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#### Summary

Today's use of medicinal plants and bioactive phytocompounds worldwide and our scientific knowledge of them comprises the modern field of the "phytosciences." The phytosciences have been created from the integration of disciplines that have never been linked before, combining diverse areas of economic, social, and political fields, chemistry, biochemistry, physiology, microbiology, medicine, and agriculture.

The field is unique among the biomedical sciences in that instead of testing a hypothesis, in the phytosciences researchers try to determine whether plants commonly used in traditional medicine brings benefits for health and, if so, what their mechanisms of action are.

Despite the common belief that phytocompounds are safe, they all have inherent risks just like synthetic compounds. Thus it is within the scope of the phytosciences to elucidate side-effects, appropriate doses, identify bioactive phytocompounds and ways of extraction and conservation. Besides these, legal aspects regarding regulation of the prescription and commercial sale of medicinal plants are a matter of debate all around the world. The varied regulations in different jurisdictions regarding the prescription and sale of these products add confusion to the formal use of phytocompounds.

As a multidisciplinary science, research in the phytosciences is almost unlimited, which makes it impossible to discuss all aspects of this emerging science in just one chapter. Therefore, we have focussed here mainly on the antimicrobial activity of bioactive phytocompounds, discussing their use against multidrug-resistant (MDR) bacteria and fungi, their mechanisms of action, and their interactions with macromolecules and potential for toxicity in mammalian cells. Technical aspects regarding the development of fast and reliable methods of extraction, highoutput screening systems, and bioautography of essential oils and crude extracts and fractions have also been discussed. Problems related to the efficacy, stability, drug delivery systems and quality control are also commented on.

Overall this chapter aims to provide a better understanding of the modern field of the phytosciences and its application in the world today.

## 1.1 Introduction

To trace the history of phytotherapy is to trace the history of humanity itself. The discovery of the curative properties of certain plants must have sprung from instinct. Primitive peoples first used plants as food and, as result of this ingestion, the link with some plant properties would have been learnt. Medicinal plants were the main source of products used to sustain health until the nineteenth century, when the German chemist Friedrich Wöhler in 1828, attempting to prepare ammonium cyanate from silver cyanide and ammonium chloride, accidentally synthesized urea. This was the first organic synthesis in history and heralded the era of the synthetic compound.



#### a)

**Fig. 1.1** Pedanius Dioscorides, *De Materia Medica* (AD 65). Greek physician Pedanius Dioscorides (c. 40–c. 90) was from Anazarbus, a small town near Tarsus in what is now south-central Turkey. As a surgeon with the Roman army of Emperor Nero, Dioscorides traveled through Italy, Gaul, Spain, and North Africa, recording the



existence and medicinal value of hundreds of plants. He compiled an extensive listing of medicinal herbs and their virtues in about AD 70. Originally written in Greek, Dioscorides's herbal was later translated into Latin as *De Materia Medica*. It remained the authority in medicinal plants for over 1500 years.

2

During the 100 years following Wöhler's discovery phytomedicine was largely forgotten by Western science. In the early 1980s, however, there was a resurgence of interest in the use of natural substances generally known today as bioactive phytocompounds. This interest can be easily understood in the light of questions concerning the safety, cytotoxicity, and side-effects of synthetic compounds, and the need to find new medicines, including new antibiotics to manage infectious diseases caused by multiresistant pathogens and substances to treat chronic diseases.

Today, the use of medicinal plants and their bioactive phytocompounds and our scientific knowledge about them comprises the modern field of the phytosciences. This is a science created from the integration of a range of disciplines that have never been linked before, combining several different areas of economic, social, and political fields, chemistry, biochemistry, physiology, microbiology, medicine, and agriculture.

The phytosciences are different from the other biomedical sciences in that instead of testing a hypothesis, researchers try to determine whether plants commonly used in traditional medicine bring benefits for health and, if so, what are their mechanisms of action. Despite the common belief that bioactive phytocompounds are safe, they have inherent risks just like all active chemical compounds. Researchers within the phytosciences are working to elucidate the side-effects, calculate appropriate dosages, identify the bioactive components, and define the best methods of extraction and conservation. Besides these, legal aspects regarding the prescription and trade in medicinal plants are a matter of debate all around the world. The varying regulations in different jurisdictions allowing the prescription and sale of these products add confusion to the formal use of bioactive phytocompounds.

As a multidisciplinary science the research in this field is almost unlimited, which makes it impractical to discuss all the aspects of this emerging science in just one chapter. Therefore, this review discusses the antimicrobial activity of bioactive phytocompounds, particularly their use against multidrug-resistant bacteria and fungi, their mechanisms of action, and their interactions with macromolecules and potential toxicity for mammalian cells. It also discusses technical aspects regarding the development of fast and reliable methods of extraction, high-output screening systems and bioauthography of essential oils and crude extracts and fractions. Problems related to the efficacy, stability, drug delivery systems and quality control will also be discussed.

#### 1.2

## Development of Fast Reliable Methods of Extraction and High-Throughoutput Screening (HTS) of Crude Plant Extracts: New Challenges

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade. Recognition of their clinical, pharmaceutical, and economic value is still growing, although this varies widely between countries. Plants are important for pharmacological research and drug development, not on-

ly when bioactive phytocompounds are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. Regulation of their exploitation and exportation is therefore essential to ensure their availability for the future [1].

Plant preparations have a very special characteristic that distinguishs them from chemical drugs: a single plant may contain a great number of bioactive phytocompounds and a combination of plants even more. This complexity is one of the most important challenges to phytoscientists attempting to identify a single bioactive phytocompound or chemical group in the enormous universe that comprises a single crude extract.

Biotechnology in the 1970s and 1980s made tremendous strides and ushered in a new era for the pharmaceutical industry. Many enzymes and receptor proteins of therapeutic interest were made available in large quantities by recombinant expression, while signal transduction pathways could be interrogated by reporter gene carrying cellular constructs. Such mechanism-based *in vitro* assays are amenable to large scales of operation, and the concept of high-throughput screening rapidly became the paradigm for lead discovery [2].

High-throughput screening, often abbreviated as HTS, is a method of scientific experimentation especially relevant to the fields of biology and chemistry. Through a combination of modern robotics and other specialized laboratory hardware, it allows a researcher to effectively conduct hundreds of scientific experiments at once. In essence, HTS uses a brute-force approach to collect a large amount of experimental data, usually observations about how some biological entity reacts to exposure to various chemical compounds in a relatively short time. A screen, in this context, is the larger experiment, with a single goal to which all this data may subsequently be applied [3].

A necessary precondition for the success of the HTS approach is a large and diverse compound collection. In the early days, this largely comprised in-house archives and natural product extracts. The former represented the efforts of chemists internally over the years, supplemented by purchase from external sources. Neither the total number of compounds, nor their chemical diversity, was appropriate to feed HTS. These deficiencies created the science of combinatorial chemistry in the late 1980s and early 1990s and an unanticipated repercussion of high-throughput chemical synthesis was a steady waning of interest in natural product screening, leading to its complete abandonment by many companies [4].

Just like drugs of synthetic origin, bioactive phytocompounds range from simple to complex structures. Either way, the evaluation of a bioactive phytocompound or a natural product leads to benefits from modern HTS for the generation of analogs [5]. Thus, paradoxically, the same combinatorial chemistry that initially caused the decline in natural product screening now promises to be an essential tool in rejuvenating it. Academic groups in particular are used to allocating significant resources of time and staff towards the total synthesis of bioactive phytocompounds. The ability to adapt such routes for the preparation of analogs is an obvious strategy for leveraging the initial expenditure, and is now increasingly evident in the literature. Because of the stricter timelines, large-scale combinatorial programs based on natural products are less common in industry, but are still practiced in the absence of more tractable synthetic leads [6].

Combinatorial chemistry has come a long way in the past two decades. Industrially, it competed with natural product extracts and purified bioactive phytocompounds for HTS resources and emerged as the preferred option. Unfortunately this technique has not produced a wealth of high-quality drug candidates. Instead, the integration of combinatorial chemistry with other mechanisms for lead generation is now rightly considered the correct strategy. A natural product lead is a legitimate starting point for combinatorial chemistry, and this process can often discover novel analogs [7]. In some cases, such compounds are more potent than the natural product or can possess superior drug-like properties. In others, the synthetic analogs display new biological activities not seen with the original molecule [4].

The ability to rapidly identify undesirable or desirable compounds in natural product extract libraries is a critical step in an efficiently run natural products discovery program. This process, commonly called dereplication [8], is important to prevent the unnecessary use of resources for the isolation of compounds of little or no value for development from extracts used in the screening process. Resources can then be focussed on samples containing the most promising leads. The recent application of HTS technologies to assay natural products extracts for biological activity has intensified the need for efficient dereplication strategies [9].

Dereplication of the bioactive phytocompounds in crude natural product extracts requires some form of feedback from the bioassay, which was initially used to detect the biological activity. This is necessary regardless of the separation technique and analytical method used. A common strategy has been to collect fractions from the high-performance liquid chromatography (HPLC) separation in deep-dish microtiter plates or tubes and then resubmit the individual fractions to the original assay. This approach requires desiccation of fractions to remove the HPLC solvents, which are usually incompatible with the bioassay, resuspending the fractions in a compatible solvent (water, DMSO, or Tween), and then individual assaying of each fraction. This process is not cost effective, being both time and labor intensive. Consequently, as a result of the increasing emphasis on the generation of new lead compounds, faster cycle times, and high efficiency, many pharmaceutical companies have moved away from the natural products area.

Currently, almost every large pharmaceutical company has established HTS infrastructures and possesses large combinatorial compound libraries, which cover a wide range of chemical diversity. However, the ability to detect the desired biological activity directly in the HPLC effluent stream and to chemically characterize the bioactive phytocompound on-line, would eliminate much of the time and labor taken in the fraction collection strategy. This way, cycle times, expenses, and the isolation of known or undesirable compounds would be reduced dramatically, allowing natural products to be screened in an efficient and cost effective manner [10].

Recently, such an on-line HPLC biochemical detection (BCD) system, in the following referred to as high-resolution screening (HRS) system, has been described for a range of pharmacologically relevant targets, such as the human estrogen receptor, cytokines, leukotrienes, and the urokinase receptor [11]. In contrast to con-

ventional microtiter-type bioassays, the interactions of the extracts and the biochemical reagents proceed at high speed in a closed continuous flow reaction detection system. When sufficient chromatographic separation is achieved, the individual contribution of the bioactive phytocompounds to the total bioactivity is obtained within a single run. Moreover, by combining on-line biochemical detection with complementary chemical analysis techniques, such as mass spectrometry (HRS-MS), chemical information that is crucial for the characterization and identification of bioactive phytocompounds is obtained in real time. Biochemical responses are rapidly correlated to the recorded MS and MS/MS data, thus providing chemical information such as molecular weight and MS/MS fingerprints [12]. Compared with traditional screening approaches of complex mixtures, which are often characterized by a repeating cycle of HPLC fractionation and biological screening, HRS-MS analysis speeds up the dereplication process dramatically. Moreover, the technology enables drug discovery programs to access the enormous chemical diversity offered by complex mixtures as a source of novel drug-like molecules [13]. The use of chromatographical assays is discussed in the next section of this chapter.

#### 1.3

# Antimicrobial Bioactive Phytocompounds from Extraction to Identification: Process Standardization

Different approaches to drug discovery using higher plants can be distinguished: random selection followed by chemical screening; random selection followed by one or more biological assays; biological activity reports and ethnomedical use of plants [14]. The latter approach includes plants used in traditional medical systems; herbalism, folklore, and shamanism; and the use of databases. The objective is the targeted isolation of new bioactive phytocompounds. When an active extract has been identified, the first task to be taken is the identification of the bioactive phytocompounds, and this can mean either a full identification of a bioactive phytocompound after purification or partial identification to the level of a family of known compounds [15].

In Fig. 1.2 an extraction-to-identification flowchart is proposed in order to optimize bioactive phytocompound identification. For screening selection, plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Initial screening of plants for possible antimicrobial activities typically begins by using crude aqueous or alcohol extractions followed by various organic extraction methods [16]. Plant material can be used fresh or dried. The aspects of plant collection and identification will be discussed further in this chapter. Other relevant plant materials related to antimicrobial activity are the essential oils. Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by hydro or steam distillation and by expression (citrus peel oils). The main constituents of essential oils (mono and sesquiterpenes), along with carbohydrates, alcohols, ethers, aldehydes, and ketones, are responsible for the fragrant and biological properties of aromatic and medicinal plants. Due to these properties, since ancient times spices and herbs have been added to food, not only as flavoring agents but also as preservatives. For centuries essential oils have been isolated from different parts of plants and are also used for similar purposes.

The activities of essential oils cover a broad spectrum. Various essential oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant, and anticancerogenic properties [17–19]. Others are biocides against a broad range of organisms such as bacteria, fungi, protozoa, insects, plants, and viruses [20–22].

The dispersion of the hydrophobic components of essential oils in the growth medium is the main problem in testing the activity of essential oils. Different or ganic solvents must be used as solubilizing agents, which may interfere with the results of antimicrobial assays. The solution to this problem is the use of nonionic emulsifiers, such as Tween 20 and Tween 80. These molecules are relatively inactive and are widely applied as emulsifying agents. Control tests must guarantee that these emulsifying agents do not interfere in the experiments.

Plants can be dried in a number of ways: in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms, or in buildings; by direct sunlight, if appropriate; in drying ovens/rooms and solar dryers; by indirect fire; baking; lyophilization; microwave; or infrared devices. Where possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials. For example, shade drying is preferred to maintain or minimize loss of color of leaves and flowers; and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances [23]. The drying conditions should be recorded. In the case of natural drying in the open air, medicinal plant materials should be spread out in thin layers on drying frames and stirred or turned frequently. In order to secure adequate air circulation, the drying frames should be located at a sufficient height above the ground. Efforts should be made to achieve uniform drying of medicinal plant materials to avoid mold formation [24].

Drying medicinal plant material directly on bare ground should be avoided. If a concrete or cement surface is used, the plant materials should be laid on a tarpaulin or other appropriate cloth or sheeting. Insects, rodents, birds and other pests, and livestock and domestic animals should be kept away from drying sites. For indoor drying, the duration of drying, drying temperature, humidity and other conditions should be determined on the basis of the plant part concerned (root, leaf, stem, bark, flower, etc.) and any volatile natural constituents, such as essential oils. If possible, the source of heat for directs drying (fire) should be limited to butane, propane or natural gas, and temperatures should be kept below 60 °C [25]. If other sources of fire are used, contact between those materials, smoke, and the medicinal plant material should be avoided.

Since researches are trying to identify bioactive phytocompounds in medicinal plant extracts generally used by local population to treat diseases and based on empiric knowledge that they have the searched bioactivity, the solvent chosen must be



8

Fig. 1.2 Standardization flowchart: from extraction to identification of bioactive phytocompounds. (1) Plants can be chosen either randomly, based on the literature or following consulation with local healers. After choosing the right material, plant collection must be followed by botanical identification and a voucher specimen must be placed in the local herbarium. All data about the collection must be observed and documented, such as climate conditions, season, geographical localization, environmental conditions, etc. in order to elucidate future differences in bioactivity compared with other results found. Any plant part can be used but consultation of the literature or with local healers is very useful to reduce research time. (2) Collected plant material can be used fresh or dried. Several studies have started extractions with both fresh and dried material in order to compare the chemical composition of the extracts. They must be ground to optimize the solvent contact during the extraction process. Weight standardization must be used (i.e. 300 g of plant material to 1000 mL of solvent). More than 90% of the studies for antimicrobial activity in the literature start extraction with methanol, ethanol or water because it is proved that ethanol extraction is more effective in isolating the bioactive phytocompound. The primary extractions methods are very variable but the idea is to research activity cited in popular use, and to choose the same extraction method. This is especially useful to corroborate the in vivo activity found in popular use. (3) After extraction the volume must be concentrated by lyophilization or another concentration technique before screening. Usually, after the lyophilization process ground powder is obtained. This must be resuspended in water at a higher concentration (i.e.  $1 \text{ g mL}^{-1}$ ) for initial drop test screening. The high concentration of the extract guarantees the identification of the bioactivity, if present. Using low concentrations in drop tests may lead to false negative results. (4) Due to the complex composition of the extract primary separation may be used to facilitate the identification process.

Micromolecules can be separated from macromolecules (proteins and carbohydrates) by very simple techniques such as ethanol precipitation (30% v/v), centrifugation (10 000g for 10 min) and filtration systems such as Centricon and Amicon (Millipore). Supernatant and precipitate phases are obtained and can be separated in drop tests. As discussed previously, antimicrobial activity is commonly present in micromolecules (supernatant) phase. (5) The antimicrobial screening by drop test (formerly disk diffusion agar assay) is the most efficient and inexpensive assay to identify antimicrobial activity. The extract is dropped (i.e. 15 µL) onto an agar surface previously inoculated with the desired microorganism. Note that is very important to count by McFarland scale or Newbauer chamber (i.e.  $10^5$  UFC mL<sup>-1</sup> for bacteria;  $10^6$  cells mL<sup>-1</sup> for fungi) the microorganism inoculums; this permits the antimicrobial activity to be compared within antibiotic controls and between different microorganism groups. (6) When antimicrobial activity is detected the minimum inhibitory concentration (MIC) must be determined to continue other antimicrobial assays of interest. The MIC is usually established by the broth dilution method. The use of 96-microwell plates to minimize costs is very effective, reducing the culture media quantities drastically and enhancing the test capacity (in one plate up to eight different extracts can be tested in 10 different concentrations plus 1 negative and 1 positive controls, also see Fig. 1.3). (7) Bioguided chromatography techniques such as bioautography preceded by solvent separation is essential to initiate the bioactive phytocompound identification process; fraction collection with HPLC or FPLC assays, preparative TLC are also valid techniques. Bio-guided fraction and purification confirms previous results leading to isolation of a bioactive phytocompound. (8) By TLC assays, Rf values can be determined and polarity or even chemical groups (use of specific dyes) elucidated (Fig. 1.3). (9) NMR, HPLC/MS, and GC/MS are used to identify a bioactive phytocompound as discussed in this chapter.

the same as that used in popular treatment. As we know, water and ethanol are by far the most commonly used, and for this reason most studies begin with water or ethanol as solvents.

Water is almost universally the solvent used to extract activity. At home, dried plants can be ingested as teas (plants steeped in hot water) or, rarely, tinctures (plants in alcoholic solutions) or inhaled via steam from boiling suspensions of the parts. Dried plant parts can be added to oils or petroleum jelly and applied externally. Poultices can also be made from concentrated teas or tinctures.

Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained initially through ethanol and water extraction [26]. Some water-soluble compounds, such as polysaccharides like starch and polypeptides, including fabatin [27] and various lectins, are commonly more effective as inhibitors of virus adsorption and would not be identified in the screening techniques commonly used [28]. Occasionally tannins and terpenoids may be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Fig. 1.2).

Another concern during the extraction phase is that any part of the plant may contain active components. For instance, the roots of ginseng plants contain the active saponins and essential oils, while eucalyptus leaves are harvested for their essential oils and tannins. Some trees, such as the balsam poplar, yield useful substances in their bark, leaves, and shoots [29]. The choice of which part to use must be based on ethnopharmacological studies and review of the literature.

For alcoholic extractions, plant parts are dried, ground to a fine texture, and then soaked in methanol or ethanol for extended periods. The slurry is then filtered and washed, after which it may be dried under reduced pressure and redissolved in the alcohol to a determined concentration. When water is used for extractions, plants are generally soaked in distilled water, blotted dry, made into slurry through blending, and then strained or filtered. The filtrate can be centrifuged (approximately 10000 g for 10 min) multiple times for clarification [30]. Crude products can then be directly used in the drop test and broth dilution microwell assays (Fig. 1.2) to test for antifungal and antibacterial properties and in a variety of assays to screen bioactivity (Fig. 1.3).

In order to reduce or minimize the use of organic solvents and improve the extraction process, newer sample preparation methods, such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE) have been introduced for the extraction of analytes present in plant materials. Using MAE, the microwave energy is used for solution heating and results in significant reduction of extraction time (usually in less than 30 min). Other than having the advantage of high extraction speed, MAE also enables a significant reduction in the consumption of organic solvents. Other methods, such as the use of SFE that used carbon dioxide and some form of modifiers, have been used in the extraction of compounds present in medicinal plants [31].

To identify the bioactive phytocompounds, liquid chromatography with an isocratic/gradient elution remains the method of choice in the pharmacopeia, and re-



Fig. 1.3 Current assays to identify bioactivity and start molecule identification. (A/B) Bioauthography technique: (A) Thin-layer chromatography (TLC) of aqueous extracts of (1) Ocimun gratissimum, (2) Anadenanthera macrocarpa, (3) Croton cajucara Benth. (4) Cymbopogon citrates, and (5) Juglans regia performed in silica gel G60 F254 aluminum plates (5 \_ 8). Plates were developed with nbutanol:acetic acid:water (8:1:1, v/v) and were visualized under ultraviolet light or after staining with cerric sulfate plate. (B) Alternatively, plates were placed inside Petri dishes and covered with over solid media (10 mL BHI with 1% phenol red). After overnight incubation for diffusion of the separated components, the plate was inoculated with Candida albicans (ATCC

51501) 10<sup>6</sup> cells per plate and incubated for 48 h at 37 °C. Growth inhibition can be seen in (1, 2, and 3) after spraying with methylthiazollyltetrazolium chloride (MTT) at 5 mg mL<sup>-1</sup>. (C) Drop test at same concentrations (200  $\mu g~mL^{-1})$  of (1) aqueous extract from Punica granatum and commercially available antifungal agents, (2) fluconazole, (3) flucytosine, and (4) anphotericin. (D) MIC microwell dilution test of (L1) Punica granatum, (L2) fluconazole, and (L3) flucytosine against Candida albicans (ATCC 51501). (C+) positive control, (C-) negative control, (1) 200  $\mu$ g mL<sup>-1</sup>, (2) 100  $\mu$ g mL<sup>-1</sup>, (3) 50 μg mL<sup>-1</sup>, (4) 25 μg mL<sup>-1</sup>, (5) 12.5 μg mL<sup>-1</sup>, (6) 6.75 μg mL<sup>-1</sup>, (7) 3.4 μg mL<sup>-1</sup>, (8) 1.7  $\mu$ g mL<sup>-1</sup>, and (9) 0.8  $\mu$ g mL<sup>-1</sup>. (+) means fungi growth.

versed octadecyl silica (C-18) and ultraviolet detection mode is the most commonly used method. Gradient elution HPLC with reversed phase columns has also been applied for the analysis of bioactive phytocompounds present in medicinal plants extracts [32].

The advantages of liquid chromatography include its high reproducibility, good linear range, ease of automation, and its ability to analyze the number of constituents in botanicals and herbal preparation. However, for the analysis of multiple bioactive phytocompounds in herbal preparations with two or more medicinal plants, coeluting peaks were often observed in the chromatograms obtained due to

the complexity of the matrix. The complexity of matrix may be reduced with additional sample preparation steps, such as liquid–liquid partitioning, solid-phase extraction, preparative LC and thin-layer chromatography (TLC) fractionation.

Capillary electrophoresis (CE) proved to be a powerful alternative to HPLC in the analysis of polar and thermally labile compounds. Reviews on the analysis of natural medicines or natural products in complex matrix by CE are well reported. Many publications showed that all variants of CE, such as capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MEKC), and capillary isoelectric focusing (cIEF), have been used for the separation of natural products. The separation in CZE is based on the differences in the electrophoretic mobilities resulting in different velocities of migration of ionic species in the electrophoretic buffer in the capillary. For MEKC, the main separation mechanism is based on solute partitioning between the micellar phase and the solution phase. Factors that are known to affect separation in CZE and MEKC include the pH of the running buffer, ionic strength, applied voltage, and concentration and type of micelle added. From the review articles, CE has been used to determine the amount of catechin and others in tea composition, phenolic acids in coffee samples and flavonoids and alkaloids in plant materials.

Chromatographic separation with mass spectrometry for the chemical characterization and composition analysis of botanicals has been growing rapidly in popularity in recent years. Reviews on the use of mass spectrometry and high-performance liquid chromatography mass spectrometry (HPLC/MS) on botanicals have been reported. The use of hyphenated techniques, such as high-resolution gas chromatography mass spectrometry (HRGC/MS), high performance liquid chromatography/mass spectrometry (HPLC/MS), liquid chromatography tandem mass spectrometry (HPLC/MS) and tandem mass spectrometry (MS/MS) to perform on-line composition and structural analyses provide rich information that is unsurpassed by other techniques.

HRGC/MS remains the method of choice for the analysis of volatile and semi-volatile components, such as essential oils and others in botanicals and herbal preparations, along with high-resolution separation with capillary column coupling with mass spectrometry using electron impact ionization (EI).

In analyzing bioactive phytocompounds, HPLC/MS has played an increasingly significant role as the technique is capable of characterizing compounds that are thermally labile, ranging from small polar molecules to macromolecules, such as peptides/proteins, carbohydrates, and nucleic acids. The most common mode of ionization in HPLC/MS includes electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Mass analyzers, such as single quadruple, triple quadruple, ion-trap, time-of-flight, quadruple time-of-flight (Q-TOF) and others, are also used. With tandem mass spectrometry, additional structural information can be obtained about the target compounds. However, methods using HPLC/MS are still limited to conditions that are suitable for MS operations. There are restrictions on pH, solvent choice, solvent additives and flow rate for LC in order to achieve optimal sensitivity.

For the identification of bioactive phytocompounds by HRGC/MS or HPLC/MS, the following conditions are useful when standards are available: a suspect peak has to show a retention time similar to the average retention time of the pure standard or control sample and mass spectra for the suspect peaks have to show relative abundance  $\pm 10\%$  (arithmetic difference) of the relative abundance of the standard analyzed that day. With HPLC/MS, applying the right separation, with the right ionization interface and mass analyzer, significant information can be obtained with regards to the target compounds. However, for the quantification of bioactive phytocompounds in plant materials, the system precision will be higher compared to that obtained using HPLC with ultraviolet detection. For on-line HPLC/MS, the internal diameter of the column selected will be an important consideration.

Another important chromatography technique is bioautography (Fig. 1.3). Bioautography is often used as an option to identify chemical groups of bioactive phytocompounds or even a single bioactive phytocompound when padrons are available. The complex chemical composition of plant extracts is generally a limiting obstacle to the isolation of antimicrobial compounds. Nevertheless, the use of bioautography agar overlay bioassays allows the detection of active components in a crude plant extract. This method permits the localization of antimicrobial active components that have been separated by TLC [33]. Precipitation with ethanol of plant aqueous extracts allows the separation of polymers, such as polysaccharides and proteins, from micrometabolites [34, 27]. By this technique, the solvation between molecules is changed, and in the same way, the interaction between molecules. Polymers (macromolecules) will be found in the water-soluble precipitate and micrometabolites in the supernatant. The precipitation of macromolecules can also be achieved by ammonium sulfate and acetone. The association of bioautography and ethanol precipitation techniques allows the detection of otherwise nondetectable bioactive phytocompounds [35].

An extremely important aspect of chromatography techniques is to identify nonnatural molecules, such as paracetamol, that may be present in or added to health supplements and commercially available herbal preparations.

#### 1.4

## Problems Associated with the Efficacy, Stability and Quality Control of Herbal Drugs Preparations

The number of reports of patients experiencing negative health consequences caused by the use of herbal medicines has increased in recent years [36]. Analysis and studies have revealed a variety of reasons for such problems. One of the major causes of reported adverse events is directly linked to the poor quality of herbal medicines, including raw medicinal plant materials. It has therefore been recognized that insufficient attention has been paid to the quality assurance and control of herbal medicines [37].

Quality control directly impacts the safety and efficacy of herbal medicinal products [38]. The implementation of good agricultural and collection practises for medicinal plants is only the first step in quality assurance, on which the safety and efficacy of herbal medicinal products directly depend, and also plays an important role in the protection of natural resources of medicinal plants for sustainable use.

Some reported adverse events following the use of certain herbal medicines have been associated with a variety of possible explanations, including the inadvertent use of the wrong plant species, adulteration with undeclared other medicines and/or potent substances, contamination with undeclared toxic and/or hazardous substances, overdosage, inappropriate use by health care providers or consumers, and interactions with other medicines, resulting in adverse drug effects [39].

The safety and quality of raw medicinal plant materials and finished products depend on factors that may be classified as intrinsic (genetic) or extrinsic (environment, collection methods, cultivation, harvest, post-harvest processing, transport, and storage practises). Inadvertent contamination by microbial or chemical agents during any of the production stages can also lead to deterioration in safety and quality. Medicinal plants collected from the wild population may be contaminated by other species or plant parts through misidentification, accidental contamination, or intentional adulteration, all of which may have unsafe consequences.

The collection of medicinal plants from wild populations can give rise to additional concerns related to global, regional, and/or local over-harvesting, and protection of endangered species. The impact of cultivation and collection on the environment and ecological processes, and the welfare of local communities should be considered [40].

It is well established that intrinsic and extrinsic factors, including species differences, organ specificity, diurnal and seasonal variation, environment, field collection and cultivation methods, contamination, substitution, adulteration, and processing and manufacturing practises greatly affect botanical quality. Intrinsically, botanicals are derived from dynamic living organisms, each of which is capable of being slightly different in its physical and chemical characters due to genetic influence.

Diurnal and seasonal variations are other intrinsic factors affecting chemical accumulation in both wild and cultivated plants. Depending on the plant, the accumulation of chemical constituents can occur at any time during the various stages of growth. In the majority of cases, maximum chemical accumulation occurs at the time of flowering, followed by a decline beginning at the fruiting stage. The time of harvest or field collection can thus influence the quality of the final herbal product. There are many extrinsic factors affecting the qualities of medicinal plants. It has been well established that factors such as soil, light, water, temperature, and nutrients can, and do, affect phytochemical accumulation in plants,

The methods employed in field collection from the wild, as well as in commercial cultivation, harvest, post-harvest processing, shipping, and storage can also influence the physical appearance and chemical quality of botanical source materials. Contamination by microbial and chemical agents (pesticides, herbicides, heavy metals), as well as by insect, animal, animal parts, and animal excreta during any of the stages of source plant material production can lead to lower quality and/or unsafe materials. Adulteration of herbal medicines with synthetic drugs represents another problem in product quality.

In the following paragraphs technical aspects of medicinal plant production will be discussed. According to the World health Organization [37] the botanical identity, scientific name (genus, species, subspecies/variety, author, and family) of each medicinal plant under cultivation should be verified and recorded. If available, the local and English common names should also be recorded. Other relevant information, such as the cultivar name, ecotype, chemotype, or phenotype, may also be provided, as appropriate. For commercially available cultivars, the name of the cultivar and of the supplier should be provided. It's essential that a voucher botanical specimen used in the experiments be placed in a regional or national herbarium for identification and further consultation by other researchers; it is almost impossible and not advised to publish without the registration numbers.

Cultivation of medicinal plants requires intensive care and management. The conditions and duration of cultivation required vary depending on the quality of the medicinal plant materials required. If no scientific published or documented cultivation data are available, traditional methods of cultivation should be followed, where feasible. Otherwise a method should be developed through research. The principles of good plant husbandry, including appropriate rotation of plants selected according to environmental suitability, should be followed, and tillage should be adapted to plant growth and other requirements. Risks of contamination as a result of pollution of the soil, air, or water by hazardous chemicals should be avoided. The impact of past land uses on the cultivation site, including the planting of previous crops and any applications of plant protection products should be evaluated.

The quality and growth of medicinal plants can also be affected by other plants, other living organisms, and by human activities. The introduction of nonindigenous medicinal plant species into cultivation may have a detrimental impact on the biological and ecological balance of the region. The ecological impact of cultivation activities should be monitored over time, where practical.

The social impact of cultivation on local communities should also be examined to ensure that negative impacts on local livelihood are avoided. In terms of local income-earning opportunities, small-scale cultivation is often preferable to largescale production, especially if small-scale farmers are organized to market their products jointly. If large-scale medicinal plant cultivation is or has been established, care should be taken that local communities benefit directly from, for example, fair wages, equal employment opportunities, and capital reinvestment.

Climatic conditions, for example, length of day, rainfall (water supply), and field temperature, significantly influence the physical, chemical, and biological qualities of medicinal plants. The duration of sunlight, average rainfall, average temperature, including daytime and night-time temperature differences, also influence the physiological and biochemical activities of plants, and prior knowledge should be considered.

The soil should contain appropriate amounts of nutrients, organic matter, and other elements to ensure optimal medicinal plant growth and quality. Optimal soil

conditions, including soil type, drainage, moisture retention, fertility, and pH, will be dictated by the selected medicinal plant species and/or target medicinal plant part. The use of fertilizers is often necessary in order to obtain large yields of medicinal plants. It is, however, necessary to ensure that correct types and quantities of fertilizers are used through agricultural research. In practise, organic and chemical fertilizers are used.

Human excreta must not be used as a fertilizer owing to the potential presence of infectious microorganisms or parasites. Animal manure should be thoroughly composted to meet safe sanitary standards of acceptable microbial limits and to destroy the germination capacity of weeds. Any applications of animal manure should be documented. Chemical fertilizers that have been approved by the countries of cultivation and consumption should be used. All fertilizing agents should be applied sparingly and in accordance with the needs of the particular medicinal plant species and supporting capacity of the soil. Fertilizers should be applied in such a manner as to minimize leaching.

Any agrochemical used to promote the growth of or to protect medicinal plants should be kept to a minimum, and applied only when no alternative measures are available. Integrated pest management should be followed where appropriate. When necessary, only approved pesticides and herbicides should be applied at the minimum effective level, in accordance with the labeling and/or package insert instructions of the individual product and the regulatory requirements that apply for the grower and the end-user countries. Only qualified staff using approved equipment should carry out pesticide and herbicide applications. Growers and producers should comply with maximum pesticide and herbicide residue limits, as stipulated by local, regional and/or national regulatory authorities.

Medicinal plants should be harvested during the optimal season or time period to ensure the production of medicinal plant materials and finished herbal products of the best possible quality. The time of harvest depends on the plant part to be used. It is well known that the concentration of biologically active constituents varies with the stage of plant growth and development. This also applies to nontargeted toxic or poisonous indigenous plant ingredients. The best time for harvest (quality peak season/time of day) should be determined according to the quality and quantity of bioactive phytocompounds rather than the total vegetative yield of the targeted medicinal plant parts. During harvest, care should be taken to ensure that no foreign matter, weeds, or toxic plants are mixed with the harvested medicinal plant materials. Medicinal plants should be harvested under the best possible conditions, avoiding dew, rain, or exceptionally high humidity. If harvesting occurs in wet conditions, the harvested material should be transported immediately to an indoor drying facility to expedite drying so as to prevent any possible deleterious effects due to increased moisture levels, which promote microbial fermentation and mold. Cutting devices, harvesters, and other machines should be kept clean and adjusted to reduce damage and contamination from soil and other materials. They should be stored in an uncontaminated, dry place or facility free from insects, rodents, birds and other pests, and inaccessible to livestock and domestic animals.

Contact with soil should be avoided as far as possible so as to minimize the microbial load of harvested medicinal plant materials. The harvested raw materials should be transported promptly in clean, dry conditions. They may be placed in clean baskets, dry sacks, trailers, hoppers, or other well-aerated containers and carried to a central point for transport to the processing facility.

# 1.5 Novel Bioactive Phytocompounds Against Multidrug-Resistant Bacteria/Fungi: The Management of Infectious and Chronic Diseases

Long before the discovery of the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, humans have used plants to treat common infectious diseases, and some of these traditional medicines are still included as part of the habitual treatment of various maladies. For example, the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*), and tee tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents. That being said, it has generally been the essential oils of these plants rather than their extracts that have had the greatest use in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal, and biliary systems, as well as on the skin. In the case of *Melaleuca alternifolia*, for example, the use of the essential oil (tee tree oil) is a common therapeutic tool to treat acne and other infectious troubles of the skin.

Antimicrobial resistance is one of the biggest challenges facing global public health. Although antimicrobial drugs have saved many lives and eased the suffering of many millions, poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil conflicts and bad governance in developing countries have tremendously limited the benefits of these drugs in controlling infectious diseases. The development of resistance in the responsible pathogens has worsened the situation, often with very limited resources to investigate and provide reliable susceptibility data on which rational treatments can be based as well as the means to optimize the use of antimicrobial agents. The emergence of multidrug-resistant isolates in tuberculosis, acute respiratory infections, and diarrhea, often referred to as the diseases of poverty, has had its greatest toll in developing countries. The epidemic of HIV/AIDS, with over 30 million cases in developing countries, has greatly enlarged the population of immunocompromised patients. The disease has left these patients at great risk of numerous infections and even greater risk of acquiring highly resistant organisms during long periods of hospitalization.

Antibiotic resistance can occur via three general mechanisms: prevention of interaction of the drug with target, efflux of the antibiotic from the cell, and direct

destruction or modification of the compound. The emergence of multidrug resistance in human and animal pathogenic bacteria as well as undesirable side-effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin.

Ahmad and Beg [41] tested alcoholic extracts of 45 traditionally used Indian medicinal plants against drug-resistant bacteria and fungi (*C. albicans*) both related to the critical prognosis and treatment of infectious diseases in immunocompromised, AIDS and cancer patients. Of these, 40 plant extracts showed varied levels of antimicrobial activity against one or more test bacteria. Anticandidal activity was detected in 24 plant extracts. Overall, broad-spectrum antimicrobial activity was observed in 12 plants (*L. inermis, Eucalyptus* sp., *H. antidysentrica, H. indicus, C. equistifolia. T. belerica, T. chebula, E. officinalis, C. sinensis, S. aromaticum* and *P. granatum*). Several other studies have also demonstrated the importance of new bioactive phytocompounds against multidrug-resistant bacteria/fungi.

Useful antimicrobial phytochemicals can be divided into several categories summarized in Table 1.1. Scientists from divergent fields are investigating plants anew with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro*. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. It would be advantageous to standardize methods of extraction and *in vitro* testing so that the search could be more systematic and interpretation of results facilitated. Also, alternative mechanisms of infection prevention and treatment should be included in initial activity screenings. Disruption of adhesion is one example of an anti-infection activity not commonly screened currently. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles.

#### 1.6

# Mode of Action of Bioactive Phytocompounds and their Interactions with Macromolecules and Toxicity

The mode of action of antimicrobial agents depends on the type of microorganism under consideration and is mainly related to their cell wall structure and the outer membrane arrangement. Gram-negative bacteria (e.g. *Pseudomonas aeruginosa*) display an intrinsic resistance to a wide variety of essential oils, which is associated with the hydrophilic surface of their outer membrane, rich in lipopolysaccharide molecules. A permeability barrier against toxic agents is formed. Small hydrophilic molecules are not prevented from passing through the outer membrane because of the action of abundant porin proteins. However, hydrophobic macromolecules, such as essential oils constituents, are unable to penetrate the barrier.

Scientific name	Compound class	Compound	Activity (most relevant)	Ref.
Allium sativum	Sulfoxide	Allicin	Broad spectrum <sup>[a]</sup>	42
Anacardium pulsatilla	Polyphenols	Salicylic acids	P. acnes	-
Anemone pulsatilla	Lactone	Anemonins	Bacteria	-
Berberis vulgaris	Alkaloid	Berberine	Protozoa and bacteria	43
Camellia sinensis	Flavonoid	Catechin	Broad spectrum <sup>[a]</sup> , viruses	44
Carum carvi	-	Coumarins	Viruses, broad spectrum <sup>[a]</sup>	45
Centella asiatica	Terpenoid	Asiatocoside	Mycobacterium leprae	-
Cinchora sp.	Alkaloid	Quinine	Plasmodium spp.	-
Citrus sinensis	Terpenoid	_	Fungi	46
Croton cajucara	Essential oil	Linalool	Leishmania amazonenis, fungi and bacteria	20
Erythroxylum coca	Alkaloid	Cocaine	Bacteria	-
Eucalyptusglobulus sp.	Polyphenol	Tannin	Bacteria and viruses	-
Gloriosa superba	Alkaloid	Colchicina	Broad spectrum <sup>[a]</sup>	_
Hydrastis canadensis	Alkaloid	Berberine	Bacteria, Giargia duodenale	47
Malus sylvestris	Flavonoid derivate	Phloretin	Broad spectrum <sup>[a]</sup>	-
Matricaria chamomilla	Phenolic acid	Anthemic acid	M. tuberculosis and S. typhimurium	-
Melissa officinalis	Polyphenols	Tannins	Viruses	48
Millettia thonningii	Flavone	Alpinum- isoflavone	Schistosoma sp.	49
Ocimum basilicum	Essential oil	Terpenoids	Bacteria, Salmonella sp.	50
Olea europaea	Aldehyde	Hexanal	Broad spectrum <sup>[a]</sup>	51
Onobrychis viciifolia	Polyphenols	Tannins	Bacteria	52
Panax notoginseng	Saponins	_	Bacteria	-
Pimenta dioica	Essential oil	Eugenol	Broad spectrum <sup>[a]</sup>	53
Piper betel	Essential oil	Cathecol	Broad spectrum <sup>[a]</sup>	50
Piper nigrum	Alkaloid	Piperine	Fungi, Lactobacillus sp.	54
Podocarpus nagi	Flavonol	Totarol	<i>P. acnes</i> and Gram- positive bacteria	55
Rabdosia trichocarpa	Terpene	Trichorabdal	Helicobacter pylori	56
Rhamnus purshiana	Polyphenols	Tannins	Viruses, broad spectrum <sup>[a]</sup>	-
Satureja montana	Terpenoid	Carvacrol	Broad spectrum <sup>[a]</sup>	
Vaccinium spp.	Monosaccharide	Fructose	Escherichia coli	57
Vicia faba	Thionin	Fabatin	Bacteria	_
Vinca minor	Alkaloid	Reserpine	Broad spectrum <sup>[a]</sup>	-
Curcuma longa	Terpenoids	Curcumin	Protozoa and bacteria	58
Aloysia tripphylla	Essential oil	Terpenoid	Ascaris sp.	-
Mentha piperita	Terpenoids	Menthol	Broad spectrum <sup>[a]</sup>	-
Artemisia dracunlus	Polyphenols	Tannins	Helminthes and viruses	48

 Table 1.1
 Plants and identified antimicrobial bioactive phytocompounds.

 $^{\rm a}$  Active against Bacteria (Gram + and Gram –) and Fungi

It has been proved that the effectiveness of the antibacterial agent generally increases with its lipophilic properties as a result of the action on cytomembranes. On the other hand, essential oils usually express low aqueous solubility, which prevents them from reaching a toxic level in cytomembranes, even if the oils have quite good affinity with the membranes. Some oil components of phenolic nature (e.g. carvacrol and thymol) cause a disruption of the lipopolysaccharide outer layer followed by partial disintegration of the outer membrane.

The mechanism of action of essential oils and other bioactive phytocompounds towards microorganisms is complex and has not yet been fully explained. It is generally recognized that the antimicrobial action of essential oils depends on their hydrophilic or lipophilic character. Terpenoids may serve as an example of lipid-soluble agents that affect the activities of membrane-catalyzed enzymes, for example their action on respiratory pathways. Certain components of essential oils can act as uncouplers, which interfere with proton translocation over a membrane vesicle and subsequently interrupt ADP phosphorylation (primary energy metabolism). Specific terpenoids with functional groups, such as phenolic alcohols or aldehydes, also interfere with membrane-integrated or associated enzyme proteins, stopping their production or activity.

Recent scientific research has shown that many plants used as food or in traditional medicine are potentially toxic, causing allergic processes, intoxication, mutagenic, and carcinogenic. The following plants are highly toxic because they cause both DNA damage and chromosomal aberrations: *Antidesma venosum* E. Mey. ex Tul. (Euphorbiaceae), *Balanities maughamii* Sprague (Balanitaceae), *Catharanthus roseus, Catunaregam spinosa* (Thunb.) Tirveng. (Rubiaceae), *Chaetacme aristata, Croton sylvaticus* Hochst. (Euphorbiaceae), *Diospyros whyteana* (Hiern) F. White (Ebenaceae), *Euclea divinorum* Hiern (Ebenaceae), *Gardênia volkensii* K. Schum. (Rubiaceae), *Heteromorpha arborescens* (Spreng.) Cham. & Schltdl. var. *abyssnica* (A. Rich.) H. Wolff (syn. *Heteromorpha trifoliata* (H.L. Wend.) Eckl., Zeyh.) (Apiaceae), *Hypoxis colchicifolia* Baker (Hypoxidaceae), *Ornithogalum longibractaetum* Jacq. (Hyacinthaceae), *Plumbago auriculata, Prunus africana* (Hook. f.) Kalkm. (Rosaceae), *Rhamnus prinoides* L'Hér. (Rhamnaceae), *Ricinus communis, Spirostachys africana* Sond. (Euphorbiaceae), *Trichelia emetica* Vahl subsp. *Emetica* (Meliaceae), *Turraea floribunda* Hochst. (Meliaceae), *Vernonia colorata* and *Ziziphus mucronata*.

In an extensive screening program of plants used in traditional medicine, researchers provided scientific evidence for their rational use in treating infections and diseases, inflammation, and disorders of the central nervous system. Using the ethnobotanical approach and bioassay-guided fractionation, several compounds with biological activity were isolated and identified. Genotoxicity studies also showed that several plants used for medicinal purposes cause damage to the genetic material and, therefore, should be used with caution.

*In vitro* screening programm, using the ethnobotanical approach, are important in validating the traditional use of herbal remedies and for providing leads in the search for new active principles. Whereas activity identified by an *in vitro* test does not necessarily confirm that a plant extract is an effective medicine, nor a suitable candidate for drug development, it does provide basic understanding of a plant's efficacy and, in some cases toxicity.

The nonprescription use of medicinal plants is cited today as an important health problem, in particular their toxicity to the kidneys. Several factors, such as active uptake by tubular cells and high concentration in the medullary interstitium, make the kidneys particularly vulnerable to toxic substances that may be present in plant preparations; the risk of kidney injury is even higher in renal patients. For instance, they may contain underestimated amounts of potassium, interact with drugs used for the treatment of renal diseases, or have vasoconstrictive properties.

The use of traditional plant remedies has been implicated in 35% of all cases of acute renal failure in Africa [59–63]. Precise identities of the culprit substances are mainly unknown, as well as the toxicological characteristics and pathogenetic mechanisms involved. Most data published are case reports, with no clear identification of the herbal product involved in the renal toxic effect. Various renal syndromes have been reported after the use of medicinal plants. They include acute tubular necrosis, acute interstitial nephritis, Fanconi's syndrome, hypokalemia, hypertension, papillary necrosis, chronic interstitial nephritis, nephrolithiasis, urinary retention, and cancer of the urinary tract. Conversely, herbal medicine also may be hazardous for renal patients because it may interact with such drugs as cyclosporine or carry significant amounts of potassium.

# 1.7 Bioactive Phytocompounds and Future Perspectives

The integration of herbal medicine into modern medical practises, including treatments for infections and cancer, must take into account the interrelated issues of quality, safety, and efficacy [64]. Quality is the paramount issue because it can affect the efficacy and/or safety of the herbal products being used. Current product quality ranges from very high to very low due to intrinsic, extrinsic, and regulatory factors. Intrinsically, species differences, organ specificity, diurnal and seasonal variations can affect the qualitative and quantitative accumulation of active chemical constituents in the source medicinal plants. Extrinsically, environmental factors, field collection methods such as cultivation, harvest, post-harvest transport, and storage, manufacturing practises, inadvertent contamination and substitution, and intentional adulteration are contributing factors to the quality of herbal medicinal products. Source plant materials that are contaminated with microbes, microbial toxins, environmental pollutants, or heavy metals; or finished products that are adulterated with foreign toxic plants or synthetic pharmaceutical agents can lead to adverse events. Substandard source materials or finished products will yield therapeutically less effective agents. Herbal medicine quality can also be attributed to regulatory practises. In a number of countries, herbal medicines are unregulated, which has led to product quality differences.

Product quality improvement may be achieved by implementing control measures from the point of medicinal plant procurement under Good Agricultural Practises (GAPs) and the manufacture of the finished botanical products under Good Manufacturing Practises (GMPs), plus postmarketing quality assurance surveillance. The lack of pharmacological and clinical data on the majority of herbal medicinal products is a major impediment to the integration of herbal medicines into conventional medical practise. For valid integration, pharmacological and especially, clinical studies, must be conducted on those plants lacking such data. Adverse events, including drug-herb interactions, must also be monitored to promote a safe integration of efficacious herbal medicine into conventional medical practises.

For the developing countries, the approval as drugs of standardized and formulated plant extracts might be the starting point of an innovative and successful local pharmaceutical industry, which can compete with the large pharmaceutical companies, not only for the treatment of minor diseases, but also for the treatment of severe and life-threatening diseases. It can be stated that the major activities of natural products research of the past decades have clearly demonstrated that natural products represent an unparalleled reservoir of molecular diversity to drug discovery and development, and are complementary to combinatorial libraries.

The major disadvantage is the time taken to isolate and to characterize the active components from the extracts. By improving the diversity and quality of sample source and screen suitability, by accelerating dereplication and by automating and standardizing early isolation steps, the effectiveness of natural products research can be enhanced. The efforts to establish collaboration between universities and local pharmaceutical companies to produce new medicines with scientific proof of safety, quality and efficacy are relevant to progress in this area. This interaction between the pharmaceutical industry and the universities has in turn stimulated the appearance of preclinical pharmacological studies and of well-controlled and randomized clinical trials to prove their worth. Furthermore, emphasis on domestication, production, and biotechnological studies, followed by genetic improvements to medicinal plants, are other fields of science that emerge from such progress in the use of medicinal plants in the world.

Scientists have dedicated significant efforts to the publishing of both basic and clinical studies on herbal medicines, and thus certainly will create the scientific basis for the physician's prescription of herbal drugs. In spite of this, so far insufficient data exist to provide an accurate assessment of the quality, efficacy, and safety of most of the herbal medicines currently available on the market. For all these reasons, a great effort in training more scientists in the relevant areas is still necessary in order to establish rational and sustainable exploitation of the world's biodiversity.

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