metal cations and the formation of multiple coordination bonds [26–28].

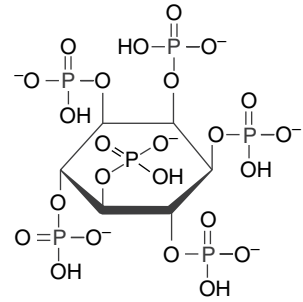

Figure 12.2 Myoinositol hexaphosphoric acid (phytic acid).

However, it must be remembered that myoinositol hexaphosphoric (phytic) acid not only chelates heavy metal ions, but also excretes calcium, magnesium, and iron, causing deficiencies in these minerals in the body [29].

12.3.3 Betalains

The detoxification properties of edible plants are attributed to polyphenolic compounds, which, because of the peculiarities of their chemical structure, are able to chelate metal ions [30, 31]. The characteristic features of many polyphenols comprise easy oxidation with the formation of highly reactive intermediates such as semiquinone radicals or orthoquinones, the ability to interact with proteins through the formation of hydrogen bonds, and the tendency to form coordination complexes with metal ions [32]. It has been shown that high antioxidant and detoxifying properties occur in betalains, which are water-soluble nitrogen-containing pigments consisting of nitrogenous structures of betalamic acid, the main compounds of which are betaine and betanin [33, 34]. To date, about 78 betalains are known [35].

Red beetroot (*Beta vulgaris* (L.)) is one of the most common and traditional food plants in Ukraine and contains betalains dissolved within the vacuole of the plant cells. Red beet is significantly superior over all other food plants in terms of betalain content [36, 37]. It is thought that betalains found in red beet are structurally and chemically different from anthocyanins since they contain nitrogen in their structure while anthocyanins do not. Betalains have been reported to be aromatic indole compounds synthesized from tyrosine [38, 39].

Betalains are divided into betacyanin (red-violet) and betaxanthin (yellow-orange) pigments [40]. A number of studies have reported that betalains exhibit high antioxidant and anti-inflammatory activities in vitro and in various animal models in vivo [41–43]. It has also been demonstrated that betanine (betanidine-5-O-β-glucopyranoside), the most common betalain with antioxidant properties, has been proven to be the most effective inhibitor of lipid peroxidation [44, 45]. The high antioxidant activity of betanine is apparently the result of its exceptional electron-donating capacity and its ability to neutralize highly reactive radicals directed at cell membranes [46].

Antioxidant and detoxification effects have been investigated in studies using beetroot juice. It has been shown that administration of beetroot juice to rats for 28 days attenuated lipid peroxidation, protein oxidation, and DNA damage caused by xenobiotic-induced liver injury [47]. In addition, the detoxifying properties of betalains are confirmed by an increase in the number of enzymes (such as glutathione-S-transferase) that play an important role in the detoxification of heavy metals and other xenobiotics by the liver [48].

Betanine is widely used in the food industry as food additive E162 (beetroot red) in powder form, which, when dissolved in water, is immediately restored to natural beetroot juice. It is authorized for application in food products in most countries of the world, including Ukraine. The potentially harmful health effects resulting from excessive daily consumption of beet in environmentally polluted areas have not been properly investigated. However, to date, there is no evidence that natural beet juice supplements pose any health risk. Further studies are still needed to evaluate the long-term safety of the dietary use of beet as a detoxifier, especially in clinical practice [48].

12.3.4 Phytochelatins

Promising substances in the search for detoxification agents for the prevention of chronic poisoning by heavy metals appear to be phytochelatins (Figure 12.3), which are synthesized in plants from their precursor glutathione by the enzyme phytochelatin synthase. Phytochelatin, under the name cadystin, was first identified in fission yeast (*Schizosaccharomyces pombe*) in 1981. The substance was then

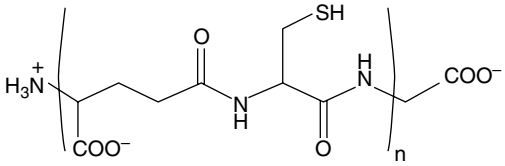

Figure 12.3 Phytochelatin.

detected in higher plants and was therefore called phytochelatin. The enzyme phytochelatin synthase was discovered in 1989 [49].

Phytochelatins act as chelators and are an important element for detoxification during the accumulation of heavy metals. They are found in plants (including foodstuffs such as cereals, legumes, and corn), fungi, nematodes, and all algae groups, including cyanobacteria. Phytochelatin synthesis depends on the type and concentration of heavy metals, the plant species, and conditions of its germination [50]. Phytochelatins are able to bind different metals via the sulfhydryl and carboxyl residues. The involvement and important role of phytochelatins in lead detoxification have been demonstrated [10]. Studies on the inhibition of phytochelatin biosynthesis by glutathione have demonstrated their fundamental role in the detoxification of metals in yeast and fungi, green algae, and some aquatic plants, as well as in cell suspension cultures and intact tissues of higher plants [51]. The primary structure of phytochelatins has been determined for many species of angiosperms from different families. They are small cysteine-rich peptides capable of binding heavy metal ions through SH groups [52, 53].

Phytochelatin synthesis can be activated in vivo by various metals. However, phytochelatin may not play a significant part in their detoxification; this may be due to the insufficient bond strength of the phytochelatin complex with the metal. Metal ions activate the enzyme phytochelatin synthase. Phytochelatins have a high affinity for heavy metal ions, which determines their important role in detoxification of heavy metals [52].

12.3.5 Ellagic Acid

One of the effective heavy metal chelators is ellagic acid (Figure 12.4), a dilactone of hexahydroxydiphenic acid, which belongs to the low-molecular-weight phenolic compounds. It occurs in both the free and bound state and is a member of the tannin family, namely ellagitannins and tannins of mixed type. Ellagic acid is contained in pomegranates, bilberries (*Vaccinium myrtillus*), strawberries, raspberries, blackcurrants, and other edible plants. Free ellagic acid is a promising compound with antitoxic properties [54–56].

Ellagic acid contributes to a significant improvement in glutathione production and a reduction in lipid peroxidation, neutralizes the toxicity of heavy metals by chelation, and also promotes their excretion, thereby protecting the liver from additional damage and oxidative stress [57]. Ellagic acid helps to maintain the balance of detoxification by inducing the production of glutathione-S-transferase, and

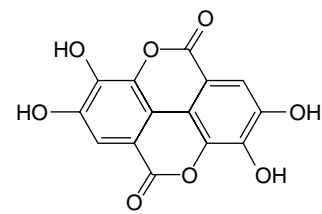

Figure 12.4 Ellagic acid.

also supports other processes of detoxification at a genetic level, directly affecting DNA molecules and protecting them from the effects of mutagens [58].

Because of its antioxidant and detoxifying potential, ellagic acid has cardioprotective properties. This is evidenced by the results of a study conducted to evaluate the protective effects of ellagic acid against lead-induced myocardial toxicity. Rats exposed to lead acetate (100 mg/l) in drinking water were treated with a high (50 mg/kg) and low (25 mg/kg) dose of ellagic acid for 12 weeks. The results of the treatment were evaluated using electrocardiographic parameters, serum biomarkers and tissue levels of antioxidants, and histological examination. There was a significant decrease in serum biomarkers and an increase in the level of antioxidants in tissues. These results indicate a positive effect of the ellagic acid treatment against lead-induced myocardial toxicity [59]. In addition, it has been found that the oil extract of pomegranate (*Punica granatum*) seeds, which contain ellagic acid, has a protective effect on toxic damage to rats caused by mercury dichloride ($HgCl_2$) [60].

12.3.6 Miscellaneous

This chapter discusses biologically active substances in several food plants. Of course, these are not the only natural compounds that have detoxifying properties. Modern research suggests that a wide range of edible plants can be used to protect the body from the toxic effects of heavy metals, radioactive substances, and other xenobiotics.

As an example, in traditional medicine, the widespread food plant artichoke (*Cynara scolymus*), which contains chlorogenic acid, cynarin, and luteolin, has long been used to protect the liver against exposure to toxic substances [61, 62]. Consumption of artichoke increases the absorption of these bioactive substances in the human body, leading to the production of useful metabolites (such as ferulic acid). Ferulic acid, chlorogenic acid, and cynarin provide antioxidant and detoxification protection against toxic chemical-induced alterations; these natural compounds also reduce the loss of cellular reserves of glutathione [63].

Tea, as the most popular food plant in the world, has detoxifying and antioxidant effects because of the polyphenolic compounds it contains. Studies in laboratory animals have shown that administration of green tea (*Camellia sinensis)* reduces the toxic effects of cadmium and lead on the body by chelating these metals. Green tea polyphenols have a protective effect, both in vivo and in vitro, in lead-related injury and toxicity to the kidneys. These polyphenols reduced the concentration of lead in the kidneys and protected mesangial kidney cells from lead-induced apoptosis [64, 65].

Coriander (*Coriandrum sativum*) leaves have been reported to enhance mercury excretion after dental amalgam removal. The use of coriander leaves in animals

reduced the absorption of lead. Studies show that coriander can protect the body as a whole, and in particular the liver, from heavy metals and toxins. However, in a study of children aged 3–7 years who were exposed to lead, coriander extract was less effective in increasing renal lead excretion (improvements in all treatment groups and placebo could be attributed to an improved diet during the intervention) [49, 66, 67].

Garlic (*Allium sativum*), a popular food plant, prevents chronic heavy metal poisoning. A study of the effects of garlic oil (250 mg/kg) found a significant decrease in the concentration of copper and zinc in the serum and liver tissue of rats. It has been found that, when administered simultaneously to rats as 60 mg/kg of garlic and 8 mg/kg of chromium chloride ($CrCl_3$), garlic inhibits the toxicity of chromium(III) chloride. The prophylactic efficacy of garlic to reduce the concentration of lead in body tissues was evaluated experimentally in goats. The simultaneous application of lead acetate and dry garlic powder significantly reduced the lead content in the tissues, indicating the potential activity of garlic against the toxic effects of lead [68].

The protective effect of an aqueous extract of saffron (*Crocus sativus*) on the neurotoxicity caused by aluminum chloride ($AlCl_3$) was revealed. A test group of mice were administered $AlCl_3$ 40 mg/kg/day and saffron extract 200 mg/kg once daily for 45 days. The authors investigated the neurotoxicity of $AlCl_3$ in mouse brain based on biochemical and molecular studies. Although the concomitant use of saffron extract did not affect the cognitive performance of mice, the herbal drug significantly improved the aluminum chloride-induced biochemical and molecular indices in brain tissues. The molecular and biochemical results obtained indicated the neuroprotective potential of aqueous saffron extract in chronic aluminum poisoning [69].

The antitoxic effects of black seed (*Nigella sativa*) and its main constituent thymoquinone against toxicities induced by various types of chemicals have been demonstrated in several in vitro and in vivo investigations [70]. Scientific data concerning the antidotal and protective activities of cinnamon (*Cinnamomum zeylanicum* and *Cinnamomum cassia*) against natural and chemical toxins have been reviewed [71].

12.4 Current State of Clinical Application of Phyto-Antidotes

Activated charcoal (carbo activatus*),* obtained from birch (*Betula* spp.) heartwood and treated with superheated steam, is traditionally applied in clinics as an effective remedy in cases of acute oral poisoning because of its ability to bind different toxins and prevent systemic toxicity [72, 73].

The tropane alkaloids atropine and scopolamine, found in Solanaceae species, are able to block the effects of anticholinesterase agents such as organophosphates at muscarinic receptor sites. For this reason, atropine has an effective application in the treatment of exposure to organophosphate and carbamate pesticides, as well as some toxic chemicals [73].

The indole alkaloid physostigmine, initially obtained from the seeds of the West African perennial shrub *Physostigma venenosum*, owing to its acetylcholinesterase inhibitory activity, is the known antidote for the treatment of anticholinergic syndrome and organophosphate toxic poisoning, and is often used in inpatient medical establishments providing emergency care. Nowadays clinical applications of physostigmine comprise the relief of central cholinergic toxicity caused by atropine, scopolamine, and belladonna alkaloids and of toxic poisoning induced by overdoses of tricyclic antidepressants [74, 75].

12.5 Further Prospects in the Search for Promising Phyto-Antidotes

The beneficial health effects of flavonoids are mainly ascribed to their antioxidant activity. Flavonoids are able to induce phase II detoxifying enzymes (e.g. NAD(P)H-quinone oxidoreductase, glutathione-S-transferase, and uridine diphosphate-glucuronosyl transferase), which are the major defense enzymes against electrophilic toxicants and oxidative stress. Data concerning the detoxifying properties of flavonoids have been presented in a review [76].

Caffeic acid phenethyl ester (CAPE), a phenolic compound found in propolis and some herbs, was able to reduce oxidative stress as well as hepatic and pancreatic damage due to acute pesticide toxic poisoning in rats [77]. Pretreatment with CAPE diminished abamectin-induced hepatotoxicity and nephrotoxicity in rats, as demonstrated by a reversal of the biochemical and histopathological changes caused by the toxin [78].

The protective effects of milk thistle (*Silybum marianum*) and its main active principle flavonolignan silymarin against numerous toxic agents of biological and chemical origin have been reviewed; the significant protective action of the plant is due to its chelating, free radical scavenging, antioxidant, antiapoptotic, and anti-inflammatory characteristics [79].

The protective effects of ginkgo (*Ginkgo biloba*) against toxicities caused by chemical and natural toxins and also by radiation have been reviewed [80]. Some special mechanisms, such as acetylcholinesterase inhibition in aluminum neurotoxicity and membrane-bound phosphodiesterase activation in triethyl tin toxicity, have been detected for ginkgo extracts [80].

A review by Dharini and Chaitra [81] summarizes the data of specific antidotes to various toxic active principles of natural origin, applied in Ayurveda; among them the following plant sources of official herbal drugs deserve particular attention: *Zingiber officinale* and *Curcuma longa*. The scientific data concerning detoxification, as well as the related hepato- and nephroprotective properties of herbal drugs, have been reviewed [68, 69].

Several medicinal plants (milk thistle, coriander, ginkgo, turmeric, green algae [*Chlorophyta*]) are considered to be promising agents for use in heavy metal poisoning because of their ability to cause a significant reduction in the absorption of these toxic substances [9]. Another review [82] describes 34 herbs that possess considerable protective activity against arsenic toxicity, demonstrated mostly in preclinical studies.

The current chapter contains scientific data on the beneficial effects of promising phyto-antidotes that exhibit detoxifying and related properties, protecting the liver as well as the urinary, respiratory, and nervous systems from the toxic effects of heavy metals and other xenobiotics. The combined impact of the biologically active substances of medicinal herbs determines their restorative effect and contributes to an increase in the capability and resistance of the human body to adverse environmental conditions that are of considerable importance for populations living in polluted areas.

12.6 Conclusions

Detoxification, as a process of elimination or neutralization of toxic substances in the human body, is an urgent global problem. Environmental pollution by heavy metals and other xenobiotics has a high risk of massive acute and chronic poisoning, which could lead to significant medical, social, and economic losses. One of the preventive measures with the purpose of detoxification of the body under everyday conditions is the search for antitoxins of natural origin among edible medicinal plants.

Summarizing the results of foreign and domestic studies, it can be concluded that some biologically active substances from food plants (pectins, phytic acids, betalains, phytochelatins, etc.) neutralize and promote the excretion of heavy metals and other xenobiotics. The advantage of using edible plants with detoxifying properties is their low cost, sufficiently high efficiency, and absence of adverse side effects.

Under the current conditions of psycho-emotional loading and poor nutrition in a considerable sector of the population in polluted territories, it is necessary to introduce qualitatively new diets based on vegetable products with known

detoxifying properties. These diets should be developed and implemented by means of comprehensive state and international programs, which should include a set of measures aimed at determining scientifically substantiated dietary supplements of vegetable origin with detoxification effects, creating conditions that fully meet the needs of different segments of the population in polluted areas with a rational detoxification diet that accords with regional ecological peculiarities, traditions, customs, age, profession, and health status.

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13

Nanoformulated Herbal Drug Delivery as Efficient Antidotes Against Systemic Poisons

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CONTENTS

13.1 Introduction

The World Health Organization (WHO) has estimated that around 35000–70000 plant species are utilized as medicines throughout the world [1]. Herbs are categorized under this broad class of plants [2]. Herbs have been widely used for medicinal purposes since ancient times [3]. Ancient medicinal systems throughout the world have utilized herbs to cure several diseases, disorders, and numerous

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infections [4]. Among a wide variety of plants, herbs caught the attention of ancient people, who used them as medicines because of their shorter growth time, which makes it easier to collect leaves or other parts, and the fact that they grow in almost all climatic conditions all over the world [5]. The herbal phytochemicals that are extracted from their specific parts, especially the leaves, are reported to possess enormous medicinal value [6]. Advancements in the extraction of phytochemicals as well as their characterization and formulation have paved the way for several herbal phytochemicals to be used in the treatment of specific diseases [7]. Herbal phytochemicals are used widely as antimicrobials [8], nutraceuticals [9], and in the treatment of cancer [10], diabetes [11], and neurovegetative diseases [12]. Even deadly diseases such as acquired immunodeficiency syndrome (AIDS) [13], severe acute respiratory syndrome (SARS) [14], and other viral diseases, such as Zika [15], have been reported to be curable using herbal phytochemical extracts. The toxicity and side effects associated with conventional, synthetic, chemical-based drugs are the reasons for choosing herbal medicines, which contain biomolecules that are biocompatible, bioavailable, and cause a negligible level of toxicity [16]. According to WHO, these advantages of herbal medicines have increased their market share, which has a value of approximately US$43 billion per year. It is noteworthy that approximately 1500 herbal medicines are sold annually in the USA alone, giving a total estimated profit of approximately US$5 billion [1]. Thus, herbal medicines have already surpassed the current synthetic medicines market, and are expected to replace them in the pharmaceutical market in the near future because of the rapid growth and efficacy of herbal medicines in curing a wide variety of diseases.

Herbal extracts are also used as antidotes for systemic poisoning conditions, which was a common practice among ancient peoples [17]. Even today, tribal populations around the world prefer herbal extracts as antidotes for the treatment of systemic poisoning conditions [18]. In recent times, herbal antidotes have attracted the attention of researchers and pharmaceutical companies because of their swift response to systemic poisons as antidotes and the reduced chance of post-treatment side effects [19]. However, the solubility of phytochemicals in biological fluids limits the delivery of the prescribed concentration of phytochemicals to the target site [20]. Thus, it is essential to formulate these herbal phytochemicals with novel encapsulations to enable their controlled and targeted delivery. Nanoformulations were introduced recently as a novel and effective means of protecting herbal phytochemical formulations from disintegration in biofluids and of delivering them to the target site [21]. Dendrimers, liposomes, micelles, nanocapsules, and polymers are the common nanoformulations that are used as carriers to encapsulate herbal phytochemicals [22]. Such nanoformulated herbal phytochemicals are gaining attention among researchers [23] as effective antidotes against systemic poisons [24]. Thus, the aim of the present chapter is to

provide an overview of herbal phytochemicals that are reported to possess antidotal ability against systemic poisons. In addition, the ability of nanoformulations to enhance the antidotal efficiency of herbal phytochemicals and the mechanisms of action of nanoformulated herbal phytochemicals as competent antidotal phytochemicals against systemic poisons are discussed.

13.2 Herbal Phytochemicals as Antidotes for Systemic Poisons

A poison is regarded as a substance that can cause illness or death when ingested in small quantities. Systemic poisons are those that affect the whole body or severely affect more than two organs. This definition excludes the multitude of materials that cause damage if ingested in large amounts. Any substance present in amounts or concentrations that are higher than required can be damaging to the body and, in some cases, life-threatening. Even oxygen and glucose, which are vital for humans to survive, are fatal at higher concentrations. There are two critical concepts to be borne in mind when describing poisoning: the degree of toxicity and the mechanism of action. The mechanism of action of different poisons is unique – some act on the cell membranes and some interfere with the synthesis of vital proteins. Poisonous substances also have the typical property of causing damage to organs, such as the liver, kidneys, brain, or central nervous system (CNS).

Several poisons that commonly affect humans exist in nature. Organic and inorganic chemicals and drugs are the most common poison types known to humans. The organic chemical poisons include ethyl alcohol, methyl alcohol, and carbon monoxide. Acute ethyl alcohol poisoning is manifest over a relatively short time after ingestion of large quantities. Similarly, methyl alcohol metabolizes to formic acid, an extremely toxic substance that affects the nerves and the eyes. Exposure to carbon monoxide even at low concentrations can result in slow accumulation, leading to a gradual toxic or fatal level. The common inorganic chemicals that are responsible for systemic poisoning include mercury, arsenic, and lead. Mercuric salts cause cell death by precipitating the proteins within the cells, which is known as coagulative necrosis. This can cause extensive tissue damage at higher concentrations.

Similarly, arsenic is known to cause severe damage to blood vessels and the CNS, triggering vascular collapse and depression, which can lead to death within hours of ingestion. The soluble salts of inorganic lead are also a potential systemic poison. They may accumulate in the body for an extended period until the toxicity reaches a threshold limit value and causes cell damage. The long-term effects of lead poisoning include lysis of the red blood cells, resulting in anemia. This can also cause

degeneration of nerve cells, which leads to depression, psychoses, convulsions, and even death. Several drug agents are also important causes of poisoning. For example, morphine is an excellent drug for the control of severe pain, but it can depress respiration and, in excess, can cause death. Barbiturates and salicylates are other major drugs that can cause severe illness from excess ingestion. They can affect the CNS and lead to coma and death.

Nature has offered us diverse curative herbs with phytochemicals that have the potential to cure poisoning conditions. The utilization of plants and their products for the treatment of various diseases dates back to the origins of humankind. Herbal phytochemicals are reported to have the ability to reduce the risk of certain disorders, such as neurodegenerative, autoimmune, and cardiovascular conditions. Several herbal compounds, including ferulic acid, quercetin, and proanthocyanidin, are well characterized to have anti-inflammatory and antioxidant properties. The significant advantages of herbal or plant-based medicines are their low adverse effects, low cost, and high efficacy. Moreover, there are also claims that a high proportion of plant extracts have valuable effects on human neurological function, particularly on human brain health. The secondary metabolites derived from plants such as alkaloids have been used for medicinal purposes since ancient times. Numerous herbal alkaloids have also been used in psychotropic medicines, social drugs, and as hallucinogens. These are also used as modulators of specific neurotransmitter targets [25].

13.2.1 Herbal Phytochemicals as Antidotes for Heavy Metal Poisoning

Heavy metal-induced neurotoxicity and poisoning can adversely affect human health, as indicated by several studies. Heavy metals are those with adverse effects on the environment or humans as they cannot be metabolized. Heavy metal poisoning occurs mostly with metals such as cadmium, lead, and arsenic, when their concentration increases in living organisms. The heavy metal-induced neurotoxicity brings about free radical production, which ultimately leads to the generation of reactive nitrogen and oxygen species. These free radicals create disturbances in the oxidative and antioxidative systems, leading to necrosis, damage to the DNA, and several disorders related to degeneration in the nervous system. Although metal chelators are the backbone of heavy metal poisoning treatment, they are usually associated with several side effects [26]. Herbal phytochemicals comprise antioxidants such as flavonoids and polyphenols that help to reduce heavy metal-induced poisoning in humans and other organisms. Argüelles-Velázquez et al. [27] evaluated the effects of *Arthrospira maxima,* commonly called *Spirulina*, on the process of cadmium-induced DNA oxidation and genotoxicity [27]. These researchers found that the plant extracts reduced cadmium-mediated genotoxicity in proportion to algal

antioxidant ability. Another recent study showed that the introduction of dietary soybean supplementation could significantly reduce arterial and cardiac injuries [28]. In addition, carotenes and vitamins C and E present in herbal phytochemicals were also proved to have a significant impact on lead and cadmium poisoning in *Chlorella* and *Spirulina* cyanobacterial species [29]. Mohammed et al. [30] demonstrated that *Nigella sativa* oil had antioxidant properties and thus protected the brain and kidneys from oxidative damage [30]. They proposed that seed oil extracted from *N. sativa* could be utilized by workers prone to cadmium poisoning risk in cement factories. Also, garlic extracts that contain allicin have been demonstrated to reduce cadmium- and lead-mediated mitochondrial injury and apoptosis in various cell lines as well as hepatic, renal, and hematic damage in rats [31].

Arsenic is another metalloid element that is omnipresent because of several complex natural as well as anthropogenic activities. Arsenic is an inorganic metallic compound that is known for its non-cancerous and cancerous effects on human health via food as the exposure pathway. According to the US Environmental Protection Agency, the maximum level of contamination for arsenic is 10 mg/l for waters supplied to the public. When exposed to arsenic, humans are prone to several health problems, such as injurious effects on several organ systems, including the respiratory, dermal, and central nervous systems [32]. Extracts of garlic have been reported to have potential antidote properties against sodium arsenite poisoning [33]. The protective mechanism of the garlic involves the thiosulfur components that form stable complexes after reacting with arsenic to mediate toxicity [34]. Arsenic poisoning was also found to decline with increasing concentrations of methanolic extracts of *Annona muricata* [35]. Moreover, several green tea plant extracts could also be useful to reduce toxicity in arsenic poisoning [36]. Other plant extracts from *Allium sativum, Aloe vera,* and *Centella asiatica,* as well as curcumin, have been evaluated as antidotes to mitigate arsenic poisoning [37].

Apart from arsenic and cadmium, lead, which is mostly used in batteries and medical appliances, is reported to be largely involved in causing biological toxicity via heavy metals [38]. A blood lead level of 10 µg/dl or above could be harmful to human health. The toxicity of lead includes dysfunction of the peripheral and central nervous systems as well as the circulatory, cardiovascular, metabolic, and reproductive systems [39]. The dispersal of lead in the entire body depends on the systemic blood flow and soluble phosphate, which retains more than 95% of lead deposited in the skeletal bones [40]. Chinthana and Anathi [41] demonstrated the effects of oral administration of *Solanum nigrum* and *Solanum trilobatum* extract, which significantly reduced lead-induced neurotoxicity in albino mice. The authors observed that there is a significant increase in the activity of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), and a reduction in lipid peroxidation [41]. Extracts from other conventional

medicinal plants such as *Moringa oleifera*, *Aloe barbadensis,* and *Centella asiatica* have been shown to have the potential to reduce oxidative stress and protect several vital human organs. These phytochemical extracts interact with cysteine and methionine, reach proteins [42], and help in the depletion of arsenic concentrations in the tissue [43].

Further, humans are also prone to exposure to mercury via air, food, and water. The primary mercury exposure route is via the ingestion of fish and fish products containing mercury compounds. These compounds lead to the formation of methylmercury (MeHg) derivatives by microbial methylation [44]. The methyl derivatives of mercury are absorbed in the intestines, travel throughout the body, and cross the blood–brain and placental barriers, causing deleterious effects to human health [45]. Several plant extracts have beneficial effects on the detoxification of MeHg. The active constituents present in *Bacopa monnieri* extract, such as alkaloids, saponins, and other steroid alcohols, have been traditionally exploited as anti-inflammatory, immunomodulatory, and antioxidant agents. The plant extracts of *B. monnieri* antagonized damage in the cerebellum caused by oxidative metabolism of MeHg and suppressed protein, free radical, and carbonyl production. Furthermore, the activities of glutathione (GSH), SOD, and CAT enzymes were also increased, which strongly suggests that the plant extracts inhibit MeHg-induced oxidative stress [46]. Similarly, the Chinese plant *Lycium barbarum* is extensively utilized as a herbal medicine and therapy. Tian et al. [47] studied the polysaccharides extracted from *L. barbarum* and their effects in reducing damage to hippocampal neural stem cells (hNSCs) caused by MeHg. The results demonstrated that the rate of differentiation of hNSCs increased significantly after *L. bararum* polysaccharide treatment, compared with treatment of hNSCs with MeHg only, indicating the neuroprotective effects of *L. barbarum* polysaccharide extracts [47]. In another study, lycopene significantly altered renal pathology and enhanced renal dysfunction [48]. However, there were no significant changes in the concentration of mercury in the urine or kidneys compared with the MeHg control group. Aqueous extracts of *Portulaca oleracea* leaves and stems were administered orally in rats for 12 days, and the results showed that there was an increase in monoamine and acetylcholinesterase activity. Thus, plant extracts are beneficial in the treatment of disorders related to the brain and nervous systems via their potential neurotransmitter protection ability [49]. *Bixa orellana,* a plant native to the USA, contains a pigment consisting of bixin, norbixin, and other carotenoids [50]. These extracts restore levels of GSH and reduce oxidative stress induced by MeHg. Carotenoids have also been reported to possess the ability to alter the redox status of MeHg [51]. It is noteworthy that the metal chelators, although being the backbone of treatment, have several drawbacks. Thus, herbal phytochemicals are gradually replacing metal chelators and other conventional strategies in the effective treatment of heavy metal poisoning.

13.2.2 Herbal Phytochemicals as Antidotes for Snake Venom Poisoning

Snakebite is a serious medical emergency throughout the world, especially in rural areas of tropical and subtropical regions. The venom contains a range of complex peptides, non-enzymatic and enzymatic proteins, and organic compounds [52]. Snakebite victims suffer severely debilitating and life-threatening effects, which can eventually lead to unresolved complications such as severe local tissue damage and consequent physical disabilities. Snake venom toxin causes tissue damage and hemostatic disturbances by interfering with the coagulation cascade or platelet functions. It exerts its actions on the coagulation cascade by means of both procoagulant and anticoagulant properties, which cause a blockage of blood flow to vital organs. Despite enormous advancements in health care, the treatment for snake envenomation remains a challenge. Although immediate antivenom therapy reduces mortality, it is entirely ineffective against local tissue damage. Moreover, antivenom therapies are expensive and require ideal storage temperatures that are not possible in developing nations. Numerous studies have demonstrated that herbal plant extracts contain crude phytochemicals such as alkaloids, flavonoids, polyphenols, and terpenoids with synergistic ability to effectively neutralize local tissue damage induced by venom toxins/enzymes. However, the purified phytochemicals have demonstrated higher neutralization efficacy.

There is a vast repository of plants with therapeutic potentials that are reported to possess antivenom activity [53]. In India alone, 520 plant varieties have been found with the potential to heal wounds and act against snake bites. Methanol extracts of the seeds of *Vitis vinifera* have shown promising effects in the treatment of viperine bites and neutralize the edema-inducing properties of the venom [54]. Similarly, *Cordia verbenacea* extracts significantly reduced the edema that is caused by the venom of the snake *Bothrops jararacussu* [55]. In another study, quercetin was used to treat the effects of *Echis pyramidum* venom in animal models. Furthermore, lipid peroxidation induced by viper venom is reported to be inhibited by methanolic extracts of *Strychnos nux-vomica* seeds. The seed extracts contain monomeric caffeic acid, which is a potential antidote for snake bite [56]. Ghosh et al. [57] described the potential of bark extracts of *Alstonia scholaris* (L.), wherein the aqueous extracts efficiently reduced the histological and biochemical changes that had occurred in the tissues after envenomation [57]. Table 13.1 gives a summary of medicinal plant parts with the ability to inhibit the local effects of snake venoms.

Apart from total plant extracts, in vitro plant tissue culture has also gained momentum in the synthesis of phytochemicals that show potential activity toward envenomation. Plant tissue cultures have several advantages over the traditional plant extraction method concerning the ease of large-scale production of metabolites, standardized techniques, and conserving rare plant species. Samkumar et al. [67] used

Table 13.1 Medicinal plants that inhibit snake venom effects.

Plant name	Parts used	Snake	Activities	Reference
Andrographis paniculata	Roots	*Naja philippinensis*	Hemorrhage	[58]
Tylophora indica	Leaves and roots	*Naja naja*	PLA2 and hemorrhage	[59]
Colocasia esculenta	Tuber	*Naja nigricollis*	SVH	[60]
Aristolochia bracteolata	Leaves and roots	*Naja naja*	PLA2 and hemorrhage	[59]
Terminalia arjuna	Bark	*Naja naja karachiensis*	PLA2	[61]
Ipomoea rubens	Seed	*Naja nigricollis*	SVH	[60]
Momordica charantia	Fruit	*Naja naja karachiensis*	PLA2	[61]
Nicotiana rustica	Leaves	*Naja nigricollis*	Proteolytic	[62]
Tabernaemontana catharinensis	Roots and bark	*Bothrops jararacussu*	Myotoxicity	[63]
Canthium parviflorum	Roots	*Naja naja*	Edema and myotoxic effects	[64]
Mikania glomerata	Leaves	*Bothrops jararaca*	Edema, hemorrhage, and peritonitis	[65]
Morus nigra (L.)	Leaves	*Bothrops jararacussu*	Edema	[66]

PLA2, snake venom phospholipase A2; SVH, snake venom hyaluronidase.

a strategy of obtaining callus from the plant material of *Euphorbia hirta*. The explants obtained from the plant were induced to produce callus, which was then treated with hormones to obtain secondary metabolites with anti-snake venom activity. The secondary metabolites mostly contained triterpenoids and taraxerol. These metabolites were found to have higher activity against the pit viper snake venoms [67].

13.3 Nanoformulated Herbal Phytochemicals as Antidotes

Plants produce and accumulate an array of phytochemicals, which are well characterized and reported in the literature. The structure and activity of most of the phytochemicals have been elucidated, and their medicinal properties have been

evaluated. These plant-derived compounds possess antibacterial, antifungal, anti-inflammatory, hepatoprotective, antidepressant, and anticancer properties because of the presence of several secondary metabolites. From ancient times, humans have benefited from these compounds by utilizing them internally or applying them externally as medicines. A large number of marketed drugs in modern medicines are formulated based on plants and their derivatives.

Several phytochemicals have been identified and characterized; however, their poor bioavailability, rapid elimination in biological systems, reduced absorption, and rapid metabolism reduce their therapeutic potential. In this context, encapsulation of these compounds or extracts in nanoparticles offers numerous advantages, including protection against degradation in the body, enhanced bioavailability, and enhanced solubility. Although this seems to be a better approach, their complex reactions and side effects lead to another set of problems in the efficient delivery of phytochemicals [68]. The literature suggests that secondary metabolites of plants, including alkaloids, flavonoids, tannins, and terpenoids, have demonstrated a better therapeutic effect after incorporating them into nanocarriers than their native forms. The nanoparticles formulated with herbal drugs can deliver the active ingredients to the target site, reduce toxicity, elevate bioavailability, and increase efficacy.

The most well-studied plant compound, curcumin, is an active ingredient of *Curcuma longa*, whose oral administration displays low levels of curcumin in plasma and tissues as well as extensive excretion and metabolism [69]. Insolubility, non-absorption, and short half-life are the limiting factors in the bioavailability of curcumin that reduce its therapeutic potential. Several nanoparticles, liposomes, nanoemulsions, and micelles have been demonstrated as potential carriers for curcumin [70]. In one study, curcumin nanoparticles were demonstrated to show antidepressant activity in rat models. The rat models were injected with reserpine to induce depression, which affected the activity of the rats. They showed significant activity after treating them with curcumin nanoparticles, as evidenced by the forced swim test and several other behavioral tests [71]. Likewise, Grama et al. [72] showed that nano-curcumin, obtained by encapsulating curcumin in poly(lactic-*co*-glycolic acid) (PLGA) polymeric nanoparticles, could be more effective in delaying cataracts caused by diabetes in experimental rats than non-encapsulated curcumin [72]. There are several other nanoformulation-based curcumin products that are available on the market for various treatments and biomedical applications, such as N-Curcusorb, Biocurcumax, Curcu-Gel, and Theracurmin. Apart from curcumin, there are several other plant-derived metabolites that are formulated using nanoparticles or nanoformulations.

Heavy metal accumulation and poisoning by cadmium have already been explained briefly in the previous section. Cadmium causes hypertension as well as inactivation of neurotransmitters such as adrenaline and noradrenaline

(epinephrine and norepinephrine). Cadmium is used to decrease the availability of the potential vasodilator nitric oxide and elevates blood pressure [73]. Kukongviriyapan et al. [74] suggested the ability of curcumin to neutralize cadmium poisoning-mediated oxidative stress and hypertension in mice. The antioxidant and antichelating properties of curcumin contributed to the neutralization [74]. Another study demonstrated the antihypertensive potential of curcumin because of its vasodilatory effects, which help to reduce the diastolic blood pressure more efficiently, leading to the prevention of hypertension during cadmium exposure [75].

Apart from herbal nanoformulations, certain drugs loaded with nanoparticles are also used as antidotes for systemic poisoning. Atropine sulfate (AS) is the primary life-saving antidote used for parathion or malathion insecticide-mediated organophosphate poisoning. An instant therapeutic antidote concentration in the blood is essential for slow physiological antagonists such as atropine. Moreover, inhalation may cause the toxicant to swiftly reach lethal concentrations. Ali et al. [24] developed a dry powder inhaler (DPI) based on nano–atropine sulfate (nano-AS) and performed clinical trials. The authors also conducted a clinical study using single DPI capsules containing lactose-suspended nano-AS (6 mg). The atropinization and bioavailability pattern in blood confirmed its ability to replace parenteral atropine after poisoning under field conditions [24].

Recent antidotes for organophosphorus (OP) compound poisoning involve carbamate-mediated pretreatment to protect against acetylcholinesterase enzyme (AChE) inhibition by OP compounds. OP poisoning leads to an accumulation of acetylcholine, which hampers the smooth working of synaptic signal transmissions. Pang et al. [76] performed a study using biomimetic nanoparticles to act as antidotes against OP poisoning. AChE on the membranes of RBCs served as an anti-OP agent, scavenging the OP compounds present in the body. The in vitro studies confirmed that the biomimetic nanoparticles retained the enzymatic membrane-bound AChE activity and possessed the ability to bind with a model OP, dichlorvos. Moreover, the nanoparticles exhibited better survival of the dichlorvos-induced mice [76]. Thus, the biocompatible nanoparticles served as a bioscavenger in treating OP-mediated poisoning and have potential as a treatment in the future.

13.3.1 Inorganic Nanoparticles

Nanoparticles of an inorganic nature have been broadly studied for their unique properties. They are mainly categorized into carbon nanoparticles, ceramics, and transition metal nanoparticles. Gold, titanium, and platinum are transition metal nanoparticles with exclusive properties that are gaining significant attention in biomedical applications. These nanosized particles have the ability to act as light radiation-excited drugs, causing damage to nucleic acids by stimulating lipid

peroxidation that resulted in cell death. This strategy is applied effectively in cancer treatment and in bioimaging applications and is highly beneficial in theranostics. Paul et al. [77] biosynthesized silver nanoparticles (AgNPs) from the leaves of *Premna serratifolia* with significant activity in carbon tetrachloride-induced liver cancer in Swiss albino (BALB/c) mice [77]. Sre et al. [78] also stated the cytotoxicity of biogenic AgNPs from extracts of *Erythrina indica* in breast and hepatocellular carcinoma cells [78]. Gold nanoparticles (AuNPs) are among the most widely used nanoparticles owing to their ease of synthesis. Although widely used in cancer therapeutics, they have also been explored as multifunctional particles in a wide range of biological fields. AuNPs of *Antigonon leptopus* extract displayed significant inhibitory activity against breast cancer cells (MCF-7) at 257.8 μg/ml [79]. Another study verified the cytotoxicity of AuNPs fabricated with *Cassia tora* leaf extract in cancerous colon cells. The study demonstrated that the activity was dependent on the different doses of *C. tora* (25, 50, and 75 μg/ml). The *C. tora* at a dose of 75 μg/ml exhibited increased anticancer activity against cancerous colon cells [80].

Nanosized iron oxide particles are also widely accepted and studied for inducing antitumor activity. Their action of mechanism involves the absorption of toxic reactive oxygen species through short wavelength radiation, i.e. near-infrared or oscillating magnetic fields. The anticancer mechanism of metallic nanoparticles involves the absorption of radiation, and then converting it to heat to damage cancer cells. Moreover, a combination of metal nanoparticles has an obvious higher ability to elicit damage in tumor cells. Bimetallic selenium–silver (Se-Ag) nanosized particles that are fabricated via gallic acid and quercetin showed exclusive antitumor activity against Dalton's lymphoma cells [81]. However, these nanosized particles have limitations pertaining to safety and systemic clearance. Although metals in their nano-regime have distinct properties, especially catalytic properties, they still have undesired effects. Because of this, there have been growing concerns about their safety, which reduces their applications.

13.3.2 Micelles and Liposomes

Micelles are spontaneous amphiphilic aggregates that are usually spherical and 5–25 nm in diameter. In polar media, they usually have a hydrophobic core, whereas inverted micelles with a hydrophilic core are formed in non-polar solvents. Micelles are excellent carriers for phytochemical-based drugs and are highly beneficial in the biomedical field. They are the preferred choice as carriers as they reduce the solubility limitations of drugs in aqueous media. However, they also have drawbacks, such as toxicity and stability, because of their dynamic nature; hence, they have a rapid clearance rate. However, these limitations can be reduced by means of polymeric micelle fabrication approaches [82]. Estakhri et al. [83] developed novel nanomicelles containing curcuminoids that showed effective activity against poisoning by

the OP pesticide diazinon. Rats poisoned with diazinon showed effective protection of the organs and reduced biomarkers of cell damage when treated with nanomicelles containing curcuminoids compared with natural curcumin extracts. Thus, micelles were found to have good potential in increasing the bioavailability of herbal phytochemicals [83]. Thymoquinone (TQ) is a liposoluble phytochemical based on benzoquinone with remarkable anticancer and antioxidant activities. Ganea et al. [84] synthesized nanosized PLGA particles loaded with TQ and assessed their physicochemical, antioxidant, and anticancer abilities. The antioxidant activity of TQ-loaded nanoparticles was higher for emulsified poly-sodium *N*-undecylenylvalinate nanosized particles than for free TQ [84].

Liposomes are 0.01–0.5 μm lipid bilayer vesicles that are synthesized by a lipid film distribution approach in an aqueous medium. There are various liposome types: small unilamellar, multilamellar, large unilamellar, multivesicle, and cochleate vesicles have been synthesized by several research groups. Liposomes have been explored as efficient carrier systems of drugs because of their stability, long shelf-life, and encapsulation ability of both polar and non-polar drugs. Moreover, their properties can be tailored with respect to size, charge, functionalization, and modification of the surface. Additionally, their biocompatibility and degradability properties make them the most preferred option for drug delivery applications. Li et al. [85] showed the protective efficacy of nanoliposomal curcumin (NLC) in dimethylhydrazine (UDMH)-induced mouse models. The poison UDMH was injected into the mouse models; these were then treated with curcumin, nanosized curcumin-β-cyclodextrin particles, or NLC. The mouse models treated with NLC showed excellent protective effects in the liver and CNS by reducing the serum alanine transaminase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase levels [85]. Thus, liposomes have been found to be effective in delivering herbal phytochemicals against systemic poisons. However, liposomes also have limitations, including their rapid circulation time. Also, although liposomes have the ability to encapsulate non-polar drugs via a hydrophobic vesicle bilayer, the formulation affects vesicle integrity, making them inappropriate for drugs of a non-polar nature. The carotenoids are highly hydrophobic in nature with excellent antioxidant ability and are extensively utilized as nutritional supplements. It is noteworthy that the mechanism of interaction between the lipid bilayer and the carotenoid plays a dynamic role in elevating their efficient delivery of drugs [86]. Several studies have indicated that carotenoid encapsulation in liposomes improved their antioxidant activity. Researchers have suggested that encapsulation using liposomes enhances their solubility and dispersion in water and reduces steric carotenoid hindrance to increase the activity of 2,2-diphenyl-1-picrylhydrazyl and ferric reducing activity potential [87]. Salvianolic acid B (SalB) is another phytochemical with enhanced chemical stability and decreased bioavailability. This compound is present in *Salvia miltiorrhiza* Bunge and is used as a functional food and a curative agent for

numerous diseases, especially coronary diseases. Polyethylene glycol-attached liposomes loaded with SalB were fabricated as drug carriers for parenteral administration. Further, a study demonstrated an increase in activity against hyperalgesia after administering liposome-encapsulated drug [88].

13.3.3 Polymeric Nanoparticles

Nanoparticles synthesized from polymers display numerous properties that are useful in cancer and other therapeutics. These nanoparticles are synthesized via biodegradable polymers and result in improved time of circulation and stability. Apart from lower toxicity, polymer nanoparticles possess advantages including controlled release of drugs and biocompatibility and support large-scale synthesis. Several novel polymers have emerged that are based on two widely studied polymers: PLGA and poly(lactic acid) (PLA). The nanoparticulate forms of these polymers, i.e. polymeric nanoparticles, are widely used for drug delivery purposes. Arsenic poisoning, leading to chronic arsenicosis, is a well-known manifestation in areas where most of the population drinks arsenic-contaminated water. Sankar et al. [89] loaded curcumin into PLGA nanoparticles and studied their efficiency in reducing arsenic-induced effects on the liver with respect to ALT, AST, and lipid peroxidation. Rats treated with these curcumin nanoparticles showed more effective protection against arsenic-mediated effects than those treated with curcumin alone [89]. Sangal et al. [90] synthesized nanosized PLGA particles to deliver the phytochemical *Achyranthes aspera* to overcome biocompatibility issues [90]. Other polymers such as sugars, proteins, nanosized gelatin particles, and several natural macromolecules can also act as nanocarriers. Natural or herbal products have been gaining wide acknowledgment since ancient times and are still being explored owing to their advantages over chemical drugs. Nowadays, the majority of the most marketed pharmaceutical drugs contain natural constituents or their derivatives. However, the major problem with delivering natural products is low bioavailability, which hinders successful clinical trials. Moreover, there have been several studies of the applications of nanotechnology in delivering herbal phytochemicals to cancer cells but there have been far fewer studies on the treatment of systemic poisoning conditions.

13.4 Mechanism of Nanoformulated Herbal Phytochemicals against Systemic Poisoning

Herbal phytochemical extracts with antidotal properties are encapsulated using nanoformulations such as liposomes, dendrimers, nanocapsules, micelles, and polymers [22], as shown in Figure 13.1. Larger nanoformulated herbal antidotes bind

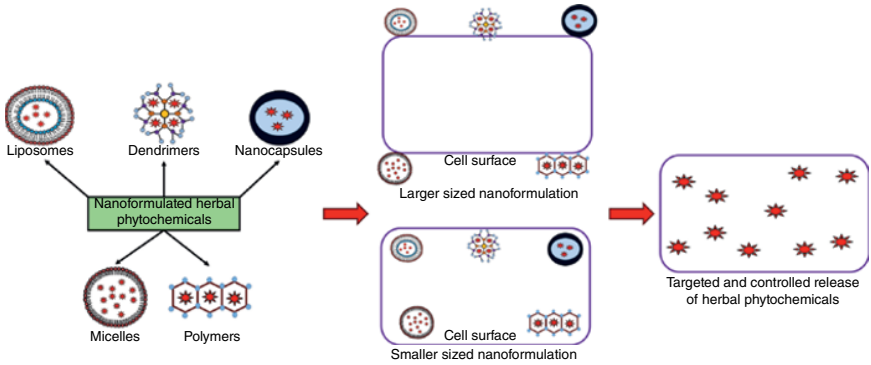

Figure 13.1 Mechanism of nanoformulation in targeted and controlled delivery of herbal phytochemicals as antidotes in cells affected by systemic poisons.

to the surface of the target cell membrane via electrostatic forces of attraction, disintegrate into ions, and infuse herbal extracts into the cell [91]. In contrast, smaller sized nanoformulations possess the potential to enter cells, disintegrate inside them, and release herbal phytochemical extracts in a controlled manner [92]. Both of these types of nanoformulations are beneficial for delivering the loaded herbal extracts to the target site in a controlled way [93]; however, larger sized nanoformulations are reported to be less toxic than smaller ones [94]. This may be the result of the increase in cellular ionic potential, while the nanoformulation disintegrates to release the phytochemicals [95]. The disintegrated monomers from the polymeric nanoformulation may lead to DNA methylation [96] and increased ionic potential may damage significant cellular components and lead to inhibition of cell growth [97]. Thus, it is vital to optimize the size, morphology, and surface charge of the nanoformulations before using them to treat systemic poisoning conditions. The released herbal phytochemicals act as an antidote for systemic poisons at the molecular level, protect cells from inhibition, and increase proliferation [98].

Each nanoformulation is utilized to deliver the loaded herbal extracts to specific cells depending on their properties. Liposomes are used to encapsulate lipophilic and amphiphilic herbal phytochemicals to deliver them to the target cells [86]. Likewise, micelles are used to encapsulate herbs that are to be delivered to fatty acid-rich cells, such as adipocytes [99]. Polymers, especially biopolymers, are used to encapsulate herbal extracts that have to be biocompatible and bioavailable for a long time in the human body [100]. Nanocapsules are similar to micelles; however, they can be manipulated to encapsulate either hydrophilic or hydrophobic herbal extracts for their controlled delivery [101]. Dendrimers have a unique nanoformulation, which can be synthesized with hydrophobic spaces inside [102]. These spaces are used to encapsulate or hold herbal extracts and deliver them to cells according to variations in pH [103]. It is also possible to incorporate different herbal phytomedicines in the void spaces of dendrimers to deliver them to the target site [104]. Thus, it is essential to study the properties of cells as well as those of herbal extracts to design appropriate nanoformulations for targeted and controlled delivery.

13.5 Future Perspectives

Currently, there is no commercial antidote for systemic poisons with nanoformulated herbal extracts. However, there are numerous herbal antidotes that are available for systemic poisons on pharmaceutical markets throughout the world, under different names. The major limitation of introducing nanoformulated herbal extracts is their approval by the US Food and Drug Administration (FDA) or any other regulatory authority [105]. Recently, several drugs with nanoformulations were approved by FDA for cancer treatment [106]. Thus, it should also be possible

to approve nanoformulated herbal extracts as antidotes for systemic poisoning conditions in the future. It is tedious to obtain approval for nanoformulations from FDA or any other regulatory board as these formulations may lead to toxic reactions in patients as well as to adverse effects on the environment [107]. This challenge can be avoided by stringent in vitro and in vivo cytotoxic analysis of nanoformulated herbal extracts using several conventional tests and the latest models [108]. In vitro analysis will be helpful in screening the nanoformulations [109], while in vivo analysis helps in evaluating their toxic reactions in live animal models [110]. There have been numerous studies on the evaluation of toxic reactions of nanoformulations and nanoparticles [111]. However, there is no regulatory authority or standard repository that maintains a standard procedure for toxicity analysis and lists toxic nanoformulations. Thus, it is highly recommended that a global repository of records should be created that lists toxic nanoparticles and their toxicity ranges to aid future researchers to design novel and more effective nanoformulations. Recently, the zebrafish has been the focus of attention from researchers evaluating toxicity, especially the genotoxicity of nanoformulations. The similarities in the genes and organs of humans and zebrafish make them advantageous in evaluating the genotoxicity of nanoformulations [112]. In addition, in silico methods using computational simulations and modeling of the cellular environment were also introduced to evaluate the toxicity of nanoformulations, which avoids the use of live animals and the associated procedures in obtaining ethics approval [113]. In future, in silico methods will take over from in vitro analysis for screening of toxic nanoparticles; this will reduce the number of animals sacrificed for toxicity analysis drastically [114]. Moreover, nanoformulated herbal extracts could also be incorporated into cloth fabrics and adhesive bandages that would inject herbal extracts when systemic poisons were encountered [115]. Smart cloths or plasters that change color in systemic poisoning conditions, indicating poison exposure in the human body, are undergoing extensive research. The cloth would also contain a micro-needle that injects the specific dosages required to act as an antidote to the poison, as shown in Figure 13.2. This will be very helpful for those persons working in areas prone to systemic poisons to safeguard them in future.

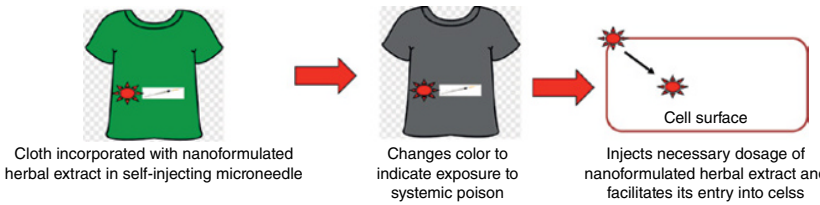

Figure 13.2 Schematic representation of the probable mechanism of action of a smart cloth incorporating a nanoformulated herbal phytochemical extract.

13.6 Conclusion

This chapter provides an overview of several herbal extracts that are utilized as antidotes against systemic poisons and nanoformulations that are beneficial in encapsulating these phytochemical extracts for their controlled delivery at the target site. Dendrimers, nanocapsules, liposomes, micelles, and polymers are the nanoformulation agents that are used to encapsulate herbal phytochemical extracts. The nanoformulations help in controlled delivery at the target site and enhance the efficacy of herbal phytochemical extracts; this will help to improve their antidotal properties against systemic poisoning conditions. Thus, it is highly recommended that nanoformulations are used to formulate effective herbal antidotes. Moreover, smart cloths and adhesive bandages with nanoformulated herbal phytochemical extracts with efficient sensing of systemic poisons and delivery of antidotes as required will be highly beneficial in the future to replace all conventional antidotes. Moreover, these smart fabrics will help to safeguard people working in places that are prone to systemic poisons.

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14

Phytochemical-Based Nanoparticles as Foes and Friends

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CONTENTS

14.1 Introduction

The utilization of nanoparticles and nanomaterials has been highlighted as one of the major emerging technologies that could solve several challenges facing mankind globally [1]. There are several methods that can be used to produce nanoparticles, including heat evaporation [2, 3], chemical reduction [4], and photochemical

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reduction [5]. It has been observed that biological techniques using biogenic sources have several merits over synthetic and physical techniques. This might be linked to the fact that they are economical, can be easily utilized for large-scale production, and are sustainable, effective, and ecofriendly. The green synthesis of nanoparticles constitutes a fascinating and more reliable field of modern science. Biosynthesized nanoparticles possess some unique features, such as stability, unique shape, and size when used for special therapeutic applications. Various types of metallic nanoparticles can be biofabricated using green technology. Examples of the typical biological techniques involve the utilization of plants or plant extracts [6, 7], microorganisms [8, 9], and enzymes [10].

The use of nanoparticles has been explored in different sectors, such as agricultural, environmental, industrial, food, and medical applications. However, nanoparticles have been utilized most extensively in the field of medicine. This might be linked to the fact that the size of the nanoparticles resembles that of biological molecules, which suggests their applicability for diverse biological activities in in vitro and in vivo research [11–14]. Generally, the application of bimetallic nanoparticles has been documented for various biological activities, the treatment of cancer, drug delivery, analysis of DNA [15], and as larvicides and pupicides [16], as well as for coatings on solar cells for use in photonics [17], staining pigments, antimicrobials [18], antimalarials [19], and catalysts [20].

Moreover, the biogenic routes of producing these bimetallic nanoparticles, especially those from plant sources, have several advantages when compared with physical and chemical production technologies [7, 21–23]. The benefits of these biogenic compounds include their shape, robustness, size, non-toxicity, effectiveness, and sustainability, while some of the drawbacks of the physical and chemical production technologies include their hazardous nature whenever they are released into the environment, difficult processes, and energy consumption as well as the fact that they are outdated and have minimal utilization in the medical–clinical field [15, 24]. Furthermore, the synthesis of ecofriendly compounds using the metabolites obtained from plants might be considered as sustainable because of the array of useful phytochemicals present in their metabolites that are responsible for the capping and reduction of different metal oxides. This could eventually lead to a reduction in the need for nanoparticles.

Numerous plants are known to produce a number of phytotoxins and phytochemicals with countless health benefits to mankind, including antibacterial, antifungal, neuroprotective, hepatoprotective, antiulcer, and anticancer properties [23, 25–32].

This chapter discusses the utilization of beneficial plants for the biofabrication of nanoparticles. The various biological applications of these nanoparticles performed both in vitro and in vivo are also highlighted. Finally, the disadvantages of these biogenic nanoparticles from plant material are also discussed.

14.2 Phytochemicals Used in the Synthesis of Nanoparticles

Plants possess numerous interesting biomolecules such as coenzymes, vitamin-based intermediates, and metabolites that contain phytochemicals such as phenol compounds, flavonoids, terpenoids, alkaloids, isoflavones, catechins, anthocyanidins, isothiocyanates, carotenoids, polyphenols, and sterols that help to reduce metal ions in nanoparticles [33]. Plant-mediated nanoparticle synthesis is a current trend in nanomedicine based on all the emerging innovations that are being discovered by various scientists from diverse fields. However, the nature of the plant is important in the synthesis of nanoparticles. Phytochemicals are the emerging potent biological resources for the synthesis of metallic nanoparticles [34, 35]. The phytochemicals that have been used in the synthesis of nanoparticles are listed in Table 14.1.

14.3 Anti-Inflammatory Effects of Nanoparticles

Inflammation is the physiological process with which the body responds to tissue injuries such as infections (microbial and viral), stress, irritants, radiation, or genetic changes [39, 55]. It can be acute or chronic; moreover, the inflammatory process depends on the nature of the stimulus to the host. The host response to inflammation leads to regeneration of cellular homeostasis and tissue structure and function. The response can be through the innate immune response, which is activated by granulocytes, phagocytes, and other cells, or through the adaptive immune response, which is specifically for eliminating pathogens. However, the duration of inflammation can be prolonged depending on the damage caused by, for example, microbial and viral effects [56].

Acute inflammation is the early non-specific response of the body to injury. It is characterized through local vasodilatation, increased capillary permeability, accumulation of fluid and blood proteins in the interstitial spaces, migration of neutrophils out of the capillaries, and release of inflammatory mediators (e.g. cytokines, lymphokines, and histamine) [57]. Other characteristics of acute inflammation include swelling, pain, increased heat, redness, and loss of function of the injured area. Generally, the actions of neutrophils thus enable macrophages to favor tissue repair and regeneration [58]. The chronic inflammatory phase occurs before the acute inflammatory phase is resolved, which leads to various diseases such as cardiovascular system (CVS) diseases, neurodegenerative diseases, diabetes, cancer, obesity, asthma, arthritis, and periodontal diseases [59]. The chronic phase is characterized by immunopathological changes such as infiltration of inflammatory cells, overexpression of proinflammatory genes,

Table 14.1 Phytochemicals used in the synthesis of nanoparticles.

Serial number	Phytochemical class	Compound	Plant source	Reference
1	Alkaloid	Berberine, theobromine, theophylline, caffeine	*Nicotiana tabacum*	[36]
2	Cardenolide	Calotropin	*Asclepias* spp.	[36]
3	Coumarin	Cleomiscosin A, B, C and D	*Dipteryx odorata*	[36]
4	Catechin	Epigallocatechin-3-gallate	*Mimosa catechu*	[36]
5	Flavonoid	Kaempferol, quercetin	*Carthamus officinalis* (L.)	[37]
6	Glycoside	Kaempferol, quercetin	*Urginea maritima*	[38]
8	Polyphenol	Ellagitannin and ellagic acid, curcumin, chlorogenic acid, ferulic acid, caffeic acid	*Sargassum tenerrimum*	[39, 40]
9	Sterol	Campesterol, β-sitosterol, and stigmasterol	*Sesbania bispinosa*	[41]
10	Tannin	Gallic acid, tannic acid	*Mimosa tenuiflora*	[42–45]
11	Terpene	Asiaticoside, lupeol, asiatic acid, jasminol, madecassic acid, cleomeolide, scabertopin, salograviolide A, ursolic acid	*Piper guineense*	[46]
12	Essential oil	Monoterpenoid or sesquiterpenoid	*Acorus gramineus* rhizome	[47]
13	Phytocannabinoid	Tetrahydrocannabinol acid	Cannabis plant	[48]
14	Carotenoid	Lutein, β-carotene	*Mauritia vinifera* Mart.	[49]
16	Anthocyanin	Cyanidin, malvidin	*Rbus cyrijuz*	[50]
17	Hydroxycinnamic acid	Chicoric acid, coumarin, ferulic acid, scopoletin	*Trigonella foenum-graecum*	[51, 52]
18	Isofavone	Daidzein, genistein	*Glycine max*	[53]
19	Lignan	Silymarin	*Morinda citrifolia*	[54]

dysregulation of cellular signaling, and loss of the barrier function [60]. However, inflammation is a precursor to repair and regeneration, resulting in the production of some mediators such as cytokines and interleukins, which are generated by specific T lymphocytes, B lymphocytes, and macrophages. Phytochemicals derived from plants have been used as anti-inflammatory applications in the treatment of ailments because of their safer toxicological profile compared with allopathic drugs [61]. Conte et al. [39] reviewed the relationships between the structure and properties of nanodrug carriers, and recent advances in anti-inflammatory research using nanocarrier-mediated drug delivery of bioactive compounds extracted from plants have also been reviewed [62]. Agarwal et al. [56] studied the differential uptake of nanoparticles into cells and the anti-inflammatory mechanism adopted by the nanoparticles synthesized by ecofriendly routes.

El-Rafie and Hamed [63] examined four *Terminalia* spp. (*Terminalia catappa, Terminalia mellueri, Terminalia bentazoe*, and *Terminalia bellerica*) that were found to contain phytochemicals such as flavonoid, phenolic, protein, and polysaccharide compounds. The nanoparticles synthesized from these plants function as powerful anti-inflammatory agents. In vitro bioactive compounds synthesized from *Avicennia marina* (Forssk.) and delivered by silver nanoparticles (AgNPs) exhibit anti-inflammatory activity. Islam et al. [64] also reported that the nano-phytoconstituents of *Prunus domestica* gum exhibit anti-inflammatory activity.

14.4 Wound-Healing Effects of Nanoparticles

The process of wound healing is a complex system of well-arranged biochemical and cellular phenomena that restore the integrity of skin and subcutaneous tissues after injury in a timely manner through three overlapping phases: inflammation, tissue formation, and remodeling [65]. Because of the low cost and availability of plant extracts, with limited side effects and the presence of diverse active metabolites, the phytoconstituents of several plants have provided safe wound-healing agents. Countless numbers of phytochemical-based compounds possess unique properties to fight infection, promote blood clotting, and accelerate the wound-healing process [7, 66, 67]. The active compounds found in plants, such as flavonoids, essential oils, alkaloids, phenolic compounds, terpenoids, fatty acids, and so on, are potential potent drugs for the treatment of wounds [68–70]. Recent advances in the understanding of the therapeutic effects and mechanisms of action of phytochemicals have demonstrated the use of phytochemicals in wound healing and skin regeneration, as suggested by Thangapazham et al. [71]. Rex et al. [67] reviewed the various types of plant-derived phytochemical compounds that promote the wound-healing process, including asiaticoside, curcumin,

chlorogenic acid, gallic acid, and quercetin. Phytochemical-based nanoparticles can facilitate and control bioavailability at the target wound site for the wound-healing process [72]. Nanotechnology techniques have been used to improve the efficiency of many herbal-based therapeutic agents. Hajialyani et al. [65] reviewed the efficacy of plant-based nanomaterials in the management of wounds and discussed the therapeutic agents involved. However, in vivo research and/or clinical data have been used to deduce the efficacy and pharmacological mechanisms of natural product-based nanostructures in different types of wounds. Thangapazham et al. [71] reviewed both the molecular targets and the mechanisms of action of some phytochemicals, such as curcumin, picroliv, and arnebin-1, and also broadly reviewed their ability to enhance wound repair and skin regeneration.

14.5 Antiparasitic, Antifungal, and Antibacterial Activities of Nanoparticles

Saad et al. [73] synthesized AgNPs and copper nanoparticles (CuNPs) and characterized the synthesized nanoparticles by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray fluorescence. The antiparasitic activities were evaluated against two of the most pathogenic strains that have been reported to spread rapidly in Egypt: *Cryptosporidium parvum* and *Entamoeba histolytica*. The results of the characterized nanoparticles show that the average sizes of the CuONPs and AgNPs were 29 and 9 nm, respectively. Evaluation of the antiparasitic activity showed that AgNPs caused a drastic reduction in the number of *C. parvum* oocysts ($p > 0.05$) and CuONPs caused a reduction in the number of *E. histolytica* cysts. Moreover, it was observed that the lethal concentration, LC_{50}, values of AgNPs for *C. parvum* and *E. histolytica* were 0.34 and 0.54 mg/l, respectively, while CuONPs showed values of 0.13 and 0.72 mg/l against *C. parvum* and *E. histolytica,* respectively. The study by Saad et al. showed that these two synthesized nanoparticles could be utilized as antiparasitic drugs for the management of *E. histolytica* and *C. parvum* parasites.

Das and Chakraborty [74] wrote a comprehensive review on the biofabrication of AgNPs and zinc oxide (ZnO) nanoparticles (ZnONPs) using various plant extracts. The antibacterial activity of the synthesized nanoparticles was evaluated against multidrug-resistant (MDR) bacteria. The authors stated that the enhanced biological activity of the AgNPs might be linked to their special characteristics, which include localized surface plasma resonance, high conductivity, catalytic activity, chemical stability, and broad-spectrum activity against MDR bacteria. Also, a reduction in the particle sizes of AgNPs enhanced their specific surface area, which consequently improved their antimicrobial activity. Also, ZnONPs have been observed to contain a non-biotoxic material with photo-oxidizing and

photocatalysis features against biological species. Furthermore, it has been stated that ZnONPs possess the ability to generate reactive oxygen species that have the tendency to react with the cell membrane of pathogenic microorganisms, which consequently leads to deactivation of the genetic material, eventually resulting in the death of these pathogens. Therefore, all these highlighted features of AgNPs and ZnONPs demonstrate their potential as ecofriendly, economical, and effective drugs for the management of numerous diseases affecting mankind.

Infectious diseases can be spread and can result in significant effects on public health globally. They are responsible for a high annual mortality rate of over 17 million people around the world, especially in developing countries. Moreover, these infectious diseases occur most commonly in those with immunocompromised systems and in children. It has been highlighted that protozoa, bacteria, and viruses are the major factors responsible for infectious diseases worldwide. The high rate of resistance to synthetic drugs as a result of their widespread use has necessitated the search for new drugs that could help to overcome all of the challenges of multidrug resistance. In view of the above, Aderibigbe [75] wrote a comprehensive review of the utilization of metal-based nanoparticles for the management of infectious diseases and their biological effects in in vitro and in vivo assays.

Parasitic diseases have been highlighted as one of the major factors affecting the lives of millions of people worldwide, especially those who live in the developing parts of the world where there are limited means of treating them. Also, most of the available drugs have been reported to cause resistance as a result of multiple usages; hence, there is a need to search for new, effective, safe, and ecofriendly drugs for the management of these dangerous parasitic diseases. Moreover, it has been observed that there is currently no vaccine available for the treatment of numerous parasitic infections; therefore, chemotherapy has been identified as the only option for the management of these diseases. The application of nanoparticles as antiparasitic drugs has been identified as a sustainable solution for the management of parasitic diseases. In view of this, Norouzi [76] wrote a comprehensive review of the application of several nanoparticles synthesized from different sources that possess cytotoxic effects and growth inhibitors against numerous parasites, including *Fasciola hepatica, Giardia, Trichinella spiralis, Leishmania*, *Toxoplasma, Plasmodium*, and helminths such as *T. spiralis, Echinococcus multilocularis,* and *F. hepatica*. Norouzi also stated that these synthesized nanoparticles could be used singly or in combination with other drugs for the effective control and prevention of all these parasites.

Zahir et al. [77] synthesized titanium dioxide (TiO_2) and silver nanoparticles (AgNPs) using the aqueous leaf extract of *Euphorbia prostrata* by exploring the synthesized drugs as antileishmanial agents. The modes of action through which these nanoparticles exhibited antileishmanial activity, especially for the induction

of cell death in *Leishmania,* were investigated. The synthesized nanoparticles were tested against promastigotes of *Leishmania donovani* with alamarBlue and propidium iodide in an in vitro assay while the antileishmanial activity on intracellular amastigotes was investigated by means of Giemsa staining. The leishmanicidal effects of the synthesized AgNPs were further affirmed by cell cycle progression, DNA fragmentation assays, and TEM of the treated parasites.

The results obtained from the TEM analysis indicated that the average particle size was 12.82 ± 2.50 nm. The two synthesized nanoparticles exhibited an inhibitory effect against *Leishmania* parasites after 24 hours of exposure with 50% inhibitory concentrations (IC_{50}) of 3.89 and 14.94 g/ml, respectively, in intracellular amastigotes and promastigotes. Moreover, the *Leishmania* parasites treated with the nanoparticles exhibited a substantial increase in the G0/G1 phase of the cell cycle with a subsequent reduction in the G2/M and S (synthesis) phases when compared with the control. The high rate of inhibition observed by the AgNPs might be linked to the enhanced length of the S phase and the reduced level of reactive oxygen species, which might be a factor in the caspase-independent shift from apoptosis (G0/G1 arrest) to massive necrosis. Also, high-molecular-weight DNA fragmentation was observed, which might be an indication of necrotic cell death. Furthermore, the trypanothione/trypanothione reductase system of *Leishmania* cells was inhibited when exposed to the synthesized AgNPs. This study signifies that the application of nanoparticles could lead to the development of safer, economical, and effective drugs that could be used for the management of visceral leishmaniasis.

Nanotechnology has been identified as a powerful technology that uses various devices and brings about innovative drugs for the management of different diseases. These nanoparticles, especially those of size 1–100 nm, have been utilized as antiparasitic, antimicrobial, antilarvicidal, and anticancer agents. Gold and silver nanoparticles have been highlighted as being among those that could be used for the management of MDR microorganisms, such as *Staphylococcus aureus*, and vector mosquitoes, including *Culex quinquefasciatus, Anopheles stephensi,* and *Aedes aegypti*. These nanoparticles have also been highlighted for use with anticancer drugs for the effective management of some cancer cell lines, such as HepG2 and MCF-7. Hence, in order to mitigate against the issue of multidrug resistance, it is essential that novel and effective nanodrugs that can perform many functions, including anticancer, antilarvicidal, and antimicrobial activity, are discovered. Therefore, in light of this, Vignesh and Moorthi [78] wrote a comprehensive review of the application of nanoparticles with a novel bioactive component that has been reported previously to be active against vectors, cell lines, and pathogens.

Jafari et al. [79] synthesized AgNPs using pennyroyal (*Mentha pulegium* (L.)), marshmallow flower (*Althaea officinalis* (L.)), and thyme (*Thymus vulgaris* (L.))

leaf extracts. They also synthesized CuNPs by reacting copper chloride ($CuCl_2$) with L-ascorbic acid. The antifungal and antibacterial properties of these synthesized nanoparticles were compared with the salt of silver nitrate ($AgNO_3$), $CuCl_2$, and synthetic antibiotics. The results indicated that the synthesized nanoparticles exhibited a higher inhibitory activity against bacteria (*Escherichia coli* and *S. aureus*) and fungi (*Aspergillus flavus* and *Penicillium chrysogenum*) than controls containing $AgNO_3$, $CuCl_2$, and synthetic antibiotics. Moreover, it was observed that the antimicrobial effectiveness of AgNPs was higher than that of CuNPs.

Nasiri and Nasiri [80] synthesized AgNPs using an extract of *Carum carvi. The* synthesized AgNPs were later characterized using X-ray diffraction (XRD) analysis, TEM, energy dispersive spectroscopy (EDX), and SEM analysis. The antifungal efficiency of the AgNPs was tested against *Candida albicans* using serial microdilution techniques. The results showed that the AgNPs consisted of 10 nm spheres. The antifungal activity showed that a concentration of AgNPs of 50 g/ml had an inhibitory effect on the tested fungal isolate.

Erick and Padmanabhan [81] synthesized AgNPs using the leaves of *Tridax procumbens*. The synthesized nanoparticles were characterized using Fourier transform infrared (FTIR), ultraviolet–visible (UV–Vis) spectrum, XRD, SEM, and EDX analysis. The characterization revealed that the size of the nanoparticles was 3.973 nm and they were either elongated or spherical in shape. The FTIR results showed that functional groups such as aliphatic amine groups, primary amides, and aromatic ketones were present in the crude extract of *T. procumbens,* which acted as both a capping and a reducing agent. Moreover, it was observed that AgNPs showed enhanced antimicrobial activity against 12 fish and human pathogens, evaluated with a 10 mm zone of inhibition, while out of the eight fungal strains evaluated only *Trichoderma virens* MTCC 794 and *Penicillium restrictum* MTCC 3391 exhibited an inhibitory effect.

Rout et al. [82] utilized an aqueous extract obtained from *Ocimum sanctum* for the synthesis of AgNPs. The synthesized nanoparticles were characterized using XRD, UV–Vis, and SEM analysis. It was shown that the maximum peak was observed at 430 nm, which became higher with increasing time, demonstrating the polydisperse nature of AgNPs. The results obtained from the XRD analysis indicated that crystallization of the bioorganic phase happened on the surface of the AgNPs. The AgNPs also exhibited enhanced antibacterial and antifungal activities against all the tested microorganisms.

Mallmann et al. [83] synthesized AgNPs by utilizing sodium dodecyl sulfate as a stabilizer and ribose as a reducing agent. The antimicrobial effectiveness of the synthesized AgNPs was also evaluated against *Candida tropicalis* and *C. albicans*. The results showed that the average sizes of the nanoparticles were 12.5 ± 4.9 nm. The nanoparticles demonstrated enhanced antimicrobial activity against all

Candida spp. and the authors suggested that these nanoparticles could serve as an alternative treatment to all the synthetic drugs used for the management of disease and infection normally caused by *Candida* spp.

14.6 Neuroprotective Effects of Nanoparticles

Hassanzadeh et al. [84] investigated nanotechnology practices and their effect on the central nervous system (CNS). They observed that, over the years, there has been a remarkable collaboration between nanotechnology approaches and tissue engineering. The combination of these two approaches has had the benefit of improving delivery systems for drug release as well as the release of bioactive proteins over a long period of time, resulting in connection across structural gaps and producing recombination of neuronal processes, controlled neurite or axonal growth in the neural circuitry, and reinstatement of a functional neuronal network. During their investigations, the authors observed that nanoscaffold systems can play a role in enhancing neural regeneration during acute CNS injuries. They observed that certain techniques coupled with advanced technologies such as atomic force microscopy (AFM) and fluorescence resonance energy transfer (FRET) create a means for neuroscientists to better understand the molecular mechanisms of neurological syndromes. To this effect, their further investigations revealed that FRET analysis was able to identify Alzheimer's disease (AD) pathogenesis through subcellular localization of amyloid-β (Aβ) plaques while imaging of Aβ fibrillogenesis was achieved with the aid of AFM scanning probes. Furthermore, their study revealed that estimation of Aβ oligomerization and patient screening was made possible through the use of restricted surface plasmon reverberation nanosensors, which had extraordinarily specific and sensitive characteristics, whereas fullerene derivatives, which are known to be effective free radical scavengers, inhibited Aβ fibrillogenesis, signifying their neuroprotective properties. Their investigations into other nanoparticles such as phospholipid nanomicelles, copper chelator-conjugated nanoparticles, and cholesterol-bearing nanogels showed that they all impeded the development or accumulation of Aβ plaques and alleviated neurotoxicity. Their investigation showed that AD induced in experimental models with cognitive deficits was ameliorated by acetylcholine-loaded single-walled carbon nanotubes (CNTs), which have the potential to cross the blood–brain barrier (BBB) to repair cognitive function. Their investigations also revealed the ability of poly(butylcyanoacrylate) (PBCA) nanoparticles to supply cholinesterase inhibitors into the brain to enhance spatial awareness and memory. Hassanzadeh et al. [84] were able to show that, with the aid of nanosensors, specific brain regions were clearly visible, showing levels of neurotransmitters, which would aid the early detection of CNS disorders. They demonstrated

the presence of catecholamine neurotransmitters that are involved during biological functions and impairments, by means of electrochemical sensors and biosensors, and that are also efficient parameters for detecting Parkinson's disease (PD). These authors observed that, in PD, nanogels facilitate the movement of antisense oligonucleotides in the brain as well as constrain monoamine oxidase B activity; these characteristics potentiate dopaminergic neurotransmission. According to their investigations, PD induced in experimental animals was treated with tyrosine hydroxylase gene transport vectors or DNA-entrapped nanoparticles containing glial cell-derived neurotrophic factor (GDNF). At the end of the treatment period, the authors observed distinct characteristics, such as improvement in and preservation of neurons in the central and peripheral nervous systems, which led to an increased striatal dopamine content, persistence of the nigrostriatal dopaminergic neurons, and enhancement of behavioral activities. The authors were able to deduce from their investigations into PD that CNTs constitute an important theragnostic tool because of their electroconductivity, apparent reactivity, and biocompatibility in conjunction with their ability to distribute drugs or biomolecules and stimulate neuronal activities and deep brain activity. Furthermore, their research into nanoparticles and PD revealed that GDNF-loaded CNTs increase the transfer of cells into the striatum, while biocompatible semiconductor nanocrystals, known as quantum dots, are known to produce brain signals or changes in channel activity with the specific function of repairing and normalizing all neuronal activities, demonstrating remarkable benefit in ameliorating brain disorders, including PD. Furthermore, they reviewed the significant effects that nanoparticles play in Huntington's disease (HD). They demonstrated that nanoparticles/nanotechnology have great relevance in neurotrophic factors owing to the fact that neurotrophins play a significant role in distinguishing between the proliferation of neurons and the survival of neurons in the CNS. In relation to this, they observed that brain-derived neurotrophic factor (BDNF) has the ability to control axonal growth, connectivity, and synaptic plasticity because it is the most profuse neurotrophin found in mammalian CNSs. These qualities were seen to have decreased in the striatum of patients with HD, which is an indication that nanoparticles play a significant role in ameliorating neurotoxins through their neuroprotective ability. Furthermore, their investigation revealed that nanoimaging plays a significant role in revealing the early stage of multiple sclerosis (MS), through the identification of cellular or subcellular levels of MS defects. Using nanoparticles layered with myelin sheath had a beneficial healing effect in ameliorating oxidative stress and axonal impairment associated with MS, which is an indication that nanomaterials can provide effective treatment of the impaired CNS. Finally, the authors showed that nanoparticles have healing properties that ameliorate neurodegeneration.

Mahmood et al. [85] investigated the beneficial effect of nanoemulsions by demonstrating their phytochemical content and how they enhance food preservation. They observed that nanoemulsions have a significant role to play in ensuring that nanoparticles aid drug delivery systems, by creating a platform through which constituents with high solubility and availability are made easily available for incorporation into drug and food substances. Furthermore, their evaluation explained the remarkable effect of nanoparticles used in the CNS for the treatment of various types of dementia or neurodegeneration. Nanocarriers were seen to be effective as a treatment for AD through a continuous supply of active nanocompounds found in the drug to the target host. The authors' study revealed that administration of nanodroplets composed of pomegranate seed oil ameliorated neurodegenerative diseases by slowing the early stages of neurodegenerative disease presentation. The authors further demonstrated that decreased lipid oxidation and neuronal loss are good indications that nanoemulsions are effective neuroprotective agents. Examples of neuroprotective nanoemulsions include curcumin, which, according to their study, had numerous biological activities but with the disadvantage of having poor water solubility as well as poor bio-accessibility. These authors observed that nanoparticles are essential in ameliorating neurodegenerative diseases.

According to Rai et al. [86], nanotechnology is seen as an increasingly successful area of medicine that has the ability to inhibit, cure, and diagnose diseases. The characteristic features of nanoparticles are that they have a relatively minute size with a high surface area to volume ratio. These characteristics can be seen in relation to curcumin, as they enable its rapid and smooth passage into biological membranes, cells, tissues, and organs. The authors observed that large molecules do not possess these characteristics and explained that, for nanoparticles to have an active and rapid response in diseases, the size, shape, and surface area of nanoparticles must be such that they have active and passive attributes to enable the drug to have its impact on the diseased cell. The significant efficacy of nanotechnology has been known to produce desired results that could be used in the treatment of CNS disorders by means of targeted drug distribution. According to the authors, nanoparticles for drug delivery have an exact action at the point where the drug is required, as well as possessing enhanced bioavailability and healing potential. To this effect, the authors identified curcumin to have employed its neuroprotective ability based on the presence of antioxidant characteristics that are essential for the treatment of neurological disorders. The authors observed that the neuroprotective function played by curcumin is restricted by poor brain availability caused by restricted BBB permeability, reduced concentration, systemic eradication, and rapid metabolism. The authors' investigation showed that curcumin is insoluble; to make it effective, curcumin nanoparticles were prepared as a water-soluble substance that is effective in ameliorating AD, making curcumin

nanoparticles a good fit for the management of CNS disorders. These authors also made use of a pharmacokinetic study into medication with oral curcumin: the nanocurcumin dosage had an effective potential that was about 20 times lower than that of the unformulated curcumin. The authors evaluated how curcumin could be used as an essential nanocurcumin formula to reduce neurotoxicity and enhance life span.

14.7 Cardioprotective Effects of Nanoparticles

Chang et al. [87] noted that myocardial infarction (MI) has been treated with several therapies, including the use of long-term angiotensin-converting enzyme inhibitors, which have deleterious side effects. Using nanomaterials with distinctive physiochemical properties offers the opportunity to improve drug delivery systems used in treating acute MI challenges. However, since cardiomyocytes are non-phagocytic in nature, the drug delivery ability of nanoparticles could be affected. During their study the authors illustrated that using poly(lactic-co-glycolic acid) (PLGA)–insulin-like growth factor (IGF) NPs extended the release of human IGF-1 in infarcted myocardium for about 24 hours; however, when IGF-1 is preserved, it has the ability to induce Akt phosphorylation, which is known to preserve the working functions of the cardiac system, inhibit ventricular alteration, and decrease infarct size. Continuous influx and storage of IGF-1 in the myocardium showed a cardioprotective effect on the heart after acute infarction. Using PLGA NPs as a channel to supply and lengthen the maintenance of molecular drugs is effective as a medical intervention. However, the authors identified the conservation of protein bioactivity as a significant difficulty when joining proteins to biomaterials for drug delivery. They observed that combining IGF-1 with PLGA NPs by means of electrostatic forces conserved the bioactivity of IGF-1, while in vitro IGF-1 was found to be bound to the surface of PLGA NPs and retained the potential to induce Akt phosphorylation in cardiomyocytes for about 24 hours. The authors acknowledged the cytotoxicity of poly(ethylenimine), which could alter the correct functioning of PLGA NPs. To avoid this, the authors carried out a clean-up process by washing PLGA NPs before combining them with IGF-1. Furthermore, their in vitro and in vivo studies on IGF-1 demonstrated the absence of any remarkable alteration of cardiomyocyte apoptosis between PLGA NP control and treatment groups. The authors observed that, at the end of MI, cardiomyocytes experienced apoptosis, which was increased in the first 24 hours after MI; this is an indication that cardiac treatment should begin within 24 hours of commencement of MI. It is inferred from the authors' observations that, following administration of free IGF-1 and IGF-1-complexed PLGA NPs into the myocardium, IGF-1 detected in the cardiac tissue after about 5 minutes consisted

Figure 14.1 Production of exosomes through the fusion of multivesicular bodies and plasma membranes.

of the same aggregates, but the authors observed that free IGF-1 could easily be removed from the myocardium while IGF-1 bonded to PLGA NPs had a greater likelihood of being retained in the myocardium. The PLGA NPs were found to be able to consistently supply IGF-1 to surrounding cells in the infarcted tissue, providing long-term antiapoptotic effects for about 24 hours post infarction. The authors observed that the reason for the long retention time of IGF-1 could be that PLGA NPs were used as a carrier, suggesting that NPs have a significant effect in crossing the BBB or epithelial layers of various organs.

Yellon and Davidson [88] confirmed that CVS diseases are a significant cause of death globally, the incidence of which is increasing daily. To this effect, the authors investigated some nanoparticles that possess cardioprotective abilities to ameliorate or arrest CVS diseases. This led to their investigations on exosomes, which are nanosized extracellular lipid particles ejected from cells; they originate from endosomal compartments and are regarded as multivesicular bodies that form intraluminal vesicles. The method by which multivesicular bodies discharge their contents is by fusion with the plasma membrane. The fusion of multivesicular bodies and plasma membranes produces exosomes (Figure 14.1).

The authors' observation led to the conclusion that exosomes can also be isolated from other cell lines and body fluids. The cardioprotective ability of exosomes was observed through a mechanism that requires stimulation of cardioprotective kinase pathways, which is regarded as preconditioning. According to the authors, exosomes can be isolated in several ways and from different sources; however, exosomes isolated from in vitro cultured murine cardiac progenitor cells have the ability to safeguard the myocardium from ischemia.

14.8 Anticancer Effects of Nanoparticles

Syaefudin et al. [89] investigated the inhibition of lung cancer with nanodrugs derived from the leaves of *Selaginella doederleinii* using a human model. The authors determined the inhibitory activity of the prepared nanoparticle extract with a dye compound (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). They

showed that at 1020 µg/ml, which is equivalent to an IC_{50} of 3%, nanoparticles containing *S. doederleinii* extract significantly prevented the growth of A549 cancer cells with flavonoids as the major bioactive constituents. It was also observed in the experiment that a dose of 1442 µg/ml, equivalent to an IC_{50} of 4%, prevented normal cell line growth [89]. The authors finally suggested that 167 µg/ml, which is equivalent to an IC_{50} of 0.5%, can effectively prevent the growth of lung cancer cells without any adverse effects on a normal cell line [89].

In the quest to prevent the development of cancer in humans, Raghunandan et al. [90] used two different nanoparticles to synthesize anticancer drugs from the extracts of clove (*Syzygium aromaticum*) and guava (*Psidium guajava*). They used four human cancer cell lines to carry out their studies. The authors also reported that a flavonoid is the most abundant secondary metabolite present in the plants studied. Furthermore, it was observed that the unevenly shaped *S. aromaticum* extract gold nanoparticles that were synthesized had more anticancer potential than the *P. guajava* extract gold nanoparticles on the various cancer cell lines studied. The AgNPs synthesized using the same extracts were devoid of anticancer activity. However, the study showed that the free radicals generated via the gold nanoparticles are responsible for the inhibition of cancer in the cell lines in a dose-dependent manner [90].

In 2018, Alsheddi et al. [91] investigated the anticancer efficacy of AgNP-incorporated *Nepeta deflersiana* extract on human cervical cancer cell lines. The authors evaluated the cell toxicity of different concentrations of the synthesized nanoparticle extract on different markers, such as mitochondrial membrane potential, reactive oxygen species generation, cell cycle arrest, necrosis, and oxidative stress markers. They reported that the results depended on the concentration of the extract. It was also revealed that there was an increase in lipid peroxidation and reactive oxygen species generation with a reduction in glutathione levels and mitochondrial membrane potential [91]. The nanoparticle extract also showed the ability to induce cell death (apoptosis) in a HeLa cancer cell line. It was therefore concluded that the nanosynthesized plant extract could be an effective treatment for cancer.

Huang et al. [92] investigated the potential of synthesized AgNPs of *Chlorophytum borivilianum* callus extract in inhibiting colon cancers in humans. The synthesized nanoparticle drug was characterized by AFM, UV–Vis spectrophotometry, FTIR spectroscopy, and XRD, with different shapes and sizes being observed before the NPs were used for cell toxicity testing. Based on the cytotoxicity assay carried out, the authors showed that different dosages of *C. borivilianum* extract delivered in AgNPs inhibited colon cancer cells at different rates in a human colon adenocarcinoma cancer cell line (HT-29). The nanoparticles with extract at a dose of 500 µg/ml showed 7% cell viability after 24 hours. It was then suggested that AgNP-incorporated *C. borivilianum* extract has the potential to halt cancer development [92].

Wang et al. [93] reported the anticancer efficacy of *Scutellaria barbata* extract incorporated in gold nanoparticles on a pancreatic cell line (PANC-1). In their study, they revealed that the synthesized *S. barbata* gold nanoparticles greatly induced the intracellular generation of reactive oxygen species in line with the concentration of the extract; the increase in the reactive oxygen species increased the potential for cell death in the pancreatic cancer cell line. It was further noted that the viability of the cancer cell line treated with *S. barbata* gold nanoparticles decreased significantly.

Jeyaraj et al. [94] evaluated the anticancer potential of AgNPs of *Sesbania grandiflora* extract on a human breast cancer cell line. In the Hoechst, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), comet (single-cell gel electrophoresis), and acridine orange/ethidium bromide (AO/EB) assays that were carried out, it was observed that gold nanoparticles with *S. grandiflora* increased the cell toxicity potential in the breast cancer cell line MCF-7. It was also found that *S. grandiflora* AgNPs induced cell death as a result of oxidative stress, apoptosis, and disruption of the membrane integrity of cells.

Kalpana et al. [95] examined the efficacy of gold nanoparticles containing three plant extracts (*Torreya nucifera, Nerium indicum,* and *Cinnamomum japonicum*) on the 3T3-L1 cancer cell line. The authors observed that the synthesized plant nanoparticles tested in vitro showed no cell toxicity in the 3T3-L1 cell line. It was therefore argued that, because of their non-toxicity, the plants would have biochemical importance.

Strasser et al. [96] investigated the antiulcerogenic efficacy of ethyl acetate, aqueous, and crude extracts of *Passiflora serratodigitata* (L.) leaves in comparison with the nanoencapsulated extract. The results revealed that, although the various leaf fractions of the extract of *P. serratodigitata* (L.) had antiulcerogenic tendency, their activity was further increased by the encapsulation of the plant extract in nanoparticles. They observed that there was a 10-fold increase in drug delivery with nanoencapsulation as well as in the physical and chemical properties of the herbal medicine. Hence, *P. serratodigitata* can be used as a component in the design of drugs intended for the prevention or treatment of ulcer.

Sreelakshmy et al. [97] studied the antiulcerogenic potential of AgNP-encapsulated extract of the root of *Glycyrrhiza glabra* in the management of gastric ulcer in vitro. The authors demonstrated the in vitro antiulcer actions of the AgNPs synthesized on an agar disk through a diffusion method and in a micro-broth through a dilution method. The agar disk method revealed that, at a concentration of 500 μg/ml, the nanoparticles exhibited an efficient cytoprotective potential against the Gram-negative bacterium *Helicobacter pylori* as an antiulcerogenic herbal formulation. It was therefore concluded that AgNPs of *G. glabra* could be an acceptable formulation in the eradication of gastric ulcers [97].

Servat-Medina et al. [98] investigated the antiulcerogenic potential of *Arrabidaea chica*–chitosan–sodium tripolyphosphate nanoparticles alongside their bioavailability and pharmacological activity. The synthesized nanoparticle extract possessed the ability to retain cell viability at minimum concentrations as well as cell proliferation at higher dosages. Also, the antiulcerogenic efficacy of *A. chica*–chitosan–sodium tripolyphosphate nanoparticles was determined in an animal model of acute gastric ulcer. It was observed that nanoparticle-encapsulated *A. chica* extract decreased the number of lesions caused by an ulcer. Thus the authors postulated that the drug could be a potential therapeutic agent for ulcers.

Lin et al. [99] reported that berberine nanoparticles suppress *H. pylori* in the gastrointestinal tract. It was revealed that interaction of berberine with nanoparticles increases the effectiveness of the nanodrug in combating infection caused by *H. pylori* on its active site.

14.9 Advantages of Nanoparticles

Nanoparticles have several merits, especially in the area of medicine. They enhance the efficacy of drugs or proteins against the action of enzymatic degradation; possess the ability to pass through sinusoidal spaces, especially those that are present in the spleen and bone marrow, which might be linked to their small sizes when compared with liposomes and microspheres; can be administered intravenously, in contrast to colloidal systems, which could block blood capillaries; possess the ability to deliver drugs to target sites more safely; possess an enhanced loading capacity, which might be linked to their higher surface area; minimize the level of toxicity in the liver; improve the solubility of drugs that are insoluble in water; enhance bioavailability by decreasing fluctuations in therapeutic ranges; and serve as alternatives to orthodox oral or intravenous techniques of administering drugs in terms of effectiveness and efficiency [100].

14.10 Disadvantages of Nanoparticles

Despite all the advantages associated with NPs, there are some disadvantages that have been highlighted, including: there is tendency for NPs to aggregate in the system, which might be linked to the fact that they possess high energy and a large surface area; following treatment there is a residual level of nanosuspension that can induce some level of toxicity; the high cost of producing some metallic nanoparticles; poor targeting; and a tendency to exhibit minimum biological half-lives [100]. Argyria has been highlighted as one of the side effects in patients exposed for a long time to silver salts or in those who consume these salts. Some of the

symptoms of argyria include black to gray staining of the skin and mucous membranes, which is caused by the deposition of silver. The deposition of silver salt might be the result of ingesting medication containing silver salts or of exposure to industrial chemicals that contain silver salts.

It was reported by Chang et al. [101] that a patient consumed colloidal silver three times in 2 years; it was believed that this consequently led to hypertension, diabetes, and hyperlipidemia. Moreover, it was reported that gold nanoparticles could result in hemolysis, immunogenic reactions, and thrombosis [102]. In addition, there are reports that the enzyme available in human saliva has the ability to change gold(0) to gold(I), which is afterwards taken up by immune cells. Furthermore, the exposure of mice to AgNPs led to the generation of dead brain cells [102]. Also, some workers have been reported to have tarnished corneas and conjunctiva as a result of inhalation [103].

Some other influences of nanoparticles include papular rash, erythema nodosum, macular rash, and allergic reactions. Also, it has been highlighted that gold nanoparticles could result in the generation of nephrotoxicity, with some signs of proteinuria. Moreover, hematological disorders are usually experienced in pregnant women who ingest gold nanoparticles. It has been observed that gold nanoparticles with densities of 0–0.001 ppm [104] could affect human health. Analyses have shown that they are present in small amounts in the following: hair (0.3 μg/g); skin (0.03 μg/g), and nails (0.17 μg/g) [105–108]. In a zebrafish model exposed for a period of 72 hours, silver, gold, and platinum nanoparticles exhibited a high level of toxicity. This led to cardiac disorders, delayed hatching, and deformed spines. It was also observed that platinum nanoparticles built up in the brain when polyvinyl alcohol was capped with particle sizes ranging from 3 to 10 nm. It has been stated that manganese and copper possess the ability to induce a high level of neurotoxicity when tested with PC-12 cell lines.

14.11 Conclusion and Future Directions

This chapter has documented the various applications of phytochemicals and plant extracts that have been utilized for the biofabrication of nanoparticles. The various biological activities that have been extensively discussed include antibacterial, antifungal, antiparasitic, antiprotozoal, anticancer, antiulcer, neuroprotective, hepatoprotective, anti-inflammatory, and wound-healing actions. The modes of action through which these nanoparticles exhibit their various biological activities were also provided in detail. Therefore, in order to obtain maximum fabrication of all these bimetallic nanoparticles, there is a need to resolve some issues, such as optimizing production parameters, producing stable compounds, and identifying recent techniques that could be used for the structural elucidation of

bioactive components that are responsible for the capping and reducing activities of various plant extracts. There is a need to utilize some metabolomics techniques to give better insight into the pool of phytochemical constituents responsible for their biological activities. Also, there is a need to introduce genetic modifications in order to enhance the activities of these bioactive compounds. This will also go a long way towards providing detailed and vital information on the molecular and cellular levels of the various phytochemicals available in different medicinal plant species in different ecological zones.

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15

Application of Metabolomics in Emergency Phytochemical Poisoning and Remediation

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CONTENTS

Poisonous Plants and Phytochemicals in Drug Discovery, First Edition.
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15.1 Introduction

Since the beginning of life on Earth, humankind has found in nature and within local habitats the most preferable sources of major needs such as food, energy, and therapeutics. Nowadays, the value of the annual world pharmaceutical market is approximately US$1.1 trillion. About 35% of these drugs are natural product-based medicaments, including products inspired by plants (25%), microorganisms (13%), and animals (about 3%) [1]. In addition, about 30% of the top-selling drugs in the world are natural products or their derivatives [2]. Moreover, of the 1562 new drugs approved by the US Food and Drug Administration (FDA) between 1981 and 2014, 64 (4%) were unaltered natural products, 141 (9.1%) were botanical drugs (defined mixture), 320 (21%) were natural product derivatives, and 61 (4%) were synthetic drugs, but with a natural product pharmacophore [1, 2]. Most of these products have proven their bioactivity against many malignant tumors and infectious diseases. In addition, the worldwide annual market for these natural products is estimated to be US$60 billion [3].

Specifically, medicinal plants have been traditionally used as, and continue to offer, exceptional scaffolds, improving quality of life and being used in the diagnosis, cure, and prevention of many ailments (e.g. malaria, dysentery, common cold, menstrual disorders, snake bite, urinary disorders). More than 90% of these crude drugs are isolated from plant sources, while the remaining 10% are from other sources, such as animal, metal, and mineral resources [4]. Prescribing of the entire medicinal herb or certain parts, such as the leaf, stem, bark, or root, was reported by traditional healers in herbal folk medicine in curing human diseases. Therefore, the ability of these herbs to treat human diseases is the result of their bioactive components distributed over the different plant organs (e.g. quinine in *Cinchona* bark, digoxin in *Digitalis* leaf, gingerol in the ginger rhizome, morphine in the *Papaver* poppy capsule or latex, pilocarpine in maranham jaborandi leaves) and their chemically modified products [5, 6].

Because of differing knowledge, beliefs, skills, and cultural habits and a wide spectrum of climatic conditions from polar, to temperate, to tropical zones on the Earth's surfaces or in marine life, several herbal-based holistic (whole body) healing systems have been developed that are specific to the world's regions, such as China, India, and South America, and are now used worldwide. Examples include the most famous and well-known Ayurvedic medicine, which was developed more than 3000 years ago in India [7, 8], and traditional Chinese medicine (TCM), which was developed more than 2500 years ago in China [9, 10]. Interestingly, Chinese traditional herbal medicine played a significant role as a drug discovery and developmental tool for the secret ingredient in Tamiflu®, which is used for the treatment of severe acute respiratory syndrome [11, 12]. The production of Tamiflu (Oseltamivir, Roche®) was based on extraction of its precursor, shikimic acid, from the fruits of the Chinese star anise (*Illicium verum* Hook.f., Schisandraceae) [13]. In addition, the medieval medicine of Western Europe has

a long history of pharmaceutical herbal remedies described by Galen. This medicine was significantly developed in the twelfth and thirteenth centuries after translation of the Arabic medical textbooks [14].

As a branch of complementary and alternative medicine, the effectiveness, accessibility, affordability, safety, biodiversity, and low cost of herbal medicine mean that it is considered the favored and major health care system in many communities, especially rural ones [15]. According to the World Health Organization (WHO), over 60% of the world's population and about 80% of developing countries depend on traditional herbal medicines for their primary health care [16]. Besides the major plant constituents, plant extracts contain many other compounds, which interact with and even damage other biological systems. In addition, there is no single technique that identifies the various phytoconstituents. Recently, metabolomics has been developed as a comprehensive tool to identify and reveal the total components within a biological system. Hence, this chapter aims to discuss metabolomics and its relevant applications as a potential technique in emergency phytochemical poisoning and remediation.

15.2 Traditional Use of Medicinal Plants

The pharmacological efficacy of traditionally used medicinal plants as therapeutics has been proven. Most of these herbal remedies are commonly taken as teas, through either a decoction or infusion process [17]. Thousands of articles have discussed their applications in different communities, districts, and folk medicines [7, 18, 19]. Herbal medicines are usually used for health promotion and therapy for chronic, as opposed to life-threatening, conditions. Nevertheless, the use of traditional remedies increases when conventional medicine has failed in the treatment of a disease, such as advanced cancer or new infectious diseases [20]. Specifically, Ayurvedic and Chinese herbal systems will be discussed in the following sections.

Ayurveda (*Ayu* + *Veda*) means the science of life. The potential medicinal plants used in the Ayurvedic system of medicine have been summarized by Murugeswaran et al. [21]. In this study, 197 plant species were identified and summarized regarding their applications, Ayurvedic terminology, curative diseases, diversity status, etc. [21].

Furthermore, an *Encyclopedia of Traditional Chinese Medicine* was developed to meet the increasing demand for TCM-related information. In 2019, Xu et al. [22] developed a database that includes multiple aspects of essential clinical and functional information on 403 TCM herb species, 3962 TCM formulae, 7274 herbal ingredients, 2266 validated or predicted drug targets, as well as 3027 related diseases.

Yuan et al. [10] published a comprehensive review article covering eight of the most practiced traditional medicines around the world. They summarized literature that dealt with the relationships among natural products, traditional medicines, and modern medicine, and showed the applied concepts and

Table 15.1 Some selected examples of well-known herbal remedies traditionally used in, especially, Ayurvedic and traditional Chinese medicine.

	Common herbal name	Major constituents	Common uses	Reference
Ayurvedic herbs	Curcuma/turmeric (*Curcuma longa*, Zingiberaceae)	Curcumin; a polyphenolic yellow substance	Anti-inflammatory, antioxidant, anti-Alzheimer's disease, and anticancer properties	[23, 24]
	Ginger (*Zingiber officinale*, Zingiberaceae)	Phenolic compounds (e.g. gingerol)	Treatment of nausea, dysentery, heartburn, flatulence, diarrhea, loss of appetite, infections, cough, and bronchitis	[25]
	Aloe vera (*Aloe vera* or *Aloe barbadensis*, Aloaceae)	Combined and free anthraquinone (e.g. aloe emodin, aloin [barbaloin], anthracene, and emodin)	Antioxidant, antitumor, and anti-inflammatory properties	[26]
	Tulsi (*Ocimum sanctum*, Lamiaceae)	Eugenol as a volatile phenolic compound	Treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, and skin diseases	[27]
	Triphala, a preparation of equal proportions of pericarps of three myrobalans, namely *Terminalia chebula* Retz. (Haritaki), *Terminalia bellerica* Roxb. (Bibhitaki), and *Emblica officinalis* Gaertn. (Amalaki)	Gallic acid, anthraquinone, tannins, and ascorbic acid	A multipurpose treatment for symptoms of dental cavities and as an antioxidant and purgative	[28]
	Ashwagandha (*Withania somnifera*, Solanaceae)	Alkaloids (e.g. isopelletierine, anaferine, cuseohygrine, anahygrine), steroidal lactones (e.g. withanolides, withaferins), and saponins	Treatment of anxiety	[29]
	Gotu kola (*Centella asiatica* (L.) Urban., Apiaceae)	Pentacyclic triterpenes, mainly asiaticoside, madecassoside, asiatic, and madecassic acids	As a brain tonic and treatment of small wounds and hypertrophic wounds as well as burns, psoriasis, and scleroderma	[30, 31]
	Guggul (*Commiphora wightii*, Burseraceae)	An oleo-gum resin containing terpenoid constituents, steroids, flavonoids, guggultetrols, and lignans	Treatment of inflammation, gout, rheumatism, obesity, and disorders of lipid metabolism	[32]
	Boswellia (*Boswellia serrata*, Burseraceae)	An oleo-gum resin containing terpenoids, such as the pentacyclic triterpene boswellic acids	Treatment of inflammation, especially osteoarthritis	[33, 34]

Chinese herbs	Ginkgo (*Ginkgo biloba,* Ginkgoaceae)	Flavone glycosides, terpene lactones (e.g. ginkgolides A, B, and C and bilobalide)	*Ginkgo biloba* leaf extract (EGb) is used for various degenerative diseases, such as cerebrovascular disease, Alzheimer's disease, and macroangiopathy	[35]
	Ginseng (*Panax ginseng,* Araliaceae)	Ginsenosides; glycosylated triterpenes	Treatment of neurological disorders, e.g. Alzheimer's disease, Parkinson's disease, and cerebral ischemia	[36, 37]
	Cinnamon (*Cinnamomum zeylanicum* and *Cinnamon cassia*, Lauraceae)	Cinnamic aldehyde as a volatile oil	Flavoring agent, spices, reduces the risk of colon cancer, increases the blood circulation in the uterus, and advances tissue regeneration	[38]
	Chili or red pepper (*Capsicum annuum*, Solanaceae)	Capsaicinoids, which include capsaicin, dihydrocapsaicin, and nordihydrocapsaicin	Treatment of rheumatism and antiseptic, counterirritant, appetite stimulator, antioxidant, and immunomodulatory properties	[39]
	Star anise (*Illicium verum*, Schisandraceae)	1,4- and 1,8-cineole and *trans*-anethole; volatile oil	Ingredient of the traditional "five-spice" powder in Chinese cooking, carminative, stomachic, stimulant, and diuretic properties	[40]
	Clove (*Syzygium aromaticum*, Myrtaceae)	Eugenol, as a volatile phenolic compound	Antioxidant, antimicrobial, and antinociceptive properties	[41]
	Astragalus (*Astragalus membranaceus*, Fabaceae)	Polysaccharides, flavonoids, and saponins	Immunomodulatory, antioxidant, and anti-inflammatory properties, as well as an anticancer herb	[42]
	Red yeast rice (the Chinese fermented product of the yeast *Monascus purpureus* grown on white rice, Gramineae)	Natural monacolins, such as monacolin K, isoflavonoids, monounsaturated fats, and sterols	Improvement of the blood circulation by decreasing cholesterol and triglyceride levels	[43]

methodologies that could be used to further develop the discovery of drugs from natural products and traditional medicines [10]. It is impossible to include all of the traditionally used herbs in the different traditional herbal medicines here; therefore, Table 15.1 provides a summary of some relevant information for common examples of herbal remedies used in Ayurvedic medicine and TCM.

15.3 Natural Products: Safety and Toxicity

There is a common belief or preference in different societies around the world that, when it comes to medicine, "natural" health products (NHPs) are better, healthier, have fewer side effects, and are safer than "unnatural" or synthetic drugs [44]. Most of these products, such as botanicals and dietary supplements derived from natural substances, are considered to be Generally Recognized as Safe (GRAS) under the US Federal Food, Drug, and Cosmetics Act [45]. What has increased this belief is that nature usually gives the best, including effectiveness, diversity, novelty, and quality. For example, in the field of medicinal herbs and drug discovery, aspirin originates from *Salix alba*, morphine from the opium poppy or *Papaver somniferum*, and quinine from *Cinchona* sp.

However, studies have proven that natural does not always means safe [46], and not all products isolated from nature have been shown to be effective, especially those used in dietary supplements. The 1994 US Dietary Supplement Health Education Act (DSHEA) created a regulatory framework for the safety and regulation of natural products involved in dietary supplements. Moreover, there are poisonous plants or natural killers, such as *Conium maculatum* and *Aconitum napellus*. One of the drawbacks of herbal medicine is that herbs may contain many still unknown chemical compounds in addition to the major bioactive constituent, requiring an ethical framework for administration of herbal medicines [46, 47]. Therefore, the aim of recent studies, including metabolomics and ecometabolomics, has been to identify most of the plant metabolites responsible for these unknown side effects [48, 49]. The following sections discuss three classes of medicinal herbs and natural products in relation to safety and toxicity.

15.3.1 Safety

Kumar et al. [8] discussed the side effects of some popular herbs mentioned in Ayurvedic herbal medicine. The article showed that improper use of some herbs could result in several undesirable effects, such as headache, constipation, and a burning sensation. In addition, others are contraindicated during pregnancy and in cases of arterial congestion; an example of this is ashwagandha (*Withania somnifera*) because it contains the alkaloid somniferin.

Other examples include kava, which is native to the Pacific Islands. This herb is often used as a dietary supplement for the treatment of anxiety, but it may also be

associated with severe liver injury, as reported by WHO [50]. In 2004, FDA banned the sale of dietary supplements containing *Ephedra* (ephedrine alkaloids), which are used for the treatment of colds and as weight-loss dietary supplements, because of concerns relating to their cardiovascular effects, including hypertension and irregular heart rhythm [51].

Furthermore, some natural products can interact with other pharmaceutical medications, resulting in serious consequences for patients. This kind of interaction is called drug–herb interaction [52]. As an example, a Canadian study found that approximately 45% of patients used natural and prescription drugs concomitantly and 7.4% described an adverse effect [53].

15.3.2 Toxicity and Natural Killers

According to WHO, natural toxins are toxic compounds that are produced by living organisms as a natural defense mechanism against predators, such as insects or microorganisms, or as a result of infection by microorganisms and in response to certain stress conditions [54]. These chemical compounds have diverse structures (Figure 15.1) and different bioactivities and toxicities and affect both humans and livestock. The clinical signs of toxicity include a range of numerous adverse health effects from allergic reactions to severe headache, nausea, vomiting, diarrhea, paralysis, and even death. Examples include aquatic biotoxins (e.g. algal toxins) [55] and mycotoxins produced by certain types of poisonous mushrooms [56]. Specifically, terrestrial medicinal plants produce a wide spectrum of phytotoxins, including the following.

- *Cyanogenic glycosides*, which can be found in at least 2500 plant species (e.g. amygdalin in apple [*Malus domestica*]; bitter almond in [*Prunus dulcis* var. *dulcis*]; and linamarin in flaxseed [*Linum usitatissimum*]) [57].

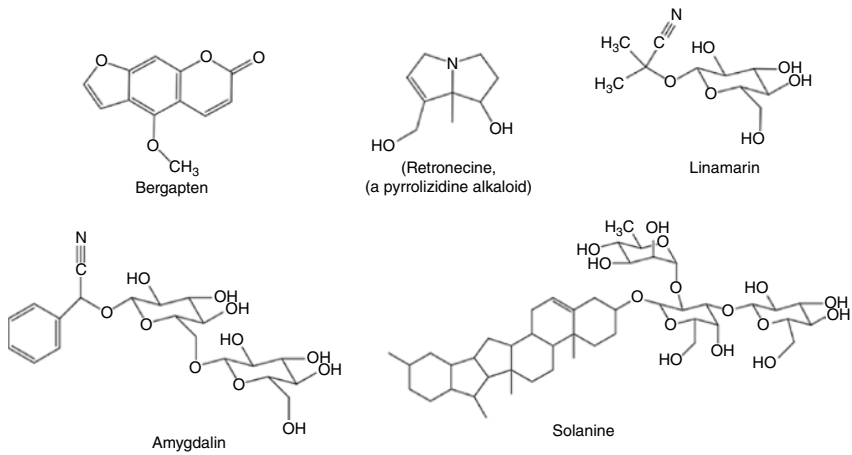

Figure 15.1 Chemical structures of some phytotoxins.

- *Furanocoumarins*, which are present in many plants, such as parsley, parsnips, celery, and citrus plants (lemon, lime, grapefruit, bergamot). They include psoralen, bergapten, isoimperatorin, oxypeucedanin, xanthoxin, trioxalen, and angelicin, which are produced under stress conditions. These compounds are also phototoxins and cause severe skin reactions with exposure to sunlight, especially ultraviolet A [58].
- *Solanines* and *chaconine* are present in solanaceous plants, especially in potato sprouts and green tomatoes. They are glycoalkaloids and are produced in response to stresses such as bruising and ultraviolet light [59].
- *Pyrrolizidine alkaloids* (PAs) are common in the Boraginaceae, Asteraceae, and Fabaceae plant families. PAs have DNA-damaging activity and can potentially lead to cancer [60].

15.4 Biological Systems in Phytochemical Poisoning and Remediation

Systems biology emerged more than 15 years ago as a new approach to decoding life [61]. Systems biology covers the comprehensive study of complex biological systems through combining and integrating data at various levels (e.g. genome, transcriptome, proteome, metabolome) [61, 62]. The suffix "-ome," of Greek origin, refers to wholeness, completion, or totality [63]. "omics" approaches are used in biology to describe the study of large-scale data to understand biological systems at different levels, such as the gene level (genomics), transcript level (transcriptomics), protein level (proteomic), and metabolic level (metabolomics) [64, 65].

Systems biology studies complex systems through a holistic approach, which explains the behavior of the system in total rather than the individual parts. The quote "The whole is greater than the sum of its parts" from Aristotle's *Metaphysics* defined the concept of holism [66]. Although reductionism-based molecular approaches are invaluable, holistic systems biology approaches are required to elucidate the complete picture of biological systems (Figure 15.2).

The main goal of systems biology is to decipher complex biological networks and to increase our understanding of the link between genotype and phenotype [67]. The basics of the systems biology research cycle include defining the biological research problem, carrying out comprehensive data analyses, and integrating data into meaningful information (Figure 15.3). Integrating various "omics" approaches that use analytical advances is a prerequisite for comprehensive systems biology. Hence, systems biology approaches can only be achieved through collaborations among biologists, mathematicians, bioinformaticians, and computer scientists.

Plant systems biology comprises the comprehensive study of plant systems in response to genetic or chemical perturbations through application of multi-omics approaches [62]. The study of such complex biological systems is achieved at various levels, including transcriptional, translational, and metabolic levels (Figure 15.4). Since a single "omics" approach is inadequate to uncover the

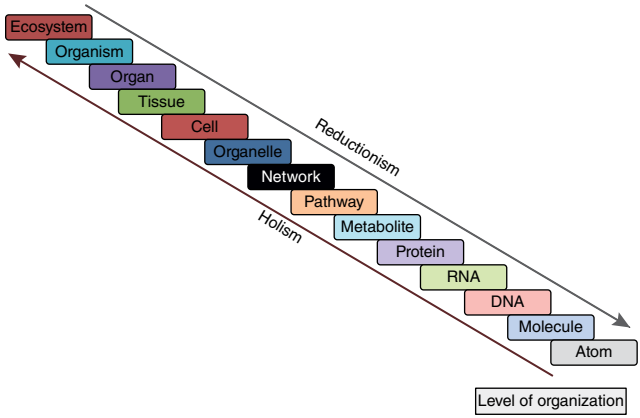

Figure 15.2 Reductionism versus holism for understanding biological systems.

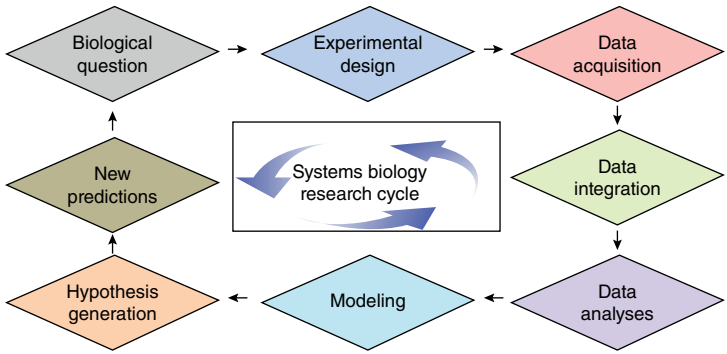

Figure 15.3 Systems biology approaches for system-wide comprehensive descriptions of complex biological processes.

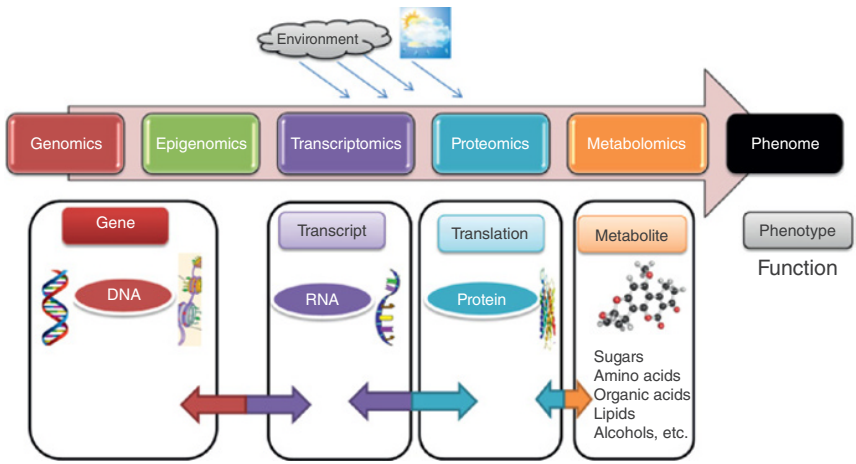

Figure 15.4 Integration of multi-omics data for system-wide understanding of the complexity of biological systems.

complexity of a biological system, the integration of multiple omics datasets provides biologically meaningful interpretation of analyzed data, giving a precise picture of the whole system [62, 68]. Deciphering the high complexity of plant systems is a major challenge in systems biology. Improving the experimental workflows and introducing better technical innovations may be vital to overcome such challenges.

15.5 Metabolomics: An Important Functional Genomics Tool

Metabolomics, the comprehensive analysis of all metabolites in an organism, has expanded greatly as an important functional genomics tool [68, 69]. The metabolome, the complete set of all metabolites, differs in some features among the genome, transcriptome, and proteome. Compared with genes or proteins metabolites are heterogeneous in terms of their physicochemical character [70–72]. The heterogeneity and complexity of metabolites are the current challenges in the field of plant metabolomics, considering that around 200 000 have been estimated to exist in plants, of which only one-fourth have been elucidated [62, 68, 73]. Additionally, the metabolome represents the output of signal integration from the genome, transcriptome, and proteome as well as their complex interactions (interactome, interaction networks) [74]. Therefore, metabolomes not only provide the metabolic components of the cell, but also reflect the functional readout of the cellular state [75]. While the genome defines indirectly what may happen, the metabolome indicates what has already happened as an endpoint of the interactions between the biological system and the environment [76]. Moreover, metabolomics data analysis is independent of the availability of organism-specific genome information, a prerequisite that has to be fulfilled for analyses of genomics, transcriptomics, and proteomics data [77]. This unique feature originates from the same molecular entity as metabolites, irrespective of the organism that makes them [78].

Since metabolites are closer to the phenotype, metabolomics studies are a cornerstone of systems biology for quantitative analysis of molecular phenotypes [70–72]. This means that metabolomics is rapidly becoming the most useful high-throughput tool for the analysis of phenotypes and the study of the cellular state of many biological systems, including mammalian [79–81], microbial [82–85], and environmental systems [86] as well as the identification and discovery of biomarkers [87]. Moreover, metabolomics studies have been used in various aspects of plant biology, such as mutation, genotyping, identification of novel metabolites, and environmental stresses (e.g. the response to biotic as well as abiotic

stresses) [70, 77, 88–92]. Metabolomics can also be considered as an indispensable tool in emergency phytochemical poisoning and remediation, which is the focus of this chapter.

15.5.1 Essential Components of a Metabolomics Workflow

Metabolomics studies include either targeted or untargeted approaches. Untargeted metabolomics studies follow a discovery-based approach that investigates previously unknown information about the response of a biological system to a certain condition. Therefore, this approach aims to analyze global metabolites (high coverage and decreased accuracy and precision) [93]. A targeted metabolomics approach is conversely hypothesis driven, in which a restricted set of predefined metabolites is absolutely quantified [94].

In metabolomics studies, the steps are quite straightforward (Figure 15.5). The biological samples are collected from different experimental groups (e.g. diseased versus healthy, wild-type versus gene mutated, control versus drug-treated, a cohort before and after being subjected to a certain stress, and so on). After collection, biological samples are extracted. The extracted samples are subjected to analytical measurements, of which mass spectrometry- and nuclear magnetic resonance (NMR)-based methods are the most common. The resulting spectra are then subjected to statistical analysis to determine the most significant features that define the specific phenotype.

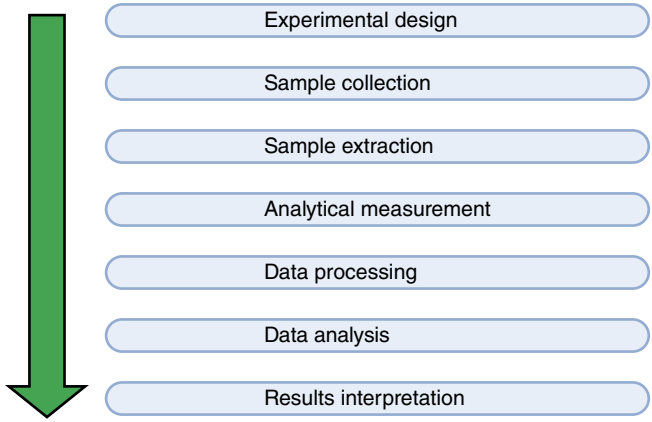

Figure 15.5 Key steps of a metabolomics study.

15.5.2 Sample Preparation

Biological samples include fluids (urine, blood, serum, etc.), cell cultures (human, animal, plant, algae, etc.), animal-derived tissues (muscle, brain, liver, etc.), and plant-derived tissues (leaves, roots, seeds, etc.). After careful sample collection, quenching and extraction protocols are applied [95, 96]. Most extraction protocols rely on a two-phase separation system for the analysis of polar or non-polar metabolites [97–104].

15.5.3 Analytical Methods in Metabolomics

Chromatographic methods such as liquid chromatography (LC) or gas chromatography (GC) hyphenated to spectroscopic techniques, mostly mass spectrometry (MS), are routinely used [105]. LC-NMR methods are also used [106]. High- or ultra-performance liquid chromatography is still the first choice in metabolomics studies. Other methods, such as including capillary electrophoresis, Raman spectroscopy, and infrared spectroscopy, have also been reported, but are less commonly used.

Compared with MS, NMR requires simple sample preparation and provides highly reproducible and quantitative data; however, its main limitation is its very low sensitivity, which restricts its application in abundant metabolites. Conversely, MS has very high sensitivity and low detection limits, enabling the detection of less abundant metabolites, even those that are invisible to NMR. Despite these advantages, the main drawback of MS is its limited ability to detect readily ionizable metabolites. Ion suppression, low reproducibility, and ionization efficiency-dependent peak intensity are also among the main limitations of MS [107].

GC, the first separation technique to be applied for metabolite analysis, is commonly used to detect and quantify volatile metabolites. GC-MS has been successfully applied in metabolomics studies [108]. Derivatization protocols are routinely applied before GC-MS analysis of non-volatile metabolites such as carbohydrates, organic acids, and amino acids. However, derivatization is time consuming and metabolites can be modified or destroyed under the high temperatures applied in GC; hence, analysis is restricted to thermally stable metabolites. Conversely, in LC analysis, metabolites can be readily detected with no modification or derivatization. Since LC analysis is routinely applied at room temperature, thermally unstable metabolites can be easily analyzed. Compared with GC, the introduction of a liquid phase in LC causes retention times to vary and results in matrix-induced ion suppression, which leads to lower resolution relative to GC [109]. Nevertheless, LC-MS has been used as the first choice in many metabolomics studies [86, 110].

15.5.4 Metabolite Identification

Metabolite identification is typically achieved in metabolomics studies by comparing the results with the published spectral libraries of known metabolites. Several databases are available for assigning structures to spectral peaks in metabolomics experiments and these have been reviewed elsewhere [111]. Unknown metabolites, lack of standards, and unavailable reference spectra are considered great challenges in metabolite identification. Plants are estimated to contain around 200 000 metabolites, of which only one-fourth have been elucidated because of a lack of many standards [62, 68, 73]. Similarly, estimates showed that the human metabolome contains around 150 000 metabolites, of which only 10 000 have been detected [112, 113]. The similarity of spectral data between different metabolites and the presence of many isomers for the same metabolite increases the ambiguities in metabolite identification.

15.5.5 Data Processing and Analysis

Huge amounts of data are usually acquired in metabolomics studies; therefore, the use of chemometric and statistical analysis is essential for data mining and visual interpretation [114]. Several free and commercial software packages have been developed for metabolomics data processing and analysis. These tools have been reviewed extensively and are easily available. Multivariate statistical analyses such as principal components analysis (PCA), hierarchical cluster analysis, partial least squares (PLS), orthogonal signal correction, and orthogonal-PLS are used in metabolomics to reduce data dimensionality [115].

15.5.6 Pathway Analysis

Following metabolite identification and data analysis, marker metabolites can be subjected to pathway analysis for understanding pathways and discovering the mechanism of action of a drug or the pathophysiology of a disease [116]. Assigning the identified metabolites to their relevant metabolic pathway from various databases can be achieved through numerous software packages [117]. Examples are: MetaboAnalyst (www.metaboanalyst.ca), Cytoscape (www.cytoscape.org), and bioconductor (https://bioconductor.org).

15.6 Assessment of Toxicity of Herbal Medicines Using Metabolomics

Metabolomics has been employed in the detection of both the safety and toxicity of herbal medicines. There are many reports on this application. For example, the mechanism by which aristolochic acid exerts its toxicity was studied using

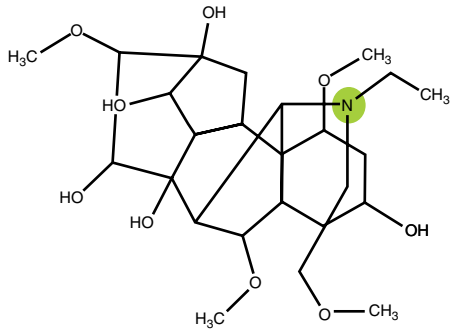

Figure 15.6 Structure of aconitine.

metabolomics-based ^{1}H NMR spectroscopic and pattern recognition (PR) methods. In this study, the urinary profiles of rats following intraperitoneal injection of aristolochic acid were compared with several toxins with known modes of action at various time intervals. Urinary ^{1}H NMR spectra were analyzed using a PR method; this showed that aristolochic acid caused a renal proximal tubular and papillary lesion and a slight hepatic lesion. Monitoring the toxicological processes from the onset, development and part recovery by PR analysis indicated that the induced renal toxicity was progressive because of dosage accumulation [118].

Aconitum carmichaelii (Hei-Shun-Pian, the processed lateral root of *A. carmichaelii* Debx., Ranunculaceae) is a TCM with analgesic, antipyretic, and anti-inflammatory effects. Aconitine, the diterpenoid alkaloid (Figure 15.6), as well as its derivatives are thought to be its active constituents. These compounds are borderline toxic based on lethal dose (LD_{50}) and effective dose (ED_{50}) (narrow LD_{50}/ED_{50}). The toxicity caused after five days of oral administration of different doses of an *A. carmichaelii* decoction in rats was assessed using metabolomics-based ^{1}H NMR of urine and plasma samples. Analysis of the ^{1}H NMR data using multivariate analysis methods, such as PCA and PLS projection to latent structures, found that the treated group had an increase in the concentration of lipids and a decrease in the concentration of phosphatidylcholine and *O*- and *N*-acetyl glycoproteins when compared with the control group. The differences in the levels of taurine, acetate, creatinine, 2-oxoglutarate, dimethylamine, and hippurate in urine were significant between the high- and medium-dose groups and the control group [119].

15.7 Application of Metabolomics in Emergency Phytochemical Poisoning and Remediation

Metabolomics plays an apparently important role in the mechanistic detection of phytochemical toxicity. It is mainly used to identify toxic metabolites, such as glutathione-conjugated metabolites [120] and *N*-oxide metabolites [121], and

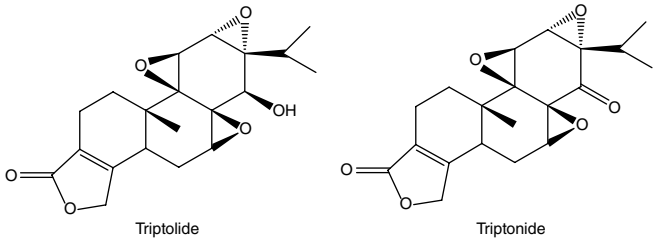

Figure 15.7 Chemical structures of triptolide and triptonide.

endogenous metabolites, such as lipids, amino acids, bile acids, dicarboxylic acids, long chain fatty acids, and acylcarnitines.

15.7.1 Hepatotoxicity of Triptolide

Triptolide (Figure 15.7) is one of the major constituents of *Tripterygium wilfordii* Hook.F. It was reported to induce severe hepatotoxicity in animals and humans [122]. Triptolide at a dose of 1 mg/kg caused a dramatic increase in the serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) in animals [120]. In contrast, triptonide (Figure 15.7), which is another major constituent of the same plant, had no hepatotoxicity in animals at the same dose [120].

Ultra-performance liquid chromatography–electrospray ionization–quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF/MS)-based metabolomics was employed to understand the difference in the effects of the two compounds in animals. Metabolomics allowed the identification of 25 drug metabolites for both triptolide and triptonide, eight of which were novel. The study showed that triptolide had a lower metabolic rate in liver microsomes than triptonide; although hydroxylation and demethylation were the major metabolic pathways for both compounds, the two compounds showed significant metabolic differences [120]. This study revealed that the hydroxyl group at C-14 in the molecular structure of triptolide plays an important role in triptonide-induced hepatotoxicity [120].

15.7.2 Hepatotoxicity of Noscapine

Noscapine (Figure 15.8) is a phthalide isoquinoline opium alkaloid that is used as an efficient cough suppressant [123]. The anticancer potential of noscapine against glioblastoma, colon cancer, and non-small cell lung cancer was investigated using various in vitro and in vivo models [124, 125]. However, the safety of noscapine was debatable because of the presence of a methylenedioxyphenyl group, which may be a structural alert for the carcinogenicity of noscapine [126, 127]. For this reason, a UPLC-ESI-QTOF/MS-based metabolomics study was

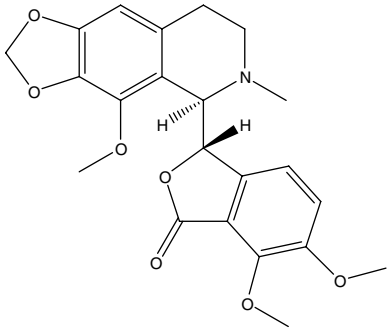

Figure 15.8 Structure of noscapine.

conducted to analyze urine and feces samples from mice treated with noscapine [126]. The study showed that, although an orthoquinone reactive intermediate was detected in the in vitro glutathione (GSH), the reactive intermediate of noscapine was not detected in vivo. The study also showed that noscapine did not induce hepatotoxicity in mice as GSH, AST, ALT, and alkaline phosphatase levels obtained from noscapine-treated mice showed no significant changes. These results provide important information on the development of noscapine for antitumor therapy because of its safety [126].

15.8 Conclusion

Metabolomics remains a vital tool for use in systems biology, and its application in determining the safety and toxicity of poisonous plants and their products is underestimated. Research institutes need to incorporate the application of metabolomics in their protocols in order to address the immediate needs of people who live and work in close proximity to poisonous plants.

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16

Methods for the Detection and Identification of Phytotoxins

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CONTENTS

Poisonous Plants and Phytochemicals in Drug Discovery, First Edition.
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16.1 Introduction

Plant toxins, also known as phytotoxins, and mycotoxins obtained from fungi have been studied in foodstuffs for decades, but little attention has as yet been paid to their detection, quantification, and occurrence in the environment. Because of the increase in awareness of the presence of micropollutants in the environment, phytotoxins and mycotoxins must be considered and investigated as part of the chemical decoctions analyzed in natural plant samples. In this chapter, we describe the biological and analytical methods used to determine the levels of important phytotoxins (i.e. protease inhibitors, phenolic acids, quinones, benzoxazinones, terpenoids, glycoalkaloids, glucosinolates, isothiocyanates, phytosterols, flavonoids, coumestans, lignans, and chalcones) and mycotoxins (i.e. resorcylic acid lactones, trichothecenes, fumonisins, and aflatoxins) in environmentally relevant matrices in plant families. The main problems encountered in many of the reviewed methods were the frequent unavailability of suitable internal standards (especially isotope-labeled analogs) and often an absent or fragmentary method of optimization and validation. Also, current, relevant, detailed, and well-structured methods pertaining to the detection of these phytotoxins were very difficult to find mainly because this area of research, dating back to the 1980s, is clearly understudied, even though several articles are available on the harmful effects of phytotoxins on plant families.

16.2 Phytotoxins

In this chapter we seek to evaluate various methods used for the detection and/or identification of phytotoxins, but before we delve into this interesting topic one may ask the following questions. What are phytotoxins? Why do we even need to worry about them?

By way of introduction, phytotoxins are basically substances that are poisonous or toxic to the growth of plants in general. Phytotoxic substances may be the result of human activity, as with herbicides, or they may be produced by microorganisms or by naturally occurring chemical reactions. The term "phytotoxin" is also used to describe toxic chemicals produced by plants themselves, which function as defensive agents against their natural predators. Phytotoxins are also defined as products of plant pathogens or of the host–pathogen interaction that directly injure plant cells and influence the course of disease development or symptoms [1].

The majority of plant diseases and injuries are caused by many of the same classes of agents as are responsible for diseases in man and animals. However, microorganisms such as fungi and bacteria are the most important causes in terms of distribution, diversity, and total damage to plants in the field as well as in storage. These microorganisms can, in part, produce phytotoxic compounds that cause disease symptoms. Many terms have been devised to describe the biology of compounds produced by parasites that are toxic to plants and that play some role

in symptom expression. One of the most classic examples relating to this definition of phytotoxins, which are also known as phytochemicals, are members of various classes of secondary metabolites, including alkaloids, terpenes, and especially phenolics, although not all such compounds are toxic or serve defensive purposes [2]. Phytotoxins have also been reported to be toxic to humans [3, 4].

The term "phytotoxin," however, does not include the phytohormones. In contrast to plant toxins, phytohormones are compounds produced by plants that adversely affect man or animals, such as the castor bean toxin ricin or the death cap mushroom toxin amanitin [5].

While a large number of plant parasites show phytotoxic activity, only a few phytotoxins have been isolated and partially or completely characterized; fewer still have been chemically synthesized and/or crystallized and subjected to X-ray analysis. The modes of action of some these phytotoxins have been elucidated. Biosynthetic studies have also been carried out on a few other phytotoxins, but the complete pathway with all intermediates and enzymes is not known for many phytotoxins.

16.2.1 Importance of Toxins

Phytotoxins play an important role in the field of medicine. Classic examples of these toxins are Botox (produced by *Clostridium botulinum*), which is used mainly commercially in facial remodeling but can also be used for the treatment of migraines, excessive perspiration, brain and spinal cord disorders, and disorders of the prostate and bladder. Toxins, to some extent, are also safe for use in agrochemicals. For instance, selenium when administered at an appropriate dosage is a powerful antioxidant, but it can easily function as a neurotoxin at higher dosages. Another classic example is the mycotoxin avermectin, which is currently used as an insecticide and for the control of nematode parasites of domestic animals [6]. Inactive toxins (toxoids) are also useful in public health as vaccines.

According to recent studies, it has been discovered that phytotoxins as a group have no common structural features. They belong to such diverse classes as peptides

Table 16.1 Common phytotoxins present in plant families.

Family	Toxin	Crop plants
Leguminosae	Protease inhibitors	Pigeon pea, chickpea, lentils, lima beans, peas, and cowpea
Solanaceae	Glycoalkaloids	Potato, eggplant, apples, bell peppers, cherries, sugar beet, and tomatoes
Rosaceae	Cyanogenic glycosides	Almond, cherry, peach, apple, plum

(or other derivatives of amino acids), terpenoids, glycosides, phenolics, polyacetates, α-pyrone derivatives, and polysaccharides, or a combination of these classes and several others. Table 16.1 shows common phytotoxins in plants by family.

16.3 Methods Generally Used for Phytotoxin Detection

There are various methods used to detect phytotoxins in different microorganisms (mainly Fungi and Bacteria). Table 16.2 shows some classic examples of detection methods for phytotoxins.

16.3.1 Biological Method Review of Detecting Phytotoxins

In this section, the methods of phytotoxin detection that have been developed and undertaken by other authors [18] are evaluated.

16.3.1.1 Bacterial Strains and Growth Conditions

The bacterial strains used in this study were *Photorhabdus* (*Xenorhabdus*) *luminescens* primary and secondary forms (provided by K. H. Nealson, University of Wisconsin, Milwaukee, WI), *Citrobacter freundii* (American Type Culture Collection 33128), *Escherichia coli* B (Coli Genetic Stock Center 5713), and clinical isolates (provided by N. Bishop, California State University, Northridge, CA) of *Staphylococcus aureus, Staphylococcus epidermidis*, and *Enterococcus faecalis*.

The strains were cultured on tryptic soy agar and were incubated for continuous proliferation for 2 days at 30°C under ambient air conditions using protease/protease inhibitor medium.

16.3.2 Chemical and Microbiological Reagents

Standard chemical reagents used in this method were from Sigma-Aldrich (St. Louis, MO) and microbiological medium components were from Becton Dickinson (Sparks, MD).

16.3.2.1 Dye-Containing Casein Medium

The casein medium was based on that described by Vijayaraghavan and Vincent [19]. The medium consisted of 5 g peptone, 1.5 g yeast extract, 1.5 g sodium chloride per liter, and 0.5% casein (instead of 1.0% [19]), dissolved as described by Montville [20, 21] in 0.02 M NaOH. Bromocresol green (BCG) (0.0015%) was added directly to the medium afterwards.

It is important to note that both BCG pre-incorporated into the medium as well as post incubation addition of a BCG dye reagent for 1–2 hours containing 0.028%

Table 16.2 Classic examples of methods used to detect phytotoxins.

Source of Toxin	Disease	Effect on plant	Type of assay	Reference
Ceratotoxin gene family	Dutch elm disease	Wilt, yellowing, death	Water conductivity in stems	[5]
Fusicoccum amygdali	Wilt of stone fruits	Wilt	Stem collapse Adverse effects on stomatal openings	[7, 8]
Helminthosporium sacchari	Eye spot disease of sugar cane	Streaks on leaves, necrosis	Leaf streaks Leakage of electrolytes	[9, 10]
Alternaria alternata f.sp. *lycopersici*	Stem canker of tomato	Cankers on stems and chlorosis of leaves	Detached leaves	[11]
Pseudomonas phaseolicola	Halo blight of beans	Chlorosis	Chlorosis on leaves Inhibition of *Escherichia coli*	[12, 13]
Phyllosticta maydis	Yellow leaf blight of corn	Yellowing, necrosis	Inhibition of root and shoot growth	[13]
Helminthosporium maydis	Southern corn leaf blight	Yellowing, necrosis	Local lesions on leaves CO_2 fixation Pollen growth inhibition Mitochondrial oxidation	[14–17]

BCG were dissolved in 0.56% (w/v) succinic acid, 0.1% (w/v) NaOH [19] with 0.6% Brij-35 [22] acidified to pH 4.2 with HCl.

This dye reagent was used following exposure to a protease inhibitor to observe bacterial growth. After primary staining and then marking the agar with a 23 gauge syringe needle dipped in India ink at a corresponding graduation mark on the plastic Petri plate, the agar was de-stained in water by completely removing it from the lower plastic dish.

Ponseau S (PS) was also used in this study for staining by flooding the plate with 5 ml of the stain mixture containing 0.1% PS and 5% acetic acid in water for 1–2 hours depending on the staining protocol used. The agar was subsequently removed from the plate and de-stained overnight in water. The plates were then viewed under a microscope for the presence and absence of stained protein.

16.3.2.2 Dye-Containing Gelatin Medium

This medium was prepared based on the method described by Medina and Baresi [23].

Tryptic soy agar powder (40 g/l) without glucose was modified to contain 8 g of gelatin (instead of 16 g [21]) per liter, and approximately 5 ml of 20% trichloroacetic acid (TCA; Thermo Fisher, Waltham, MA) was used for protein precipitation per Petri plate. The developer of this medium preparation protocol also worked with pre-incorporated BCG, or post-incubation BCG dye reagent and PS for the casein plates, each as described above.

16.4 Protease Inhibition Detection Protocol

This comprehensive protocol was developed by Quintero and Bermudes [18] in a series of three main steps, as described below. Because the protocol destroys the assayed plate, a duplicate plate should also be used and retained for further analysis of the strains if necessary.

16.4.1 Exposure of the Protease Detection Plate to a Protease Inhibitor or Bacterial Growth (Step 1)

We used either commercially available protease inhibitor peptides or growth of bacterial strains on the protease detection plates in this experiment. The protease inhibitors were α_2-macroglobulin (7.8 mg/ml in sterile water; Thermo Fisher), aprotinin (0.3 mM in sterile distilled water [dH_2O]; Thermo Fisher), leupeptin (10 mM in sterile dH_2O; Thermo Fisher), and bestatin (1 mM in methanol; Thermo Fisher). These were pipetted as 2.0 µl drops onto the surface of the protein-containing plates and allowed to be absorbed into the plate and diffuse for 1 hour.

The bacterial strains *P. (Xenorhabdus) luminescence* strain Hm primary and secondary forms, *E. coli* B, *C. freundii*, *S. aureus*, *S. epidermidis*, and *E. faecalis* were allowed to grow for 2 days at 30°C. Following incubation, the bacterial strains were washed off the plate using a gentle stream of water in order to remove the colonies and eliminate their potential for surface inhibition effects on diffusion of the protease and/or dyes.

16.4.2 Exposure to a Protease-Containing Solution (Step 2)

Preliminary studies showed that casein-containing plates required a higher concentration of trypsin to achieve clearing: we investigated trypsin concentrations as high as 2.5 mg/ml. For most of the experiments, the plates were flooded with either 1.0 ml of sterile-filtered 0.625 mg/ml trypsin or 0.0625 mg/ml trypsin in 10 mM Tris 1 mM ethylenediaminetetraacetic acid (pH 8.0) for casein and gelatin, respectively. Following trypsin adsorption (20–60 minutes), an additional 1 ml of sterile filtered water containing 2.5 mg carbenicillin and 750 μg streptomycin was also allowed to absorb into the plate to stop any further bacterial growth on plates that had contained bacterial colonies. The trypsin or trypsin/antibiotics were then incubated at 37°C overnight.

16.4.3 Detecting Zones of Protease Inhibition (Step 3)

The zones of protease inhibition were observed by the presence of opaque or dye-stained zones surrounded by clear zones as detected by TCA precipitation, by BCG previously incorporated into the medium, or by the addition BCG or PS dyes. Radial diffusion was measured in triplicate, entered into Microsoft Excel, plotted, and then analyzed using linear regression.

16.5 Isolation of Phytotoxins from Microorganisms

Phytotoxins are isolated from microorganisms that are grown under culture conditions. In culture, fungi and bacteria produce trace amounts of phytotoxins of up to 2 g of toxin per liter under optimum conditions. Ideally, synthetic media are the most desirable as a starting source for extracting toxins since various plant or animal concoctions in the medium affect the purification procedures. On the other hand, plant extracts are sometimes necessary for optimum fungal growth and toxin production [9].

16.5.1 Detection of Phytotoxins Isolated from Fungi

The pathogenic fungi and bacteria of many plants produce one or more phytotoxins. Some of the most notable fungal toxin producers are species of *Alternaria*, *Helminthosporium, Rhynchosporium, Fusicoccum, Ceratocystis,* and *Stemphyllium*. Toxins have not been isolated and characterized from the fungi that cause rust, smut, and mildew diseases [9]. *Xanthomonas, Pseudomonas, Rhizobium*, and *Corynebacterium* are the most common bacteria associated with plants that produce phytotoxins. Whether a toxin is involved in a disease can be determined by examining symptoms developing in the plant. Yellowing, wilting, brightly colored lesions, and necrosis are commonly caused by phytotoxins. If symptoms are produced at sites far removed from the pathogen, a phytotoxin could be involved in the disease.

16.5.2 Purification of the Extracted Phytotoxins

Purification of a phytotoxin to homogeneity relies heavily upon the availability of a quantitative bioassay technique. Once the parasite is removed from the culture medium by filtration or centrifugation, substances can be separated on the basis of their molecular size by solvent precipitation or chromatography over molecular sieves. For relatively small phytotoxins, direct solvent extraction with subsequent purification on high-performance liquid chromatography, thin layer chromatography, or other chromatographic techniques can be used. Most often, the biological activity of the molecule is the principal means by which it is followed through the steps of purification until some idea of its chemical nature is obtained, at which time appropriate colorimetric and chemical detection procedures can be employed. The biological activity of a phytotoxin can be measured in a number of ways depending on the apparent effect it has on the plant. The more quantitative assays, and usually the most preferred, are those in which the toxin lesion is measured directly, e.g. by plasmalemma disruption as determined by electrolytic leakage or membrane depolarization. Many of the assays in which leaf wilt, necrosis, discoloration, or chlorosis are measured provide only semiquantitative estimates of biological activity.

16.6 Conclusion

Research literature on the detailed methods pertaining to the detection of phytotoxins is very difficult to find, mainly because this area of research is clearly understudied even though several articles are available on the harmful effects of phytotoxins on plant families. Even articles such as those on bacterial cultures for

the determination of the production of secreted protease inhibitors and the detection of phytotoxins isolated from fungi are not well reviewed for clarity and implementation in research laboratories.

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17

Categorization, Management, and Regulation of Potentially Weaponizable Toxic Plants

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CONTENTS

17.1 Introduction

Phytochemicals (from Greek *phyto*, meaning "plant") are the chemical compounds produced naturally by plants for their own requirements. They play a part in plant growth and defense. Plants produce some toxins naturally for self-defense against insect consumers and predators. They also produce toxins for protection against

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microbial and fungal infestation in variable and extreme climatic conditions such as drought or humidity [1]. Waterbodies are another important natural source of toxins to humans and land animals, but not to aquatic organisms such as fish and shellfish. The microscopic algae and plankton in lakes, ponds, and oceans produce toxic chemical compounds that are consumed by fish and aquatic animals. When these fish and aquatic animals are, in turn, eaten by humans, they can cause toxicity that can lead to serious morbidity and mortality [2]. Phytochemicals are considered as research chemicals that are not essential nutrients as no research has proven their health benefits [3]. Phytochemicals are categorized into two major groups, i.e. carotenoids and polyphenols, which include flavonoids, stilbenes/lignans, and phenolic acids. These two major groups can be further divided into groups based on similarities in chemical structure. Flavonoids are classified as flavanones, anthocyanins, isoflavones, flavones, and flavanols. Flavanols also have subclasses, such as catechins, epicatechins, and proanthocyanidins [4]. For study purposes, researchers extract and then isolate phytochemicals from plants. Then, structural studies are performed in laboratory model systems, which include culture of cells and in vitro or in vivo experimentation. There are many challenges in this process, including compound isolation and the specific structural determination of the compounds as well as identifying bioactives [2]. Phytochemicals are not only used as nutrient supplements and in traditional medicine; they are also poisons. Various phytochemicals are proven toxins that cause lethal effects in humans, e.g. a carcinogenic phytotoxin – aristolochic acid – causes cancers even at low doses. Some are pro-oxidant phytochemicals, such as polyphenols and flavonoids in high doses. Others inhibit nutrient absorption, and thus are labeled as anti-nutrients [3].

17.2 Management of Weaponized Natural Food Agents

The World Health Organization (WHO), in collaboration with the Food and Agriculture Organization of the United Nations (FAO), is responsible for assessing the risk of natural toxins to humans by monitoring food contamination; these organizations are also responsible for protecting aquatic life against toxins. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) assesses the risk of natural toxins to food and its recommendations are followed by governments as well as by the intergovernmental standard-setting body for food, the Codex Alimentarius Commission. The Codex standards are international standards governing the provision of food at the national level as well as food trading. These standards are used to ensure safety and good quality standards in food for purchase and consumption, regardless of the food production area.

Groups of scientific experts, such as JECFA or ad hoc FAO/WHO committees, include internationally recognized professionals who perform scientific reviews

of research studies and their related data on particular natural toxins. The outputs of these health risk estimations include the highest tolerable ingestion (exposure) level and, in contrast, raising health concerns, including suggestions on risk management procedures to avoid and manage contamination, as well as control activities, monitoring, and analytical methods.

Exposure to natural toxins should be avoided and/or regulated to keep people safe. Natural toxins not only present a risk to the well-being of humans and livestock, but also affect food safety and nutritional value by exposing consumers to unsafe food. National authorities should be motivated by WHO to supervise and ensure that levels of those food substances that contain some natural toxins are kept below the levels stated in their respective local and regional specifications and legislation.

17.3 Techniques Used for Extraction, Segregation, and Decontamination of Phytochemicals

It is essential that people are trained to use the correct processes and equipment to detect and identify toxic agents. Safety measures and health security need to be provided by laboratories. Moreover, awareness regarding health and safety is essential [5]. Each program should consist of: (i) designated health and safety officers, (ii) formal written health and safety plans, (iii) ongoing training programs, and (iv) periodic inspections of emergency equipment and safety violations.

17.3.1 Solvent-Based Extraction of Phenolic Compounds

Researchers have examined the effects of various kinds of solvents, such as methanol and ethyl alcohol, to extract antioxidant compounds from different parts of plants. For example, methanol is a polar molecule and thus has a higher affinity for antioxidants than other solvents. Many solvents can be used to obtain phytochemicals, and commonly biologically active components are extracted from the dry powder form of plants. To extract bioactive compounds from plants, the solvents are selected on the basis of their affinity for the solute that is to be separated. When the polarity of the solute and the solvent are the same, a larger proportion of the solute will dissolve in the solvent. The polarity of some solvents is: hexane < chloroform < ethyl acetate < acetone < methanol < water.

17.3.2 Microwave-Associated Extraction

Microwaves are a form of electromagnetic radiation with frequencies between 300 MHz and 300 GHz and wavelengths between 1 cm and 1 m. Objects are, initially, heated by microwaves because the objects absorb the

electric and magnetic waves and convert them into heat. Microwave-based extraction is a method that minimizes the destruction of bioactive compounds and is especially useful for medicinal plants. Also, this form of extraction is more useful than conventional methods because it is faster and uses less solvent [6]. The purpose of microwave-associated extraction (MAE) is to heat up the solute and solvent and then extract the active ingredients from medicinal herbs.

The effectiveness of MAE can be altered by changing some conditions, such as temperature, solvent, and time of extraction. For example, it has been found that 170 °C is a very suitable temperature for extraction of phenolic substances from Chinese tea. MAE has multiple benefits and has a high affinity for many compounds of interest, as described by Williams et al. [7]

17.3.3 Ultrasound-Assisted Extraction

Ultrasonic techniques are used in food processing technology to obtain active ingredients from plants [7]. Plant cell walls are damaged by ultrasound waves with a frequency higher than 20 kHz. This increases the ability of solvents to access the cells and also increases the amount of bioactive compound extracted. Ultrasound-assisted extraction is a very easy and simple technique. In this approach a macerated sample is mixed with the appropriate solvent and placed in an ultrasonic bath; the extraction time and temperature are monitored [8]. Usually, an ultrasonic bath and ultrasonic probe system are used in this method. Albu et al. [9] used an ultrasound-based technique to obtain phenolic compounds from rosemary. The outcomes proved that ultrasound-assisted extraction is a very successful method of extraction.

17.4 Techniques for Identification of Bioactive Compounds

17.4.1 Ultraviolet–Visible Spectroscopy

Ultraviolet (UV)–visible spectroscopy can be used to identify the category of bioactive compounds, but mostly it is used to assess the quality of aromatic compounds. Phenolic molecules such as anthocyanins and tannins can bind with iron, enabling them to be identified by (UV–Vis) spectroscopy [10]. UV–Vis spectroscopy is also used to assess the total phenolic extraction yield. This is a time-saving approach and is less expensive than nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and mass spectroscopy, among other techniques [11].

17.4.2 Infrared Spectroscopy

In this process, some of the frequencies within the infrared spectrum are absorbed by organic molecules subjected to infrared radiation. In contrast, other frequencies are transferred through the organic compounds, so no absorption takes place. Absorption of infrared radiation is associated with vibrational alterations that occur in the compounds. This is why infrared spectroscopy is also called vibrational spectroscopy. Different bonds (C—C, C═C, C≡C, C—O, C═O, O—H, and N—H) have different frequencies of vibration. If these bonds are present in organic compounds, they can be identified by assessing the specific frequency absorption band in the infrared spectrum [11]. FTIR spectroscopy is a method used to recognize the chemical species in a compound.

17.4.3 Nuclear Magnetic Resonance Spectroscopy

NMR uses the magnetic features of some atomic nuclei, such as the hydrogen atom nucleus, carbon, and carbon isotopes. NMR spectroscopy is used to identify molecules by evaluating the variations among the different magnetic nuclei. It also indicates which atoms are found in nearby groups. Different methods such as thin-layer chromatography, liquid chromatography, and column chromatography were used in the past to segregate phenolic compounds, but the structures of these compounds were identified by NMR [10].

17.4.4 Mass Spectrometry

In mass spectrometry bioactive compounds are bombarded by electrons. This changes atoms into ions, which have high energy. A mass spectrum is a graphical representation of the relative amounts of disjointed ions as well as the mass to charge ratio of such ions. It is possible to determine molecular weight and molecular formula by mass spectrometry, providing structural information about compounds. The combined use of high-performance liquid chromatography and mass spectrometry provides a faster and more reliable method of detection of chemical substances in medicinal plants.

17.5 Types of Natural Phytotoxins

17.5.1 Aquatic Biotoxins

Algae inhabit fresh water as well as oceans and other waterbodies and produce toxins called algal phytotoxins. When some particular species of algae produce blooms over the surface of waterbodies, they prevent the evaporation of chemical

substances produced underwater by aquatic organisms and the absorption of compounds into the water from the air. This means that absorbing and producing chemical substances is the mechanism by which algae produce toxins, e.g. ciguatoxins produced by dinoflagellates can contaminate fish. Aquatic animals ingest these dissolved toxins, especially scallops, mussels, and oysters; the toxins are also ingested by fish but in comparatively smaller amounts. When these aquatic animals are, in turn, consumed by humans, the toxins gain entry into the human body and cause toxicity such as diarrhea, tingling, vomiting, and even paralysis.

17.5.2 Glycosides

Cyanogenic glycosides are among the phytotoxins that occur in about 2000 species of plants, with the majority of these plants being used as food by humans in many parts of world; for example, almonds, stone fruits, sorghum, bamboo roots, and cassava are commonly consumed items containing cyanogenic glycosides. The concentration of cyanide present in a cyanogenic plant is related to its toxicity in humans. The signs and symptoms of cyanogenic toxicity are hypotension, headache, dizziness, dyspnea, cyanosis, confusion, and even coma and death at high-dose ingestion [12].

17.5.3 Other Common Phytotoxins

Parsnips, citrus plants, celery roots, and various medicinal plants contain toxins known as furocoumarins. These toxins are secreted by plants during damage or injury to the plant or during stress. Furocoumarins disturb the normal function of the digestive tract and also cause topical reactions when in contact with the skin or during exposure to sunlight in susceptible people. Lectins are another group of phytotoxins found in beans; the highest concentrations are in kidney beans, especially the red ones [13]. Using beans after drying, overnight soaking, and boiling prevents lectin poisoning. Canned beans are safe and do not contain lectins.

Edible species of mushrooms are also poisonous and produce toxins such as muscimol and muscarine. Clinical features such as vomiting, confusion, diarrhea, salivation, visual disturbances, and hallucinations are present within 6–24 hours of ingestion. If symptoms are delayed, then ingestion of these toxins can be fatal as they can cause damage to the kidneys, liver, and central nervous system [14]. Mycotoxins are toxic chemical substances produced by fungi, especially molds [15]. Because of their extensive hyphae and the resistance of their spores to such conditions as heat, molds have widespread and successful growth on food materials such as fruits, breads, cereals, and even spices. They can attack crops in fields or stores, particularly in the humid, warm conditions that occur in the rainy season. Being stable and resistant to heat treatment, they are very difficult to remove from foodstuffs. Mycotoxins can cause acute symptoms, and sometimes even lead to death [16]. Numerous natural chemical compounds that are present in various commonly consumed plants are potent tumor promoters or carcinogens. Examples

include capsaicin, found in capsicum; cycasin, found in the fruits, seeds, and roots of cycad plants; phytoestrogens, found in red clover, fava beans, and soybeans; and ptaquiloside, found in bracken fern [17].

17.6 Conclusion

The chemical substances produced by plants, known as phytochemicals, have been demonstrated to promote good health in humans when they are consumed as, for example, fruits and vegetables. Research studies have revealed the anticancer and anti-inflammatory potential of phytochemicals, and especially their antioxidant properties. In addition to the valuable advantages of phytochemicals, they can also have negative effects. They may be toxic to humans and livestock, such as carcinogens or tumor promoters; they may cause mental instability; and they may cause high rates of mortality and morbidity. Research organizations should investigate such phytochemicals and issue warnings about any hazards associated with their use or consumption. Consumers should take account of the safety and toxicity of the constituents of these products, and manufacturers should not make claims about the safety of supplements containing phytotoxins without any post-market surveillance.

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18

In Silico Modeling as a Tool to Predict and Characterize Plant Toxicity

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18.1 Introduction

The search for new lead compounds in drug discovery has recently focused on plants as a natural reservoir. However, the drug development pipeline has high attrition rates for the following reasons. First, a vast number of compounds are dropped before the preclinical stage of development; in fact, less than 5% of those compounds that undergo preclinical trials become registered medicines [1, 2]. Second, the costs of drug development are very high; for example, in 2013, the estimated cost of successful drug development ranged from US$1.3 to US$1.7 billion [3]. Third, the average time taken to fully develop a drug is about 12 years, which is a long time, and failures can occur in very late stages. Therefore, there is a need to employ a new technology that will prevent all the aforementioned adverse effects.

By determining the toxicity of compounds among other properties in the early stages of drug development, money and time can be saved on a project that might eventually fail. In vitro and in vivo experimental approaches have traditionally been used to determine the toxicity of compounds. Animals and other organisms or tissue cells are utilized to observe the toxic effects of a compound or drug; these experimental approaches take place in wet laboratories. Most electronic library repositories and databases used in biology and biological chemistry contain experimental data that have been derived through experiments carried out in wet laboratories. There are several factors that are associated with in vitro and in vivo toxicity assessment, the most important of these are the need to restrict the use of animals in toxicity studies, the high cost implications, and the long periods of time required to carry out these investigations [4]. These hindrances encountered in wet laboratories can be addressed by the use of computational in silico experiments. Experiments carried out on computers gave rise to the term dry laboratories.

The basic definition of the term "in silico" is any process carried out on or by means of a computer. In silico toxicity, therefore, is a method of assessing the harmful effects of chemical compounds in animals through modeling, visualization, simulation, and prediction of experimental data on computers [5, 6]. Therefore, this chapter elucidates the novel techniques that can be used as alternative toxicity methods and that have enormously reduced the need to use animals in toxicity studies. Moreover, the advantages and disadvantages of these in silico techniques as well as some recommendations are discussed.

18.2 Components of In Silico Toxicity Methods

Robust in silico toxicity models are built using a blend of several computer platforms with different strengths and weaknesses to ensure an output model that best describes the toxicity of the compound in question. The following components are the minimum requirements to consider when designing an in silico toxicity model.

18.2.1 Databases

In silico methods rely on large volumes of chemical compound and toxicity data, which are stored in various databases. Different databases exist for different purposes: in silico plant toxicity modeling uses databases that store plant toxicity data. These include compounds that have had their toxicity endpoints experimentally determined as well as chemical data on those compounds from the literature. The databases may also include structural information on chemical compounds. An example of a database defined to contain toxic plant data is the "Toxic Plants–Phytotoxins" database. This database currently stores thousands of phytotoxins, which are characterized by ecotoxicological relevance mainly in Central Europe and linked to 844 plant species [7]. ChemSpider® is an example of a useful database that enables users to access over 65 million structures across different libraries. Structural information also includes other properties such as physical and chemical properties, stereochemistry, spectral data, and three-dimensional visualizations.

18.2.2 Molecular Descriptors

The properties of a chemical compound are determined by its chemical structure, physical attributes, chemical profile, and biological properties [8]. The characterization of these properties and how they relate to toxicity is what forms the descriptors of the compound under study [9]. Algorithms and software have been built to calculate molecular descriptors. Table 18.1 includes some common free software (web and standalone) that can be used to calculate molecular descriptors of different types.

18.2.3 Toxicity Models and Modeling Software

In toxicity assessment, prediction models constitute the major basis for the determination of an accurate description of toxic endpoints associated with a compound of interest. While different models exist in toxicology, developing prediction in assessing plants depends on the stage at which the plant is being studied. Modeling methods most applicable in prediction plant toxicities are discussed later in the chapter.

18.2.4 Simulation Packages

Models are simulated to approximate observations that are then used to make an accurate prediction. Simulations are run on computer-aided software and programing languages. The choice of simulation package depends on the model and type of dataset fitted into the model. Pharmacokinetics–pharmacodynamics (PKPD) models use mathematical equations to describe the biological fate of drugs, e.g. Berkeley Madonna. These models require geographical simulations. In silico models discussed in this chapter do not require robust geographical visualizations

Table 18.1 Workflow involved when designing in silico toxicity prediction models.

Software	Number of descriptors	Types of descriptors
ADAPT	Over 260	Topological, geometrical, electronic, physicochemical
ADMET Predictor	297	Constitutional, functional group counts, topological E-state, Moriguchi descriptors, Meylan flags, electronic properties, 3D descriptors, H-bonding, acid–base ionization, empirical state of quantum descriptors
ADRIANA Code	1244	Global physicochemical descriptors, atom-weighted 2D and 3D autocorrelations and RDF, surface property-weighted autocorrelations
CODESSA	1500	Constitutional, topological, geometric, charge-related, semiempirical, thermodynamic
DRAGON	5270	Constitutional, topological, 2D autocorrelations, geometric, RDF, functional group properties, 2D binary and 2D frequency fingerprints
GRID	Undefined	Molecular interaction fields
ISIDA Fragmenter	Structural data	Substructural molecular fragments, property-labeled fragments
JOE Lib	Over 40	Counting, topological, geometric properties
MARUIN Beans	Over 499	Physicochemical, topological, geometric, fingerprints
MOE	Over 300	Topological, physical properties, structural keys
MOLCONN-Z	Over 40	Topological
MOLGEN-QSPR	707	Constitutional, topological, geometric
PADEL-descriptor	863	Constitutional, WHIM, topological, fingerprints
POWER MV	Over 1000	Constitutional, atom pairs, fingerprints
PreADMET	955	Constitutional, topological, geometric, physicochemical
SARCHITECT	1084	Constitutional, 2D and 3D descriptors

2D, two-dimensional; 3D, three-dimensional; RDF, radial distribution functions.

but simple standalone software. There are different simulation packages available to use for computational toxicity tests.

18.3 Modeling Methods

In silico modeling methods that enable the prediction of plant toxicities are those that can ably predict the toxicities of plant metabolites individually and synergistically. Pharmaceutical research on plants has focused on metabolite characterization, compound isolation, structural elucidation, and the evaluation of antimicrobial, antimalarial, and antioxidant activities to identify lead compounds for clinical testing. The toxicity of an identified lead compound is a check point for continuity in the quest for new drugs [10]. In silico modeling methods of compounds require simple modeling approaches to provide toxicity endpoint predictions while advanced and complex modeling approaches such as PKPD and concentration–time-based models best fit a clinical trial set-up in which plant toxicity information is required to guide plant-based drug discovery research in clinical trials.

18.4 Structural Alerts/Rule Based

The in silico models are based on toxic fragments called toxicophores. They work on the principle that the toxicophores are directly linked to a specific toxicity endpoint and a specific compound that contains such toxicophores [8]. Structural alerts may be connected to a single chemical or more than one chemical. Structural alerts are integrated in rule-based models: the "IF THEN" conditional computer programming code is a simple analogy of how structural alerts are used in rule-based models. A rule could be set that "IF" a specific chemical is found in a substance "THEN" a specific toxicity endpoint is likely to be an outcome.

There are two types of rule-based models, depending on how they are generated. Human-based rules (HBRs) are derived from literature mining or from experts' recommendations. Induction-based rules (IBRs) are computationally derived from datasets. IBRs demand a high application of statistics and probabilities; from this perspective, HBRs are more accurate. However, IBRs can be generated more rapidly and, with advances in machine language and deep learning, the predictions obtained using IBRs will eventually attain a high level of accuracy in predicting toxicity endpoints from datasets. The applicability of structural alerts and rule-based models in plant toxicity is limited to metabolites. For plants whose metabolites have been isolated in pure form, a combination of HBRs and IBRs would be ideal to guide the direction of the search for novel drug molecules.

It has been discovered that structural alerts are easy to understand and implement [11]. They play a crucial role in the design of drugs and help to decide whether a drug should be changed or modified in order to minimize the level of toxicity. The application of structure-based techniques in prediction enables the structure of significant metabolites to be recognized [4]. The disadvantages of structural alerts include the utilization of binary features, such as the absence or presence of chemical structures and quantitative endpoints that may be non-carcinogenic or carcinogenic [12]. Moreover, it has been discovered that structural alerts do not provide a clear understanding of the biological pathways at the level of the toxicity, and so they might not be adequate for the determination of toxicity. Also, with the availability of some particular chemical properties, toxicity may reduce or increase [11]. Furthermore, the total number of rules and structural alerts may not be sufficient, which might lead to a large number of false-negative results for toxic chemicals indicated as non-toxic during predictions [11–13].

It is very important during the development of models to ensure that rules and structural alerts are comprehensive and sophisticated, especially in the presence of more experimental data. It is very important to maintain a balance between the rule and the list of structural alerts [14]. Furthermore, the level of diversity in the structural alerts and rules varies: they can be utilized for numerous chemicals but there is a tendency to enhance the number of false-positive results obtained, which may result in the prediction of non-toxic chemicals as being toxic. However, they may be utilized for a minimal group of chemicals if the model capacity is too narrow, which may also lead to a high number of negative results.

Some of the examples of structural alerts enlisted for skin sensitization are available while the prediction of mutagenicity and carcinogenicity was suggested by Ashby and Tennant [15–17] in 1988 in the Organisation for Economic Co-operation and Development (OECD), Toxtree, Quantitative Structure–Activity Relationship tool box. The structural alerts and rule-based models were also created for skin sensitization [16, 18], hepatotoxicity [19], irritation/corrosion of the skin [6] and eyes [20], and cytotoxicity [21]. Some other models include several systems referred to as expert systems. These give structural alerts and pre-built rule-based lists; for example, Hazard Expert [11, 22] and Toxtree [11, 23–27]. There is some other software used to review the level of toxicity present in the structural alerts, including categorical–structure activity relationships [28], computer-assisted structure elucidation [29], and prediction of activity spectra for substances [30]. Moreover, some other algorithms that might be used to remove the most frequent molecular substructures are Apriori, which depends on a breadth-first search, and Pattern Growth, which depends on a depth-first search. Some other algorithms include Graph/Sequence/Tree Extraction [31], graph-based substructure pattern mining [32], fast frequent subgraph mining [33], and molecular fragment miner [2].

18.5 Statistical Structure-Based Activity Relationship Models

The quantitative structure–activity relationship (QSAR) model is the most popular and widely used model, and many research papers and reviews have been published on it. The methodology is mathematical in nature. It uses a statistical model derived from a large dataset comprising a training set and test data of example chemicals [34]. The model uses logit regression modeling, in which predictions are made according to two states of yes or no, active or not active, or positive or negative data structures. At a certain level of probability, the model decides to make a prediction between the two states, based on mathematical calculations and molecular descriptors that are used to establish a relationship between the descriptors and the toxicological outcome. The data to be used in the training set include chemicals that were experimentally determined to be toxic or non-toxic in traditional toxicology tests. Molecular descriptors are integrated for the test dataset chemicals and then the model generates a prediction. To achieve an acceptable prediction, the test chemicals must possess properties that are congruent with the training set chemicals. With access to large reliable databases containing chemicals and their toxicities, the toxicities of plant metabolites may be predicted by means of this method using packages such as R and Python. R and Python software and programming languages offer powerful packages and modules to work with large datasets that are easily manipulated and modeled using codes that allow the incorporation of model parameters; in this case, molecular descriptors. Other existing QSAR software includes the TOPKAT and ADMET predictors. QSAR models are used by regulatory organizations such as the European Chemicals Agency and OECD, with the latter being a consortium of over 34 countries [9, 35].

18.5.1 Read-Across

The read-across (RA) method has two key parts: analogs, or the reference used as the source of toxicity information, and target, the compound being predicted. RA screens through a pool of chemicals to predict the toxicity of a query chemical. The methods establish a structural resemblance between the analog chemical and the query chemical; in most cases, the structure of the chemicals and their biological fate are co-variates. RA takes experimental data from analogs to ascribe similarity to a query chemical in terms of their chemical reactivity, mode of action, structure, physicochemical properties, and biological fate.

RA can be either quantitative [1, 12, 14] or qualitative, provided the level of the toxicity endpoint is qualitative. Also, the utilization of interpolation, which deals with the source chemical in relation to the targeted chemical, is preferable to extrapolation from one side [36, 37].

The recognition of chemicals that have the same configuration can be carried out in two different ways, including exhibiting chemicals as vectors of chemical features and then evaluating the similarity between the chemicals. The first step involves performing holographic fingerprints, which is based on the frequency of properties of chemicals, while a binary fingerprint indicates the absence or presence of certain chemical groups [38, 39]. Some chemical features such as melting points could be determined as well. A hierarchy of categories and subcategories exhibited better performance than a single feature vector. Each stage of the hierarchy exhibits features that are used in the formation of a category. The category is later separated into subcategories by utilizing some other features that are available [2]. This hierarchy enables the evaluation of features and can streamline model elucidation [38]. The statistical similarity between two different chemicals can be assessed by using various types of distances such as Tanimoto, Hamming, Cosine, Mahalanobis, Euclidean, or linear or nonlinear relationships between the structures [38, 40]. Some merits of RA are that it includes different types of descriptors, similarity measures can be utilized to show common features between chemicals [38], it is transparent [41], implementation and interpretation are very easy [39], and it can be used to model qualitative and quantitative toxicity endpoints [39].

Some of the disadvantages of RA include that it makes use of only small datasets in comparison with other techniques such as QSAR because of the presence of only a few analogs for a given chemical [38]. The level of accuracy is determined by the similarity metrics, the chemical properties, the number and choice of analogs, the category boundaries, and the strength of similarity between the chemicals. These features are very independent, endpoint-specific, and mutually dependent, where also the opinion of a specialist in this area may be required [37–40, 42]. This technique does not give accurate information about the biological levels of toxicity. The level of accuracy in this technique might be unreliable if the analogs have conflicting toxicity profiles [43] or if the number of analog chemicals is not adequate; in this case, QSAR gives a more accurate result [9, 12, 39, 40, 42, 43].

RA could be used to predict environmental toxicity [44], carcinogenicity [45], skin sensitization [46], aquatic toxicity [47], reproductive toxicity [48], and hepatoxicity [19]. Examples of tools that utilize RA include AmbitDiscovery [49], ChemIDplus [50], OECD QSAR Toolbox [51], DSSTox [52], Toxmatch [53], AIM [54], and Toxtree [23].

18.6 Conclusion

This chapter has discussed how in silico plant toxicity predictions can reinforce traditional toxicity testing methods, which are deemed expensive, invasive, and time-consuming. The application and consumption of plant-based products as fully processed or semiprocessed medicinal herbs and nutraceuticals have risen

dramatically, driven by a "natural is safe" misconception, so regulation on the use of plant-based products according to toxicity information is long overdue. A gap exists in toxicity assessment that the in silico methods discussed in this chapter are now starting to fill. Further, the in silico prediction of plant toxicity is essential in guiding drug discovery from plants. There is a need for governments to support the policy and the application of bioinformatics as sustainable tools that could minimize the huge amounts of money used during the validation of drug toxicity. This will be in the form of training young scientists, the use of software that deals with the in silico evaluation of plant toxicities, and the creation of curricula that will cover various applications of bioinformatics, especially for the determination of plant toxicities. Moreover, combining all the advantages of dry and wet laboratories will address several challenges encountered during toxicity testing and validation of the potency of drugs, especially those derived from plants.

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