



Chapter 1.

INTRODUCTION

1. INTRODUCTION

Since ancient times humanity has depended on the diversity of plant resources for food, clothing, shelter, and traditional medicine to cure myriads of ailments. Early humans recognized their dependence on nature in both health and illness. Medicinal plants constitute a source of raw materials for both traditional systems of medicine (e.g. Ayurvedic, Chinese, Unani, Homeopathy, and Siddha) and modern medicine. Physical evidence of the use of herbal remedies has been found from some 60,000 years ago in a burial site of a Neanderthal man uncovered in 1960 in a cave in northern Iraq¹. The first written records detailing the use of herbs in the treatment of illness are in the form of Mesopotamian clay tablet writings and Egyptian papyrus². Herbal medicine is the oldest form of health care known to humanity and has been used in all cultures throughout history.

Nowadays, plant materials are employed throughout the industrialized and developing world as home remedies, over-the-counter drugs, and ingredients for the pharmaceutical industry. Medicinal plants have played a key role in world health. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants. Many pharmacological classes of drugs include a natural product prototype. Aspirin, atropine, morphine, quinine are just a few of the drugs that were originally discovered through the study of traditional cures and folk knowledge of indigenous people.

During the past decade, there has been increasing acceptance and public interest in natural therapies in both developing and developed countries. Due to poverty and limited access to modern medicine, about four billion people, 80% of the world's population living in developing countries uses herbal medicine as their source of primary health care³⁻⁶. In these communities, traditional medical practice is often viewed as an integral part of their culture. This recent resurgence of interest in plant remedies has been spurred on by several factors:

- The effectiveness of plant medicines.
- The preference of consumers for natural therapies, a greater interest in alternative medicines and a commonly held erroneous belief that herbal products are superior to manufactured products.

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

Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Traditional medicine evolved over centuries, depending on local flora, culture, and religion⁷⁻⁹. Indeed, well into the twentieth century, much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native people. This knowledge of plant-based drugs developed gradually and was passed on, thus laying the foundation for many systems of traditional medicine all over the world. Herbal medicine can broadly be classified into a few basic systems:

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

Ayurvedic herbalism has developed into highly sophisticated system of diagnosis and treatment over the centuries. Atharveda (around 1200 BC), Charak Samhita and Sushrut Samhita (100 - 500 BC)¹⁰⁻¹¹ are the main classics that have given detailed descriptions of over 700 herbs. Researches on pharmacognosy, chemistry, pharmacology and clinical therapeutics have been carried out on ayurvedic medicinal plants and many of the major pharmaceutical corporations have renewed their strategies in favour of natural products drug discovery. Numerous drugs have entered the International

Pharmacopoeia through the study of ethnopharmacology and ayurvedic medicine¹².

Herbal products often contain a variety of biochemicals found naturally in the plants and many different biochemicals contribute to a plant's medicinal benefit. Chemicals known to have medicinal benefits are referred to as "active ingredients," and their presence depends on the plant species, the way the herb is prepared, the time and season of harvest, the type of soil, etc.

While some are safe and effective for specific uses, others are not. The general perception that herbal drugs are very safe and free from side effects is not true.

Herbs can produce undesirable side effects and can be toxic. A particular plant part will have many constituents and some of them may be toxic. However, it may take more to cause toxicity, because herbs usually are not as potent as manufactured drugs, and compared with synthetic drugs the adverse effects of most herbal drugs are relatively infrequent¹³⁻¹⁴.

As such, they represent a substantial proportion of the global drug market. Although most medicinal herbs, in their natural state are not fit for administration, preparations suitable for administration are made according to pharmacopoeial directions. The therapeutic potential of herbal drugs depends on their form: whether parts of a plant, or simple extracts, or isolated active constituents. Herbal remedies consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically. The herbal drug preparation in its entirety is regarded as the active substance and the constituents are either of known therapeutic activity or are chemically defined substances or group of substances generally accepted to contribute substantially to the therapeutic activity of the drug. Phytochemical screening involves botanical identification, extraction with suitable solvents, purification, and characterization of the active constituents of pharmaceutical importance. Qualitative chemical examination employing different analytical techniques is conducted to detect and isolate the active constituent(s). In general, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being efficacious and safe. Ultimate proof of these can only be achieved by some form of clinical research.

A defined and constant composition of the drug is therefore one of the most important prerequisites for any kind of clinical experiment.

The World Health Organization has recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Proven agro-industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines¹⁵. It is necessary to develop methods for rapid, precise and accurate identification and estimation of active constituents in order to bring out consistency of important constituents in the formulations.

1.1) WHO Guidelines for Assessment of Herbal Medicines

Every herbal formulation must be standardized as per WHO guidelines. WHO collaborates and assists health ministries in establishing mechanisms for the introduction of traditional plant medicines into primary healthcare programmes, in assessing safety and efficacy and in ensuring adequate supplies and the quality control of raw and processed materials. According to WHO guidelines less stringent selection procedures could be applied for the screening, chemical analysis, clinical trials and regulatory measures but the procedure for pure phytochemicals for quality control should be identical to that of synthetic drugs according to WHO guidelines¹⁶.

The World Health Organization (WHO) has recently defined traditional medicine as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. The traditional preparations comprise medicinal plants, minerals, organic matter, etc.

Some of the important parameters are stability testing, safety assessment, specific therapeutic activity, analysis and estimation of the active constituents in plant raw material and finished products. The objective of WHO guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of herbal medicines and therefore to assist national regulatory authorities, scientific organizations, and manufacturers to undertake an assessment of the documentation in respect of such products.

A method of identification, and where possible quantification of the plant material in the finished product should be defined. If the identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances (e.g., "chromatographic fingerprint") to ensure consistent quality of the product¹⁷. According to WHO, "Herbal Medicines" should be regarded as, "Finished, labeled medicinal products that contain as active ingredients aerial or underground parts of plants, or other plant material, or combinations thereof, whether in the crude state or as plant preparations. Plant material includes juices, gums, fatty oils, essential oils, and any other substance of this nature.

Multi-component botanical formulations can be standardized with newer techniques such as high pressure thin layer chromatography (HPTLC), liquid chromatography, mass spectroscopy. The value of animal testing to establish safety and toxicity is also critical for the botanicals used in traditional forms prepared using drugs with a narrow therapeutic index. Nevertheless, all the critical pharmacopoeial tests such as dissolution time, microbial, pesticide and heavy metals contamination etc. must be in accordance with global standards and all the Ayurvedic medicines manufactured must be in accordance with current good manufacturing procedures for herbs¹⁸.

1.2) Analysis of Raw Materials from Plants

Raw material can be defined as starting material or any intermediate which will be utilized for further processing. Before finished pharmaceutical dosage forms are produced, the identity, purity and quality of raw materials as per specifications for impurities and other related substances present must be established with use of suitable test methods. Pharmacopoeias and formularies of various countries provide standardized test methods for the most common and widely used materials in their monographs¹⁹.

1.3) Quality Control of Herbal Drugs

Quality control for efficacy and safety of herbal products is of paramount importance. Quality can be defined as the status of a drug that is determined by identity, purity, content, and other chemical, physical, or biological

properties, or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. For the quality control of a traditional medicine, the traditional methods are procured and studied, and documents and the traditional information about the identity and quality assessment are interpreted in terms of modern assessment²⁰⁻²¹.

In general, all medicines, whether they are of synthetic or of plant origin, should fulfill the basic requirements of being efficacious and safe, and this can be achieved by suitable clinical trials. This applies both to the multinational pharmaceutical company conducting a multi-center, double-blind placebo-controlled study with a herbal extract, and to the health practitioner in a rural village who applies a locally produced herbal mixture.

Natural products in medicine constitute a vast array of "raw materials," making clear definitions important. Quality criteria are based on clear scientific definitions of the raw material. The term "herbal drugs" denotes plants or plant parts that have been converted into phyto-pharmaceuticals by means of simple processes involving harvesting, drying, and storage. Hence they are capable of variation. This variability is also caused by differences in growth, geographical location, and time of harvesting. A practical addition to the definition is also to include other crude products derived from plants, which no longer show any organic structure, such as essential oils, fatty oils, resins, and gums. Derived or isolated compounds in the processed state such as extracts or even isolated purified compounds or mixtures of compounds are, as a rule, not included in the definition. Combinations with chemically defined active substances or isolated constituents, and homeopathic preparations which frequently contain plants, are not regarded as herbal medicines. Their production is already based on adequate quality control of the respective starting materials. The following paragraphs will focus on quality control of herbal drugs in compliance with the above definition²²⁻²³.

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

- Identity: Is the herb the one it should be?
- Purity: Are there contaminants, e.g., in the form of other herbs which should

not be there?

- Content or assay: Is the content of active constituents within the defined limits?

It is obvious that the content is the most difficult one to assess, since in most herbal drugs the active constituents are unknown. Sometimes markers can be used which are, by definition, chemically defined constituents that are of interest for control purposes, independent of whether they have any therapeutic activity or not. To prove identity and purity, criteria such as type of preparation sensory properties, physical constants, adulteration, contaminants, moisture, ash content and solvent residues have to be checked. The correct identity of the crude herbal material, or the botanical quality, is of prime importance in establishing the quality control of herbal drugs²⁴.

Identity can be achieved by macroscopical and microscopical examinations. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification. At times an incorrect botanical quality with respect to the labeling can be a problem.

Purity is closely linked with the safe use of drugs and deals with factors such as ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation also includes microbial contamination, aflatoxins, radioactivity, and pesticide residues²⁵.

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

- Herbal drugs are usually mixtures of many constituents.
- The active principle(s) is (are), in most cases unknown.
- Selective analytical methods or reference compounds may not be available commercially.
- Plant materials are chemically and naturally variable.
- Chemo-varieties and chemo cultivars exist.
- The source and quality of the raw material are variable.

- The methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) have an effect.

Strict guidelines have to be followed for the successful production of a quality herbal drug. Among them are proper botanical identification, phytochemical screening, and standardization. Quality control and the standardization of herbal medicines involve several steps. The source and quality of raw materials, good agricultural practices and manufacturing processes are certainly essential steps for the quality control of herbal medicines and play a pivotal role in guaranteeing the quality and stability of herbal preparations²⁶.

The quality of a plant product is determined by the prevailing conditions during growth, and accepted Good Agricultural Practices (GAP) can control this. These include seed selection, growth conditions, use of fertilizers, harvesting, drying and storage. In fact, GAP procedures are, and will be, an integral part of quality control. Factors such as the use of fresh plants, age and part of plant collected, period, time and method of collection, temperature of processing, exposure to light, availability of water, nutrients, drying, packing, transportation of raw material and storage, can greatly affect the quality, and hence the therapeutic value of herbal medicines. Apart from these criteria, factors such as the method of extraction, contamination with microorganisms, heavy metals, and pesticides can alter the quality, safety, and efficacy of herbal drugs. Using cultivated plants under controlled conditions instead of those collected from the wild can minimize most of these factors. Sometimes the active principles are destroyed by enzymic processes that continue for long periods from collection to marketing, resulting in a variation of composition. Thus proper standardization and quality control of both the raw material and the herbal preparations should be conducted²⁷.

1.4) Standardization of Herbal Formulations

Standardization involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations. Standardized extracts are high-quality extracts containing

consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes.

No regulatory definition exists for standardization of dietary supplements. As a result, the term “standardization” may mean many different things. Some manufacturers use the term standardization incorrectly to refer to uniform manufacturing practices; following a recipe is not sufficient for a product to be called standardized. Therefore, the presence of the word “standardized” on a supplement label does not necessarily indicate product quality. When the active principles are unknown, marker substance(s) should be established for analytical purposes and standardization.

Marker substances are chemically defined constituents of a herbal drug that are important for the quality of the finished product. Ideally, the chemical markers chosen would also be the compounds that are responsible for the biological effects in the body.

There are two types of standardization:

In the first category, “true” standardization, a definite phytochemical or group of constituents is known to have activity. These products are highly concentrated and no longer represent the whole herb, and are now considered as phytopharmaceuticals. In many cases they are vastly more effective than the whole herb.

The other type of standardization is based on manufacturers guaranteeing the presence of a certain percentage of marker compounds; these are not indicators of therapeutic activity or quality of the herb.

Single or multiple markers can be used to ensure that the concentration and ratio of components in an herbal mixture are present in reproducible levels in raw materials, manufacturing intermediates, and in the final dosage forms. In this way, multiple markers or chromatographic fingerprints give information assisting manufacturing control and assuring batch-to-batch consistency²⁸.

1.4.1) Chemical marker for herbal standardization²⁹

It is important to understand that a plant extract consists of established classes of chemical compounds. These include the primary metabolites, secondary metabolites and inorganic salts and metals. Primary metabolites are

compounds like carbohydrates, proteins, lipids which are essential for the plant physiology. Secondary metabolites are compounds which are not essential for the plant physiology as such but are formed as byproducts in the biochemical pathways. Selection of chemical markers is crucial for the quality control of herbal medicines, including authentication of genuine species, harvesting the best quality raw materials, evaluation of post-harvesting handling, assessment of intermediates and finished products, and detection of harmful or toxic ingredients. Ideal chemical markers should be the therapeutic components of herbal medicines. However, for most herbal medicines, the therapeutic components have not been fully elucidated or easily monitored. Bioactive, characteristic, synergistic, correlative, toxic and general components may be selected. These include very interesting and useful classes of compounds like alkaloids, flavonoids, coumarins, terpenoids, anthocyanins, etc, and we can utilize these secondary metabolites for the identification of plant material as our knowledge of chemistry has advanced sufficiently and through sophisticated analytical techniques we can measure these compounds qualitatively and quantitatively.

Liquid chromatography like HPLC which is useful only when a marker compound is known and can be used as reference compound; and finally planar chromatography like TLC and more advanced version like HPTLC which accounts for all the variations found in TLC. The extracts obtained similarly from each batch are loaded onto a TLC plate and are developed in suitable solvent system. We may use different solvent systems to develop a better TLC pattern. This developed plate is then studied under different conditions in the scanner which utilizes a laser beam and detects and quantifies distinct spots due to different compounds on the plate. The different conditions which can be used for detecting spots on plate include UV at different wavelength, derivitisation of plate to obtain distinct colored spots which are then detected by the software program and a characteristic graph obtained. There is a very distinct graphic pattern for each plant material and the extracts from different batches usually give overlapping graphic patterns. So, basically we can optimize this pattern to give a distinct HPTLC profile for each fractionated plant part .

1.4.2) Finger-printing of Ayurvedic Drugs

Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration and variation in composition. Therefore, quality control of herbal medicines offers a host of problems. A marker can be defined as a chemical entity, in the plant material which may or may not be chemically defined and serves as a characteristic fingerprint for that plant. In other ways through various analytical techniques like TLC, HPLC and HPTLC we can visualize the presence of this compound in the plant and also quantify it to ascertain the limits. A biomarker on the other hand is a chemical component which in addition of being unique for that plant material also correlates with the biological efficacy³⁰. Batch to batch variations start from the collection of raw material itself in the absence of any reference standards for proper identification, and multiply during storage and further processing. So the need arises to lay standards by which the right material is selected and incorporated into the formulation.

1.5) Parameters for Quality Control of Herbal Drugs

1.5.1) Microscopic Evaluation

Quality control of herbal drugs has traditionally been based on appearance and today microscopic evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, and detection of foreign matter and adulterants. A primary visual evaluation, which seldom needs more than a simple magnifying lens, can be used to ensure that the plant is of the required species, and that the right part of the plant is being used. At other times, microscopic analysis is needed to determine the correct species and/or that the correct part of the species is present. For instance, pollen morphology may be used in the case of flowers to identify the species, and the presence of certain microscopic structures such as leaf stomata can be used to identify the plant part used. Although this may seem obvious, it is of prime importance, especially when different parts of the same plant are to be used for different treatments³¹.

1.5.2) Determination of Foreign Matter

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

Furthermore, when foreign matter consists, for example, of a chemical residue, TLC is often needed to detect the contaminants³².

1.5.3) Determination of Ash

The limits of the inorganic contents have been prescribed by WHO depending upon the properties of the plant material. If the inorganic content is found to exceed the limits, it may be concluded that the plant material contains sand and siliceous material in excess which may be due to improper method of cultivation and harvesting. To determine ash content the plant material is burnt and the residual ash is measured as total and acid-insoluble ash. Total ash is the measure of the total amount of material left after burning and includes ash derived from the part of the plant itself and acid-insoluble ash. The latter is the residue obtained after boiling the total ash with dilute hydrochloric acid, and burning the remaining insoluble matter. The second procedure measures the amount of silica present, especially in the form of sand and siliceous earth³¹.

1.5.4) Determination of Heavy Metals

Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such as mercury, lead, cadmium, and arsenic in herbal remedies can be attributed to many causes, including environmental pollution, and can pose clinically relevant dangers for the health of the user and should therefore be limited. The potential intake of the toxic metal can be estimated on the basis of the level of its presence in the product and the recommended or estimated dosage of the product. Instrumental analyses have to be employed when the metals are present in trace quantities, in admixture,

or when the analyses have to be quantitative. The main methods commonly used are atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP) and neutron activation analysis (NAA) ³³⁻³⁴.

1.5.5) Determination of Microbial Contaminants and Aflatoxins

Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi, and viruses. Inevitably, this microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations. Herbal drugs normally carry a number of bacteria and molds, often originating in the soil. Poor methods of harvesting, cleaning, drying, handling, and storage may also cause additional contamination, as may be the case with *Escherichia coli* or *Salmonella* spp³⁵.

Laboratory procedures investigating microbial contaminations are laid down in the well-known pharmacopeias, as well as in the WHO guidelines. Limit values can also be found in the sources mentioned. In general, a complete procedure consists of determining the total aerobic microbial count, the total fungal count, and the total Enterobacteriaceae count, together with tests for the presence of *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, and *Pseudomonas aeruginosa* and *Salmonella* spp³⁶.

The presence of fungi should be carefully investigated and/or monitored, since some common species produce toxins, especially aflatoxins. Aflatoxins in herbal drugs can be dangerous to health even if they are absorbed in minute amounts [65, 68]. Aflatoxin-producing fungi sometimes build up during storage. Procedures for the determination of aflatoxin contamination in herbal drugs are published by the WHO. After a thorough clean-up procedure, TLC is used for confirmation³⁷.

1.5.6) Determination of Pesticide Residues

Even though there are no serious reports of toxicity due to the presence of pesticides and fumigants, it is important that herbs and herbal products are free of these chemicals or at least are controlled for the absence of unsafe levels [38]. Herbal drugs are liable to contain pesticide residues, which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation, and administering of fumigants during storage. However, it may be

desirable to test herbal drugs for broad groups in general, rather than for individual pesticides. Many pesticides contain chlorine in the molecule, which, for example, can be measured by analysis of total organic chlorine. In an analogous way, insecticides containing phosphate can be detected by measuring total organic phosphorus. Samples of herbal material are extracted by a standard procedure, impurities are removed by partition and/or adsorption, and individual pesticides are measured by GC, MS, or GC/MS³⁹.

1.5.7) Determination of Radioactive Contamination

There are many sources of ionization radiation, including radionuclides, occurring in the environment. Hence a certain degree of exposure is inevitable. Dangerous contamination, however, may be the consequence of a nuclear accident. The WHO, in close cooperation with several other international organizations, has developed guidelines in the event of a widespread contamination by radionuclides resulting from major nuclear accidents. At present, no limits are proposed for radioactive contamination³⁵.

1.5.8) Analytical Methods

Published monographs in a pharmacopoeia are the most practical approach for quality control of herbal drugs. When pharmacopoeial monographs are unavailable, development and validation of analytical procedures have to be carried out by the manufacturer. The best strategy is to follow closely the pharmacopoeial definitions of identity, purity, and content or assay. Valuable sources for general analytical procedures are included in the pharmacopoeias, in guidelines published by the WHO. Additional information, especially on chromatographic and/or spectroscopic methods can be found in the general scientific literature. The plant or plant extract can be evaluated by various biological methods to determine pharmacological activity, potency and toxicity. A simple chromatographic technique such as TLC may provide valuable additional information to establish the identity of the plant material. This is especially important for those species that contain different active constituents. Qualitative and quantitative information can be gathered concerning the presence or absence of metabolites or breakdown products. TLC fingerprinting is of key importance for herbal drugs made up of essential oils, resins, and gums, which are complex mixtures of constituents that no longer have any

organic structure. It is a powerful and relatively rapid solution to distinguish between chemical classes, where macroscopy and microscopy will fail. Chromatograms of essential oils, for example, are widely published in the scientific literature, and can be of invaluable help in identification.

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

HPLC is the preferred method for quantitative analysis of more complex mixtures. Though the separation of volatile components such as essential and fatty oils can be achieved with HPLC, it is best performed by GC or GC/MS. The quantitative determination of constituents has been made easy by recent developments in analytical instrumentation. Recent advances in the isolation, purification, and structure elucidation of naturally occurring metabolites have made it possible to establish appropriate strategies for the determination and analysis of quality and the process of standardization of herbal preparations. Classification of plants and organisms by their chemical constituents is referred to as chemotaxonomy. TLC, HPLC, GC, quantitative TLC (QTLC), and high-performance TLC (HPTLC) can determine the homogeneity of a plant extract. Over-pressured layer chromatography (OPLC), infrared and UV-VIS spectrometry, MS, GC, liquid chromatography (LC) used alone, or in combinations such as GC/MS, LC/MS, and MS/MS, and nuclear magnetic resonance (NMR), electrophoretic techniques, especially by hyphenated chromatographies, are powerful tools, often used for standardization and to control the quality of both the raw material and the finished product. The results from these sophisticated techniques provide a chemical fingerprint as to the nature of chemicals or impurities present in the plant or extract⁴⁰.

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

for evaluating fingerprints are all powerful tools for quality control of herbal products⁴¹.

1.5.9) Validation

The validation of herbal products is a major public health concern both in developed and resource-poor countries, where fake business selling adulterated herbal medicines are common. In this regard, there is no control by the government agencies, despite the existence of certain guidelines in some individual countries and those outlined by the WHO. If the herbal products are marketed as therapeutic agents, and irrespective of whether the products really have any positive effects to cure and reduce the severity of the disease, it is necessary to ensure scientific validation and periodic monitoring of the quality and efficacy by drug control administrators. It is feasible that the introduction of scientific validation would control the production of impure or adulterated herbal products and would eventually ensure their rational use. This could also lead to the regulation of the industry so that only qualified physicians and health providers are allowed to prescribe the medication. Several of the principal pharmacopoeias contain monographs outlining standards for herbal drugs. The major advantage of an official monograph published in a pharmacopoeia is that standards are defined and available, and that the analytical procedures used are fully validated. This is of major importance, since validation can be a rather time-consuming process. By definition, validation is the process of proving that an analytical method is acceptable for its intended purpose for pharmaceutical methods. Guidelines from the United States Pharmacopeia (USPC, 1994–2001), the International Conference on Harmonization (ICH), and the US Food and Drug Administration (FDA) provide a framework for performing such validations. In general, validation investigations must include studies on specificity, linearity, accuracy, precision, range, detection, and quantitative limits, depending on whether the analytical method used is qualitative or quantitative⁸⁰. Also of utmost importance is the availability of standards. For macroscopic and microscopic procedures in general this means that reliable reference samples of the plant must be available. A defined botanical source (e.g. voucher specimens) will normally solve this problem. Standards for chromatographic procedures are less easy to

obtain. Characteristic plant constituents, either active or markers, are seldom available commercially. Sometimes an LC/MS approach can be referred to as a mode of characterization. Going one step further, after isolation of such a compound, elucidations to prove its definite structure will not be easy. The method often employed is to use readily available compounds that behave similarly in the chosen chromatographic systems, and to calculate retention values and/or times towards these compounds as a standard. Qualitative chemical examination is designed to detect and isolate the active ingredient (s). TLC and HPLC are the main analytical techniques commonly used. In cases when active ingredients are not known or too complex, the quality of plant extracts can be assessed by a "fingerprint" chromatogram⁴²⁻⁴³.

1.6) Adulteration of Herbal Drugs

Direct or intentional adulteration of drugs usually includes practices in which a herbal drug is substituted partially or fully with other inferior products. Due to morphological resemblance to the authentic herb, many different inferior commercial varieties are used as adulterants. These may or may not have any chemical or therapeutic potential. Substitution by "exhausted" drugs entails adulteration of the plant material with the same plant material devoid of the active constituents. This practice is most common in the case of volatile oil-containing materials, where the dried exhausted material resembles the original drug but is free of the essential oils. Foreign matter such as other parts of the same plant with no active ingredients, sand and stones, manufactured artifacts, and synthetic inferior principles are used as substitutes.

Deterioration may contribute to indirect adulteration, and crude drugs are often prone to deterioration, especially during storage, leading to the loss of the active ingredients, production of metabolites with no activity and, in extreme cases, the production of toxic metabolites. Physical factors such as air (oxygen), humidity, light, and temperature can bring about deterioration directly or indirectly. These factors, alone or in combination, can lead to the development of organisms such as molds, mites, and bacteria. Oxidation of the constituents of a drug can be brought about by oxygen in the air, causing some products, such as essential oils, to resinify or to become rancid. Moisture or humidity and

elevated temperatures can accelerate enzymatic activities, leading to changes in the physical appearance and decomposition of the herb⁴⁴.

1.7) Contamination of Herbal Drugs and Herb-Drug Interactions

Conventional synthetic pharmaceuticals such as synthetic corticosteroids, nonsteroidal anti-inflammatory drugs and other prescription drugs, potent drugs such as phenylbutazone, in fact examples of almost every therapeutic drug class have been found in certain herbal remedies as contaminants. A recent study suggested that potent corticosteroids had been deliberately added to herbal creams in order to increase their efficacy. These "adulterated" herbal medicines sometimes result in serious ailments such as acute renal failure. A number of clinically significant interactions between prescribed and herbal medicines have been identified. When these medications are used together, they can interact in the body, causing changes in the way the herbs and/or the drug works. Such changes are called herb-drug interactions. Concurrent use of herbal or homeopathic remedies alongside prescribed or over the-counter medicines are frequent, and may mimic, magnify, or oppose the effect of the drug. Herb-drug interactions are not chemical interactions between a drug and a herbal component to produce something toxic. Instead, the interactions generally cause either an increase or decrease in the amount of drug in the bloodstream. As with conventional medicines, herbal medicines interact with drugs in two general ways: pharmacokinetically and pharmacodynamically. *Pharmacokinetic* interactions result in alterations in the absorption, distribution, metabolism, or elimination of the drug or natural medicine. These interactions affect drug action by quantitative alterations, either increasing or decreasing the amount of drug available to have an effect. *Pharmacodynamic* interactions cause alterations in the way a drug or natural medicine affects a tissue or organ system. These actions affect drug action in a qualitative way, either through enhancing or antagonizing effects. Herb-drug interactions change the effectiveness of the treatment, sometimes resulting in potentially dangerous side effects, possibly leading to toxicity, and/or reduced benefits. They can modify the mode of action of the drug, leading to unexpected complications or enhancement of the therapeutic effect, possibly leading to

overmedication and an impact on health. Controlled clinical studies are needed to clarify and determine their clinical importance and more research is required to define them⁴⁵⁻⁴⁷.

1.8) Toxicity of Herbal Drugs

For several reasons it is not possible to establish absolute safety standards for herbal preparations based solely on epidemiological studies. First, these types of studies would be costly. Second, there is little published data in countries where the major use of medicinal plants occurs and thus general standards based on a limited number of reports would have little meaning. Third, the exact identification of the products implicated in side effects claimed for medicinal plants is usually lacking. In spite of these inadequacies, there are a number of general comments that can be made with regard to avoiding potential serious side effects from herbal medicines⁴⁸.

The definition of "toxic" is ultimately a matter of viewpoint. Traditionally, herbs and herbal products have been considered to be nontoxic and have been used by the general public and traditional medicinal doctors worldwide to treat a range of ailments. The fact that something is natural does not necessarily make it safe or effective. The active ingredients of plant extracts are chemicals that are similar to those in purified medications, and they have the same potential to cause serious adverse effects. Whilst the literature documents severe toxicity resulting from the use of herbs, on many occasions the potential toxicity of herbs and herbal products has not been recognized. Herbs and herbal preparations can cause toxic adverse effects, serious allergic reactions, adverse drug interactions, and can interfere with laboratory test⁴⁹

Two kinds of side effects have been reported for herbal medicines. The first, considered to be intrinsic to herbal drugs themselves, is mainly related to predictable toxicity due to toxic constituents of the herbal ingredients and overdosage, and the second is allergy. Many cases of allergic reactions have been reported for herbal drugs. It is impossible to completely eliminate the possibility of any substance, including prescription drugs, herbal remedies, or cosmetics, producing an allergic response in people exposed to them. Perhaps the major problem with regard to the safety of herbal medicines is related to the

manufacturing practice, including contamination, substitution, incorrect preparation and dosage, intentional addition of unnatural toxic substances, interactions involving synthetic prescriptions, drugs, and herbal medicines, either intentional or unintentional mislabeling, and the presence of natural toxic contaminants⁵⁰.

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

- The *food herbs* – medicines such as peppermint, ginger, garlic, hawthorn, nettles, lemon, and balm are gentle in action, have low toxicity, and are unlikely to cause any adverse response. They can be consumed in substantial quantities over long periods of time without any acute or chronic toxicity. However they may bring about allergic reactions in certain individuals.
- The *medicinal herbs* – these are not daily “tonics” and need to be used with greater knowledge (dosage and rationale for use) for specific conditions (with a medical diagnosis) and usually only for a limited period. They have a greater potential for adverse reactions and in some cases drug interactions. They include aloe vera, black cohosh, comfrey, echinacea, ephedra, ginkgo biloba, ginseng, kava kava, milk thistle, and senna.
- The *poisonous herbs* – have a strong potential for either acute or chronic toxicity and should only be prescribed by trained clinicians who understand their toxicology and appropriate use. Fortunately, the vast majority of these herbs are not available to the public and are not sold in health food or herbal stores. Aconite, *Arnica* spp., *Atropa belladonna*, digitalis, datura, male fern, gelsemium, and veratrum are some examples⁵¹⁻⁵³.

1.9) Screening of Herbal Drugs

Once the botanical identity of a herb is established, the next step is phytochemical screening, which involves bioassays, extraction, purification, and characterization of the active constituents of pharmaceutical importance. The herb or herbal drug preparation in its entirety is regarded as the active substance. These constituents are either of known therapeutic activity or are chemically defined substances or a group of substances generally accepted to contribute substantially to the therapeutic activity of a herbal drug. In any

program in which the end product is to be a drug, some type of pharmacological screening, or evaluation, must obviously be done.

Pharmacological screening programs are not without problems. Ideally the active principles should be isolated, preferably using bioassay guided isolation processes, which can be problematic. The ideal pharmacological screen would be to identify those extracts or pure compounds that are highly active and nontoxic. There are many pharmacological screening tests available. An extension of this procedure is to isolate metabolites or "active compounds" from the plant that had shown most promising activity and subject them to pharmacological tests. In another approach, plants containing specific types or classes of chemical compounds, for example alkaloids, are tested. Simple tests such as color reactions are carried out on various parts of the plant in the field, and assays are carried out in the laboratories .

In vitro screening methods, though restricted to the detection of defined activities, are simpler and more useful⁵⁴. Recently, biochemical and receptor-ligand binding assays have gathered momentum. This has been made possible by the increasing availability of human receptors from molecular cloning, and extracts and compounds can be tested for binding directly to the presumed therapeutic target protein⁵⁵.

1.10) Labelling of Herbal Products

The quality of consumer information about the product is as important as the finished herbal product. Warnings on the packet or label help to reduce the risk of inappropriate uses and adverse reactions⁵⁶. The primary source of information on herbal products is the product label. Currently, there is no organization or government body that certifies an herb or a supplement as being labeled correctly. It has been found that herbal remedy labels often cannot be trusted to reveal what is in the container. Studies of herbal products have shown that consumers have less than a 50% chance of actually getting what is listed on the label, and published analysis of herbal supplements have found significant differences between what is listed on the label and what is in the bottle. The word "standardized" on a product label is no guarantee of higher product quality, since there is no legal definition of the word "standardized."

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

1.11) Policies and Regulations.

Unlike conventional drugs, herbal products are not regulated for purity and potency and this could cause adverse effects and can even lead to drug interactions⁵⁷. Herbals are frequently adulterated with prescription drugs. In certain countries, herbal products used for diagnosis, cure, mitigation, treatment, or prevention of disease are normally treated as drugs, and hence regulated by legislation.

However, in most countries, including the United States, such legislation does not exist and in fact, most botanical products are marketed as dietary supplements. Herbal products categorized as nutritional or dietary supplements are not regulated⁵⁸⁻⁶². It is clear that the herbal industry needs to follow strict guidelines and that regulations are needed. The food and drug administrations that regulate prescription drugs only review a herbal product if the item is suspected of being harmful or if the label contains a medical claim.

Before any new chemical or herbal drug is approved, research must prove that it is both safe and effective. As a result of these restrictions, packages of herbal

medicines are labeled as food supplements in a number of countries, which do not require pre-approved testing⁶³⁻⁶⁵.

1.12) Trends and Developments

The rationalization of the new multi-drug and multi-target concept of therapy in classical medicine is likely to have great implications on the future basic research in phytomedicine and evidence-based phytotherapy. It requires concerted cooperation between phytochemists, molecular biologists, pharmacologists, and clinicians, with the aim of using modern high-tech methods for standardization of phyto-preparations, of integrating new molecular biological assays into the screening of plant extracts and plant constituents, and of increasing studies on the efficacy proof of phytopreparations using controlled clinical trials. This should be paralleled or followed by pharmacokinetic and bioavailability studies. One major concern will be the investigation of the multivalent and multi-target actions of plant constituents and standardized extracts, with the aim of rationalizing the therapeutic superiority of many plant extracts over single isolated constituents. Increased effort in three major research areas will be crucial: (1) efforts to develop suitable standardization methods for phytopreparations; (2) the integration of molecular biological assays into the screening of plant extracts, single isolated compounds thereof and phytopreparations; and (3) the performance of further placebo- controlled, mono- or double-blind, clinical trials, paralleled or followed by pharmacokinetic and bioavailability studies. Standards should be developed for each natural health product and the same regulatory standards that apply to manufactured pharmaceuticals should apply equally to herbal products as well.

The World Health Assembly in resolutions WHA31.33 (1978), WHA40.33(1987), and WHA42.43 (1989) has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards⁶⁶⁻⁶⁷. These resolutions describe a series of tests for assessing the quality of medicinal plant materials.

It has now become evident that there is need for a holistic approach to health care, and the untapped potential of traditional medicines should be utilized.

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

The legislators at the national level should continue to press for effective laws to protect consumers from potentially harmful herbal drugs. Quality control for efficacy and safety of herbal products is of utmost importance. The assurance of the safety of a herbal drug requires monitoring of the quality of the finished product as well as the quality of the consumer information on the herbal remedy⁶⁸.

1.13) Rasashastra

Historically, *Rasa Shastra* or "Vedic Chemistry" is an offshoot of Ayurveda that developed around the period when Buddha existed, more than 2500 years ago⁶⁹. This special branch of medicine is called *Rasa Shastra*. *Rasa Shastra* describes the use of metals, gems, minerals and poisons to produce special formulations that combat acute conditions or serious diseases. *Rasa Shastra* is Ayurvedic pharmaceuticals, which deals with the drugs of mineral origin, their varieties, characteristics, processing techniques, properties and their therapeutic uses. This science is often referred to as "alchemy" and the resultant medications are called *rasas*. This means they are able to carry the herbs mixed with them faster to the desired site and start the action immediately⁷⁰. They act as catalysts and increase the bioavailability of the herbs to the cell. After performing the desired action, they are eliminated through our excretory systems, specifically via *mutra* and *mala* (urine and stool). Ayurvedic chemists have evolved various procedures like sublimation, oven treatment, controlled heat incineration, grinding, mixing, churning etc. to inculcate the therapeutic properties in the minerals for which many specific types of *yantras* (instruments) are designed. When it comes to using any metal,

mineral or a natural product directly or as a formulation, the alchemic scientists (*Raja Siddhas*) state that, barring a few exceptions everything should be purified/detoxified first (*Shodhana*) and then must be then incorporated in the formulations. The ancient chemists after classifying mercury as the *rasa* after which this branch has been named, classified the other metals, minerals and gems into *dhatu*, *upadhatu*, *ratna*, *upratna*, *maharasa*, *uprasa*, *sadhrana rasa* and *sudha varga*⁷¹.

1.13.1) *Shodhana*

In Ayurveda, purification is called *shodhana* which is the process through which the external and internal impurities of metals and minerals are removed.

Chemical purification is different from medicinal purification. In chemical purification it is only elimination of foreign matter, whereas in medicinal purification the objects are involved in the

- 1) Elimination of harmful matter from the drug.
- 2) Modification of undesirable physical properties of the drug.
- 3) Conversion of some of the characteristics of the drug to different stages.
- 4) Enhancement of the therapeutic action.

The purification is done by various processes of mixing, boiling of the raw drug with other substances with specific properties, Drying etc.

There are two kinds of *shodhana*. The first type, *Samanya shodhana* (general purification), is applicable to the large number of metals or minerals as heating the thin sheets of metals and immersing them in oil (*Taila*), extract (*Takra*), cow urine (*gomutra*), and other materials. The second type, *Vishesha Shodhana* (special purification), is applicable only to specific metals, minerals and in certain preparation. *Vishesha shodhana* includes *bhavana*, *svedana*, *nirvapana* and *marana*.

After the purification of the metals and minerals, they are then turned into ash or calcinated powders and are ready to be used as medicines. In traditional Ayurveda, the duration and intensity of the heat is regulated by the size of the pile of dung cakes called a *puta* in Sanskrit.

According to modern Ayurvedic medicine different metals have different healing effects. Mercury is antibacterial and antisyphilitic; sulphur is used against scabies and skin diseases, rheumatoid arthritis, spasmodic asthma, jaundice,

blood poisoning and internally as a stool softener; gold is effective against rheumatoid arthritis, and as a nervine tonic, an antidote, and a sexual stimulant; arsenic cures all fevers, asthma, and anaemia; copper is used to treat leprosy, skin diseases, and to improve the blood; and iron is effective against anaemia, jaundice, and as a general tonic for toning the body.

Despite the irrefutable scientific evidence that shows most of these minerals and metals to be toxic to the human body, ayurvedic practitioners continue to use them in their every day treatment of patients. They claim that their respective traditions have provided special techniques to detoxify the metals and minerals and to render them safe and extremely potent⁷².

A golden triangle consisting of Ayurveda, modern medicine and science will converge to form a real discovery engine that can result in newer, safer, cheaper and effective therapies. Globally there is a positive trend towards holistic health, integrative sciences approaches in drug discovery and therapeutics which is the unique feature of Ayurveda. Ayurveda and modern medicine techniques must be coupled in order to bring out high quality herbal products with rapid onset of action and good bioavailability. Herbal drug development is possible only through the development of standardized herbal products with reference to their active phytoconstituents present for commercialization, correct identification and supply of raw material and to avoid adulteration. Since some botanicals have undergone changes in their physical characteristics, the concept of active markers needs a flexible approach in favour of the complex nature of these materials. The ultimate goal of ethnopharmacology must be to identify drugs to eliminate human illness by a thorough analysis of plants through out the world.