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 ±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 large-scale 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.

**Table 1.1** Plants and identified antimicrobial bioactive phytocompounds.

| *Scientific name* | *Compound class* | *Compound* | *Activity (most relevant)* | *Ref.* |
|---|---|---|---|---|
| *Allium sativum* | Sulfoxide | Allicin | Broad spectrum[a] | 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[a], viruses | 44 |
| *Carum carvi* | – | Coumarins | Viruses, broad spectrum[a] | 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[a] | – |
| *Hydrastis canadensis* | Alkaloid | Berberine | Bacteria, *Giargia duodenale* | 47 |
| *Malus sylvestris* | Flavonoid derivate | Phloretin | Broad spectrum[a] | – |
| *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[a] | 51 |
| *Onobrychis viciifolia* | Polyphenols | Tannins | Bacteria | 52 |
| *Panax notoginseng* | Saponins | – | Bacteria | – |
| *Pimenta dioica* | Essential oil | Eugenol | Broad spectrum[a] | 53 |
| *Piper betel* | Essential oil | Cathecol | Broad spectrum[a] | 50 |
| *Piper nigrum* | Alkaloid | Piperine | Fungi, *Lactobacillus* sp. | 54 |
| *Podocarpus nagi* | Flavonol | Totarol | *P. acnes* and Gram-positive bacteria | 55 |
| *Rabdosia trichocarpa* | Terpene | Trichorabdal | *Helicobacter pylori* | 56 |
| *Rhamnus purshiana* | Polyphenols | Tannins | Viruses, broad spectrum[a] | – |
| *Satureja montana* | Terpenoid | Carvacrol | Broad spectrum[a] | |
| *Vaccinium* spp. | Monosaccharide | Fructose | *Escherichia coli* | 57 |
| *Vicia faba* | Thionin | Fabatin | Bacteria | – |
| *Vinca minor* | Alkaloid | Reserpine | Broad spectrum[a] | – |
| *Curcuma longa* | Terpenoids | Curcumin | Protozoa and bacteria | 58 |
| *Aloysia tripphylla* | Essential oil | Terpenoid | *Ascaris* sp. | – |
| *Mentha piperita* | Terpenoids | Menthol | Broad spectrum[a] | – |
| *Artemisia dracunlus* | Polyphenols | Tannins | Helminthes and viruses | 48 |

[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.

## References


1 Newman D.J., Cragg, G.M., Snader, K.M. *J. Nat. Prod.* **2003**, *6*, 1022–1037.
2 Ganesan, A. *Drug Discov. Today* **2002**, *7*, 47–55.
3 Mehta, G., Singh, V. *Chem. Soc. Rev.* **2002**, *31*, 324–334.
4 Breinbauer R., Vetter, I.R., Waldmann, H. *Angew. Chem. Int. Ed.* **2002**, *41*, 2879–2890.
5 Lee M.L., Schneider, G. *J. Comb. Chem.* **2001**, *3*, 284–289.
6 Bajorath, J. *J. Comput. Aided Mol. Des.* **2002**, *16*, 431–439.
7 Hall, D.G., Manku, S., Wang, F. *J. Comb. Chem.* **2001**, *3*, 125–150.
8 Hook, D.J., Pack, E.J., Yacobucci, J.J., Guss, J. *J. Biomol. Screen.* **1997**, *2*, 145–152.
9 Wolfender, J-L., Rodriguez, S., Hostettmann, K. *J. Chromatogr.* **1998**, *A794*, 299–316.
10 Silva, C.J., Brian, P., Peterson, T. *Drugs Pharmac. Sci.* **2001**, *114*, 357–382.
11 Kuiper, G.G.J.M., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., van der Burg, B., Gustafsson, J-Ä. *Endocrinology* **1998**, *139*, 4252–4263.
12 Corley, D.G., and Durley, R.C. *J. Nat. Prod.* **1994**, *57*, 1484–1490.
13 Pannell, L.K., Shigematsu, N. *Am. Lab.* **1998**, *30*, 28–30.
14 Eloff, J.N. *J. Ethnopharmacol.* **1998**, *60*, 1–8.
15 Miles, D.H., Nguyen, C.L., Miles, D.H. *Curr. Med. Chem.* **1998**, *5*, 421–440.
16 Rojas, A., Hernandez, L., Pereda–Miranda, R., Mata, R. *J. Ethnopharmacol.* **1992**, *35*, 275–283.
17 Mazzanti, G., Battinelli, L., Salvatore, G. *Flav. Fragr. J.* **1998**, *13*, 289–294.
18 Lopes, D., Bizzo, H.R., Sobrinho, A.F.S., Pereira, M.V.G. *Oil Res.* **2000**, *12*, 705–708.
19 Grynberg, N. F., Echevarria, A., Lima, J.E., Pamplona, S.S., Pinto, A.C., Maciel, M.A. *Planta Med.* **1999**, *65*, 687–689.
20 Rosa, M.S.S., Mendonça Filho, R.R., Bizzo, H.R. Rodrigues, I.A., Soares, R.M.A., Souto-Padrón, T., Alviano, C.S., Lopes, A.H.C.S. *Antimicrob. Agents Chemother.* **2003**, *47*, 1895–1901.
21 Tassou, C.C., Drosinos, E.H., Nychas, G.J.E. *J. Appl. Bacteriol.* **1995**, *78*, 593–600.
22 Esquenazi, D., Wigg, M.D., Miranda, M.M.F.S., Rodrigues, H.M., Tostes, J.B.F., Rozental, S., Da Silva, A.J.R., Alviano, C.S. *Res. Microbiol.* **2002**, *153*, 647–652.
23 Ibanez, E., Kubatova, A., Senorans, F.J., Cavero, S., Reglero, G., Hawthorne, S.B. *J. Agric. Food Chem.* **2003**, *51*, 375–382.
24 Ju, Z., Howard, L. *J. Agric. Food Chem.* **2003**, *51*, 5207–5213.
25 Awad, R., Arnason, J.T., Trudeau, V.L., Bergeron, C., Budzinski, J.W., Foster, B.C., Merali, *Z. Phytomed.* **2003**, *10*, 640–649.
26 Lewis, W.H., Elvin-Lewis, M.P. *Ann. Mo. Bot. Gard.* **1995**, *82*,16–24.
27 Zhang, Y., Lewis, K. *FEMS Microbiol. Lett.* **1997**, *149*, 59–64.
28 Balzarini, J., Schols, D.J., Neyts, E. Van Damme, W. Peumans., E. De Clercq. *Antimicrob. Agents Chemother.* **1991**, *35*, 410–416.
29 Hui, K.M., Wang, X.H., Xue, H. *Planta Med.* **2002**, *66*, 91–93.
30 Kubo, I., Muroi, H., Himejima, M. *J. Nat. Prod.* **1993**, *56*, 220–226.
31 Moerman, D.E. *J. Ethnopharmacol.* **1996**, *52*, 1–22.
32 Heimhuber, B., Galensa, R., Herrmann, K. *J. Chromatogr.* **1988**, *739*, 481–483.
33 Saxena, G., Towers, G.H.N., Farmer, S., Hancock, R.E.W. *Phytochem. Anal.* **1995**, *6*, 125–129.
34 Wu, A.M., Jiang, Y.J., Hwang, P.Y., Shen, F.S. *Biochim. Biophys. Acta* **1995**, *1243*, 157–160.
35 Schmourlo, G., Mendonça–Filho, R.R., Alviano, C.S., Costa, S.S.C. *J. Ethnopharmacol.* **2005**, *96*, 563–568.
36 Farnsworth N.R. *Herbal Gram* **1993**, *29*, 36A–367H.
37 World Health Organization, *WHO Guidelines for Methodologies on Research and Evaluation of Traditional Medicine*. World Health Organization, Geneva, **2001**.
38 Bannerman, R., Burton, J., Chen, W.C. eds. *Traditional Medicine and Health Care Coverage*. World Health Organization, Geneva, **1983**.

**39** Awang, D.V.C. *Food Drug Law J*. **1997**, *52*, 341–344.

**40** World Health Organization, Good Manufacturing Practices for pharmaceutical products: main principles. In *WHO Expert Committee on Specifications for Pharmaceutical Preparations*. Thirty-seventh report. WHO Technical Report Series, No. 908. World Health Organization, Geneva, **2003**, Annex 4.

**41** Ahmad, I., Beg, A.Z. *J. Ethnopharmacol*. **2001**, *74*, 113–123.

**42** Naganawa, R., Iwata, N., Ishikawa, K., Fukuda, H., Fujino, T., Suzuki, A. *Appl. Environ. Microbiol*. **1996**, *62*, 4238–4242.

**43** Omulokoli, E., Khan, B., Chhabra, C.S. *J. Ethnopharmacol*. **1997**, *56*, 133–137.

**44** Ooshima, T., Minami, T., Aono, W., Izumitani, A., Sobue, S., Fujiwara, T., Kawabata, S., Hamada, S. *Caries Res*. **1993**, *27*, 124–129.

**45** Bose, P.K. *J. Indian Chem. Soc*. **1958**, *58*, 367–375.

**46** Stange, R.R., Midland, S.L., Eckert, J.W., Sims, J.J. *J. Nat. Prod*. **1993**, *56*, 1627–1629.

**47** Freiburghaus, F., Kaminsky, R., Nkunya, M.H.H., Brun, R. *J. Ethnopharmacol*. **1996**, *55*, 1–11.

**48** Wild, R. (ed.) *The Complete Book of Natural and Medicinal Cures*. Rodale Press, Emmaus, PA, **1994**.

**49** Perrett, S., Whitfield, P.J., Sanderson, L., Bartlett, A. *J. Ethnopharmacol*. **1995**, *47*, 49–54.

**50** Wan, J., Wilcock, A., Coventry, M.J. *J. Appl. Microbiol*. **1998**, *84*, 152–158.

**51** Kubo, A., Lunde, C.S., Kubo., I. *J. Agric. Food Chem*. **1995**, *43*, 1629–1633.

**52** Jones, G.A., McAllister, M.A., Muir, A.D., Cheng, K.J. *Appl. Environ. Microbiol*. **1994**, *60*, 1374–1378.

**53** Martinez, M.J., Betancourt, J., Alonso-Gonzalez, N., Jauregui, A. *J. Ethnopharmacol*. **1996**, *52*, 171–174.

**54** Ghoshal, S., Krishna Prasad, B.N., Lakshmi, V. *J. Ethnopharmacol*. **1996**, *50*, 167–170.

**55** Kubo, I., Muroi, H., Kubo, A. *J. Nat. Prod*. **1994**, *57*, 9–17.

**56** Kadota, S., Basnet, P., Ishii, E., Tamura, T., Namba, T. *Zentbl. Bakteriol*. **1997**, *286*, 63–67.

**57** Ofek, I., Goldhar, J., Sharon, N. *Adv. Exp. Med. Biol*. **1996**, *408*, 179–183.

**58** Apisariyakul A., Vanittanakom, N., Buddhasukh, D. *J. Ethnopharmacol*. **1995**, *49*, 163–169.

**59** Seedat, Y.K. *South Afr. Med. J*. **1978**, *54*, 427–431.

**60** Ojogwu L.I., Anah C. *East Afr. Med. J*. **1983**, *60*, 478–484.

**61** Kadiri S., Ogunlesi A., Osinfade K., Akinkugbe O. *Afr. J. Med. Med. Sci*. **1992**, *21*, 91–96.

**62** Randeree, I.G., Czarnocki, A, Moodley, J., Seedat, Y. *Ren. Fail*. **1995**, *17*, 147–153.

**63** Adelekun, T.A., Ekwere, T.R., Akinsola, A. *West Afr. J. Med*. **1999**, *18*, 60–63.

64 Schwartsmann, G., Ratain, M.J., Cragg, G.M., Wong, J.E., Saijo, N., Parkinson, D.R., Fujiwara, Y., Pazdur, R., Newman, D.J., Dagher, R., DiLeone, L. *J. Clin. Oncol*. **2002**, *20* (Suppl. 18), 47S–59S.