

Review

Standardization of herbal medicines - A review

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There is increasing awareness and general acceptability of the use of herbal drugs in today's medical practice. Although, most of these applications are unorthodox, it is however a known fact that over 80% of the world population depends on herbal medicines and product for healthy living. This rise in the use of herbal product has also given rise to various forms of abuse and adulteration of the products leading to consumers' and manufacturers' disappointment and in some instances fatal consequences. The challenge is innumerable and enormous, making the global herbal market unsafe. This review seeks to enlighten stakeholders in herbal medicine on the need to establish quality parameters for collection, handling, processing and production of herbal medicine as well as employ such parameters in ensuring the safety of the global herbal market. The processes of good quality assurance and standardization of herbal medicines and products were also discussed.

Key words: Herbal medicine, standardization, quality control.

INTRODUCTION

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history (Barnes et al., 2007). Early humans recognized their dependence on nature for a healthy life and since that time humanity has depended on the diversity of plant resources for food, clothing, shelter, and medicine to cure myriads of ailments. Led by instinct, taste, and experience, primitive men and women treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or inactive, and also which combinations or processing methods had to be used to gain consistent and optimal results. Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopeias. Physical evidence of the use of herbal remedies some sixty thousand years ago has been found in a burial site of a Neanderthal man uncovered in 1960

in a cave in northern Iraq (Solecki, 1975).

Indeed, well into the twentieth century, much of the pharmacopeia of scientific medicine was derived from the herbal lore of native people. The 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. In some communities herbal medicine is still a central part of their medical system.

Medicinal plants are widely distributed throughout the world but most abundantly in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants (WHO, 2005, 2002a,b, 1999a,b, 1998a,b, 1990, 1981, 1979; De Smet, 1995; Duke and Martinez, 1994; Majno, 1975; Ackerknecht, 1973). Thus, herbal medicine has led to the discovery of a number of new drugs, and non-drug substances.

HERBAL MEDICINE

An herb is a plant or part of a plant valued for its medicinal, aromatic, or savoury qualities. Herbs can be

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viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal remedies or medicines consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically. Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value. They may come from any part of the plant but are most commonly made from leaves, roots, bark seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied topically to the skin. Herbal products often contain a variety of naturally-occurring biochemicals from plants, many of which contribute to the plant's medicinal benefits. Chemicals known to have medicinal benefits are referred to as "active ingredients" or "active principles" and their presence depends on a number of factors including the plant species, the time and season of harvest, the type of soil, the way the herb is prepared, etc.

During the past decade, there has been increasing public interest and acceptance of natural therapies in both developing and developed countries. Due to poverty and limited access to modern medicine, about 80% of the world's population, especially in the developing countries uses herbal medicine as their source of primary healthcare (Bodeker et al, 2005; Mukherjee, 2002; Farnsworth et al., 1985; Bisset, 1994). In these communities, traditional medical practice is often viewed as an integral part of their culture. In the West, people are attracted to herbal therapies for many reasons, the most important reason being that, like our ancestors, it is believed they will help us live healthier lives. Herbal medicines are often viewed as a balanced and moderate approach to healing. Individuals who use them as home remedies and over-the-counter drugs spend billions of dollars on herbal products. As such, they represent a substantial proportion of the global drug market (WHO, 2005, 2002a, 1999a and b, 1990; Blumenthal, 2000; Blumenthal et al., 1998; Roberts and Tyler, 1997; Farnsworth et al., 1985).

To achieve the desired benefit from herbal preparations, an individual must take the required dose over a certain length of time. Although it is generally believed that most herbal preparations are safe for consumption, some herbs like most biologically active substances could be toxic with undesirable side effects (Bisset, 1994).

The variability of the constituents in herbs or herbal preparations due to genetic, cultural and environmental factors has made the use of herbal medicines more challenging than it would necessarily have been. For instance, the availability and quality of the raw materials are frequently problematic, the active principles are diverse and may be unknown, and quality of different batches of preparation may be difficult to control and ascertain. In most countries, herbal products are launched into the market without proper scientific evaluation, and without any mandatory safety and

toxicological studies. There is no effective machinery to regulate manufacturing practices and quality standards. Consumers can buy herbal products without a prescription and might not recognize the potential hazards in an inferior product. A well-defined and constant composition of the drug is therefore, one of the most important prerequisites for the production of a quality drug. Given the nature of products of plant origin, which are not usually constant and are dependent on and influenced by many factors, ensuring consistent quality of products is vital for the survival and success of the industry (Bauer, 1998).

QUALITY CONTROL AND STANDARDIZATION OF HERBAL MEDICINES – CONCEPT AND SCOPE

Generally, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being safe and effective (EMA, 2005; WHO, 2002c, 1998c, 1996, 1991a,b, 1990, 1988). The term "herbal drugs" denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage (EMA, 1998). Hence they are capable of variation. This variability is also caused by differences in growth, geographical location, and time of harvesting.

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control process.

Several problems not applicable to synthetic drugs often influence the quality of herbal drugs. For instance:

1. Herbal drugs are usually mixtures of many constituents.
2. The active principle(s) is (are), in most cases unknown.
3. Selective analytical methods or reference compounds may not be available commercially.
4. Plant materials are chemically and naturally variable.
5. Chemo-varieties and chemo cultivars exist.
6. 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.) also affect herbal quality.

At present no official standards are available for herbal preparations. Those manufacturers, who are currently

doing some testing for their formulations, have their own parameters, many of which are very preliminary in nature. Presently it is very difficult to identify the presences of all the ingredients as claimed in a formulation. Hence the first important task is to evolve such parameter by which the presence of the entire ingredient can be identified, various chromatographic and spectrophotometric methods and evaluation of physicochemical properties can be tried to evolve pattern for identifying the presence of different ingredient. Wherever possible these methods can be applied for quantitative estimation of bioactive group of compounds like alkaloids, flavonoids, polyphenolic components or estimation of particular compound (Wani, 2007)

The need for standardization – Producers’ and consumers’ perspective

In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine are getting more apparent.

It is the cardinal responsibility of the regulatory authorities to ensure that consumers get the medication, which guarantees purity, safety, potency and efficacy. The regulatory authorities rigidly follow various standards of quality prescribed for raw materials and finished products in pharmacopoeias, formularies and manufacturing operation through statutory imposed good manufacturing practices. These procedures logically would apply to all types of medication whether included in modern system of medicine or one of the traditional systems.

Though herbal products have become increasingly popular throughout the world, one of the impediments in its acceptance is the lack of standard quality control profile. The quality of herbal medicine that is, the profile of the constituents in the final product has implications in efficacy and safety. However, due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish quality control parameter though modern analytical technique are expected to help in circumventing this problem. Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown or only partly explained. This is further complicated by the use of combination of herbal ingredients as being used in traditional practice. It is common to have as many as five different herbal ingredients in one product. Thus batch to batch variation starts from the collection of raw material itself in the absence of any reference standard for identification. These variations multiply during storage and further processing. Hence for herbal drugs and products, standardization should encompass the entire field of study from cultivation of medicinal plant to its clinical application.

Plant materials and herbal remedies derived from them represent substantial portion of global market and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary.

Standardization and quality control of herbal crude drugs – Processes and procedures

According to WHO (1996a and b, 1992), standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Attention is normally paid to such quality indices such as:

1. Macro and microscopic examination: For Identification of right variety and search of adulterants.
2. Foreign organic matter: This involves removal of matter other than source plant to get the drug in pure form.
3. Ash values: These are criteria to judge the identity and purity of crude drug – Total ash, sulphated ash, water soluble ash and acid insoluble ash etc.
4. Moisture content: Checking moisture content helps reduce errors in the estimation of the actual weight of drug material. Low moisture suggests better stability against degradation of product.
5. Extractive values: These are indicative weights of the extractable chemical constituents of crude drug under different solvents environment.
6. Crude fibre: This helps to determine the woody material component, and it is a criterion for judging purity.
7. Qualitative chemical evaluation: This covers identification and characterization of crude drug with respect to phytochemical constituent. It employs different analytical technique to detect and isolate the active constituents. Phytochemical screening techniques involve botanical identification, extraction with suitable solvents, purification, and characterization of the active constituents of pharmaceutical importance.
8. Chromatographic examination: Include identification of crude drug based on the use of major chemical constituents as markers.
9. Quantitative chemical evaluation: To estimate the amount of the major classes of constituents.
10. Toxicological studies: This helps to determine the pesticide residues, potentially toxic elements, safety studies in animals like LD50 and Microbial assay to establish the absence or presence of potentially harmful microorganisms.

The processes mentioned above involves wide array of scientific investigations, which include physical, chemical and biological evaluation employing various analytical

methods and tools. The specific aims of such investigation in assuring herbal quality are as varied as the processes employed.

Physical evaluation

Each monograph contains detailed botanical, macroscopic and microscopic descriptions of the physical characteristics of each plant that can be used to ensure both identity and purity. Each description is accompanied by detailed illustrations and photographic images which provide visual documentation of accurately identified material.

Microscopic evaluation

Full and accurate characterization of plant material requires a thorough physical examination. Microscopic analyses of plants are invaluable for assuring the identity of the material and as an initial screening test for impurities.

Chemical evaluation

This covers screening, isolation, identification and purification of the chemical components. Chemical analysis of the drug is done to assess the potency of vegetable material in terms of its active principles. The chemical screening or tests may include colour reaction test, which help to determine the identity of the drug substance and possible adulteration.

Biological evaluation

Pharmacological activity of certain drugs has been applied to evaluate and standardize them. The assays on living animal and on their intact or isolated organs can indicate the strength of the drug or their preparations. These assays are known as Biological assays or Bioassay.

Purity determination

Each monograph includes standards for purity and other qualitative indices already mentioned above.

Analytical methods

Critical to compliance with any monograph standard is the need for appropriate analytical methods for determining identity, quality, and relative potency. There

are a plethora of analytical methods available. However, it is often difficult to know which is the most appropriate to use, but critical among known analytical tools in monograph standardization is chromatography.

Chromatography

Chromatography is the science which studies the separation of molecules based on differences in their structure and/or composition. In general, chromatography involves moving a preparation of the materials to be separated, "the test preparation", over a stationary support. The molecules in the test preparation will have different interactions with the stationary support leading to separation of similar molecules. Test molecules which display tighter interactions with the support will tend to move more slowly through the support than those molecules with weaker interactions. In this way, different types of molecules can be separated from each other as they move over the support material. Chromatographic separations can be carried out using a variety of supports, including immobilized silica on glass plates (thin layer chromatography), very sensitive High Performance Thin Layer Chromatography (HPTLC), volatile gases (gas chromatography), paper (paper chromatography), and liquids which may incorporate hydrophilic, insoluble molecules (liquid chromatography). High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC, the same analysis can be viewed collectively in different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific type of analysis.

Quantitative analysis

The most appropriate quantitative analytical method with accompanying chromatograms is desirable. The primary goal of the methods is to provide validated methods to be used to quantify the compounds most correlated with pharmacological activity or qualitative markers (Wani, 2007).

Control of starting material

Control of the starting materials is essential in order to ensure reproducible quality of herbal medicinal products (De Smet, 2004; Gaedcke and Steinhoff, 2003; WHO, 2002b; Phillipson, 1993). The following points are to be considered in the control of starting materials:

Authentication and reproducibility of herbal ingredients

The problems associated with unregulated herbal products highlight the major public health issues that can arise when their herbal ingredients have not been authenticated correctly. Herbal ingredients must be accurately identified by macroscopic and microscopic comparison with authentic material or accurate descriptions of authentic herbs (Houghton, 1998). It is essential that herbal ingredients are referred to by their binomial Latin names of genus and species; only permitted synonyms should be used. Even when correctly authenticated, it is important to realise that different batches of the same herbal ingredient may differ in quality due to a number of factors such as:

1. Inter- or intra-species variation: The variation in constituents is mostly genetically controlled and may be related to the country of origin.
2. Environmental factors: The quality of a herbal ingredient can be affected by environmental factor like climate, altitude and other conditions under which it was cultivated.
3. Time of harvesting: For some herbs the optimum time of harvesting should be specified as it is known that the concentrations of constituents in a plant can vary during the growing cycle or even during the course of a day.
4. Plant part used: Active constituents usually vary between plant parts and it is not uncommon for a herbal ingredient to be adulterated with parts of the plant not normally utilised. In addition, plant material that has been previously subjected to extraction and is therefore 'exhausted' is sometimes used as adulterants to increase the weight of a batch of herbal ingredient.
5. Post-harvesting factors: Storage conditions and processing treatments can greatly affect the quality of a herbal ingredient. Inappropriate storage after harvesting can result in microbial contamination, and processes such as drying may result in a loss of thermo-labile active constituents.

Adulteration/substitution

There are instances when herbal remedies have been adulterated with other plant material and conventional medicines. Reports of herbal products devoid of known active constituents have reinforced the need for adequate quality control of herbal remedies.

Identity and purity

In order to try to ensure the quality of licensed herbal medicines, it is essential not only to establish the botanical identity of a herbal ingredient but also to ensure batch-to-batch reproducibility. Thus, in addition to

macroscopic and microscopic evaluation, identity tests are necessary. Such tests include simple chemical tests, e.g. colour or precipitation and chromatographic tests. Thin-layer chromatography is commonly used for identification purposes but for herbal ingredients containing volatile oils, a gas-liquid chromatographic test may be used. Although the aim of such tests may be to confirm the presence of active principles, it is frequently the case that the nature of the active principle has not been established. In such instances chemical and chromatographic tests help to provide batch-to-batch comparability and the chromatogram may be used as a 'fingerprint' for the herbal ingredient by demonstrating the profile of some common plant constituents such as flavonoids, alkaloids and terpenes.

Identity and purity ask the most important question "is the herb the one it should be?" In answering this, a lot of quality determinants are critically examined. Such determinant as purity and chemical constituents are very important. 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. Identity can be achieved by macro- and microscopical examinations. Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification (De Smet, 1999). At times an incorrect botanical quality with respect to the labelling can be a problem. For example, in the 1990's, a South American product labelled as "Paraguay Tea" was associated with an outbreak of anticholinergic poisoning in New York. Subsequent chemical analysis revealed the presence of a class of constituents that was different from the metabolites normally found in the plant from which Paraguay tea is made.

Assaying for those herbal ingredients with known active principles is another method of ensuring product's identity and purity. An assay should be established in order to set the criterion for the minimum accepted percentage of active substances. Such assays should, wherever possible, be specific for individual chemical substances, and high-pressure liquid chromatography and gas-liquid chromatography are the methods of choice. Where such assays have not been established, then non-specific classical methods such as titration or colorimetric assays may be used to determine the total content of a group of closely related compounds.

Purity is closely linked with the safe use of drugs and deals with factors such as values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation also includes microbial contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods such as photometric analysis, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and

gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations. Depending on whether the active principles of the preparation are known or unknown, different concepts such as "normalization versus standardization" have to be applied in order to establish relevant criteria for uniformity.

Content assay is the most difficult area to perform in quality control since in most herbal drugs the active constituents are not known. Sometimes markers can be used. In all other cases, where no active constituent or marker can be defined for the herbal drug, the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeias. The choice of the extracting solvent depends on the nature of the compounds involved, and might be deduced from the traditional uses. For example, when an herbal drug is used to make a tea, the hot water extractable matter, expressed as milligrams per gram of air-dried material, may serve this purpose (WHO, 1998b, 1996b). A special form of assay is the determination of essential oils by steam distillation. When the active constituents (for example, sennosides in Senna) or markers (for example, alky amides in Echinacea) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy (UV/VIS), TLC, HPLC, GC, mass spectrometry (MS), or a combination of GC and MS (GCMS), can be employed (Watson, 1999).

Good agricultural/Manufacturing practices

Quality control and the standardization of herbal medicines also involve several other steps like source and quality of raw materials, good agricultural practices and good manufacturing practices. These practices play a pivotal role in guaranteeing the quality and stability of herbal preparations (WHO, 2004, 2003, 2000, 1992, 1988b; EMEA, 2002; Blumenthal et al., 1998; Roberts and Tyler, 1997). 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, fertilizers application, harvesting, drying and storage. In fact, GAP procedures are 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 (Eskinazi et al., 1999; Blumenthal et al., 1998; Bauer, 1998). 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 conducted.

Contaminants of herbal ingredients

Herbal ingredients of high quality should be free from insects, animal matter and excreta. It is usually not possible to remove completely all contaminants, hence specifications should be set in order to limit them:

1. Ash values: Incineration of a herbal ingredient produces ash which constitutes inorganic matter. Treatment of the ash with hydrochloric acid results in acid-insoluble ash which consists mainly of silica and may be used to act as a measure of soil present. Limits may be set for ash and acid-insoluble ash of herbal ingredients.

2. Foreign organic matter: It is not possible to collect a herbal ingredient without small amounts of related parts of plant or other plants. Standards should be set in order to limit the percentage of such unwanted plant contaminants.

3. Microbial contamination: Aerobic bacteria and fungi are normally present in plant material and may increase due to faulty growing, harvesting, storage or processing. Herbal ingredients, particularly those with high starch content, may be prone to increased microbial growth. Pathogenic organisms including Enterobacter, Enterococcus, Clostridium, Pseudomonas, Shigella and Streptococcus have been shown to contaminate herbal ingredients. It is essential that limits be set for microbial contamination and the European Pharmacopoeia now gives non-mandatory guidance on acceptable limits (Barnes et al., 2007).

4. Pesticides: Herbal ingredients, particularly those grown as cultivated crops, may be contaminated by DDT (dichlorodiphenyltrichloroethane) or other chlorinated hydrocarbons, organophosphates, carbamates or polychlorinated biphenyls. Limit tests are necessary for acceptable levels of pesticide contamination of herbal ingredients. The European Pharmacopoeia includes details of test methods together with mandatory limits for 34 potential pesticide residues (Barnes et al., 2007).

5. Fumigants: Ethylene oxide, methyl bromide and phosphine have been used to control pests which contaminate herbal ingredients. The use of ethylene oxide as a fumigant with herbal drugs is no longer permitted in Europe (Barnes et al., 2007).

6. Toxic metals: Lead, cadmium, mercury, thallium and arsenic have been shown to be contaminants of some

herbal ingredients. Limit tests for such toxic metals are essential for herbal ingredients.

7. Radioactive contamination: There are many sources of ionization radiation, including radionuclides, occurring in the environment. Hence, a certain degree of exposure is inevitable. (AOAC, 2005; WHO, 2000; De Smet, 1992).

8. Other contaminants: As standards increase for the quality of herbal ingredients it is possible that tests to limit other contaminants such as endotoxins and mycotoxins will be utilized to ensure high quality for medicinal purposes (Barnes et al., 2007).

Standardization of herbal medicines

This involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations. Botanical extracts made directly from crude plant material show substantial variation in composition, quality, and therapeutic effects. Standardized extracts are high-quality extracts containing consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes. No regulatory definition exists for standardization of dietary supplements. As a result, the term “standardization” may mean many different things. Some manufacturers use the term standardization incorrectly to refer to uniform manufacturing practices, but 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 substances 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 pharmacological effects in the body. There are two types of standardization. In the first category, “true” standardization, a definite phytochemical or group of constituents is known to have activity. Ginkgo with its 26% ginkgo flavones and 6% terpenes is a classic example. These products are highly concentrated and no longer represent the whole herb, and are now considered as phytopharmaceuticals. In many cases they are vastly more effective than the whole herb. However the process may result in the loss of efficacy and the potential for adverse effects and herb–drug interactions may increase. The other type of standardization is based on the guarantee of the manufacturers for the presence of a certain percentage of marker compounds which are not indicators of therapeutic activity or quality of the herb.

In the case of herbal drug preparations, the production and primary processing of the medicinal plant or herbal

drug has a direct influence on the quality of the active pharmaceutical ingredients (APIs). Due to the inherent complexity of naturally growing medicinal plants and the limited availability of simple analytical techniques to identify and characterize the active constituents solely by chemical or biological means, there is a need for an adequate quality assurance system. This assurance is also required during cultivation, harvesting, primary processing, handling, storage, packaging, and distribution. Deterioration and contamination through adulteration, especially microbial contamination, can occur at any one of these stages. It is extremely important to establish good agricultural, harvesting, and manufacturing practices for herbal starting materials in order to minimize these undesirable factors. In this regard producers, processors, and traders of medicinal plants or herbal drugs have an obligation and a role to play. The manufacturers and suppliers of herbal products should adhere to quality control standards and good manufacturing practices. Currently, only a few manufacturers adhere to complete quality control and good manufacturing procedures including microscopic, physical, chemical, and biological analysis. Organizations, such as, National Agency for Food and Drugs Administration and Control (NAFDAC) help safeguard Nigerians’ health, and Health Canada help safeguard Canadians’ health by carrying out premarket reviews of all drugs before they are authorized for sale. The products available in the market are analyzed regularly to ensure that they are free of unsafe ingredients and that the products actually contain the ingredients indicated on the labels.

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

CRITICAL FACTORS AFFECTING THE QUALITY CONTROL OF HERBAL DRUGS

Microscopic evaluation

Quality control of herbal drugs has traditionally been based on the appearance and today microscopic evaluation is indispensable in the initial identification of herbs, as well as, in identifying small fragments of crude or powdered herbs, and detection of foreign matter and adulterants. A primary visual evaluation, which seldom

needs more than a simple magnifying lens, can be used to ensure that the plant is of the required species, and that the right part of the plant is being used. At other times, microscopic analysis is needed to determine the correct species and/or that the correct part of the species is present. For instance, pollen morphology may be used in the case of flowers to identify the species, and the presence of certain microscopic structures such as leaf stomata can be used to identify the plant part used. Although this may seem obvious, it is of prime importance, especially when different parts of the same plant are to be used for different treatments. Stinging nettle (*Urtica urens*) is a classic example where the aerial parts are used to treat rheumatism, while the roots are applied for benign prostate hyperplasia (AOAC, 2005).

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 insect, including excreta and visible contaminant such as sand and stones, poisonous and harmful foreign matter and chemical residues. Animal matters such as insects and "invisible" microbial contaminants, which can produce toxins, are also among the potential contaminants of herbal medicines (WHO, 2004, 2003; EMEA, 2002). Macroscopic examination can easily be employed to determine the presence of foreign matter, although, microscopy is indispensable in certain special cases (for example, starch deliberately added to "dilute" the plant material). Furthermore, when foreign matter consists, for example, of a chemical residue, TLC is often needed to detect the contaminants (AOAC, 2005; WHO, 1999a, 1998a).

Ash content

To determine ash content, the plant material is burnt and the residual ash is measured as total and acid-insoluble ash. Total ash is the measure of the total amount of material left after burning and includes ash derived from the part of the plant itself and acid-insoluble ash. The latter is the residue obtained after boiling the total ash with dilute hydrochloric acid, and burning the remaining insoluble matter. The second procedure measures the amount of silica present, especially in the form of sand and siliceous earth (AOAC, 2005).

Heavy metals

Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such as mercury, lead, copper, cadmium, and arsenic in herbal

remedies can be attributed to many causes, including environmental pollution, and can pose clinically relevant dangers for the health of the user and should therefore be limited (AOAC, 2005; WHO, 1998c; De Smet, 1992). The potential intake of the toxic metal can be estimated on the basis of the level of its presence in the product and the recommended or estimated dosage of the product. This potential exposure can then be put into a toxicological perspective by comparison with the so-called Provisional Tolerable Weekly Intake values (PTWI) for toxic metals, which have been established by the Food and Agriculture Organization of the World Health Organization (FAO-WHO) (De Smet, 1999; WHO, 1981, 1979). A simple, straightforward determination of heavy metals can be found in many pharmacopoeias and is based on colour reactions with special reagents such as thioacetamide or diethyldithiocarbamate, and the amount present is estimated by comparison with a standard (WHO, 1988a). Instrumental analyses have to be employed when the metals are present in trace quantities, in admixture, or when the analyses have to be quantitative. Generally, the main methods commonly used are atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP) and neutron activation analysis (NAA) (Watson, 1999).

Microbial contaminants and aflatoxins

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

Herbal drugs normally carry a number of bacteria and molds, often originating in the soil. Poor methods of harvesting, cleaning, drying, handling, and storage may also cause additional contamination, as may be the case with *Escherichia coli* or *Salmonella* spp. while a large range of bacteria and fungi are from naturally occurring microflora, aerobic spore-forming bacteria that frequently predominate. Laboratory procedures investigating microbial contaminations are laid down in the well-known pharmacopoeias, as well as, in the WHO guidelines (WHO, 2000, 1998a). Limit values can also be found in the sources mentioned. Generally, a complete procedure consists of determining the total aerobic microbial count, the total fungal count, and the total Enterobacteriaceae count, together with tests for the presence of *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, and *Pseudomonas aeruginosa* and *Salmonella* spp. The European Pharmacopoeia also specifies that *E. coli* and *Salmonella* spp. should be absent from herbal preparations.

Materials of vegetable origin tend to show much higher levels of microbial contamination than synthetic products and the requirements for microbial contamination in the European Pharmacopoeia allow higher levels of microbial contamination in herbal remedies than in synthetic pharmaceuticals. The allowed contamination level may also depend on the method of processing of the drug. For example, higher contamination levels are permitted if the final herbal preparation involves boiling with water. 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 (WHO, 2000). Aflatoxin-producing fungi sometimes build up during storage (De Smet, 1992). Procedures for the determination of aflatoxin contamination in herbal drugs are published by the WHO (2000). After a thorough clean-up procedure, TLC is used for confirmation. In addition to the risk of bacterial and viral contamination, herbal remedies may also be contaminated with microbial toxins, and as such, bacterial endotoxins and mycotoxins, at times may also be an issue (Sinha, 1998; De Smet et al., 1997; De Smet, 1992). There is evidence that medicinal plants from some countries may be contaminated with toxigenic fungi (*Aspergillus*, *Fusarium*). Certain plant constituents are susceptible to chemical transformation by contaminating micro-organisms. Withering leads to enhanced enzymic activity, transforming some of the constituents to other metabolites not initially found in the herb. These newly formed constituent(s) along with the molds such as *Penicillium nigricans* and *P. jensi* may then have adverse effects (De Smet, 1992).

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 (De Smet, 1992). 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-MS. Some simple procedures have been published by the WHO and the European

Pharmacopoeia has laid down general limits for pesticide residues in medicine (WHO, 1996a, 1998a, 2000; De Smet, 1999; AOAC, 2005)

Radioactive contamination

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 wide spread contamination by radionuclides resulting from major nuclear accidents. These publications emphasize that the health risk, in general, due to radioactive contamination from naturally occurring radio nuclides is not a real concern, but those arising from major nuclear accidents such as the nuclear accident in Chernobyl and Fukushima may be serious and depend on the specific radionuclide, the level of contamination, and the quantity of the contaminant consumed. Taking into account the quantity of herbal medicine normally consumed by an individual, is unlikely to be a health risk. Therefore, at present, no limits are proposed for radioactive contamination (AOAC, 2005; WHO, 2000; De Smet, 1992).

Analytical methods

Published monographs in a pharmacopoeia are the most practical approach for quality control of herbal drugs and there are many available (EMA, 2005; WHO, 1998a,b, 1996a, 1998a, 1981). When pharmacopoeia 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 pharmacopoeia 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 (AOAC, 2005; WHO, 2000). 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 of products (AOAC, 2005). 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 may 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-Visible determinations are easy to operate, and validation procedures are straightforward but at the same time precise. Although measurements are made rapidly, sample preparation can be time consuming and works well only for less complex samples, and those compounds with absorbance in the UV-Visible 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-Visible spectrometry, MS, GC, liquid chromatography (LC) used alone, or in combinations such as GC-MS and LC-MS, and nuclear magnetic resonance (NMR), electrophoretic techniques, especially by hyphenated chromatographic techniques, are powerful tools, often used for standardization and to control the quality of both the raw material and the finished product. The results from these sophisticated techniques provide a chemical fingerprint as to the nature of chemicals or impurities present in the plant or extract (WHO, 2002c). Based on the concept of photo equivalence, the chromatographic fingerprints of herbal medicines can be used to address the issue of quality control. Methods based on information theory, similarity estimation, chemical pattern recognition, spectral correlative chromatograms (SCC), multivariate resolution, the combination of chromatographic fingerprints and chemometric evaluation for evaluating fingerprints are all powerful tools for quality control of herbal products.

Validation

The validation of herbal products is a major public health concern both in developed and resource-poor countries, where fakers 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 Pharmacopoeia (USPC, 1994 to 2001), the International Conference on Harmonization (ICH), and the US Food and Drug Administration (FDA) provide a framework for performing such validations. Generally, validation investigations must include studies on specificity, linearity, accuracy, precision, range, detection, and quantitative limits, depending on whether the analytical method used is qualitative or quantitative (De Smet et al., 1997). 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 ingredients. TLC and HPLC are the main analytical techniques commonly used. In cases when active ingredients are not known or too complex, the quality of plant extracts can be assessed by a "fingerprint" chromatogram (De Smet et al., 1997).

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 will help to reduce the risk of inappropriate uses and adverse reactions (De Smet et al., 1997). The primary source of information on herbal products is the product label. Currently, there is no organization or government body that certifies herb or a supplement as being labelled correctly. It has been found that herbal remedy labels often cannot be trusted to reveal what is in the container. Studies of herbal products have shown that consumers have less than a 50% chance of actually getting what is listed on the label, and published analyses of herbal supplements have found significant differences between what is listed on the label and what is in the bottle. The word “standardized” on a product label is no guarantee of higher product quality, since there is no legal definition of the word “standardized.” Consumers are often left on their own to decide what is safe and effective for them and the lack of consistent labelling on herbal products can be a source of consumer frustration. Certain information such as “the product has been manufactured according to Pharmacopoeia standards,” listing of active ingredients and amounts, directions such as serving quantity (dosage) and frequency of intake of the drug, must be in the label.

CONCLUSIONS

Plant materials are used throughout the developed and developing world as home remedies, in over-the-counter drug products, and as raw material for the pharmaceutical industry, and they represent a substantial proportion of the global drug market. Therefore, it is essential to establish internationally recognized guidelines for assessing their quality. Certain herbs have become popular over the years, but the general public, medical practitioners and the media still have a poor understanding of safe and effective use of herbal medicine. Evidence is emerging on the dangers of indiscriminate use of some of these herbs. As in most situations, the truth lies hidden under the media hype, poorly understood science, an exaggerated claim. The need for standardization of herbals is now very essential given the global acceptance of herbal products as remedies for various diseases and ailments.

The deployment of modern analytical tools in testing the various quality parameters for an effective quality control herbal product cannot be over emphasized. The assurance of the safety and efficacy of a herbal drug requires monitoring of the quality of the product from collection through processing to the finished packaged product. It is recommended that various government agencies should follow a more universal approach to herbal quality by adopting the WHO guidelines and also developing monographs using the various quality parameters outlined above. This will strengthen the regulatory process and minimize quality breach.

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