EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 10
121-132
Year: 2021

STANDARDIZATION OF HERBAL MEDICINES

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Article Received on 10/08/2021 **Article Revised on 31/08/2021** **Article Accepted on 21/09/2021**

ABSTRACT
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. Herbal formulations are widely accepted as a therapeutic agents for several diseases. The development of authentic analytical methods which can reliably identifies the phytochemical composition, including quantitative analyses of marker/ bioactive compounds and other major constituents, is a major challenge to scientists. Standardization is an important parameter for the establishment of a consistent biological activity or simply to maintain the quality of production and manufacturing of herbal drugs. 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 products 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. Herbal medicine on the need to establish quality parameters with the help of advanced analytical tools and well defined standardization methods in ensuring the safety of the global herbal market. The processes of good quality assurance and standardization of herbal medicines and products using various spectroscopic, chromatographic and electrophoretic methods were also discussed.

KEYWORDS: spectroscopic, chromatographic and electrophoretic.

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. 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. 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.

Standardization of herbal formulations is essential in order to assess of quality drugs, based on the concentration of their active principle, physical, chemical, physcio-chemical standardization and in vitro, in-vivo parameters. Natural products have been our single most successful source of medicines. Each plant is like factory capable of synthesizing unlimited no of highly complex and unusual chemical substance whose structure covered otherwise escape the imagination forever. It is necessary to maintain reproducible efficacy and safety of phyto pharmaceutical therefore if phytopharmaceutical have to regard as rational drug should be standardized and pharmaceutical quality must be approved.

1.1 Herbal Medicines
An herb is a plant or part of a plant valued for its medicinal, aromatic, or savoury qualities. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal remedies or medicines consist of portions of plants or unpurified plantextracts 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-occuring 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. 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. 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.

1.1.1 Herbal Drugs

Herbal drugs are of two types

A. Single/ crude drug
B. Multiple herbal formulations

A. Single /crude drugs.

- All mainly whole, fragment or cut plant, plant parts usually dried forms, but sometimesfresh.
- It also includes algae, fungi and lichen.

B. Multiple herbal formulations.

- Formulations are obtained by subjecting herbal ingredients to various manufacturingprocess such as extraction, distillation, expression, fractions, partition, chromatography and formulations.

1.2 STANDARDIZATION

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 medicines. Hence standardization is a tool in the quality control process. American Herbal Product association defines: "Standardization refers to the body of information and control necessary to product material of reasonable consistency. This achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes. "Standardization" expression is used to describe all measures, which are taken during the manufacturing process and quality control leading to a reproducible quality. It also encompasses the entire field of study from birth of a plant to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or a respectively by adding excipients or by mixing herbal drugs or herbal drug preparations.

1.2.1 Need of Standardization

In olden days Vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. In almost all the traditional system of medicine, the quality control aspect has been considered from its inspection of its Rishis, Vaidyas and Hakims. Unlike in olden times where traditional practitioners prepared and tested the qualities of herbal medicines, the problem faced today are these of economics of industrial scale production, shelf life and distribution to long distances. These have necessitated development of modern and objective standards for evaluating the safety, quality and efficacy of these medicines. People are also becoming aware of the potency and side effect. To gain public trust and to bring herbal product into mainstream of today health care system, the researchers, the manufacturers and the regulatory agencies must apply rigorous scientific methodologies to ensure the quality and lot to lot consistency of the traditional herbal products.

Need of Quality control and standardization of herbal products can be summarized as follows.

1. When traditional medicines were developed technology and concept of standardization was quite different.
2. During past thousand years dynamic process of evolution may have changed the identity of plant material.
3. Due to commercialization, supply of genuine raw material has become a challenge.
4. Properties of botanicals may have undergone change due to time and environmental factors

1.2.2 The Need For Standardization 'Procedures And Consumers' Perspective

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.

1.3 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.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.

2. CONCEPT AND SCOPE

Generally, all medicines, whether they are synthetic or of plant origin, should fulfill the basic requirements of being safe and effective. 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 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. Safety in herbal drugs- Major difference in the assessment of quality, safety and efficacy would hinder free circulation of herbal medicinal products may represent a risk for consumers. The complexity of herbal drug preparation and the interpretation of bibliographic data on safety and efficacy reflecting the experience gathered during long-term use are best addressed by involving specific expertise and experience. Safety and efficacy of complex biological products, such as herbal medicines products, are directly linked to pharmaceutical details such as the way of production and the specification of extracts. A

consideration quality of herbal drugs may need more detailed information on aspects of agriculture products. The selection of seeds, condition of cultivation, and harvesting represent an important aspect in producing a reproducible quality of herbal drugs. Ongoing discussion on good agriculture practices [GAP] for medicinal plants should be monitored regularly. 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.

3. GUIDELINES FOR HERBAL DRUG STANDARDIZATION

WHO Guidelines

The subject of herbal drug standardization is massively wide and deep. The guidelines set by WHO can be summarized as follows.

1. Reference to the identity of the drug. Botanical evaluation- sensory characters, foreign organic matter, microscopical, histological, histochemical evaluation, quantitative measurements etc.
2. Refers to the physicochemical character of the drug. Physical and chemical identity, chromatographic fingerprints, ash values, extractive values, moisture content, volatile oil and alkaloidal assays, quantitative estimation protocols etc.
3. A reference to the pharmacological parameters, biological activity profiles, bitterness values, hemolytic index, astringency, swelling factor, foaming index etc.
4. Toxicity details- pesticide residues, heavy metals, microbial contamination like total viable count, pathogens like *E. coli, Salmonella, P. aeruginosa, S. aureus, Enterobacteria* etc.
5. Microbial contamination.
6. Radioactive contamination.

4. Standardization And Quality Control of Herbal Medicine–

4.1. Processes And Procedures.

According to WHO, 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 drugin 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 actualweight 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.

4.2 Classification of standardization techniques are as follows.

A. Physical Evaluation
B. Microscopical Evaluation
C. Chemical Evaluation
D. Biological Evaluation

A. 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. Physical evaluation is very crucial factor in the standardisation of crude drugs. This method helps in evaluation of crude drugs with reference to moisture content, viscosity, melting point, pH etc.

Significance - Physical evaluation is important tool for

determination of Quality Quantity and purity of crude drugs.

A.1. Ash value
The residue remaining after incineration is the ash content of the drug.

Significance- Ash value is an important parameter to prove acceptability and purity in case of drugs that are collected or stored by incorrect way. High ash value is indicative of contamination, substitution, adulteration in crude drug.

E.g. Inorganic salts, naturally occurring in drug in the form of adulteration. Ash value is determinant of identity or purity of drug.

The ash value is determined by three methods total ash, acid insoluble ash, water soluble ash.

Method: Incinerate 2 to 3 g of the ground drug in a tared silica dish at a temperature not exceeding 450ºc cool and weigh. Calculate the % of ash with reference to the air-dried drug.

A.1.1. Acid insoluble ash
It determines amount of silica present, especially as sand siliceous earth **Procedure:** Boil the ash with 25 ml of dil. HCL. Collect the insoluble matter in a crucible. Wash with hot water and ignite to constant weight. Calculate the % acid insoluble ash with reference to the air dried drug.

A.1.2. Water soluble ash
Method: Boil the ash for 5 minutes with 25 ml of water. Collect insoluble matter in a crucible wash and ignite for 15 minutes at a temp not exceeding 450ºc. Calculate the percentage of water- soluble ash with reference to the air dried drug. (Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash).

A.2. Refractive index
Refractive index gives idea about purity. When a ray of light passes from through a rarer medium to denser medium it is bent and this bending of light is called as refraction. Thus, the ratio of velocity of light in vacuum to its velocity in a substance is known as refractive index of the second medium. It is constant for a liquid for particular purity value that's why it is considered as important tool for the standardization. It can be affected by wave length of the incident light, temperature and pressure.

A.3. Determination of pH.
The pH value may be defined as the negative logarithm of hydrogen ion concentration to the base
10. Potentiometrically pH value determine by a glass electrode and a suitable pH meter. The pH for most of the extract ranges from 5 to 7 and can be considered as one of the quality indicator.

A.4. Volatile oil content
Odorous and volatile principal of drug is known as volatile oils such crude drugs are standardized on the basis of their volatile content.

A.5. Viscosity
Viscosity of liquid is constant for that particular liquid at a given temperature and is an index of its composition, that's why it is an important tool for standardizing liquid drugs.

Significance-It gives idea about composition of drug and stability.

A.6. Melting point
Melting point for pure chemicals or phytochemicals is constant, but the crude drugs from animal or plant origin contain the mixed chemicals, that's why they are described with certain range of melting point.

Significance-It is one of the parameter to judge the purity and stability of crude drugs.

B. 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.

Significance- This method allows detailed examination of drug and it is tool for identification of standard drug. It is considered as crucial factor for qualitative evaluation of organised crudedrugs.

B.1. Determination of leaf constant
B.1.1. Stomatal number
It is average number of stomata per square mm of the epidermis of the leaf.
e.g. Digitalis perpuria: 25-50

B.1.2. Stomatal index
The Stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells each stoma being counted as one cell.
Stomatal index= S / E+S×100
Where: S= Total number of stomata in a given area of leaf E= Number of epidermal cells in the same area of leaf.
e.g. Digitalis perpuria: 1.3-3.5

B.1.3. Vein islet number
The vein-islet number is average number of veinislet per square mm of leaf surface midway between midrib and margin. Various species of drugs are distinguished by vein-islet number. E.g. Alexandrian senna and Indian senna are distinguished because of their difference in vein islet numbers which are 27 and 22 respectively.

Vein islet number of various drugs.
Datura metal 19-22
Datura stramonium 12-16
Datura fastuosa 18-24
Cannabis sativa 20-30

B.1.4. Vein termination number.
It is defined as the no. of veinlet termination per sq. mm of the leaf surface midway between midrib and margin.

B.1.5. Palisade ratio
It is defined as average no. of palisade cells beneath each epidermal cell.

E.g. Cassia angustifollia 5.5-10.5 Cassia acutifollia 4.5-9.5 Digitalis perpuria: 3.7-4.2.

B.1.6. Trichomes
The elongated outgrowth of leaf called as trichomes and they are also known as plant hairs.

Types of trichomes
1. Covering trichomes
a. Unicellular trichomes e.g. Nuxvomica,caanabis
b. Multicellular-unbranched trichomes
c. Multicellular branched trichomes- e.g. Verbascum Thapsus

2. Glandular trichome
a. Unicellular glandular trichome- e.g.Vasaka
b. Multicellular glandular trichome- e.g. Digitalis purpurea

3. Hydathode trichome – e.g. Piper betal

C. 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.Many of the crude drugs have definite chemical constituents and biological or pharmacological activity depends on these chemical constituents. The chemical evaluation of crude drug helpful to identify certain drug orto test their purity.

Name of Test Performed for

Borax test	Aloes
Borntrager's test	Anthraquinone glycosides (Senna)
Baudouin's test	Sesame oil
Carr-Price reaction	Vitamin A
Saponification Claud test	bees wax
Ehrlich test	Ergot alkaloids
Fiehe's test	Invert sugar
Fluorescence test	Pale catechu
Foam test	Saponins
Frohde's test	Ipecac
Goldbeater's Skin test	Tannins
Grignard reaction	Cyanogenetic glycosides
Haemolysis test	Saponins
Halphen test	Cottonseed oil
Kedde's test	Cardiac glycosides
Keller-Kiliani test	Digitoxose
Klunge's / Cupraloin test	Aloes (isobarbaloin)
Kreis test	Rancidity of fats and oils

D. 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.

D.1. 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 pharmacopeias, as well as, in the WHO guidelines. 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 Entero-bacteriaceae 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. 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 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. 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.

5. Analytical Methods For Standardization of Herbal Medicines -

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 know analytical tools in monograph standardization is chromatography. A simple chromatographic technique such as TLC may provide valuable additional information to establishthe 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. 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. The instruments for UV-Visible determinations are easy to operate, and validation procedures are straight forward 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 chemicalconstituents 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.

5.1 Chromatographic Evaluation

5.1.1 Thin Layer Chromatography

TLC is one of the most important tool for separation of compound. It is widely used technique of chromatography. It is based on principle of adsorption. In this method stationary phase is a finely
divided solid and it is applied as a thin layer on supporting plate and the mobile phase is a liquid which is allowed to flow on the surface of the plate by capillary action.
Common adsorbent material used- Silica gel, Alumina, Kieselguhr.

5.1.2 HPTLC

In HPTLC a layer thickness of 100-150micron is used to achieve separation. HPTLC uses open layers of adsorbents on plates or foils to separate component of

samples.

Significance of HPTLC: Identification and detection of adulterants in herbal product and it is also important in identification of pesticide content, mycotoxins and in quality control of herbs and health foods.

Examples

I) Carvone

Stationary Phase: Silica gel
Mobile Phase: Chloroform : acetone (100:2)
Detection: By dipping in anisaldehyde sulphuric acid reagent, heating at 800 for about 10 min. Quantification: UV absorbance 410nm.

II) Aloin:

Stationary Phase: Silica gel
Mobile Phase: Ethyl acetate : formic acid: Water (17: 2: 3)Quantification: UV absorbance 350 nm.

5.1.3 HPLC

HPLC is useful for isolation and purification of herbal compounds. There are two types of preparative- HPLC: low pressure HPLC (near about 5 bar) and high pressure HPLC (>20 bar). This is very important in pharmaceutical industry because it is efficient purification technique and it spend less time on the synthesis conditions.

5.1.4 Gas liquid chromatography & gc-ms:

Significance: GC is an important tool for detection of volatile substances. GLC separate the volatile substances by percolating a gas stream over a stationary phase. The basic of separation in GLC is the partitioning of sample in and out of the film of liquid spread over an inert solid. The nitrogen and helium are most common gases used in GC. Advantages of these methods are their high sensitivity, stability and high efficiency. Especially, the hyphenation with MS provides reliable information for the qualitative analysis of the complex constituents.

Examples

i. Analysis of anethole in Fennel oil

Test sample: Fennel oil Stationary phase: FFAP Carrier gas: Helium Sample size: 0.1micro litre

ii. Analysis of eugenol in clove oil

Test sample: Clove oil Stationary phase: FFAP Carrier gas: Helium
Sample size: 0.20 micro litre.

5.2 Spectroscopial Analysis

5.2.1 Ultra Violet Spectroscopy

UV spectroscopy is important technique in the analysis of herbal as well as synthetic drugs. It play important role and gives idea about purity of the substance. Its detection capability depends on Beer-Lamberts Law that is absorbance is directly proportional to the concentration and path length.

5.2.2 Infrared spectroscopy

Significance -It is analytical technique for detection of functional groups. IR Spectra may be measured on plant substances in an automatic recording IR spectrophotometer. The sampling of the solid sample is done by using mulling technique.
Some important IR Frequencies Amines -3300-3500 cm-1 Alkanes-2940-2860 cm-1 Carboxylic acid-3520 cm-1 Cynide-2225 cm-1
Hydroxyl-3400-3500 cm-1**.**

5.2.3 Mass Spectrometry

It gives idea about molecular weight and molecular formula. The idea of molecular weight is generated from molecular ion peak. The index of hydrogen deficiency is useful for prediction of validated molecular formula and no of unsaturation.

5.2.4 NMR Spectroscopy

It is spectroscopic technique which gives idea of no and types of protons present in particular structure of compound.

6. 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. 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 oreven 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.

7. 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.

8. 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.

9. 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.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 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 conducted.

10. 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 limitsbe set for microbial contamination and the European Pharmacopoeia now gives non-mandatory guidance on acceptable limits.
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.
5. 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.
6. Radioactive contamination: There are many sources of ionization radiation, including radionuclides, occurring in the environment. Hence, a certain degree of exposure is inevitable.
7. 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.

11. CRITICAL FACTORS AFFECTING THE QUALITY CONTROL OF HERBAL DRUGS -

11.1 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.

11.2 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 insec, 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. Macroscopic examination can easily be employed to determine the presence of foreign matter, although, microscopy is indispensable in certain special cases Furthermore, when foreign matter consists, for example, of a chemical residue, TLC is often needed to detect the contaminants.

11.3 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.

11.4 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. 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). 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. 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).

11.5 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. 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.

11.6 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.

11.7 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. This could also lead to the regulation of the industry so that only qualified physicians and health providers are allowed to prescribe the medication. By definition, validation is the process of proving that an analytical method is acceptable for its intended purpose for pharmaceutical methods. 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.

11.8 Labelling of herbal products

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. 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 nolegal 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.

12. CONCLUSION

The subject of herbal drug standardization is massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function. For the purpose of research work on standardization of herbal formulations, a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of utmost importance. 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 WHOguidelines. This will strengthen the regulatory process and minimize quality breach.

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