**Standardization of natural products**

Natural products, either as a pure compounds or standardized plant extracts, provide extensive opportunities for new drug leads because of unmatched availability chemical diversity.

Phytotherapeutic agents are standardized herbal preparation consisting of complex mixtures of one or more plants which are used in most countries for the management of various diseases. (Garg et al.,2012)

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 healthcar

(Bodeker et al, 2005; Mukherjee, 2002; Farnsworth et al., 1985; Bisset, 1994).

***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.

Hence for herbal drugs and products, standardization should encompass the entire field of study from cultivation of medicinal plant to its clinical application. **(Kunle et al., 2012** )

***Standardization***

* Standardization of drug means confirmation of its identity and determination of its quality , purity and detection of nature of adulterants by various parameters morphological, microscopical, physical , chemical and biological observations.
* 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.

(Kunle et al., 2012)

Standardization of plant- derived drugs involve the collection of information and application of stringent quality control measures at every step of process from the growing of medicinal plant to finished therapeutic substance.

It include a complete description of starting drug , control and monitoring of numerous factors, namely, growing conditions, harvesting time, part of plant harvested, , absence of toxic pesticides or other contaminants , drying methods , freshness, storage ,extraction process and analytical controls which are required for providing the consistency of the quality of extract. (Garg et al.,2012)

**Good Practices/Techniques in Herbal Products**

* Quality control methods for medicinal plant materials. (WHO ,1998.)
* • Good agricultural and collection practices (GACP) for medicinal plants (WHO ,2003).
* • International pharmacopoeia, 4th ed. (WHO ,2006).
* Good manufacturing practices: main principles for pharmaceutical products (WHO ,2007).
* • Good manufacturing practices: supplementary guidelines for the manufacture of herbal medicinal products. (WHO, 2003 , 2007)
* • Guide to good storage practices for pharmaceuticals. (WHO,2003).
* Good trade and distribution practices (GTDP) for pharmaceutical starting materials. (WHO,2004).
* • General guidelines for methodologies on research and evaluation of traditional medicine. (WHO,2000).
* • Guidelines for assessment of herbal medicines. WHO,1996).
* • WHO monographs on selected medicinal plants. (WHO ,1999; WHO, 2000).

1. **Taxonomical identification**

Medicinal plant undergoing standardization must be taxanomicaly identfied .

1. **NOMENCLATURE** Complete information about its nomenclature
2. **Botanical evaluation**

**Macroscopic and microscopic analysis**

For Identification of right variety and search of adulterants.

* **Morphology and organoleptic evaluation:**

In case of whole drug macroscopic and sensory characters e.g, ., color ,order ,taste , feel of drug, appearance etc. observe with sensory organs. **Microscopic evaluation:**

These are valuable for powder and ungrounded drugs e.G., Epidermal parenchyma, stomata , trichome , vessels, fibers, calsium oxalate crystals etc. (Garg et al., 2012)

* **Powder Analysis**

Fruits are powdered and then passed through sieve no. 60 and examined for its microscopic characters.( Kokate et al., 2005)

The powder of the drug is boiled with chloral hydrate and then mounted on the glass slides using glycerin, covered with a cover slip and viewed under microscope.

Powder of the fruits is studied for chemical analysis. (WHO ,1998)

The powders are also stained with staining reagent like safranin (1%), fast green (0.2%), phloroglucinol and examined under electron microscope. Drawings are made with the help of camera Lucida. (Iyengar MA. 2002)

1. **Physical evaluation**

* **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. These are criteria to judge the identity and purity of crude drug to detect the excess of sandy and earthy matter and determined as – Total ash, sulphated ash, water soluble ash and acid insoluble ash etc.

* **Crude fiber**: This helps to determine the woody material component, and it is a criterion for judging purity.
* **Physical constants :**such as specific gravity, refractive index, optical rotation, viscosity are specially important for oils, fats ,oleoresins, balsams and similar substances.
* **Moisture content:**

Moisture content must be determined and controlled so as to prevent the decomposition of plant material. Methods which are commonly employed for the determination of MC are loss on drying, toluene distillation, GC, Karl fisher and spectroscopic methods.

* **Volatile content:**

Pharmaceutical significance of aromatic drugs is due to their odorifirous principles. These drugs are standardized on the basis of their volatile oil content.

* **Bitterness value:**

Bitterness value of plant materials is usually determined by comparing the threshold bitter concentration of extract of the material with that of dilute solution of quinine hydrochloride.bitterness value is expressed in unit equivalent to the bitterness of a solution containing 1g of quinine hydrochloride in 2000 ml solution.

* **Swelling index:**

Usually determined for gums, and herbal drugs containing mucilage, hemicelluloses. Swelling index is volume in milliliter taken up by the swelling of 1g of the plant material under specified conditions.

* **Solubility:** is useful for the examination of many oils and oleoresins and presence of adulterant could be indicated by solubility studies.

(Garg et al.,2012)

* **Determination of extractive value:**

Determination of extractable matter refers to amount of constituents present in a plant material which is extracted by a specific solvent.

**Types of extraction:**

* Cold maceration method
* Soxhlet extraction
* Soniation
* Supercritical Fluid Extraction
* Coupled SFE-SFC
* Coupled SFE-GC and SFE-LC
* Microwave-Assisted Extraction
* Solid phase extraction (Patil et al., 2010)

1. **Chemical methods for the standardization of herbal products**

This covers **chemical 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.

* **Identification and characterization**

Due to the fact that plant extracts usually occur as a combination of various type of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds.

A number of different separation techniques such as **TLC, HPTLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC**,

* Liquid Chromatography- Mass Spectroscopy (LC-MS) should be used to obtain pure compounds.
* Liquid Chromatography- Nuclear Magnetic Resonance (LC-NMR)
* Gas Chromatography Fourier Transform Infrared spectrometry
* Supercritical Fluid Chromatography (SFC)  **(Patil et al., 2010)**

The pure compounds are then used for the determination of structure and biological activity. Beside that, **non-chromatographic techniques such as immunoassay, which use monoclonal antibodies**  (MAbs), phytochemical screening assay, Fourier-transform infrared spectroscopy (FTIR), can also be used to obtain and facilitate the identification of the bioactive compounds.



**DNA finger printing**

* This technique is useful for the identification of phytochemically indistinguishable genuine drug from substituted or adulterated drug. DNA fingerprint genome remains same irrespective of plant part used while phytochemical constituent will vary with plant part used ,physiology and environment. (Garg et al.,2012)

1. **TOXOLOGICAL STANDARDIZATION**

**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).

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).

Generally, the main methods commonly used are **atomic absorption spectrophotometry (AAS),** inductively coupled plasma (ICP) and **neutron activation analysis** (NAA) (Watson, 1999).

**Pesticide residues**

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.

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.

For the detection of pesticide fosrisil R grade (60/100) column is used.

Acceptable daily intake of aldrin and dieldrin is not more than 0.0001 mg/kg. As per WHO guidelines for the determination of pesticides HCH and DTH should not be more than .005mg/kg. (Garg et al.,2012)

**Determination of radioactive contaminants**

* **Method of measurement**

Following a severe nuclear accident, the environment may be contaminated with airborne radioactive materials. These may deposit on the leaves of medicinal plants. Their activity concentration and the type of radioactive contamination can be measured by the radiation monitoring laboratories of most of the WHO Member States. The activity concentration of radioisotopes in herbs should be assessed by the **competent national radiohygiene laboratories** taking into account the relevant recommendations of international organizations, such as Codex Alimentarius, the International Atomic Energy Agency (IAEA), FAO and WHO.

Since radionuclides from accidental discharges vary with the type of facility involved, a generalized method of measurement is not yet available.

However, should such contamination be a concern, suspect samples can be analysed by a competent laboratory.

**(WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues)**

**Aflatoxin**

Aflatoxins are known as waste products of microbial world and they are toxic to human health. Even a minute quantity could cause great harm. Aflatoxins are being tested using TLC method and compared with standards using visual inspection and under UV light.

The WHO guidelines indicate that aflatoxins like B1,B2,G1,G2 should be nil in crude drug samples.

(Garg et al.,2012)

**Foreign matter**

Foreign matter found in a sample of herbs and herbal materials should not exceed limits set in national, regional or international pharmacopoeias. Foreign matter includes insects and other animal contamination including animal excreta, as well as other species of plants In general, any substance other than the acceptable sample of good quality medicinal plant material is regarded as foreign matter.A pure sample is seldom found and there is always some foreign matter present. However no poisonous, dangerous or otherwise harmful foreign matter should be allowed. Thus following the GACP should help to ensure that contamination is kept to a minimum.

Removal of larger pieces of foreign matter from whole and cut plants is often done by hand-sorting after macroscopic examination. Finished products should also be examined for foreign materials.

1. **Biological Evaluation**

**Microbial contaminants:**

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.

**Microbial contamination limits in herbal materials, preparations and finished products**

Different limits are set according to the intended use of the herbal material and the medicines themselves. **(WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues)**

* **Biological assays**

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.

**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.

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.

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. **(Kunle et al., 2012)**

**Labelling of herbal products**

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.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. **(Kunle et al., 2012)**

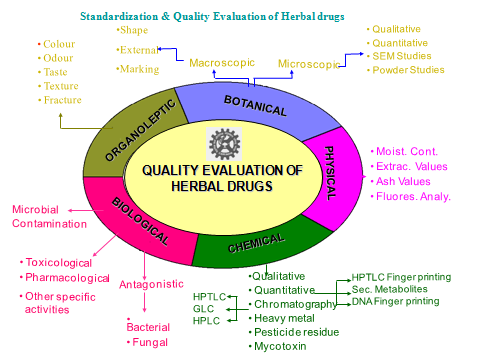
**The most established information with regard to the use of herbal preparations currently available in the public domain is in the form of pharmacopoeial monographs.** These documents publish traditional and standardized therapeutic uses of herbs and provide a foundation for clinical practice.

**Monographs consist of a description of the herb, including botanical information, laboratory analysis, therapeutic indications, and drug interactions (if relevant).** Although the monographs may be tedious to read, they include specific information that may not be available in other references. Several national and international organizations have adopted the monograph format.

**References**

1. AOAC (2005). Official Methods of Analysis of AOAC International, 18th edn. AOAC International, Gaithersburg, MD.
2. Bodeker C, Bodeker G, Ong CK, Grundy CK, Burford G, Shein K (2005). WHO Global Atlas of Traditional, Complementary and Alternative Medicine. World Health Organization, Geneva.
3. Bisset NG (1994). Herbal Drugs and Phytopharmaceuticals. CRC Press, Boca Raton, FL.
4. Bigoniya P., SinghC S., Srivastava B. Pharmacognostical and physico-chemical standardization of Syzygium cumini and Azadirachta indica seed. Asian Pacific Journal of Tropical Biomedicine (2012)S290-S295
5. De Smet PAGM, Keller K, Hansel R, Chandler RF (1992). Aristolochia species In: Adverse Effects of Herbal Drugs, Springer-Verlag, Heidelberg. 1.
6. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z (1985). World Health Organ., 63: 965.
7. Garg V., Dhar V.J., Sharma A.,Dutt R.Facts about standardization of herbl medicine: a review. Journal of chinese integrative medicine, October 2012,Vol,10. No,10.
8. Iyengar MA. Study of Crude Drugs, Manipal, Edition 10th, 2001, pp. 110-114.
9. Kokate CK, Purohit AP, Gokhale SB. Analytical Pharmacognosy: Pharmacog. 30th edition, Feb. 2005, pp. 1, 99.
10. Kunle, Folashade O., Egharevba, Omoregie H. and Ahmadu, Ochogu P. Standardization of herbal medicines - A review International Journal of Biodiversity and Conservation Vol. 4(3), pp. 101-112, March 2012
11. Mukherjee PW (2002). Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. Business Horizons Publishers, New Delhi, India.
12. Patil P.S., Shettigar R. An Advancement of Analytical Techniques in Herbal Research. J. Adv. Sci. Res, 2010, 1(1); 08-14 Published on 10th Aug 2010
13. S. Sasidharan, Y. Chen, D. Saravanan, K.M. Sundram, L. Yoga Latha.Extraction, isolation and characterization of bioactive compounds from plants’ extracts. Afr J Tradit Complement Altern Med. (2011) 8(1):1-10
14. WHO(2006). International pharmacopoeia, 4th ed., Vol. 1. Geneva, World Health Organization.
15. WHO(2006). International pharmacopoeia, 4th ed., Vol. 2. Geneva, World Health Organization.
16. WHO (1998). Quality control methods for medicinal plant materials. Geneva, World Health Organization.
17. WHO (1998c). Basic Tests for Drugs, Pharmaceutical Substances, Medicinal Plant Materials and Dosage Forms. World Health Organization, Geneva.
18. WHO (2003) guidelines on good agricultural and field collection practices (GACP) for medicinal plants. Geneva, World Health Organization.
19. WHO (2007).Good manufacturing practices for pharmaceutical products: main principles. WHO Expert Committee on Specifi cations for Pharmaceutical Preparations. Thirty-seventh report. Geneva, World Health Organization, 2003 (WHO Technical Report Series, No. 908) Annex 4. (These guidelines are also included in Quality assurance of pharmaceuticals: a compendium of guidelines and related materials, Vol. 2, 2nd updated ed.: good manufacturing practices and inspection. Geneva, World Health Organization, 2007.) (These guidelines are also extracted and published as: WHO guidelines for Good Manufacturing Practices (GMP) for herbal medicines. Geneva, World Health Organization.
20. WHO(2007).Good manufacturing practices: updated supplementary guidelines for manufacture of herbal medicines. WHO Expert Committee on Specifi cations for Pharmaceutical Preparations. Fortieth report. Geneva, World Health Organization, 2006 (WHO Technical Report Series, No. 937) Annex 3. (These guidelines are also included in Quality assurance of pharmaceuticals: a compendium of guidelines and related materials, Vol. 2, 2nd updated ed.: good manufacturing practices and inspection.Geneva, World Health Organization, 2007.) (These guidelines are also extracted and published as: WHO guidelines for Good Manufacturing Practices (GMP) for herbal medicines. Geneva, World Health Organization.
21. WHO (2007).Good manufacturing practices for pharmaceutical products: main principles. WHO Expert Committee on Specifi cations for Pharmaceutical Preparations. Thirty-seventh report. Geneva, World Health Organization, 2003 (WHO Technical Report Series, No. 908) Annex 4. (These guidelines are also included in Quality assurance of pharmaceuticals: a compendium of guidelines and related materials, Vol. 2, 2nd updated ed.: good manufacturing practices and inspection. Geneva, World Health Organization, 2007.) (These guidelines are also extracted and published as: WHO guidelines for Good Manufacturing Practices (GMP) for herbal medicines. Geneva, World Health Organization.
22. WHO(2003).Guide to good storage practices for pharmaceutical products. WHO Expert Committee on Specifi cations for Pharmaceutical Preparations. Thirty-seventh report. Geneva, World Health Organization. (WHO Technical Report Series, No. 908) Annex 9.
23. WHO(2004).Good trade and distribution practices for pharmaceutical starting materials. WHO Expert Committee on Specifi cations for Pharmaceutical Preparations. Thirty-eighth report. Geneva, World Health Organization. (WHO Technical Report Series, No. 917) Annex 2.
24. WHO(2000).General guidelines for methodologies on research and evaluation of traditional medicine. Geneva, World Health Organization, 2000 (WHO/EDM/TRM/2000.1).
25. WHO(1997).Guidelines for assessment of herbal medicines. WHO Expert Committee on Specifi cations for Pharmaceutical Preparations. Thirty-fourth report. Geneva, World Health Organization, 1996 (WHO Technical Report Series, No. 863) Annex 11. (These guidelines are also included in Quality assurance of pharmaceuticals: a compendium of guidelines and related materials, Vol. 1. Geneva, World Health Organization.
26. WHO(1999). monographs on selected medicinal plants, Vol. 1. Geneva, World Health Organization.
27. WHO(2002). monographs on selected medicinal plants, Vol. 2. Geneva, World Health Organization.
28. Watson DG (1999). Pharmaceutical Analysis. Churchill Livingstone, Edinburgh.
29. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues

.

 **ELEMENTS OF STANDARDIZATION**