

## 2

## Quality Control, Screening, Toxicity, and Regulation of Herbal Drugs

*Wickramasinghe M. Bandaranayake*

### Summary

Medicinal plants constitute a source of raw materials for both traditional systems of medicine (e.g. Ayurvedic, Chinese, Unani, Homeopathy, and Siddha) and modern medicine. Nowadays, plant materials are employed throughout the industrialized and developing world as home remedies, over-the-counter drugs, and ingredients for the pharmaceutical industry. As such, they represent a substantial proportion of the global drug market. Most rural populations, especially in the developing world, depend on medicinal herbs as their main source of primary health care. Although most medicinal herbs are not, in their natural state, fit for administration, preparations suitable for administration are made according to pharmacopeia directions. The therapeutic potential of a herbal drug depends on its form: whether parts of a plant, or simple extracts, or isolated active constituents. Herbal remedies consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically.

The herbal drug preparation in its entirety is regarded as the active substance and the constituents are either of known therapeutic activity or are chemically defined substances or group of substances generally accepted to contribute substantially to the therapeutic activity of the drug. Phytochemical screening involves botanical identification, extraction with suitable solvents, purification, and characterization of the active constituents of pharmaceutical importance. Qualitative chemical examination employing different analytical techniques is conducted to detect and isolate the active constituent(s). In general, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being efficacious and safe. Ultimate proof of these can only be achieved by some form of clinical research. A defined and constant composition of the drug is therefore one of the most important prerequisites for any kind of clinical experiment.

Quality control for the efficacy and safety of herbal products is essential. The quality control of phytopharmaceuticals may be defined as the status of a drug, which is determined either by identity, purity, content, and other chemical, physical or biological properties, or by the manufacturing process. Compared with syn-

thetic drugs, the criteria and the approach for herbal drugs are much more complex.

Phytopharmaceuticals are always mixtures of many constituents and are therefore very variable and difficult to characterize. The active principle(s) in phytopharmaceuticals are not always known. The quality criteria for herbal drugs are based on a clear scientific definition of the raw material. Depending on the type of preparation, sensory properties, physical constants, moisture, ash content, solvent residues, and adulterations have to be checked to prove identity and purity. Microbiological contamination and foreign materials, such as heavy metals, pesticide residues, aflatoxins, and radioactivity, also need to be tested for. To prove the constant composition of herbal preparations, appropriate analytical methods have to be applied and different concepts have to be used in order to establish relevant criteria for uniformity.

Are there rigorous trials to show that herbal treatments work? With many of these herbal medicines we do not fully understand how they work. Nor do we always know which component is pharmacologically active. Even though herbal remedies may be effective, do their benefits outweigh their risks? In some countries herbal remedies are sold as food supplements, thus evading safety regulations. Can herbal medicines save money? Not all plant-based medicines are cheap.

Even though global herbal resources have a great potential as natural drugs and are of great commercial importance, they are very often procured and processed without any scientific evaluation, and launched onto the market without any mandatory safety and toxicology studies because there is no effective machinery to regulate manufacturing practices and quality standards. Although some herbal medicines are efficacious, there is unquestionably a need for more reliable information, a demand that must be met adequately by doctors, pharmacists, and other health care professionals.

Policy and regulation in their use, are two of the most sensitive aspects of developing and using plant-based medicines and health products. At present there is almost no policy worth its name to regulate the procurement and sale of medicinal plants in developing countries. Neither are the products derived from medicinal plants subject to control.

Stringent quality control should be enforced. Growing evidence of effectiveness is counterbalanced by inadequate regulation. The present review will address some of these issues.

## 2.1 Introduction

Since ancient times humanity has depended on the diversity of plant resources for food, clothing, shelter, and traditional medicine to cure myriads of ailments. Early humans recognized their dependence on nature in both health and illness. Physical evidence of the use of herbal remedies has been found from some 60 000 years ago in a burial site of a Neanderthal man uncovered in 1960 in a cave in northern

Iraq [1]. Here, scientists found great quantities of plant pollen, some of which came from medicinal plants still used today. The first written records detailing the use of herbs in the treatment of illness are in the form of Mesopotamian clay tablet writings and Egyptian papyrus [2]. Led by instinct, taste, and experience, primitive men and women treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Herbal medicine is the oldest form of health care known to humanity and has been used in all cultures throughout history. Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or nonactive, and also which combinations or processing methods had to be used to gain consistent and optimal results. Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopeias. Traditional medicine evolved over centuries, depending on local flora, culture, and religion [3–5]. Indeed, well into the twentieth century, much of the pharmacopeia of scientific medicine was derived from the herbal lore of native people. This knowledge of plant-based drugs developed gradually and was passed on, thus laying the foundation for many systems of traditional medicine all over the world.

Herbal medicine can broadly be classified into a few basic systems:

- Ayurvedic herbalism (derived from the Sanskrit word *ayurveda*, meaning “the science of life”), which originated in India more than 5000 years ago and was also practiced in neighboring countries such as Sri Lanka.
- Chinese herbalism, which is a part of traditional oriental medicine.
- African herbalism.
- Western herbalism, which originated from Greece and Rome and then spread to Europe and North and South America.

Chinese and Ayurvedic herbalism have developed into highly sophisticated systems of diagnosis and treatment over the centuries. Both have a long and impressive history of effectiveness. Western herbalism today is primarily a system of folk medicine. A European healing tradition, sometimes called the “wise woman” also focuses primarily on herbal healing.

Medicinal plants have played a key role in world health. They are distributed worldwide, but they are most abundant in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants [6–23].

By definition, a herb is a plant or a part of a plant valued for its medicinal, aromatic, or savoury qualities. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value. They are also referred to as botanicals, biomedicines, or herbal supplements. Herbal drugs range from parts of plants to isolated, purified active constituents. They may come from any part of the plant but are most commonly made from leaves, roots, bark seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied to the skin [24].

Typically, there is no one single herb that is recommended for a given health disorder; and there is no one single health disorder linked with just one single herb. Herbal products often contain a variety of biochemicals found naturally in the plants and many different biochemicals contribute to a plant's medicinal benefit. Chemicals known to have medicinal benefits are referred to as "active ingredients," and their presence depends on the plant species, the way the herb is prepared, the time and season of harvest, the type of soil, etc. Most herbal products contain plant parts or plant materials in the crude or processed state as active ingredients and certain excipients, such as solvents, diluents, or preservatives. In most cases, the active principles responsible for their pharmacological action are unknown.

A herb might be considered a "diluted" drug. To achieve the desired benefit, an individual must take an adequate amount over a certain length of time. Each herb is different. While some are safe and effective for specific uses, others are not. The general perception that herbal drugs are very safe and free from side effects is not true. Herbs can produce undesirable side effects and can be toxic. A particular plant part will have many constituents and some of them may well be toxic. However, it may take more to cause toxicity, because herbs usually are not as potent as manufactured drugs, and compared with synthetic drugs the adverse effects of most herbal drugs are relatively infrequent [25–27].

Herbal medicines are very different from well-defined synthetic drugs. For example, the availability and quality of the raw materials are frequently problematic; the active principles are frequently unknown; and standardization, stability, and quality control are feasible but not easy. In comparison with modern medicine, herbal medicines cost less, are more often used to treat chronic diseases, and the occurrence of undesirable side effects seems to be less frequent.

A vast number of plants have medicinal properties; in fact, many pharmaceutical drugs were originally derived from plants. Ethnopharmacology – the scientific study of indigenous medicines – is an interdisciplinary science practiced all over the world. Phytotherapeutic agents or phytomedicines are standardized herbal preparations that contain, as active ingredients, complex mixtures of plant materials in the crude or processed state. One basic characteristic of phytotherapeutic agents is the fact that they normally do not possess an immediate or strong pharmacological action. For this reason, these agents are not suitable for emergency treatment.

During the past decade, there has been increasing acceptance and public interest in natural therapies in both developing and developed countries. Due to poverty and limited access to modern medicine, about four billion people, 80% of the world's population, living in developing countries use herbal medicine as their source of primary health care [25, 28–30]. In these communities, traditional medical practice is often viewed as an integral part of their culture.

In the West, people are attracted to herbal therapies for many reasons, the most important reason being that, like our ancestors, we believe they will help us live healthier lives. Herbal medicines are often viewed as a balanced and moderate approach to healing. Individuals who use them as home remedies and over-the-counter drugs spend billions of dollars on herbal products. As such, they represent a substantial proportion of the global drug market [16, 19–21, 23, 24, 27, 28, 31–36].

This recent resurgence of interest in plant remedies has been spurred on by several factors [21, 23, 26, 31]:

- The effectiveness of plant medicines.
- The preference of consumers for natural therapies, a greater interest in alternative medicines and a commonly held erroneous belief that herbal products are superior to manufactured products.
- A dissatisfaction with the results from synthetic drugs and the belief that herbal medicines might be effective in the treatment of certain diseases where conventional therapies and medicines have proven to be inadequate.
- The high cost and side effects of most modern drugs.
- Improvements in the quality, efficacy, and safety of herbal medicines with the development of science and technology.
- Patients' belief that their physicians have not properly identified the problem; hence they feel that herbal remedies are another option.
- A movement towards self-medication.

Medicinal plants provide the raw materials for the pharmaceutical industry. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Many pharmacological classes of drugs include a natural product prototype. Aspirin, atropine, morphine, quinine are just a few of the drugs that were originally discovered through the study of traditional cures and folk knowledge of indigenous people [37]. Herbal therapies, on the other hand, consist of the chemical components of a plant as they occur naturally [8]. Some are made from plant extracts, others are synthesized to mimic a natural plant compound. Pharmaceutical drugs derived from plants are made by isolating the active chemicals and concentrating them to the medication. Pharmacognosy is the scientific study of drugs from natural products.

In most countries herbal products are launched into the market without proper scientific evaluation, and without any mandatory safety and toxicological studies. There is no effective machinery to regulate manufacturing practices and quality standards. Consumers can buy herbal products without a prescription and one might not recognize the potential hazards in an inferior product. A well-defined and constant composition of the drug is therefore one of the most important prerequisites for the production of a quality drug. Given the nature of products of plant origin, which by definition are never constant and are dependent on and influenced by many factors, quality control plays a significant role for the industry to thrive and be successful [38, 39].

## 2.2

### Preparation of Herbal Drugs

Herbal therapies are usually prepared by grinding or steeping the parts of a plant that are believed to contain medicinal properties. The ground plant matter is called the "macerate." The macerate is soaked in a liquid referred to as the "menstruum" in order to extract the active ingredients. Herbal infusions are prepared by treating

the herb with water or alcohol (ethanol) or mixtures of the two; coarsely bruised drug boiled in water for a definite period is known as a decoction and tinctures are solutions of the active principles of the drug in alcohol and water. This extraction process leads to the production of the herbal preparations in the form of fresh juice, hot and cold infusions, decoctions, tinctures, pastes, and powders referred to as “pulverata.” The resulting therapies come in several forms, including oral tablets, capsules, gel caps, extracts, and infusions. Solid or powdered extracts are prepared by evaporation of the solvents used in the process of extraction of the raw material. Some phytotherapeutic agents are greatly concentrated in order to improve their therapeutic efficacy. In this process, it is possible to remove some secondary metabolites present in the plants, which may produce undesirable side effects [40]. The extracts also contain marker compounds which are, by definition, chemically defined constituents that are of interest for control purposes, independent of whether they have any therapeutic activity or not.

### 2.3

#### Quality Control of Herbal Drugs

Quality control for efficacy and safety of herbal products is of paramount importance [14–16, 19, 20, 41–45]. Quality can be defined as the status of a drug that is determined by identity, purity, content, and other chemical, physical, or biological properties, or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. For the quality control of a traditional medicine, the traditional methods are procured and studied, and documents and the traditional information about the identity and quality assessment are interpreted in terms of modern assessment. In general, all medicines, whether they are of synthetic or of plant origin, should fulfill the basic requirements of being efficacious and safe, and this can be achieved by suitable clinical trials. This applies both to the multinational pharmaceutical company conducting a multi-center, double-blind placebo-controlled study with a herbal extract, and to the health practitioner in a rural village who applies a locally produced herbal mixture.

Natural products in medicine constitute a vast array of “raw materials,” making clear definitions important. Quality criteria are based on clear scientific definitions of the raw material. The term “herbal drugs” denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage [46]. Hence they are capable of variation. This variability is also caused by differences in growth, geographical location, and time of harvesting. A practical addition to the definition is also to include other crude products derived from plants, which no longer show any organic structure, such as essential oils, fatty oils, resins, and gums. Derived or isolated compounds in the processed state such as extracts or even isolated purified compounds (e.g. strychnine from *Strychnos nux-vomica*) or mixtures of compounds (e.g. abrin from *Abrus precatorius*) are, as a rule, not included in the definition. Combinations with chemical-

ly defined active substances or isolated constituents, and homeopathic preparations which frequently contain plants, are not regarded as herbal medicines. Their production is already based on adequate quality control of the respective starting materials. The following paragraphs will focus on quality control of herbal drugs in compliance with the above definition.

In general, quality control is based on three important pharmacopeial definitions:

- Identity: Is the herb the one it should be?
- Purity: Are there contaminants, e.g., in the form of other herbs which should not be there?
- Content or assay: Is the content of active constituents within the defined limits?

It is obvious that the content is the most difficult one to assess, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used which are, by definition, chemically defined constituents that are of interest for control purposes, independent of whether they have any therapeutic activity or not [46, 47]. To prove identity and purity, criteria such as type of preparation sensory properties, physical constants, adulteration, contaminants, moisture, ash content and solvent residues have to be checked. The correct identity of the crude herbal material, or the botanical quality, is of prime importance in establishing the quality control of herbal drugs.

Identity can be achieved by macro- and microscopical examinations. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification [40, 48]. At times an incorrect botanical quality with respect to the labeling can be a problem. For example, in the 1990s, a South American product labeled as “Paraguay Tea” was associated with an outbreak of anticholinergic poisoning in New York. Subsequent chemical analysis revealed the presence of a class of constituents that was different from the metabolites normally found in the plant from which Paraguay tea is made [49].

Purity is closely linked with the safe use of drugs and deals with factors such as ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation also includes microbial contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods such as photometric analysis, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Depending upon whether the active principles of the preparation are known or unknown, different concepts such as “normalization versus standardization” have to be applied in order to establish relevant criteria for uniformity.

Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are not known. Sometimes markers can be used. In all other cases, where no active constituent or marker can be defined for the herbal drug, the percentage extractable matter with a solvent may be used

as a form of assay, an approach often seen in pharmacopeias. The choice of the extracting solvent depends on the nature of the compounds involved, and might be deduced from the traditional uses. For example, when a herbal drug is used to make a tea, the hot water extractable matter, expressed as milligrams per gram of air-dried material, may serve this purpose [18, 50].

A special form of assay is the determination of essential oils by steam distillation. When the active constituents (e.g. sennosides in *Senna*) or markers (e.g. alkydamides in *Echinacea*) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy (UV/VIS), TLC, HPLC, GC, mass spectrometry (MS), or a combination of GC and MS (GC/MS), can be employed [51].

Several problems not applicable to synthetic drugs influence the quality of herbal drugs:

- Herbal drugs are usually mixtures of many constituents.
- The active principle(s) is (are), in most cases unknown.
- Selective analytical methods or reference compounds may not be available commercially.
- Plant materials are chemically and naturally variable.
- Chemo-varieties and chemo cultivars exist.
- The source and quality of the raw material are variable.
- The methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) have an effect.

Strict guidelines have to be followed for the successful production of a quality herbal drug. Among them are proper botanical identification, phytochemical screening, and standardization. Quality control and the standardization of herbal medicines involves several steps. The source and quality of raw materials, good agricultural practices and manufacturing processes are certainly essential steps for the quality control of herbal medicines and play a pivotal role in guaranteeing the quality and stability of herbal preparations [32, 35, 36, 47, 52–56].

The quality of a plant product is determined by the prevailing conditions during growth, and accepted Good Agricultural Practices (GAP) can control this. These include seed selection, growth conditions, use of fertilizers, harvesting, drying and storage. In fact, GAP procedures are, and will be, an integral part of quality control. Factors such as the use of fresh plants, age and part of plant collected, period, time and method of collection, temperature of processing, exposure to light, availability of water, nutrients, drying, packing, transportation of raw material and storage, can greatly affect the quality, and hence the therapeutic value of herbal medicines. Apart from these criteria, factors such as the method of extraction, contamination with microorganisms, heavy metals, and pesticides can alter the quality, safety, and efficacy of herbal drugs. Using cultivated plants under controlled conditions instead of those collected from the wild can minimize most of these factors [36, 38, 57–59].

Sometimes the active principles are destroyed by enzymic processes that continue for long periods from collection to marketing, resulting in a variation of compo-

sition. Thus proper standardization and quality control of both the raw material and the herbal preparations should be conducted.

Standardization involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations. Botanical extracts made directly from crude plant material show substantial variation in composition, quality, and therapeutic effects. Standardized extracts are high-quality extracts containing consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes. No regulatory definition exists for standardization of dietary supplements. As a result, the term “standardization” may mean many different things. Some manufacturers use the term standardization incorrectly to refer to uniform manufacturing practices; following a recipe is not sufficient for a product to be called standardized. Therefore, the presence of the word “standardized” on a supplement label does not necessarily indicate product quality. When the active principles are unknown, marker substance(s) should be established for analytical purposes and standardization. Marker substances are chemically defined constituents of a herbal drug that are important for the quality of the finished product. Ideally, the chemical markers chosen would also be the compounds that are responsible for the botanical’s effects in the body.

There are two types of standardization. In the first category, “true” standardization, a definite phytochemical or group of constituents is known to have activity. Ginkgo with its 26% ginkgo flavones and 6% terpenes is a classic example. These products are highly concentrated and no longer represent the whole herb, and are now considered as phytopharmaceuticals. In many cases they are vastly more effective than the whole herb. However the process may result in the loss of efficacy and the potential for adverse effects and herb–drug interactions may increase. The other type of standardization is based on manufacturers guaranteeing the presence of a certain percentage of marker compounds; these are not indicators of therapeutic activity or quality of the herb.

In the case of herbal drug preparations, the production and primary processing of the medicinal plant or herbal drug has a direct influence on the quality of the active pharmaceutical ingredients (APIs). Due to the inherent complexity of naturally growing medicinal plants and the limited availability of simple analytical techniques to identify and characterize the active constituents solely by chemical or biological means, there is a need for an adequate quality assurance system. This assurance is also required during cultivation, harvesting, primary processing, handling, storage, packaging, and distribution. Deterioration and contamination through adulteration, especially microbial contamination, can occur at any one of these stages. It is extremely important to establish good agricultural, harvesting, and manufacturing practices for herbal starting materials in order to minimize these undesirable factors.

In this regard producers, processors, and traders of medicinal plants or herbal drugs have an obligation and a role to play. The manufacturers and suppliers of herbal products should adhere to quality control standards and good manufactur-

ing practices. Currently, only a few manufacturers adhere to complete quality control and good manufacturing procedures including microscopic, physical, chemical, and biological analysis. Organizations such as Health Canada help safeguard Canadians' health by carrying out premarket reviews of all drugs before they are authorized for sale. The products available in the market are analyzed regularly to ensure that they are free of unsafe ingredients and that the products actually contain the ingredients indicated on the labels.

The potency and quality of an individual herbal product may be unclear because of lack of regulation. It is obvious that for a given plant product its quality will also be determined by the prevailing conditions during the growth cycle of the plant. Therefore, for cultivated plants the GAP system has been introduced, under which each step, including seed selection, growing conditions, use of fertilizers, and optimization of harvest time, harvesting, and drying, has to adhere to a set of criteria. It is likely that GAP procedures will become an integral part of quality control in the near future.

### 2.3.1

#### **Parameters for Quality Control of Herbal Drugs**

##### **2.3.1.1 Microscopic Evaluation**

Quality control of herbal drugs has traditionally been based on appearance and today microscopic evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, and detection of foreign matter and adulterants. A primary visual evaluation, which seldom needs more than a simple magnifying lens, can be used to ensure that the plant is of the required species, and that the right part of the plant is being used. At other times, microscopic analysis is needed to determine the correct species and/or that the correct part of the species is present. For instance, pollen morphology may be used in the case of flowers to identify the species, and the presence of certain microscopic structures such as leaf stomata can be used to identify the plant part used. Although this may seem obvious, it is of prime importance, especially when different parts of the same plant are to be used for different treatments. Stinging nettle (*Urtica urens*) is a classic example where the aerial parts are used to treat rheumatism, while the roots are applied for benign prostate hyperplasia [60].

##### **2.3.1.2 Determination of Foreign Matter**

Herbal drugs should be made from the stated part of the plant and be devoid of other parts of the same plant or other plants. They should be entirely free from moulds or insects, including excreta and visible contaminant such as sand and stones, poisonous and harmful foreign matter and chemical residues. Animal matter such as insects and "invisible" microbial contaminants, which can produce toxins, are also among the potential contaminants of herbal medicines [54–56]. Macroscopic examination can easily be employed to determine the presence of foreign matter, although microscopy is indispensable in certain special cases (for example,

starch deliberately added to “dilute” the plant material). Furthermore, when foreign matter consists, for example, of a chemical residue, TLC is often needed to detect the contaminants [17, 19, 60].

#### 2.3.1.3 Determination of Ash

To determine ash content the plant material is burnt and the residual ash is measured as total and acid-insoluble ash. Total ash is the measure of the total amount of material left after burning and includes ash derived from the part of the plant itself and acid-insoluble ash. The latter is the residue obtained after boiling the total ash with dilute hydrochloric acid, and burning the remaining insoluble matter. The second procedure measures the amount of silica present, especially in the form of sand and siliceous earth [60].

#### 2.3.1.4 Determination of Heavy Metals

Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such as mercury, lead, copper, cadmium, and arsenic in herbal remedies can be attributed to many causes, including environmental pollution, and can pose clinically relevant dangers for the health of the user and should therefore be limited [42, 60–62]. The potential intake of the toxic metal can be estimated on the basis of the level of its presence in the product and the recommended or estimated dosage of the product. This potential exposure can then be put into a toxicological perspective by comparison with the so-called Provisional Tolerable Weekly Intake values (PTWI) for toxic metals, which have been established by the Food and Agriculture Organization of the World Health Organization (FAO-WHO) [14, 15, 48].

A simple, straightforward determination of heavy metals can be found in many pharmacopeias and is based on color reactions with special reagents such as thioacetamide or diethyldithiocarbamate, and the amount present is estimated by comparison with a standard [41]. Instrumental analyses have to be employed when the metals are present in trace quantities, in admixture, or when the analyses have to be quantitative. The main methods commonly used are atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP) and neutron activation analysis (NAA) [63, 51, 64].

#### 2.3.1.5 Determination of Microbial Contaminants and Aflatoxins

Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi, and viruses. Inevitably, this microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations. Risk assessment of the microbial load of medicinal plants has therefore become an important subject in the establishment of modern Hazard Analysis and Critical Control Point (HACCP) schemes.

Herbal drugs normally carry a number of bacteria and molds, often originating in the soil. Poor methods of harvesting, cleaning, drying, handling, and storage may also cause additional contamination, as may be the case with *Escherichia coli* or *Salmonella* spp. While a large range of bacteria and fungi are from naturally occurring microflora, aerobic spore-forming bacteria frequently predominate.

Laboratory procedures investigating microbial contaminations are laid down in the well-known pharmacopeias, as well as in the WHO guidelines [17, 65]. Limit values can also be found in the sources mentioned. In general, a complete procedure consists of determining the total aerobic microbial count, the total fungal count, and the total Enterobacteriaceae count, together with tests for the presence of *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, and *Pseudomonas aeruginosa* and *Salmonella* spp. The European Pharmacopoeia also specifies that *E. coli* and *Salmonella* spp. should be absent from herbal preparations [66]. However it is not always these two pathogenic bacteria that cause clinical problems. For example, a fatal case of listeriosis was caused by contamination of alfalfa tablets with the Gram-positive bacillus *Listeria monocytogenes* [67].

Materials of vegetable origin tend to show much higher levels of microbial contamination than synthetic products and the requirements for microbial contamination in the European Pharmacopoeia allow higher levels of microbial contamination in herbal remedies than in synthetic pharmaceuticals. The allowed contamination level may also depend on the method of processing of the drug. For example, higher contamination levels are permitted if the final herbal preparation involves boiling with water [66].

The presence of fungi should be carefully investigated and/or monitored, since some common species produce toxins, especially aflatoxins. Aflatoxins in herbal drugs can be dangerous to health even if they are absorbed in minute amounts [65, 68]. Aflatoxin-producing fungi sometimes build up during storage [61]. Procedures for the determination of aflatoxin contamination in herbal drugs are published by the WHO [65]. After a thorough clean-up procedure, TLC is used for confirmation.

In addition to the risk of bacterial and viral contamination, herbal remedies may also be contaminated with microbial toxins, and as such, bacterial endotoxins and mycotoxins, at times may also be an issue [61, 69–72]. There is evidence that medicinal plants from some countries may be contaminated with toxigenic fungi (*Aspergillus*, *Fusarium*). Certain plant constituents are susceptible to chemical transformation by contaminating microorganisms.

Withering leads to enhanced enzymic activity, transforming some the constituents to other metabolites not initially found in the herb. These newly formed constituent(s) along with the molds such as *Penicillium nigricans* and *P. jensi* may then have adverse effects [61].

#### 2.3.1.6 Determination of Pesticide Residues

Even though there are no serious reports of toxicity due to the presence of pesticides and fumigants, it is important that herbs and herbal products are free of these chemicals or at least are controlled for the absence of unsafe levels [61]. Her-

bal drugs are liable to contain pesticide residues, which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation, and administering of fumigants during storage. However, it may be desirable to test herbal drugs for broad groups in general, rather than for individual pesticides. Many pesticides contain chlorine in the molecule, which, for example, can be measured by analysis of total organic chlorine. In an analogous way, insecticides containing phosphate can be detected by measuring total organic phosphorus.

Samples of herbal material are extracted by a standard procedure, impurities are removed by partition and/or adsorption, and individual pesticides are measured by GC, MS, or GC/MS. Some simple procedures have been published by the WHO [17, 43, 65] and the European Pharmacopoeia has laid down general limits for pesticide residues in medicine [48, 60, 66, 73, 74].

#### 2.3.1.7 Determination of Radioactive Contamination

There are many sources of ionization radiation, including radionuclides, occurring in the environment. Hence a certain degree of exposure is inevitable. Dangerous contamination, however, may be the consequence of a nuclear accident. The WHO, in close cooperation with several other international organizations, has developed guidelines in the event of a widespread contamination by radionuclides resulting from major nuclear accidents. These publications emphasize that the health risk, in general, due to radioactive contamination from naturally occurring radio nuclides is not a real concern, but those arising from major nuclear accidents such as the nuclear accident in Chernobyl, may be serious and depend on the specific radionuclide, the level of contamination, and the quantity of the contaminant consumed. Taking into account the quantity of herbal medicine normally consumed by an individual, they are unlikely to be a health risk. Therefore, at present, no limits are proposed for radioactive contamination [60, 61, 65].

#### 2.3.1.8 Analytical Methods

Published monographs in a pharmacopeia are the most practical approach for quality control of herbal drugs and there are many available [15, 17, 18, 41, 43, 45, 55, 75]. When pharmacopeial monographs are unavailable, development and validation of analytical procedures have to be carried out by the manufacturer. The best strategy is to follow closely the pharmacopeial definitions of identity, purity, and content or assay. Valuable sources for general analytical procedures are included in the pharmacopeias, in guidelines published by the WHO [60, 65, 76]. Additional information, especially on chromatographic and/or spectroscopic methods can be found in the general scientific literature. The plant or plant extract can be evaluated by various biological methods to determine pharmacological activity, potency, and toxicity. A simple chromatographic technique such as TLC may provide valuable additional information to establish the identity of the plant material. This is especially important for those species that contain different active constituents.

Qualitative and quantitative information can be gathered concerning the presence or absence of metabolites or breakdown products [60].

TLC fingerprinting is of key importance for herbal drugs made up of essential oils, resins, and gums, which are complex mixtures of constituents that no longer have any organic structure. It is a powerful and relatively rapid solution to distinguish between chemical classes, where macroscopy and microscopy will fail. Chromatograms of essential oils, for example, are widely published in the scientific literature, and can be of invaluable help in identification.

The instruments for UV-VIS determinations are easy to operate, and validation procedures are straightforward but at the same time precise. Although measurements are made rapidly, sample preparation can be time consuming and works well only for less complex samples, and those compounds with absorbance in the UV-VIS region.

HPLC is the preferred method for quantitative analysis of more complex mixtures. Though the separation of volatile components such as essential and fatty oils can be achieved with HPLC, it is best performed by GC or GC/MS.

The quantitative determination of constituents has been made easy by recent developments in analytical instrumentation. Recent advances in the isolation, purification, and structure elucidation of naturally occurring metabolites have made it possible to establish appropriate strategies for the determination and analysis of quality and the process of standardization of herbal preparations. Classification of plants and organisms by their chemical constituents is referred to as chemotaxonomy. TLC, HPLC, GC, quantitative TLC (QTLC), and high-performance TLC (HPTLC) can determine the homogeneity of a plant extract. Over-pressured layer chromatography (OPLC), infrared and UV-VIS spectrometry, MS, GC, liquid chromatography (LC) used alone, or in combinations such as GC/MS, LC/MS, and MS/MS, and nuclear magnetic resonance (NMR), electrophoretic techniques, especially by hyphenated chromatographies, are powerful tools, often used for standardization and to control the quality of both the raw material and the finished product. The results from these sophisticated techniques provide a chemical fingerprint as to the nature of chemicals or impurities present in the plant or extract [44, 77–79].

Based on the concept of photoequivalence, the chromatographic fingerprints of herbal medicines can be used to address the issue of quality control. Methods based on information theory, similarity estimation, chemical pattern recognition, spectral correlative chromatograms (SCC), multivariate resolution, the combination of chromatographic fingerprints and chemometric evaluation for evaluating fingerprints are all powerful tools for quality control of herbal products.

#### 2.3.1.9 **Validation**

The validation of herbal products is a major public health concern both in developed and resource-poor countries, where a fake businesses selling adulterated herbal medicines are common. In this regard, there is no control by the government agencies, despite the existence of certain guidelines in some individual countries

and those outlined by the WHO. If the herbal products are marketed as therapeutic agents, and irrespective of whether the products really have any positive effects to cure and reduce the severity of the disease, it is necessary to ensure scientific validation and periodic monitoring of the quality and efficacy by drug control administrators.

It is feasible that the introduction of scientific validation would control the production of impure or adulterated herbal products and would eventually ensure their rational use. This could also lead to the regulation of the industry so that only qualified physicians and health providers are allowed to prescribe the medication.

Several of the principal pharmacopeias contain monographs outlining standards for herbal drugs. The major advantage of an official monograph published in a pharmacopeia is that standards are defined and available, and that the analytical procedures used are fully validated. This is of major importance, since validation can be a rather time-consuming process.

By definition, validation is the process of proving that an analytical method is acceptable for its intended purpose for pharmaceutical methods. Guidelines from the United States Pharmacopeia (USPC, 1994–2001), the International Conference on Harmonization (ICH), and the US Food and Drug Administration (FDA) provide a framework for performing such validations. In general, validation investigations must include studies on specificity, linearity, accuracy, precision, range, detection, and quantitative limits, depending on whether the analytical method used is qualitative or quantitative [80]. Also of utmost importance is the availability of standards. For macroscopic and microscopic procedures in general this means that reliable reference samples of the plant must be available. A defined botanical source (e.g. voucher specimens) will normally solve this problem. Standards for chromatographic procedures are less easy to obtain. Characteristic plant constituents, either active or markers, are seldom available commercially. Sometimes an LC/MS approach can be referred to as a mode of characterization. Going one step further, after isolation of such a compound, elucidations to prove its definite structure will not be easy. The method often employed is to use readily available compounds that behave similarly in the chosen chromatographic systems, and to calculate retention values and/or times towards these compounds as a standard.

Qualitative chemical examination is designed to detect and isolate the active ingredient(s). TLC and HPLC are the main analytical techniques commonly used. In cases when active ingredients are not known or too complex, the quality of plant extracts can be assessed by a “fingerprint” chromatogram [81–87].

## 2.4 Herbal Supplements

A botanical is a plant or part of a plant valued for its medicinal or therapeutic properties, flavor, and/or scent. Herbs are subsets of botanicals. To be classified as a dietary supplement, a botanical must meet the following criteria:

1. It is intended to supplement the diet.
2. It contains one or more dietary ingredients (including amino acids, vitamins, minerals, herbs, or other botanicals, etc.).
3. It is intended to be taken orally as a pill, capsule, tablet, or liquid.
4. It is labeled as being a dietary supplement.

A herbal supplement labeled “Natural” does not mean it is safe or without any harmful effects. Herbal products can act the same way as drugs. Their safety depends on factors such as their chemical make-up, how they work in the body, method of preparation, and dosage. In the US, the FDA regulates herbal and other dietary supplements. This means that they do not have to meet the same standards as drugs and over-the-counter medications, they are not required to be standardized, and no legal or regulatory definitions exist for standardization. As a result, manufacturers are not required to demonstrate the safety and effectiveness of their products before they reach the market. In addition, they do not have to adhere to any of the quality control measures applicable to drugs; hence the composition may vary greatly from one batch to another.

The use of some herbal supplements has been reported to be associated with ailments such as oral manifestations, including swelling, irritation, and bleeding of the tongue. These potential effects of herbal supplements, in conjunction with factors related to regulation restrictions, suggest that the use of these products may be associated with various adverse reactions that can affect health. The active ingredient(s) in many herbal supplements are not known, and some have been found to be contaminated with metals, unlabeled prescription drugs, and microorganisms. Under its current regulatory authority, the FDA can remove a herbal supplement from the market only after it has been shown to be unsafe. There has been an increase in the number of Internet websites that sell and promote herbal supplements. Unfortunately, some of them make inaccurate claims and statements regarding their products and claim unsubstantiated effects in curing disease and disease conditions. In the US, distributors of herbal products are under the jurisdiction of the Federal Trade Commission (FTC), which monitors advertising for truthful statements that do not mislead.

## 2.5

### **Adulteration of Herbal Drugs**

Direct or intentional adulteration of drugs usually includes practices in which a herbal drug is substituted partially or fully with other inferior products. Due to morphological resemblance to the authentic herb, many different inferior commercial varieties are used as adulterants. These may or may not have any chemical or therapeutic potential. Substitution by “exhausted” drugs entails adulteration of the plant material with the same plant material devoid of the active constituents. This practice is most common in the case of volatile oil-containing materials, where the dried exhausted material resembles the original drug but is free of the

essential oils. Foreign matter such as other parts of the same plant with no active ingredients, sand and stones, manufactured artifacts, and synthetic inferior principles are used as substitutes [29].

The practice of intentional adulteration is mainly encouraged by traders who are reluctant to pay premium prices for herbs of superior quality, and hence are inclined to purchase only the cheaper products. This encourages producers and traders to sell herbs of inferior quality. Rarity of a herbal product is another factor that influences adulteration. Sometimes sale of inferior products may be unintentional. In the absence of proper means of evaluation, an authentic drug partially or fully devoid of the active ingredients may enter the market. Factors such as geographical sources, growing conditions, processing, and storage are all factors that influence the quality of the drug. Deterioration may contribute to indirect adulteration, and crude drugs are often prone to deterioration, especially during storage, leading to the loss of the active ingredients, production of metabolites with no activity and, in extreme cases, the production of toxic metabolites. Physical factors such as air (oxygen), humidity, light, and temperature can bring about deterioration directly or indirectly [88]. These factors, alone or in combination, can lead to the development of organisms such as molds, mites, and bacteria. Oxidation of the constituents of a drug can be brought about by oxygen in the air, causing some products, such as essential oils, to resinify or to become rancid. Moisture or humidity and elevated temperatures can accelerate enzymatic activities, leading to changes in the physical appearance and decomposition of the herb.

Dried herbs are particularly prone to contamination with spores of bacteria and fungi present in the air. Bacterial growth is usually accompanied by the growth of molds, whose presence is evidenced by changes in appearance, break down of the plant material, and smell. Mites, nematode worms, insects/moths, and beetles can also destroy herbal drugs during storage.

Control measures to protect against deterioration include the use of airtight containers made of materials that will not interact physically or chemically with the material being stored. Storage in ventilated, cool, dry areas and periodic spraying of the stored area with insecticides will help to prevent the spread of infestation. Sterilization of crude drugs is achieved by treatment of bulk consignments with ethylene oxide, and methyl bromide under controlled conditions and complying with acceptable limits for toxic residues [29, 47, 88]. World markets from time to time experience wild fluctuations in the price of herbals. One reason for this is indiscriminate harvesting which leads to the extinction of natural populations – still the only source of bioresources. This in turn encourages producers to replace the required herb with other supplements.

## 2.6

### Contamination of Herbal Drugs and Herb–Drug Interactions

Conventional synthetic pharmaceuticals such as synthetic corticosteroids, nonsteroidal anti-inflammatory drugs and other prescription drugs, potent drugs such as

phenylbutazone, in fact examples of almost every therapeutic drug class have been found in certain herbal remedies as contaminants. A recent study by Ramsay et al. found that potent corticosteroids had been deliberately added to herbal creams in order to increase their efficacy [89]. This problem is widespread, and occurs in both Oriental and European countries [90–94]. These “adulterated” herbal medicines sometimes result in serious ailments such as acute renal failure [10, 95–99].

Many people, especially those living with HIV/AIDS, use both herbal medicines and prescription drugs. A number of clinically significant interactions between prescribed and herbal medicines have been identified. When these medications are used together, they can interact in the body, causing changes in the way the herbs and/or the drug works. Such changes are called herb–drug interactions. Concurrent use of herbal or homeopathic remedies alongside prescribed or over-the-counter medicines are frequent, and may mimic, magnify, or oppose the effect of the drug [100].

Herb–drug interactions are not chemical interactions between a drug and a herbal component to produce something toxic. Instead, the interactions generally cause either an increase or decrease in the amount of drug in the bloodstream. As with conventional medicines, herbal medicines interact with drugs in two general ways: pharmacokinetically and pharmacodynamically. *Pharmacokinetic* interactions result in alterations in the absorption, distribution, metabolism, or elimination of the drug or natural medicine. These interactions affect drug action by quantitative alterations, either increasing or decreasing the amount of drug available to have an effect. *Pharmacodynamic* interactions cause alterations in the way a drug or natural medicine affects a tissue or organ system. These actions affect drug action in a qualitative way, either through enhancing or antagonizing effects.

Herb–drug interactions change the effectiveness of the treatment, sometimes resulting in potentially dangerous side effects, possibly leading to toxicity, and/or reduced benefits. They can modify the mode of action of the drug, leading to unexpected complications or enhancement of the therapeutic effect, possibly leading to overmedication and an impact on health. Drug interactions are a significant problem in association with the use of St John’s wort [101, 102].

The risk of herb–drug interactions is not limited to synthetic drugs. Herbal supplements and certain foods can interact with medications. Unfortunately very little is known about these interactions and there is little available scientific research on herb–drug interactions. When combining herbal therapies with other medications, it is important to watch for potential symptoms and to inform health care providers. It is essential to train doctors to appreciate that drug interactions exist and to emphasize the importance of the need for physicians and naturopathic doctors to work together.

Currently, there is very little information published on herb–drug interactions [103–109]. Controlled clinical studies are needed to clarify and determine their clinical importance and more research is required to define them.

## 2.7

### Toxicity of Herbal Drugs

For several reasons it is not possible to establish absolute safety standards for herbal preparations based solely on epidemiological studies. First, these types of studies would be costly. Second, there is little published data in countries where the major use of medicinal plants occurs and thus general standards based on a limited number of reports would have little meaning. Third, the exact identification of the products implicated in side effects claimed for medicinal plants is usually lacking. In spite of these inadequacies, there are a number of general comments that can be made with regard to avoiding potential serious side effects from herbal medicines.

The definition of “toxic” is ultimately a matter of viewpoint. Traditionally, herbs and herbal products have been considered to be nontoxic and have been used by the general public and traditional medicinal doctors worldwide to treat a range of ailments. The fact that something is natural does not necessarily make it safe or effective. The active ingredients of plant extracts are chemicals that are similar to those in purified medications, and they have the same potential to cause serious adverse effects. Whilst the literature documents severe toxicity resulting from the use of herbs, on many occasions the potential toxicity of herbs and herbal products has not been recognized [108]. In certain countries, such as Taiwan, herbs can be obtained from temples, night markets, street vendors, herbal stores, neighborhoods, or relatives, and from traditional medicine practitioners. Ordinary people recommend the medicines to others without safety considerations. The general public and many practitioners also believe that the herbs are nontoxic. Apparently, this cultural style/concept needs more attention in terms of drug safety education. Herbs and herbal preparations can cause toxic adverse effects, serious allergic reactions, adverse drug interactions, and can interfere with laboratory tests [110–117]. High-risk patients such as the elderly, expectant mothers, children, those taking several medications for chronic conditions, those with hypertension, depression, high cholesterol or congestive heart failure, should be more cautious in taking herbal medicine.

It is axiomatic that pregnancy should be a time of minimal medical intervention, and herbalists in particular regard pregnancy as a “contraindication” to taking herbal medicines [106, 110, 118, 119].

Two kinds of side effects have been reported for herbal medicines. The first, considered to be intrinsic to herbal drugs themselves, is mainly related to predictable toxicity due to toxic constituents of the herbal ingredients and overdosage, and the second is allergy. Many cases of allergic reactions have been reported for herbal drugs. It is impossible to completely eliminate the possibility of any substance, including prescription drugs, herbal remedies, or cosmetics, producing an allergic response in people exposed to them. Herbal medicines do not present any more of a problem in this respect than any other class of widely used foods or drugs.

Based on published reports, the side effects or toxic reactions associated with herbal medicines in any form are rare. This could be due to the fact that herbal

medicines are generally safe, that adverse reactions following their use are under-reported, or because the nature of the side effects or minor allergic reactions are such that they are not reported.

Perhaps the major problem with regard to the safety of herbal medicines is related to the manufacturing practice, including contamination, substitution, incorrect preparation and dosage, intentional addition of unnatural toxic substances, interactions involving synthetic prescriptions, drugs, and herbal medicines, either intentional or unintentional mislabeling, and the presence of natural toxic contaminants. Many ordinary foods contain constituents that could be regarded as poisonous. Alpha gliadin produced by gluten in wheat, oats, and rye, the cyanogenic glycosides in many fruit skins and seeds, thiocyanates of the brassica vegetables, and lectins of many pulses including soya and red kidney bean are such examples. Cyanogenetic glycosides present in the kernel of many fruits can undergo gastric hydrolysis, resulting in the release of hydrogen cyanide. Viscotoxins, which are constituents of mistletoe, are both cytotoxic and cardiotoxic [101, 120]. Nonetheless, these foods are generally regarded as safe. Similarly, both water and oxygen can kill in excessive amounts! So quantity is often an important consideration.

A number of cases have been reported in the literature in which herbal medicines, used for a number of years with safety, suddenly appear to be unsafe, and to date there has been no satisfactory explanation for these adverse effects.

In this context herbs can be broadly classified into three major categories:

- The *food herbs* – medicines such as peppermint, ginger, garlic, hawthorn, nettles, lemon, and balm are gentle in action, have low toxicity, and are unlikely to cause any adverse response. They can be consumed in substantial quantities over long periods of time without any acute or chronic toxicity. However they may bring about allergic reactions in certain individuals.
- The *medicinal herbs* – these are not daily “tonics” and need to be used with greater knowledge (dosage and rationale for use) for specific conditions (with a medical diagnosis) and usually only for a limited period. They have a greater potential for adverse reactions and in some cases drug interactions. They include aloe vera, black cohosh, comfrey, echinacea, ephedra, ginkgo biloba, ginseng, kava kava, milk thistle, and senna.
- The *poisonous herbs* have a strong potential for either acute or chronic toxicity and should only be prescribed by trained clinicians who understand their toxicology and appropriate use. Fortunately, the vast majority of these herbs are not available to the public and are not sold in health food or herbal stores. Aconite, *Arnica* spp., *Atropa belladonna*, digitalis, datura, male fern, gelsemium, and veratrum are some examples [116].

There are herbs such as *Lobelia* and *Euonymus* spp. that have powerful actions, often causing nausea or vomiting, although they are safe under appropriate conditions. There is also an idiosyncratic grouping of herbs that have been alleged, with some scientific support, to exhibit specific kinds of toxicity. The best known example is the hepatotoxicity of pyrrolizidine alkaloid-containing plants such as *Symphy-*

tum (comfrey), *Dryopteris* (male fern), *Viscum* (mistletoe), and *Corynanthe* (Yohimbe) [9, 121].

## 2.8

### Screening of Herbal Drugs

Once the botanical identity of a herb is established, the next step is phytochemical screening, which involves bioassays, extraction, purification, and characterization of the active constituents of pharmaceutical importance [17, 44, 50, 76]. The herb or herbal drug preparation in its entirety is regarded as the active substance. These constituents are either of known therapeutic activity or are chemically defined substances or a group of substances generally accepted to contribute substantially to the therapeutic activity of a herbal drug. In any program in which the end product is to be a drug, some type of pharmacological screening, or evaluation, must obviously be done.

Pharmacological screening programs are not without problems. Ideally the active principles should be isolated, preferably using bioassay guided isolation processes, which can be problematic. The ideal pharmacological screen would be to identify those extracts or pure compounds that are highly active and nontoxic. Such a screen is rare to find. Failure to duplicate pharmacological results is another problem.

There are many pharmacological screening tests available [87]. In the random selection program of the National Cancer Institute (NCI) in the US, plants are randomly selected, extracted, and the extracts are evaluated against one or more *in vitro* tumor systems and *in vitro* cytotoxicity tests. An extension of this procedure is to isolate metabolites or “active compounds” from the plant that had shown most promising activity and subject them to pharmacological tests. In another approach, plants containing specific types or classes of chemical compounds, for example alkaloids, are tested. Simple tests such as color reactions are carried out on various parts of the plant in the field, and assays are carried out in the laboratories [87]. In terms of cost–benefit ratio, these “shotgun” approaches are considered to be very unsatisfactory.

Another method involves random collection of plants and subsection of their extracts to several broad screening methods and pharmacological tests. The success of this method depends on the number of samples assayed, adequate funding, and appropriate predictable bioassay protocols. Broad-based empirical screening, which is time consuming and expensive, can detect novel activities but is not suited for screening large numbers of samples [29, 81, 82, 122, 123].

Diagnosis by observation, a method introduced by the “father” of medicine, Hippocrates, is still one of the most powerful tools of today’s physicians. *In vitro* screening methods, though restricted to the detection of defined activities, are simpler and more useful [124]. Recently, biochemical and receptor–ligand binding assays have gathered momentum. This has been made possible by the increasing availability of human receptors from molecular cloning, and extracts and compounds can be tested for binding directly to the presumed therapeutic target pro-

tein. Clone receptors can be expressed in a functional state linked to receptor proteins in cells such as yeast, and this has been made possible by applications of molecular biology. Combined with automated instrumentation and computer databases, hundreds of such assays can be completed in relatively short periods of time [83, 88, 125–129]. These screening processes are successfully used by international agencies such as the National Cancer Institute (NCI) in the United States and the Central Drug Research Institute in India [29, 124, 130].

The technology of plant medicinal screening processes has even advanced to enzyme isolation. The enzymes that cause the disease are first isolated and the plant extracts are tested to determine if they block enzyme action [131]. An enzyme immunoassay for the quantification of femtomole quantities of therapeutically important alkaloids has been established [132]. Ethanolic extracts, tinctures, and pure plant compounds from commercially available herbs have been analyzed for their *in vitro* cytochrome P450 3A4 (CYP3A4) inhibitory capability via a fluorometric microtiter plate assay. These studies indicate that high-throughput screening methods for assessing CYP3A4 inhibition by natural products have important implications for predicting the likelihood of potential herb–drug interactions [133].

Higher plants contain both mutagens and antimutagens and are susceptible to mutagenesis, but screening programs for the detection of antimutagenesis rarely employ higher plant systems. However, using modified screening tests to detect antimutagenic agents, higher plants have been shown to contain a variety of structurally novel antimutagenic agents [134–136]. Short-term bacterial and mammalian tissue culture systems are the standard methods employed.

## 2.9

### Labeling of Herbal Products

The quality of consumer information about the product is as important as the finished herbal product. Warnings on the packet or label will help to reduce the risk of inappropriate uses and adverse reactions [70]. The primary source of information on herbal products is the product label. Currently, there is no organization or government body that certifies an herb or a supplement as being labeled correctly. It has been found that herbal remedy labels often cannot be trusted to reveal what is in the container. Studies of herbal products have shown that consumers have less than a 50% chance of actually getting what is listed on the label, and published analyses of herbal supplements have found significant differences between what is listed on the label and what is in the bottle. The word “standardized” on a product label is no guarantee of higher product quality, since there is no legal definition of the word “standardized.” Consumers are often left on their own to decide what is safe and effective for them and the lack of consistent labeling on herbal products can be a source of consumer frustration.

Certain information such as “the product has been manufactured according to Pharmacopoeia standards,” listing of active ingredients and amounts, directions such as serving quantity (dosage) and frequency of intake of the drug, must be in-

cluded on the labels of all herbal products and packages. The label should also indicate the method of extraction and relative amount of macerate and menstruum used, and possible side effects. It should indicate that the product's content has been standardized to contain a particular amount of a specified biochemical constituent. Standardization gives the buyers a measure of potency by which to judge the quality of the product and to compare dosage with those indicated by clinical trials. This will also ensure that the correct herb has been used. In addition to the above information, the label should include the name and origin of the product, its intended use, net quantity of contents, other ingredients such as herbs and amino acids, and additives, for which no daily values have been established, storage conditions, shelf life or expiry date, warnings, disclaimer, and name and address of manufacturer, packer or distributor.

A herb categorized as a nutritional supplement cannot claim any health benefits or "disease claims" on the label, leaving the consumer with little information [137]. Marketing plays a big role in the use of herbal products and the media help significantly to provide information about natural health products. One of the problems with mass media "propaganda" is scientific inconsistency. Unless the packaging contains a medical claim, herbal products are not reviewed by any government agency. Food and drug administrations that regulate prescription drugs only review a herbal product if the item is suspected of being harmful or if the label contains medical claims. Scientists use several approaches to evaluate botanical dietary supplements for their potential health benefits and safety risks, including their history of use and laboratory studies using cell or animal models. Studies involving people can provide information that is relevant as to how botanical dietary supplements are used.

## 2.10 Policies and Regulations

It is a widely held myth that modern drugs are dangerous foreign chemicals with side effects, while herbals are natural, gentle and safe. The truth is that some herbs can be dangerous and can bring about serious diseases and even lead to death. Unlike conventional drugs, herbal products are not regulated for purity and potency and this could cause adverse effects and can even lead to drug interactions [138, 139]. There are fewer studies on herbal medicines than on conventional drugs, mainly because, unlike synthetic chemicals, herbs cannot be patented, so there is little money to be made by funding such research.

It is important that consumers are made aware of interactions herbs might have with other drugs they are taking. Unfortunately this information is not available with herbals. Herbals are also frequently adulterated with prescription drugs. In certain countries, herbal products used for diagnosis, cure, mitigation, treatment, or prevention of disease are normally treated as drugs, and hence regulated by legislation. However, in most countries, including the United States, such legislation does not exist and in fact, most botanical products are marketed as dietary supple-

ments. Herbal products categorized as nutritional or dietary supplements are not regulated [139–142]. In many countries these medicines are not required to pass any regulatory analysis to be sold as health food supplements.

It is clear that the herbal industry needs to follow strict guidelines and that regulations are needed. The food and drug administrations that regulate prescription drugs only review a herbal product if the item is suspected of being harmful or if the label contains a medical claim. Although research is being done, it is very limited and only a few herbal drugs have been studied adequately by well-controlled clinical trials. Even though evidence should always be presented to support claims of products, most herbs are still marketed with little or no research [24, 36, 54, 137, 143, 144]. To be registered as drugs, these products need to be tested to prove their safety and clinical efficacy. However, so far, few programs have been established to study the safety and efficacy of herbal medicines as originally proposed in the WHO guidelines for the assessment of herbal medicines [27, 44, 53, 146, 147].

The future of herbal drugs is overshadowed by the pervading lack of regulatory control [145, 148–151]. In 1993, the WHO sponsored a symposium on the use of medicinal plants. The result was a standard guideline for the assessment of herbal medicines and a recommendation that governments of the world should protect medicinal plants, improve regulation of herbal medicines, and respect traditional medicine approaches [50, 91–93, 146, 151–153].

More recently the Health Directorate of Canada developed a new regulatory framework for natural health products, which came into effect in January 2004. Among other things, the new regulations call for improved labeling, good manufacturing practices, product and site licensing, and provision of a full range of health claims that will be supported by evidence. However, even in Canada, the only regulatory requirements enforced are that all products intended for medicinal use, including natural health products, are issued a Drug Identification Number (DIN). These numbers are not required for raw materials such as bulk herbs.

In the US, access to herbal medicines is restricted by FDA regulations. Before any new chemical or herbal drug is approved, research must prove that it is both safe and effective. As a result of these restrictions, packages of herbal medicines are labeled as food supplements, which do not require pre-approved testing. Food supplements cannot make any healing claims or issue warnings about potential risks. In the US, plant-based derivatives already appear in a quarter of the prescription medicines produced. However, many other plants with healing properties are shunned by the medical community despite scientific data from other countries showing their effectiveness. The misconception that herbs are old fashioned and unscientific has helped to promote a general distrust of phytotherapy. The American Botanical Council contends that, in many cases, herbal medicines are safer than prescription drugs. According to the Council, herbal medicines react more slowly and often include their own antidotes to counteract any toxic effects [135].

With proper enforcement of regulations, more products that are legitimate will enter the market and the consumers will see justifiable claims on labels. In fact, it is predicted that appropriate regulations will rejuvenate the market in response to growing concerns about the regulatory environment for herbal remedies.

## 2.11

### Trends and Developments

The rationalization of the new multidrug and multitarget concept of therapy in classical medicine is likely to have great implications on the future basic research in phytomedicine and evidence-based phytotherapy. It requires concerted cooperation between phytochemists, molecular biologists, pharmacologists, and clinicians, with the aim of using modern high-tech methods for standardization of phytopreparations, of integrating new molecular biological assays into the screening of plant extracts and plant constituents, and of increasing studies on the efficacy proof of phytopreparations using controlled clinical trials. This should be paralleled or followed by pharmacokinetic and bioavailability studies.

One major concern will be the investigation of the multivalent and multitarget actions of plant constituents and standardized extracts, with the aim of rationalizing the therapeutic superiority of many plant extracts over single isolated constituents.

Increased effort in three major research areas will be crucial: (1) efforts to develop suitable standardization methods for phytopreparations; (2) the integration of molecular biological assays into the screening of plant extracts, single isolated compounds thereof and phytopreparations; and (3) the performance of further placebo-controlled, mono- or double-blind, clinical trials, paralleled or followed by pharmacokinetic and bioavailability studies [154].

Herbs are still marketed without sufficient research but evidence must always be shown to consumers to support claims of products [24, 36, 54, 137, 143, 144]. More clinical studies are needed and doctors, along with other professionals, should work towards on untangling this herbal maze. Standards should be developed for each natural health product and the same regulatory standards that apply to manufactured pharmaceuticals should apply equally to herbal products as well. Unlike conventional drugs, herbal products are not regulated for purity and potency and this could cause adverse effects and drug interactions [108]. Herbal manufacturing processes should be refined in order to improve the purity, safety and quality of products and the herbal industry needs to follow strict guidelines, for herbal products are now classified as medicines. Manufacturers and producers tend to resist these laws because such laws will increase cost, which will have to be passed on to consumers, and thus the appeal or herbal drugs might then be lost. The media help significantly to provide information about natural health products to consumers. One of the biggest problems with many mass media stories today is scientific inconsistency. With proper enforcement of regulations, more products that are legitimate will come to the market and the consumer will see justifiable claims on labels and these regulations will rejuvenate the market. Herbal medicines still have value because they have a long history.

Finally, it is sometimes asked whether natural health food stores require legislation. The answer should be yes. Promoting herbal products for medical conditions should be regulated in a similar fashion to shops that dispense pharmaceutical products.

## 2.12

**Conclusions**

Plant materials are used throughout the developed and developing world as home remedies, in over-the-counter drug products, and as raw material for the pharmaceutical industry, and they represent a substantial proportion of the global drug market. Therefore, it is essential to establish internationally recognized guidelines for assessing their quality. Certain herbs have become popular over the years, but the public, medical practitioners, and the media still have a poor understanding of herbal medicine. Evidence is emerging on the dangers of herbs. As in most situations, the truth lies hidden under the media hype, poorly understood science, and exaggerated claims. Seeing herbal medicines as either panaceas or poisons blinds us to the reality that in most cases they are neither! Lack of experience, information, and education about herbs make consumers, physicians, and other orthodox health care providers easy victims of market exploitation and herbal myths.

There is no rational reason behind the tendency to equate “natural” with “harmlessness.” The fact that something is natural does not necessarily make it safe or effective. In addition, a lack of knowledge of phytochemistry leads to misinterpretation and misunderstanding. It is very likely that some herbs will have side effects, interact with other medications, and be toxic. Information on isolated constituents should not be applied directly to the whole herb and studies on *in vitro* forms should not be confused with oral administration. The gold standard for proof of efficacy for a medication is the controlled double-blind trial, which can offer proof of activity and effectiveness. In addition to this, well-designed unblinded and clinical trials, epidemiological, animal, and phytochemical studies can provide useful information on the herbal drug. It is not uncommon for studies to be carried out on animals and the results extrapolated to humans even though they have different metabolic processes. Many herbs have not been subjected to this type of study. We do not fully understand how many of these herbal medicines work, nor do we know which component is pharmaceutically active. Even though herbal remedies may be effective, do their benefits outweigh the risks?

With rationing looming in virtually all health care systems, the question whether herbal medicines can save money is important. Not all plant medicines are cheap. Botanicals are not patentable (they can be patented for use); hence herbal remedies are not viable candidates for the existing drug approval processes. Pharmaceutical companies will not risk a loss, and herbal producers, especially in developing countries, lack the financial resources even to consider conducting research or seeking approval. In contrast to the United States, many European and Asian countries have taken a more holistic approach to researching the efficacy of herbal remedies.

Companies supplying standardized extracts with the greatest degree of quality control typically offer the highest quality products. Most standardized extracts are currently made under strict guidelines set forth by individual members of the European Community (EC) as well as those proposed by the EC. The EC production of standardized extracts serves as a model for quality control processes for all forms

of herbal preparations. Herbal products and nutritional supplements are not the same. Most herbal remedies in the United Kingdom and the United States are sold as food supplements [138]. Thus, they evade regulation of their safety.

The possibility of herb–drug interactions is important but “under-research” is an issue. The World Health Assembly in resolutions WHA31.33 (1978), WHA40.33 (1987), and WHA42.43 (1989) has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards [42, 148, 149]. These resolutions describe a series of tests for assessing the quality of medicinal plant materials. The tests are designed primarily for use in national drug quality control laboratories in developing countries, and complement those described in the international pharmacopeia, which provide quality specifications only for the few plant materials that are included in the WHO Model List of Essential Drugs. This manual does not constitute a herbal pharmacopeia, but a collection of test procedures to support the development of national standards based on local market conditions, with due regard to existing national legislation and national and regional norms [14, 15].

The test procedures cannot take account of all possible impurities. Common sense and good pharmaceutical practice should be applied in deciding whether an unusual substance not detectable by the prescribed tests can be tolerated. The international pharmacopeia provides quality specifications only for the few plant materials that are included in the WHO Model List of Essential Drugs [14, 15, 52, 146].

There is a lack of open interpretation in the area of safety and efficacy, especially for bibliographic studies. Such interpretations are particularly relevant for herbal medicinal products because they have been used for long periods of time, sometimes over centuries, and a wealth of literature is available. It is desirable that this documented knowledge is exploited in order to avoid unnecessary tests with animals and clinical trials. Scientific evaluation of the traditional knowledge is needed. In many societies much of the knowledge resides in the hand of the healers, where oral transmission of information is the unwritten rule. In most cases, the information is not documented. As a result, in many regions, this knowledge is endangered because the younger generation is unwilling to carry on the profession of the elders. Knowledge that has been refined over thousands of years of experimentation with herbal medicine is being lost. A major research opportunity in this field would be to catalogue information on herbal medicines by traditional healers in cultures where these skills are normally transmitted through an apprentice system [141].

Opinion about the safety, efficacy, and the appropriateness of medicinal herbs varies widely among medical and health professionals in countries where herbal remedies are used. In most cases the safety and efficacy of drugs of herbal origin cannot be attributed to one single chemical constituent. Various pharmaceutical particulars, including the production and collection of the starting material and the extraction procedures, need to be assessed. Some professionals, however, accept historical, empirical evidence as the only necessary criterion for the efficacy of herbal medicines. Others would ban all herbal remedies as dangerous or of question-

able value. Herbal medicines have the potential for improving public health at low cost. Phytomedicines, if combined with preventive medical practice, could be a cost-effective, practical way to shift modern health care from treatment to prevention.

Manufacturers and distributors should attempt to certify that the herbal medicines available to the public meet certain standards by answering questions such as: Does the product meet recognized standards of quality? Does the label accurately reflect what is in the product? Is the product reasonably free of contaminants such as heavy metals or pesticides? Was the product produced and packaged under clean and safe conditions? Good housekeeping is required to prove that a product is safe and effective. To obtain this certification, a manufacturer must submit research-based evidence that the product does what it claims to do and that it does so without harming the consumer. Clinical trials should be conducted to establish facts such as average effective dose for any drug, as well as potential side effects a compound may cause. Recommendations on product information such as dosage limits and any warnings should also be supplied to the consumer [69–71].

Two paradigm shifts in medicine characterize the beginning of the twenty-first century: the gradual renunciation of the long-standing reliance on monosubstance therapy in favor of a multidrug therapy and the transition to a new kind of multitarget therapy, through which the interference of drugs with protective, repair, and immunostimulatory mechanisms of the human body, rather than with single disease-causing agents, gains more and more importance. Phytomedicine research has a good chance of contributing to these new strategies through the development of new and better drugs for an evidence-based and rational phytotherapy. One major concern will be to investigate the multivalent and multitarget actions of plant constituents and standardized extracts, with the aim of rationalizing the therapeutic superiority of many plant extracts over single isolated constituents. Phytomedicine and chemosynthetic pharmaceutical research find themselves in a race to develop new medicines, with fewer or no side effects, for therapeutic and preventive application in illness for which causality-based treatments are nonexistent or imperfect [154].

It has now become evident that there is need for a holistic approach to health care, and the untapped potential of traditional medicines should be utilized. However, this will not be easy, as it requires a thorough search for medicinal plants, proper guidelines for their identification, validation of the scientific methods of isolation of active ingredients, preclinical evaluation of their pharmacological and toxicological profiles, and clinical evidence of their usefulness. Clinical trials should be conducted to establish facts such as the average effective dose for any drug, as well as potential side effects a compound may cause. In short, these herbal drugs need to be analyzed in the same way as any modern drug, that is with randomized controlled clinical trials.

As doctors and researchers continue to explore the safety and effectiveness of herbal medicines, more is learned about both their promises and their pitfalls. At the same time, legislators at the national level should continue to press for effective laws to protect consumers from potentially harmful herbal drugs. In the mean

time, your own scrutiny and curiosity are your best protection. Quality control for efficacy and safety of herbal products is of utmost importance. The assurance of the safety of a herbal drug requires monitoring of the quality of the finished product as well as the quality of the consumer information on the herbal remedy.

## References

- 1 Solecki, R.S., Shanidar IV, *Science* **1975**, *190*, 880.
- 2 Bensky, D., Gamble, A. *Chinese Herbal Medicine: Materia Medica*. Eastland Press, Seattle, **1993**.
- 3 Cassileth, B.R. *The Alternative Medicine Handbook*. W.W. Norton and Co., New York, **1998**.
- 4 Lans, C. Harper, T. Georges K., Bridgewater, E. *BMC Complement. Altern. Med.* **2001**, *10*, 1
- 5 Cragg, G.M., Newman. D.J. *Ann. NY Acad. Sci.* **2001**, *953*, 3.
- 6 Ackerknecht, E.H. *Therapeutics: from the Primitives to the Twentieth Century*. Hafner Press, New York, **1973**.
- 7 Majno, G.M. *Healing Hand: Man and Wound in the Ancient World*. Harvard University Press, Cambridge, MA, **1975**.
- 8 Farnsworth, N.R., Morris, R.W. *Am. J. Pharm. Educ.* **1976**, *148*, 46.
- 9 Duke, J.A., Martinez, R.V. *Handbook of Ethnobotanicals (Peru)*. CRC Press, Boca Raton, FL, **1994**.
- 10 De Smet, P.A.G.M. *Drug Safety* **1995**, *13*, 81.
- 11 Cragg, G.M. Newman, D.J. Snader, K.M. *J. Nat. Prod.* **1997**, *60*, 52.
- 12 Shu. Y.Z. *J. Nat. Prod.* **1998**, *61*, 1053.
- 13 Liu, Z., Liu, Y. *J. Herbal Phytother.* **2001**, *1*, 25.
- 14 WHO. *The International Pharmacopeia*, Vol. 1: *General Methods of Analysis*, 3rd edn. World Health Organization, Geneva, **1979**.
- 15 WHO. *The International Pharmacopeia*, Vol. 2: *Quality Specifications*, 3rd edn. World Health Organization, Geneva, **1981**.
- 16 WHO. *The Use of Essential Drugs*. Eighth report of the WHO Expert committee. World Health Organization, Geneva, **1990**.
- 17 WHO. *Quality Control Methods for Medicinal Plant Materials*, World Health Organization, Geneva, **1998**.
- 18 WHO. *Guidelines for the Appropriate Use of Herbal Medicines*. WHO Regional Publications, Western Pacific Series No 3, WHO Regional office for the Western Pacific, Manila, **1998**.
- 19 WHO. *Quality Control Methods for Medicinal Plant Materials*. World Health Organization, Geneva, **1999**.
- 20 WHO. *WHO Monographs on Selected Medicinal Plants*, Vol. 1, World Health Organization, Geneva, **1999**.
- 21 WHO. *WHO Monographs on Selected Medicinal Plants*, Vol. 2, World Health Organization, Geneva, **2002**.
- 22 WHO. *Ancient Remedies, New Disease*. World Health Organization, Geneva, **2002**.
- 23 WHO. *WHO Global Atlas of Traditional, Complementary and Alternative Medicine*. 2 vol. set. Vol. 1 contains text and Vol. 2, maps. World Health Organization, Geneva, **2005**.
- 24 Akerele, O. *HerbalGram*. **1993**, *28*, 13.
- 25 Bisset, N.G. (ed.). *Herbal Drugs and Phytopharmaceuticals*. CRC Press, Boca Raton, FL, **1994**.
- 26 Calixto, J. B. *Braz. J. Med. Biol. Res.* **2000**, *33*, 179.
- 27 Pal, S.K., Shukla, Y. *Asian J. Cancer Prev.* **2003**, *4*, 281.
- 28 Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., Guo, Z. *World Health Organ.* **1985**, *63*, 965.
- 29 Mukherjee, P.W. *Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals*. Business Horizons Publishers, New Delhi, India, **2002**.
- 30 Bodeker, C., Bodeker, G., Ong, C.K., Grundy, C.K., Burford, G., Shein, K. *WHO Global Atlas of Traditional, Complementary and Alternative Medicine*. World Health Organization, Geneva, **2005**.
- 31 Kong, J.M., Goh, N.K., Chia, L.S., Chia, T.F. *Acta Pharm. Sinic.* **2003**, *24*, 7.

- 32 De Smet, P. A. G. M. *Drugs*, 1997, 54, 801.
- 33 Blumenthal, M. *HerbalGram* 1999, 45, 68.
- 34 Grunwald, J. *HerbalGram* 1995, 34, 60.
- 35 Roberts, J.E., Tyler, V.E. *Tyler's Herbs of Choice. The Therapeutic Use of Phytomedicinals*. The Haworth Press, New York, 1997.
- 36 Blumenthal, M., Brusse, W.R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C. W., Rister, R.S. *The Complete German Commission E Monographs. Therapeutic Guide to Herbal Medicines*. The American Botanical Council, Austin, TX, 1998.
- 37 Midley, J.M. *Pharm. J.* 1988, 241, 358.
- 38 Bauer, R., Tittel, G. *Phytomedicine*. 1996, 2, 193.
- 39 Baur, R. *Drug Inf. J.* 1998, 32, 101.
- 40 Schier, W., Sachsa, B., Schultze, W. *Dtsch Apoth. Ztg.* 1994, 134, 4569.
- 41 WHO. *The International Pharmacopeia*, Vol. 3: *Quality Specifications for Pharmaceutical Substances, Excipients, and Dosage Forms*, 3rd edn. World Health Organization, Geneva, 1988.
- 42 WHO. *Basic Tests for Drugs, Pharmaceutical Substances, Medicinal Plant Materials and Dosage Forms*. World Health Organization, Geneva, 1998.
- 43 WHO. *Quality Assurance of Pharmaceuticals: A Compendium of Guidelines and Related Materials*, Vol. 2: *Good Manufacturing Practices and Inspection*. World Health Organization, Geneva, 1996.
- 44 WHO. *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine*. World Health Organization, Geneva, 2002.
- 45 EMEA. *Guidelines on Quality of Herbal Medicinal Products/Traditional Medicinal Products*, EMEA/CVMP/81400 Review. European Agency for the Evaluation of Medicinal Products (EMA), London, 2005.
- 46 EMEA. *Quality of Herbal Medicinal Products. Guidelines*. European Agency for the Evaluation of Medicinal Products (EMA), London, 1998.
- 47 WHO. *Quality Control Methods for Medicinal Plant Materials*. World Health Organization, Geneva, 1992.
- 48 De Smet, P.A.G.M. *Drug Inf. J.* 1999, 33, 717.
- 49 Hsu, C.K., Leo, P., Shastry, D., Meggs, W., Weisman, R., Hoffman, R.S. *Arch. Intern. Med.* 1995, 155, 2245.
- 50 WHO. *Guidelines for the Assessment of Herbal Medicines*. WHO Technical Report Series, No 863. World Health Organization, Geneva, 1996.
- 51 Watson, D.G. *Pharmaceutical Analysis*. Churchill Livingstone, Edinburgh, 1999.
- 52 WHO. *Quality Control Methods for Medicinal Plant Materials*. World Health Organization, Geneva, 1988.
- 53 WHO. *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine*. World Health Organization, Geneva, 2000.
- 54 WHO. *WHO Guidelines on Good Agricultural and Collection Practices (GACP)*. World Health Organization, Geneva, 2003.
- 55 WHO. *Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants*. World Health Organization, Geneva, 2004.
- 56 EMEA. *Points to Consider on Good Agricultural and Collection Practice for Starting Materials of Herbal Origin*. EMEA/HMPWP/31/99 Review. European Agency for the Evaluation of Medicinal Products (EMA), London, 2002.
- 57 Eskinazi, D., Blumenthal, M., Farnsworth, N., Riggins, C.W. *Botanical Medicine: Efficacy, Quality, Assurance, and Regulation*. Mary Ann Liebert, New York, 1999.
- 58 Keller, K. *Drug Inf. J.* 1996, 30, 933.
- 59 Li, F., Sun, S., Wang, J., Wang, D. *Biomed. Chromatogr.* 1998, 12, 78.
- 60 AOAC. *Official Methods of Analysis of AOAC International*, 18th edn. AOAC International, Gaithersburg, MD, 2005.
- 61 De Smet, P.A.G.M., Keller, K. Hansel, R., Chandler, R.F. *Adverse Effects of Herbal Drugs*, Vol. 1. Springer-Verlag, Heidelberg, 1992.
- 62 Lazarowich, N., Pekos, P. *Drug Inf. J.* 1998, 32, 497.
- 63 Sarmani, S. B., Abugassa, I., Hamzah, A., Yahya, M.D. *Biol. Trace Elem. Res.* 1999, 71–72, 365.
- 64 Boumans, P.W.J.M. *Inductively Coupled Plasma-emission Spectroscopy Part 1*. John Wiley & Sons, New York, 1999.

- 65 WHO. *The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2000–2202* (WHO/PCS/01.5). International Programme on Chemical Safety, World Health Organization, Geneva, 2000.
- 66 Anonymous. *European Pharmacopoeia*, 3rd edn. Strasbourg Council of Europe, 1996.
- 67 Farber, J.M., Carter, A.O., Varughese, P.V., Ashton, F.E., Ewan, E.P. *New Engl. J. Med.* 1990, 322, 338.
- 68 Kneifel, W., Czech, E., Kopp, B *Planta Med.* 2002, 68, 5.
- 69 De Smet, P.A.G.M., in *Adverse Effects of Herbal Drugs*, De Smet, P.A.G.M., Keller, K., Hansel, R., Chandler, R.F. (eds), Vol. 3. Springer-Verlag, Heidelberg, 1997, p. 137.
- 70 De Smet, P.A.G.M., in *Adverse Effects of Herbal Drugs*, De Smet, P.A.G.M., Keller, K., Hansel, R., Chandler, R.F. (eds), Vol. 3. Springer-Verlag, Heidelberg, 1997, p. 145.
- 71 De Smet, P.A.G.M., in *Adverse Effects of Herbal Drugs*, De Smet, P.A.G.M., Keller, K., Hansel, R., Chandler, R.F. (eds), Vol. 3. Springer-Verlag, Heidelberg, 1997, p. 129.
- 72 Sinha, K.K. (ed.). *Mycotoxins in Agriculture and Food Safety*. Marcel Dekker, New York, 1998.
- 73 Anonymous. *Deutsches Arzneibuch 2000*. Deutscher Apotheker Verlag, Stuttgart, 2000.
- 74 Anonymous. *Momooptischen Arzneibuch 2000*. Deutscher Apotheker Verlag, Stuttgart, 2000.
- 75 van Valkenburg, J.L.C.H., Bunyapraphatsara, N. (eds). *Plant Resources of South-East Asia 12 (2): Medicinal and Poisonous Plants 2*. Backhuys Publ., Leiden, 2001.
- 76 WHO. *The International Pharmacopoeia*, Vol. 4: *Tests Methods, and General Requirements*, 3rd edn. World Health Organization, Geneva, 1988.
- 77 Rózylo, J.K., Zabinska, A., Matysiak, J., Niewiadomy, A. *J. Chromatogr. Sci.* 2002, 40, 581.
- 78 Bilia, A.R., Bergonzi, M.C., Lazari, D., Vincieri, F.F. *J. Agric. Food Chem.* 2002, 50, 5016.
- 79 Ebel, S., Gigalke, H.J., Voelkl, S. *AMD-HPTLC Analysis of Medicinal Plants*. Proceedings of 4th International Symposium of Instrumental HPTLC, Selvino/Bargamo at Italy, 1987, p. 113.
- 80 Ko, R.J., Greenwald, M.S., Loscutoff, S.M., Au, A.M., Appel, B.R., Kreutzer, R.A., Haddon, W.F., Jackson, T.Y., Boo, F.O., Presicek, G. *West. J. Med.* 1996, 164, 71.
- 81 Farnsworth, N.R., Henry, L.K., Svoboda, G.H., Blomster, R.N., Yates, M.J., Euler, K.L. *Lloydia* 1966, 29, 101.
- 82 Farnsworth, N.R., Henry, L.K., Svoboda, G.H., Blomster, R.N., Fong, H.H.S., Quimby, M.W., Yates, M.J. *Lloydia* 1968, 35, 35.
- 83 WHO. *The International Pharmacopoeia*, Vol. 4: *Test Methods and General Requirements. Quality Specifications for Pharmaceutical Substances, Excipients, and Dosage Forms*, 3rd edn. World Health Organization, Geneva, 1994.
- 84 WHO. *The International Pharmacopoeia*, Vol. 2: *Quality Specifications*, 3rd edn. World Health Organization, Geneva, 1983.
- 85 *The International Pharmacopoeia*, Vol. 4: *Test Methods and General Requirements. Quality Specifications for Pharmaceutical Substances, Excipients, and Dosage Forms*, 3rd edn. World Health Organization, Geneva, 1994.
- 86 IPCS. *Pyrrolizidine alkaloids, Health and Safety Guide*. Standards on Chemical Safety Health and Safety Guide No. 26. International Programme on Chemical Safety, Geneva, 1989.
- 87 Farnsworth, N.R., Bingel, A.S., in *New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutic Activity*. A.H. Wagner, P. Wolff (eds), Proceedings of the First International Congress on Medicinal Plant Research. Springer-Verlag, Berlin, Heidelberg, New York, 1977.
- 88 Evans, W.C. *Br. J. Phytother.* 1990, 1, 16.
- 89 Ramsay, H.M., Goddard, W., Gill, S., Moss, C. *Arch. Dis. Child.* 2003, 88, 1056.
- 90 Segasothy, M., Samad, S. *Nephron* 1991, 59, 199.
- 91 Chan, T.Y., Chan, J.C., Tomlinson, B., Critchley, J.A. *Lancet* 1993, 342, 1532.

- 92 Anonymous. *Evaluation of Certain Food Additives and Contaminants*. 22nd Report of the Joint FAO/WHO Expert committee on Food Additives. Technical Report Series 631. World Health Organization, Geneva, 1978.
- 93 Anonymous. *Evaluation of Certain Food Additives and Contaminants*. 33rd Report of the Joint FAO/WHO Expert committee on Food Additives. Technical Report Series 776. World Health Organization, Geneva, 1989.
- 94 Anonymous. *Evaluation of Certain Food Additives and Contaminants*. 41st Report of the Joint FAO/WHO Expert committee on Food Additives. Technical Report Series 837. World Health Organization, Geneva, 1993.
- 95 Ross, J.J.W., Pelders, M.G., De Smet, P.A.G.M. *Pharm. World Sci.* **1999**, *21*, 44.
- 96 Abt, A.B., Oh, J.Y., Huntington, R.A., Burkhart, K.K. *Arch. Intern. Med.* **1995**, *155*, 211.
- 97 Nelson, L., Shih, R., Hoffman, R. *J. Toxicol-Clin. Toxicol.* **1995**, *33*, 467.
- 98 Gertner, E., Marshall, P.S., Filandrinos, D., Potek, A.S., Smith, T.M. *Arthritis Rheum.* **1995**, *38*, 614.
- 99 Van der Stricht, B.I., Parvais, O.E., Vanhaelen-Fastre, R.J., Vanhaelen, M.H. *BMJ* **1994**, *308*, 1162.
- 100 Pitetti, R., Singh, S., Hornyak, D. *Pediatr. Emerg. Care.* **2001**, *17*, 165.
- 101 Herr, S.M. *Herb-Drug Interaction Handbook*, 3rd edn. ISBN 0967877326, **2005**.
- 102 Barnes, J., Anderson, L.A., Phillipson, J.D. *J. Pharm. Pharmacol.* **2001**, *53*, 583.
- 103 Smolinske, S.C. *J. Am. Med. Womens Assoc.* **1999**, *54*, 191.
- 104 Fugh-Berman, A. *Lancet* **2000**, *355*, 144.
- 105 Meneilly, G. Top ten tips for avoiding drug-herb interactions. *Living +* **2000**, *6*. <http://www.bcpwa.org/issue6/tentips.htm>. Accessed January 2004.
- 106 Ernst, E. *Eur. J. Pediatr.* **2003**, *162*, 72.
- 107 Janetzky, K., Morreale, A.P. *Am. J. Health Syst. Pharm.* **1997**, *54*, 692.
- 108 Jowell, T. *Herbal Medicines*. House of Commons official report (Hansard). **1999**, *26*, 426.
- 109 Mathews, J. *Holisticonline.com* 1998–2005. [http://www.holistic-online.com/alt\\_medicine/altmed\\_definitions.htm](http://www.holistic-online.com/alt_medicine/altmed_definitions.htm). Accessed August 2005.
- 110 Moore, B., Adler, R. *Pediatrics.* **2000**, *106*, 600.
- 111 Florido-Lopez, F., Gonzalez-delgado, P., Desanpedro, B.S., Perez-Miranda, C., De Saavedra, J.M.A., Marinpozo, J.F. *Int. Arch. Allergy Immun.* **1995**, *108*, 170.
- 112 D'Arcy, P.F. *Adverse Drug React. Toxicol. Rev.* **1991**, *10*, 189.
- 113 D'Arcy, P.F. *Adverse Drug React. Toxicol. Rev.* **1993**, *12*, 147.
- 114 Rosenblatt, M., Mindei, J. *N. Engl. J. Med.* **1997**, *336*, 1108.
- 115 Pillans, P.I. *N. Z. Med. J.* **1995**, *108*, 469.
- 116 Winston, D., Nvwoti. in *American Herbalism*, Tierra, M. (ed.). The Crossing Press, Freedom, CA, **1992**.
- 117 McRae, S. *Can. Med. Assoc. J.* **1996**, *155*, 293.
- 118 Marcus, D.M., Snodgrass, W.R. *Obstet. Gynecol.* **2005**, *105*, 1119.
- 119 Marcus, D.M., Snodgrass, W.R. *Obstet. Gynecol.* **2005**, *106*, 640.
- 120 Tomassoni, J., Simone, K. *Curr. Opin. Pediatr.* **2001**, *13*, 162.
- 121 Brinker, F. *An Introduction to the Toxicology of Common Botanical Medicines*. NCNM (National College of Naturopathic Medicine), Oregon, **1983**. AMA. *AMA Handbook of Poisonous and Injurious Plants*. American Medical Association, Chicago, **1985**.
- 122 Sharma, M.L., Chandokhe, N., Ray Ghatak, B.J., Jamwal, K.S., Gupta, O.P., Singh, G.B., Ali, M.M., Thakur, R.S., Handa, K.L., Rao, P.R., Jamwal, P.S., Sareen, Y.K. *Indian J. Exp. Biol.* **1978**, *16*, 228.
- 123 Aswal, B.S., Bhakuni, D.S., Goel, A.K., Kar, K., Mehrotra, B.N. *Indian J. Exp. Biol.* **1984**, *22*, 487.
- 124 Boyd, N.G. *Princ. Pract. Oncol.* **1989**, *3*, 1.
- 125 Farnsworth, N.R. *HerbalGram* (Special Suppl.), **1993**, *29*, 36A–36H.
- 126 Farnsworth, N.R., Loub, W.D., in *Plants: the Potential for Extracting Proteins, Medicines, and Other Useful Chemicals*. Workshop proceedings. OTA-BP-F-23. US Congress, Office of Technology Assessment, Washington, DC, **1983**.
- 127 Loub, W.D., Farnsworth, N.R., Soejarto, D.D., Quinn, M.L. *J. Chem. Inf. Comput. Sci.* **1985**, *25*, 99.
- 128 Strosberg, A.D., Marullo, S. *Trends Pharmacol. Sci.* **1992**, *13*, 95.

- 129 Hobden, A.N., Harris, T.J.R. *Proc. R. Soc. Edin. B.* **1992**, 99, 37.
- 130 Cragg, G.M., Boyd, M.R. Drug development at the national cancer institute, in Balick, M.J., Elisabetsky, E., Laird, S.A. (eds) *Medicinal Resources of the Tropical Forest*. Columbia University Press, New York, **1999**.
- 131 Hallowell, C. *Time* **1997**, 150, 16.
- 132 Poulev, A., Deus-Neumann, B., Zenk, M.H. *Planta Med.* **1993**, 59, 442.
- 133 Budzinski, J.W., Foster, B.C., Vandenhoeck, S., Arnason J.T. *Phyto-medicine* **2000**, 7, 273.
- 134 Mitscher, L.A., Telikepalli, H., Wang, P.B., Kuo, S., Shankel, D.M., Stewart, S. *Mutat. Res.* **1992**, 267, 229.
- 135 Williamson, N. *Am. Hort.* **1995**, 74.
- 136 Tona, L., Kambu, K., Mesia, K., Cimanga, K., Apers, S., De Bruyne, T., Pieters, L., Totte, L., Vlietinck, A.J. *Phytomedicine*. **1999**, 6, 59.
- 137 Gaedcke, F., Steinhoff, B. *Herbal Medicinal Products. Scientific and Regulatory Basis for Development, Quality Assurance and Marketing Authorisation*. CRC Press, Boca Raton, **2003**,
- 138 Snider, S. *FDA Consum.* **1991**, 31.
- 139 Drew, A., Myers, S.P., *Med. J. Aust.* **1997**, 166, 538.
- 140 Wichtl, N.G., Wichtl, M. (eds). *Asian J. Can. Prev.* **2000**.
- 141 Arvigo, R., Balick, M. *Rainforest Remedies: 100 Healing Herbs of Belize*. Lotus Press, Twin Lakes, WI, **1993**.
- 142 Rousseaux, C.G., Schachter, H. *Birth Defects Res. B: Dev. Reprod. Toxicol.* **2003**, 68, 505.
- 143 Zeisel, S.H. *Science* **1999**, 285, 1853.
- 144 Petrovick, P.R., Marques, L.C., Paula, I.C., *J. Ethnopharmacol.* **1999**, 66, 51.
- 145 Haden, B.L., Haden, C.R. *Alternative Health Care: The Canadian Directory*. Nobel Agnes Publishing, Toronto, **1997**.
- 146 Mukherjee, P.K., Verpoorte R. (eds) *GMP for Botanicals: Regulatory and Quality Issues on Phytomedicines*. Business Horizons, New Delhi, **2003**.
- 147 WHO. *Legal Status of Traditional Medicine and Complementary/Alternative Medicine*. World Health Organization, Geneva, **2002**.
- 148 WHO. *Bulletin of the World Health Organization. Regulatory Situation of Herbal Medicines. A Worldwide Review*. World Health Organization, Geneva, **1998**.
- 149 WHO. *Regulatory Situation of Herbal Medicines: a Worldwide Review*. World Health Organization, Geneva, **1998**.
- 150 WHO. *Guidelines for the Regulatory Assessment of Medicinal Products for Use in Self Medication*. World Health Organization, Geneva, **2002**.
- 151 WHO. *Research Guidelines for the Safety and Efficacy of Herbal Medicines*. World Health Organization, Geneva, **1993**,
- 152 Chaudhury R.R. *Herbal Medicine for Human Health*. SEARO, No. 20. World Health Organization, New Delhi, **1992**.
- 153 Lueng, A. *HerbalGram* **2000**, 48, 63.
- 154 Wagner, H. Natural products chemistry and phytomedicine research in the new millennium: new developments and challenges. [http://www.arkat-usa.org/ark/journal/2004/107\\_Antus/SA-1008R/1008R.asp](http://www.arkat-usa.org/ark/journal/2004/107_Antus/SA-1008R/1008R.asp). Accessed 26 September 2005, **2004**.

