



# A SYSTEMATIC REVIEW ON RECENT ADVANCES IN STANDARDIZATION OF HERBAL DRUG

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**Abstract:** Herbs are the plants in which aromatic properties that are used as a flavoring & garnishing food, medicinal purposes or for various fragrances. Generally, herbs which are used in before the invention of writing and the documented history of medicine. It is also called as herbalism means which is a plant-based medicine. It is also called as a phytomedicine means which is produce from herbs. Herbal medicine which is produced from herbs is the oldest & still the most widely used systems in the current scenario. It is differing from conventional medicine. Today, herbal medicine is in great demand in developing as well as developed country for healthcare not because they are not costly/inexpensive but because they have wide range of acceptability, better compatibility & less side effects on the human. Hence, people which are from several countries are more attracted towards traditional Indian herbal medicines which is pure plant-based medicine. The herbal industry which shares with the growth of potential worldwide is about US\$100 billion<sup>1</sup>, the annual growth rate of about 15%. For last decades research efforts have been intensified in both developed and developing countries to evaluate using clinical trials on herbal drugs.

In these communities, traditional medical practice is quite important & viewed as an integral part of their culture. So herbal medicines are often viewed as a balanced and moderate approach to healing the disease. Persons who use them as a medicine made with ingredients available at home as well as over-the-counter drugs will spend billions of dollars on herbal products & herbal medicines. They will represent a substantial proportion of the global drug market,<sup>1-5</sup> to achieve the desired benefit from herbal preparations or the herbal products, an individual must take the required dose over a certain length of time period. So, it is generally believed that most of the herbal preparations which is safe & effective for consumption & some herbs which are most biologically active substances could be toxic with undesirable side effects on human body.<sup>6</sup>

**Index Terms** - Herbs, Herbal products, Standardization, Quality control, Evaluation

## I. INTRODUCTION:

As we all known in our Ayurvedic system of medicines drug standardisation of Ayurvedic formulation or herbal formulation is a biggest challenge. Since ancient times herbal drugs have been used for the treatment of various diseases. In world health the herbal medical plants have played a key role. There are great advances observed in modern medicines in recent decades in spite of that plants still make an important contribution to health care.<sup>7</sup>

Herbs as a medicine which is used in all cultures throughout history and it is the oldest form of healthcare.<sup>8</sup> the term “herbal drugs” means a plant or a part of plants that have been converted into phytopharmaceuticals by simply using the processes involving collection or harvesting, drying and storage.<sup>9</sup>

India is a mother hub for development of Ayurveda, Unani, Siddha, Homoeopathy and other natural herbs-based health science (Ayush). Confirmation of drug means identify the drug, quality and purity throughout all phases of its cycle *i.e.* shelf-life, storage, distribution and use by various parameters known as standardization of drug.<sup>10</sup>

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal preparations. It includes various parts of crude plant material, like leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be either whole, fragmented or in powdered form. In addition to herbs, herbal materials in which fresh juices, gums, fixed oils, essential oils, resins & dry powders of herbs are also involved. Herbal medicines are used very commonly in various health practices as well as therapies of Traditional Medicines like Chinese medicine, Ayurveda, Unani, Naturopathy, Osteopathy and Homeopathy.<sup>11</sup>

So clear cut guidelines have not been developed. that's why it is necessary to promote ISM manufacturing industry people for the drug standardization work. So, Ministry of Ayush, Government of India recently established Pharmacopeial Commission of Indian medicines and Homoeopathy medicines (PCIM and H) for setting up drug standard of ASU and H Medicines.<sup>12</sup> World Health Organization (WHO) gives the importance of the qualitative as well as quantitative methods for characterizing the samples, quantification of the biomarkers or chemical markers and the fingerprint profiles etc. If active component is known, it is most logical to quantitate this compound. Where active ingredients which gives to therapeutic efficacy are known botanical preparations should be standardized to these compounds. & where the active ingredients are not known a marker substance which should be specific for the botanical could be chosen

for analytical purpose.<sup>13</sup> So, for standardization of herbal medicines, assurance of safety, quality and efficacy of medicinal plants and herbal products has become an important issue. The herbal raw material has a lot of variation due to several factors like, the important ones being the identity of the plants & seasonal variation (which has a bearing on the time of collection), the ecotypic, genotypic and chemo typic variations, drying as well as storage conditions and the presence of xenobiotics.<sup>13</sup> In some countries, different types of local procedures have been used to process these materials, such as steaming, roasting, or stir-baking with honey, alcoholic beverages or other materials. Herbal preparations are produced by extraction, fractionation, purification, concentration, or by other physical or biological processes. Herbal preparations also made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in other materials.<sup>14</sup>

The use of herbs as a medicine or as a herbal products is the oldest form of healthcare known to humanity and has been used in all cultures throughout history in the world.<sup>15</sup> During the past decade, there has been increasing public interest and acceptance of natural therapies for herbal products 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 which uses herbal medicine as their source of primary healthcare section.<sup>16-19</sup>

## II. Standardization:

Standardization means the process of making something conform to a standard (Definition from Oxford language)

American Herbal Product association defined by Standardization as: "The Standardization refers to the information & control which is necessary to reasonable consistency of product material. This can be achieved through minimizing the inherent variation of natural products composition through in which quality assurance practices applied to agricultural as well as the manufacturing processes."<sup>20</sup>

Due to commercialization of the herbal medicines has been happened, assurance of safety, quality and efficacy of herbal products and medicinal plants has become an important issue. Due to the several factors, herbal raw material is susceptible to a lot of variations, the important factor is the identity of plants and seasonal variations, the ecotypic, genotypic and chemo typic variations, presence of xenobiotics, drying and storage conditions.<sup>21</sup> By minimizing the inherent variations of natural product composition through quality assurance practices this has been achieved.<sup>22</sup> It is the process of developing and concurring upon technical standards. The process of prescribing a set of characteristics exhibited by the particular herbal medicines are worked out by experimentation and observations which would lead by specific standards. Hence the process of standardization is an important tool in the quality control process.<sup>23-24</sup>

In the recent years, there has been a great demand for plant derived products in developed as well as undeveloped countries. These products are increasingly being sought out as medicinal products, nutraceuticals, cosmetic & Pharmaceuticals etc.<sup>25</sup> In order to have a good coordination between the quality of raw materials, in process materials as well as the final products, it has become essential to develop reliable, specific and sensitive quality control & quality assurance methods using a combination of classical and modern instrumental method of analysis. Standardization is basically essential measurement for ensuring the quality control of the herbal drugs or the herbal products.<sup>26</sup>

The process of standardization of herbal medicines which is a set of standards also it has the inherent characteristics, constant parameters, and qualitative as well as quantitative values that carry an assurance of quality, efficacy, purity, safety, reproducibility & effectiveness. So, it is the process of developing and agreeing upon technical standards for herbal products. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of inherent characteristics exhibited by the particular medicines. Hence standardization is a tool in the quality control process<sup>27</sup>

The term "Standardization" which is used to describe all measures, which are taken during the manufacturing process as well as quality control which produces quality & effectiveness in the product. It also encompasses the entire field of study from birth of a plant means from growth to its clinical application. It also means adjusting the herbal drug preparation to a defined content of a constituent or by adding an excipients or by mixing herbal drugs as well as herbal drug preparations.<sup>28</sup> "Evaluation" of a drug is the term which means confirmation of its identity, quality, purity, potency & detection of its nature of adulteration.<sup>29</sup> Different aspects have been taken into consideration that helps in the assessment of herbal drugs, correct identity of sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (includes ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Guideline to the phytochemical profile of the drug in ensuring the quality has been served by fingerprint profile whereas the quantification of the marker compound/s would serve as an additional parameter in assessing the quality of the sample.

Phytochemical standardization encloses all possible information generated concerning the chemical constituents present in an herbal drug. Hence, the phytochemical evaluation for standardization purpose encompass the following:

1. Introductory testing for the presence of different chemical groups.
2. Quantify chemical groups of interest in herbal drugs (e.g., total alkaloids, total phenolics, total triterpene acids, total tannins). Establishment of fingerprint profiles.
3. Multiple marker-based fingerprint profiles.
4. Quantification of important chemical constituents.<sup>30</sup>

The authentication of herbal drugs and identification of adulterants from genuine medicinal herbs are essential for both pharmaceutical companies as well as public health, to ensure reproducible quality of herbal medicines.<sup>31</sup>

## III. Authentication of medicinal plants:

DNA-based techniques have been widely used for corroboration of plant species of medicinal importance. This technique is especially useful in case of those that are frequently substituted or adulterated with other species or varieties that are phytochemically identical and/or morphologically identical.<sup>32</sup>

III. Dried fruit samples of *Lycium barbarum* were discriminate from its related species using RAPD markers. The RAPD technique has also been used for adjudge the components of a Chinese herbal prescription, yu-pingfeng san. In this study the presence of three herbs (*Astragalus membanaceus* (Fisch.) Bge, *Ledebouriella seseloides* Wolff and *Atractylodes macrocephala* Koidz) in the formulation have been detected using a single RAPD primer.<sup>33</sup>

#### IV. STANDARDIZATION OF HERBAL FORMULATION:

Standardization of herbal formulation requires execution of Good Manufacturing Practices (GMP).<sup>34-35</sup> Furthermore, study of various parameters which considered as essential includes pharmacodynamics, pharmacokinetics, dosage, stability, shelf-life, toxicity evaluation, chemical profiling of the herbal formulation.<sup>36</sup>

Other equivalently prime factors are pesticides residue, aflatoxin content, heavy metals contamination, Good Agricultural Practices (GAP) in herbal drug standardization.<sup>37</sup>

#### V. NEED OF STANDARDIZATION:

To gain the public trust and to bring herbal product or herbal medicines into mainstream of today in health care system the researchers, the manufacturers and the regulatory agencies must apply rigorous scientific methodologies & experimentation to ensure the quality and lot to lot consistency of the traditional herbal products.<sup>38</sup>

Experimental data, toxicity studies and human clinical studies are the parameters in which modern system of medicine is based on. However, Pharmacopeial standards of raw material / finished products are not available yet. cGMP for herbal industry are not well defined nor the simple or essential minimum standards of medicinal plant products are maintained or regulated.

Since deficiency of quality standards has resulted in mild to serious adverse effects ranging from hepato toxicity to death. And due to this reason herbal ingredients require different tools which helps in determining identity, purity and quality of herbal preparations and these tools should be technically sufficient, rapid and cost effective with GMP requirements.

For the determination of safety, efficacy and quality of herbal medications, WHO has set specific guidelines. care should be taken starting from the proper identification of plants, season, area of collection, their extraction and purification, in order to obtain quality herbal product.

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#### 5.1 Need of quality control & standardization of herbal products:

1. When traditional medicines were developed technology and concept of standardization was quite different in manner.
2. During thousand years ago dynamic process of evolution may have changed the identity of plant material.
3. Due to commercialization, supply of genuine raw material has become a biggest challenge.
4. Properties of botanicals may have undergone change due to time variation & environmental factors.<sup>40</sup>

#### 5.2 SOME ANCIENT METHODS FOR STANDARDISATION:

Collection time for herbal plant:

Rutu: some drugs are seasonal as well as some parts of herb should collect in specific Rutu.

Desha: Availability of some drugs in specific area like Himalaya, so geographical distribution is important one.

Nakshatra: Collection of some drugs should be done on specific Nakshatra which Indicate using dry drugs & wet drugs.<sup>41</sup>

#### VI. WHO GUIDELINES FOR QUALITY STANDARDIZED HERBAL FORMULATIONS:

- 1) Quality control of herbal drug
  - 2) Stability assessment and shelf life
  3. Safety assessment
  - 4) Assessment of efficacy by ethno- medical information and biological activity evaluations. Along with the chromatographic fingerprints (TLC, HPTLC, HPLC, and GC), the bioactive extract should be standardized on the basis of active principles or major compounds.
- In general, all medicines, whether they are synthetic or of plant origin, should satisfy the basic requirement of being safe and effective.<sup>42-43</sup>

#### 6.1. Quality Control of Herbal Drugs –

Quality control is a process that involved in maintaining the quality and validity of a finished product. In general, three important pharmacopeial aspects on which quality control is based on -

- a. Identity or authenticity- it should have one herb
- b. Purity – it should not have any contaminant other than herb
- c. Assay or Content -the active constituents should be within the defined limits.

Assessment of identity of the herb can be achieved by macro and microscopic examination. Furthermore, identity test includes simple chemical tests or precipitation. Chromatographic tests are also necessary. These chemical and chromatographic tests help to procure batch to batch comparability and the chromatogram may be used as a 'fingerprint' for the herbal ingredient by signifying the profile of some common plant constituents such as flavonoids, alkaloids and terpenes. To estimate the identity and purity, criteria such as type of preparations, sensory properties, physical constants and adulteration, contaminants, moisture, ash constants and solvent residues have to be examined. Voucher specimens are reliable reference sources. Eruption of diseases among plants may result in alterations to the physical appearance of the plant and lead to incorrect identification.<sup>44-45</sup>

Purity is closely associated with safe use of drugs and deals with factors such as ash values, contaminants and heavy metals. Analytical methods such as photometric analysis, Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), and Gas chromatography (GC) can be utilized in order to demonstrate the constant composition of herbal preparations. Since in most herbal drugs the active constituents are unknown, Content or assay is the most difficult area of quality control to perform. Sometimes markers can be used. As per the approach often seen in pharmacopeia, in all other cases where no active constituents or marker can be interpreted for herbal drugs the percentage extractable matter with a solvent may be used as a form of assay.<sup>46-47</sup> A special form of assay is the determination of essential oils by steam distillation. When active constituents (e.g. sennosides in senna) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy (UV/VIS), TLC, HPLC, HPTLC, GC, mass spectrometry, or a combination of GC and MS(GC/MS), can be employed.<sup>48</sup>

#### 6.2. Stability Assessment and Shelf Life:

Prolonged and apparently monotonous use of a substance usually offers testimony of its safety. In a few instances, investigation of the potential toxicity of naturally occurring substances widely used as ingredients in these preparations causes systematic toxicity, carcinogenicity and teratogenicity. Regulatory authorities need to be quickly and reliably informed of these findings. They should also have the authority to respond, either by withdrawing or varying the licenses of registered products containing suspect substances, or by rescheduling the substances to limit their use to medical prescription.

### 6.2.1 Assessment Quality:

All procedures should be accomplished in accordance with good manufacturing practices.

### 6.2.2 Crude Plant Material

The botanical definition of crude plant, including its genus, species, description, part of the plant, active and characteristics constituents of the crude plant should be specified in a proper manner and, if possible, limits of content should be defined. Foreign matter, impurities and microbial content should be defined or limited. Voucher specimens, should be authenticated by a qualified botanist and should be stored for at least a 10-year period which represent each lot of plant material.

### 6.2.3 Plant Preparations:

The manufacturing procedure should be described in detailed manner. In order to adjust the plant preparation to a certain level of active constituents or for any other purpose, if other substances are added during manufacturing and these added substances should be mentioned in the manufacturing procedures. If identification of an active principle is not possible and to ensure consistent quality of the preparation, it should be sufficient to identify a characteristic substance or mixture of substances.

### 6.2.4 Finished Product:

The manufacturing procedure and formula, including the number of excipients, should be described in detailed manner. To ensure consistent quality of the product, a finished product specification should be defined. The finished product should comply with general requirements for particular dosage forms.

### 6.2.5 Stability:

The physical and chemical stability of the product should be tested under defined storage conditions (container in which it is marketed) and the shelf-life should be established.

## 6.3. Safety Assessment:

Herbal medicines are generally regarded as safe based on their long-established use in various cultures. However, there are many serious cases of adverse events after administration of herbal products have been reported. In a lot of cases, the toxicity has been traced to contaminants and adulteration. However, there are some of the plants used in herbal medicines are highly toxic. As a whole, herbal medicines can have a risk of adverse effects and they may show drug-drug interaction and drug-food interactions if not properly assessed. The first priority in herbal research is estimation of the safety of herbal products. These are various approaches used for the evaluation of safety of herbal medicines.

Detailed Phyto-chemical and pharmacological studies required for evaluation of the toxic effects of plant constituents of herbal formulation. However, based on human experiences in various cultures, now we can assume that the use of toxic plant ingredients has already been largely eliminated.<sup>49</sup>

Safety and many other factors (like cost of raw material) cause problem for availability of genuine or true drug, which encourages the adulteration of plant by substitution with inferior commercial varieties, artificially manufactured substances, exhausted drugs or cheaper plant or by another vegetative part.<sup>50</sup> Several reports suggest that many herbal products contain undisclosed pharmaceuticals and heavy metals.<sup>51</sup>

### 6.3.1 Assessment of Toxicity:

Toxicity investigation will also be required because the analysis alone is hard to reveal the contributions to toxicity itself. While assessing toxicity of an herbal medicine, the dose chosen is very important.<sup>52</sup> Estimation of toxicity involves one or more of the following techniques- In vivo techniques, in vitro techniques, cell line techniques, micro- array and other modern techniques also used.

## 6.4. Assessment of Efficacy:

Comparing with conventional pharmacological treatments herbal medicines are immanently different, at present there is no way to estimate their efficacy other than by currently used conventional clinical trial methodologies, in which efficacy is traditionally estimate by clinical, laboratory, or diagnostic results:

Clinical outcomes include parameters such as improved morbidity, decline pain or discomfort, improved appetite and weight gain, depletion of blood pressure, reduction of tumour size or extent, and improved quality of life.

Laboratory /other diagnostic results include parameters such as depletion of blood glucose, depletion of opacity as measured by radiological or imaging techniques, refinement of haemoglobin status, and improvement in electrocardiogram (ECG) findings.

Standardization, however, may sometimes be conflict with the existing judicial framework and awareness is needed regarding the ethical implications of such studies. Although randomized clinical trials (with double blind trials as the gold standard) are relatively difficult to be implemented in the case of herbal medicine, they are not ruled out per se in assessing the efficacy of these products. Data from case series studies may provide sufficient scientific and ethical validity to conduct such trials, but acceptance of this protocol needs a paradigm change in the methodology of drug evaluation as understood in conventional medicine. Standardization and Quality control of herbal drugs involve wide variety of scientific investigations, which include physical, chemical and biological evaluation using various analytical method and tools.

### 6.4.1 Physical Evaluation-

For accurate identification of the crude material, each monograph contains detailed botanical, macroscopic and microscopic descriptions of crude drug with detailed photographic images. A microscopic analysis assures the identity of the material and as an initial screening test for impurities.

**6.4.2 Chemical Evaluation-** Chemical evaluation of the crude drug is performed to estimate the potency of the material in terms of its active constituents. It covers screening, isolation, identification, and purification of the chemical components. This helps to determine the identification of the drug substance and possible adulteration.

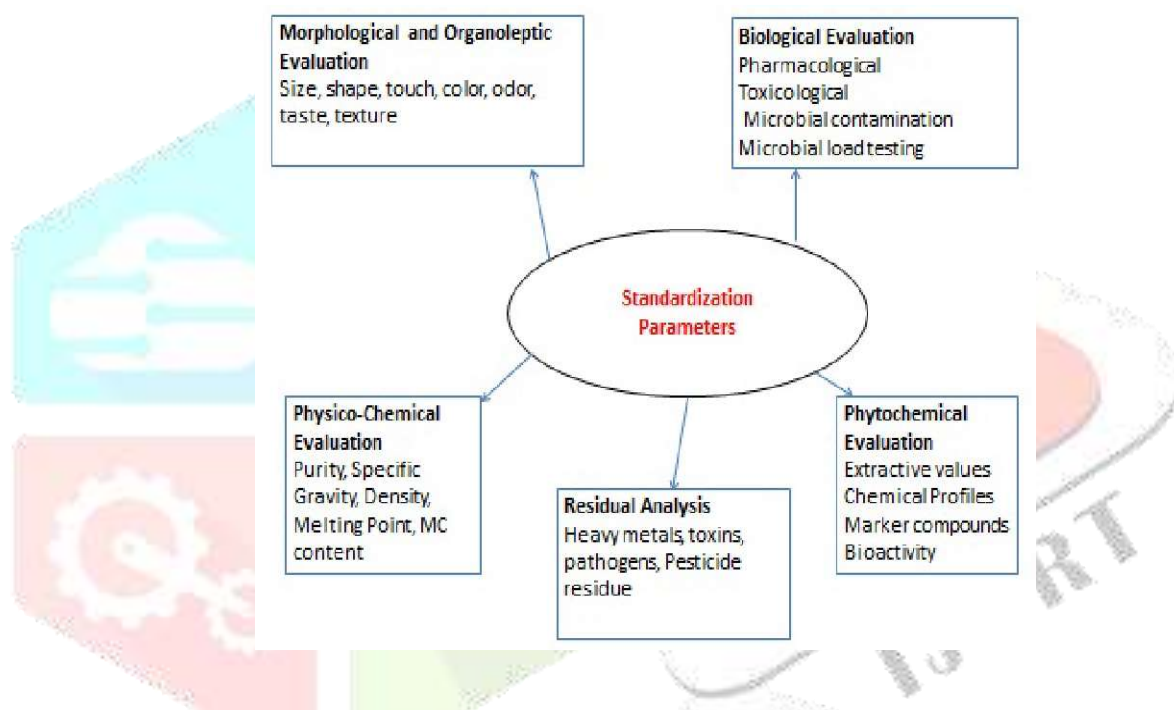
**6.4.3 Biological Evaluation-** Biological evaluation of crude drug is important to estimate the pharmacological activity of certain drugs in order to evaluate and standardize them.

**6.4.4 Analytical Methods-** It helps in determining identity, quality and relative potency.<sup>53-54</sup>

### VII. The active principal of identification and standardization:

The identification of purely active moiety or active component is an important requirement for quality control and dose determination of plant related drugs. A medicinal herbal plant can be checked as an artificial laboratory as it produces and contains various chemical moiety. That moiety, is responsible for medicinal activity of the herbal plant, are secondary metabolites. for example, Alkaloids are nitrogenous principle an organic moiety combine with acid to form crystalline salt & also herbal plants contains Resin, Oleoresins, lactones, saponin and volatile oils etc. Complete phytochemical screening of most of the medicinally essential herbs which are not done in India. this would be helpful in standardization as well as dose determination of herbal drugs.<sup>55</sup>

Medicinal plants have played an important role in world health section. They are circulated worldwide, but they are most rich in tropical countries as well. It is noted that about 25% of all modern medicines are directly or indirectly obtained from higher plants. World Health Organization (WHO) has a specific set of guidelines for the evaluation of the safety, efficacy and quality of herbal drugs or herbal medicines. WHO find out that 80% of the people in the world currently use herbal medicine or drugs for the most important health cares. Except in some countries herbal drugs may also use by traditional, natural or inorganic active constituents, which is the not plant source. Herbal drugs are a main constituent in usual medicine and a general ingredient in Homeopathic, Ayurvedic, Naturopathic as well as in other medicine system. Herbs are usually measured as safe toxicity, potency, side effects of allopathic drugs, has led to more increased in number of herbal drugs manufacturers. For the past few years, herbal drugs or herbal medicines have been mostly used by the people with no prescription. Leaves, stem, bark, flower, seeds, roots and extract of all these have been used in herbal drugs over the thousand's years ago.<sup>56-57</sup>



### VIII. Parameters for standardization & quality Control of herbal drugs with their Morphological or Organoleptic evaluation:

It includes the evaluation of herbal drugs by their size, shape colour, odour, taste and particular characteristics like touch, texture etc. This is a technique of qualitative evaluation which related to the study of morphological properties and sensory report of whole drugs. e.g. fractured surfaces in cascara bark, cinchona bark, quillia bark as well as quassia wood have essential characteristics. Umbelliferous fruits have aromatic odour likewise liquorice have sweet taste are the examples of this type of evaluation. Shape of drug may be varying from conical (aconite), subcylindrical (podophyllum), cylindrical (sarsapilla), fusiform (jalap). Size represents thickness, length, breadth and diameter as well. Colour represents external colour which various from white to brownish black are essential for diagnostic features. Taste which is a specific type of sensation feel by which epithelial layer of tongue. taste may be sweetish (saccharin), sour (acidic), salt like (saline), bitter or tasteless as well.<sup>58-60</sup>

Physicochemical evaluation of crude drug, such as selection and handling of crude material, safety, documentation of safety and risk based on experience, efficacy and stability evaluation of finished product, provision of product particulars to consumer and product promotion is carried out by the process of standardization of herbal drugs.

Important quality indices include:

- **Morphology and organoleptic evaluation:**

In case of whole drug morphological characters are important for differentiating purpose. It mainly comprises colour, odour, taste, shape, size etc. Detail attribute includes fractures, texture, etc.

- **Microscopic and histologic evaluation:**

These are valuable in both entire as well as powdered drug. It mainly includes study of attributes like parenchyma, trichomes, calcium oxalate crystals, vascular bundle arrangements, stomata, fibers etc.

- **Quantitative microscopic study:**

Microscopic evaluation such as vein islet number, stomatal index, stomatal number, vein termination number, size of fibers, palisade ratio. Such study helps in distinction of closely allied species.

● **Physical evaluation:**

Determination of various physical parameters like moisture content, solubility, viscosity, refractive index, melting point, optical rotation, ash values, extractives and foreign organic matter.

● **Qualitative chemical evaluation:**

In accordance with phytochemical constituent, this covers identification and characterization of crude drug. It utilizes different analytical technique to identify and segregate the active constituents. One technique which known as phytochemical screening is the process which involve purification, and characterization of the active constituents of pharmaceutical importance, botanical identification, extraction with suitable solvents.

● **Quantitative chemical evaluation:**

Estimation of the amount of the major classes of constituents in herbal drug.

● **Toxicological studies:**

Toxicological studies are the study of the adverse effects of chemical substances.

● **Microbiological parameters:**

It includes the full content of viable, total mould count, total coliforms count. the total amount of impurities, such as reagents used in the extraction of various herbs, impurities directly from the manufactured product and solvents etc are determine and control by the limiters. It can be used as a quantitative tool or semi-quantitative tool.<sup>61-63</sup>

**8.1. The detailed evaluation are as follows:**

**8.1.1. Microscopic Evaluation:**

In Microscopic Evaluation it involves the detailed assessment of the herbal drugs or the drug product & it is used to recognize the organized drugs on the basis of their known histological characters. It is regularly used for the qualitative analysis of organized crude drugs in form of total or powder under the microscope. The inner pseudo parenchyma cells are round or the oval shapes. They contain protein as well as fixed oil. The crude drugs are identified by taking thin Transverse section, Longitudinal Section of a bark, wood as well as leaf under the microscope. The various parameters are included in microscopy are given bellow.

I. Stomata II. Trichomes III. Leaf Content IV. Quantitative Microscopy

Some Microscopic Identification test are given below:<sup>64-66</sup>

**8.1.2. Chemical Evaluation:**

The most of drug contain definite chemical constituents to which their pharmacological and biological activity depended on the specific characteristics. Qualitative chemical test used to identify drug quality as well as purity. The identification, isolation & purification of active chemical constituents is depending on chemical methods of evaluation. phytochemical investigation is also a part of Preliminary chemical evaluation. Some qualitative chemical test for chemical evaluation of crude drug like saponification value & acid value etc.<sup>67-69</sup>

Sr. No.	Name of Constituents	Identification Test
1	Volatile oil	1.Ester value 2. Acetyl value
2	Balsams	1.Acid value 2.Saponification value 3. Ester value
3	Resins	1.Sulphated Ash 2. Acid value
4	Gums	1.Methoxy determination 2. Volatile acidity

**8.1.3. Determination of Foreign Matter:**

Herbal drugs should be prepared from various confirmed part of the plant. They should be totally free from insects as well as mould, which includes visible and excreta contaminant such as stones, sand, harmful, poisonous foreign matter & chemical residues. Animal objects such as insects & various invisible microbial contaminants, which produces toxins as well as the harmful potential contaminants of herbal medicines. Macroscopic evaluation can easily use to determine the presence of foreign matter as well as the contaminants, although microscopy is essential in certain special cases for e.g. starch intentionally added to “dilute” the plant material.<sup>70-71</sup>

$$\text{percentage of foreign Organic Matter} = N \times W \times 94,100 \times 100/S \times M \times P$$

Where; n = No. of chart particles in 25 fields.

S = No. of spores in the same area of the 25 fields.

W = Wt. in mg of lycopodium was taken.

M= Wt. of the sample in mg.

P= No. of characteristics particles/mg of the pure foreign matter.

94,000= No. of spores per mg of lycopodium<sup>70,72</sup>

#### 8.1.4. Determination of Extractive Values

The extract obtained by exhausting crude drugs which are indicative of approx. the measure of their chemical constituents/Ingredients. The various solvents which is used for determination of extractive values.<sup>73-74</sup>

These are classified as follows.

1. Water Soluble extractives.
2. Alcohol Soluble extractives.
3. Ether Soluble extractives.

#### 8.1.5. Determination Of heavy Metals:

In general, quantitative & limit tests accurately determine the concentration of heavy metals in the form of impurities as well as contaminants. The heavy metals like Arsenic, mercury, lead, thallium & cadmium have been shown to be contaminants of few herbal constituents. The determination of heavy metals can be found in many pharmacopoeias which are available & it is based on colour reaction with special reagents such as diethyl dithiocarbonate or thioacetamide & amount is determined by the comparison with a standard one. So, the methods commonly used for analysis are inductive coupled plasma (ICP), Neutron activation analysis (NAA), Atomic Absorption Spectrophotometry (AAS).<sup>75-77</sup>

#### 8.1.6. Radioactive contamination:

The microbial growth in herbal drugs which is usually avoided by the irradiation. Dangerous contamination, which may be the consequence of a nuclear accident. The WHO, in close with cooperation of several other international organizations, has been developed guidelines in the event of a wide spread contamination by radio nuclides resulting from major nuclear accidents. The nuclear accident in Chernobyl & Fukushima may be serious as well as depend on the specific radionuclide, the stage of contamination & the quantity of the contaminant consumed. E.g. of such radionuclides include long lived & short-lived fission products, actinides & activation products. Therefore, at current no limits are proposed for the radioactive contamination.<sup>78-81</sup>

#### 8.1.7. Determination of specific optical rotation:

Specific rotation determination formula  $-D_{25} = 100 \times \phi / c$

Where:  $\phi$  = corrected observed rotation in drug at  $-25^\circ$

**D** = sodium light of line d

**l** = length of polarimeter tube in done.

**c** = conc. of substance in percent w/v.<sup>74</sup>

#### 8.1.8. Pesticides Residue:

Pesticide residues are the term in which any particular matter in food, various animal feed as well as agricultural commodities resulting from the use of a pesticides which are used in herbal drugs. Herbal drugs which contain pesticide residue, which are gathered from agricultural practices, like Spraying, behavior of soil during cultivation & addition of fumigants during the storage. Many times, Pesticides contains chlorine in the molecules, which can be determined by the analysis of chlorine with chemical test & can be detected by measuring with total organic phosphorus for insecticides containing phosphate. The various methods are used to measure pesticides by GC, MS, OR GCMS etc. WHO and European pharmacopeia published Some simple methods are also has in general limits for pesticides residue in medicines.<sup>82-84</sup>

## 8.2. DISCUSSION:

### 8.2.1. Methods of standardization:

It should take into consideration that all aspects which are contribute to the quality of the herbal drug, identity of particular sample, pharmacognostic evaluation, evaluation of organoleptic properties, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing as well as biological activity. The phytochemical profile is the important criteria since it has a direct bearing on the activity of the herbal drugs or the drug product. The fingerprint profiles which gives as guideline to the phytochemical profile of the drug which ensures that the quality, while quantification of the marker compounds would give as an additional parameter in assessing the quality of the sample.

Hence, the phytochemical evaluation for standardization purpose which includes the following parameters:

1. Preliminary testing is done for the presence of different chemical groups.
2. Quantification of chemical groups (e.g., total alkaloids, total phenolics, total triterpene acids, total tannins). Establish the fingerprint profiles.
3. estimate marker-based fingerprint profiles.
4. Quantification of important chemical ingredients.<sup>85</sup>

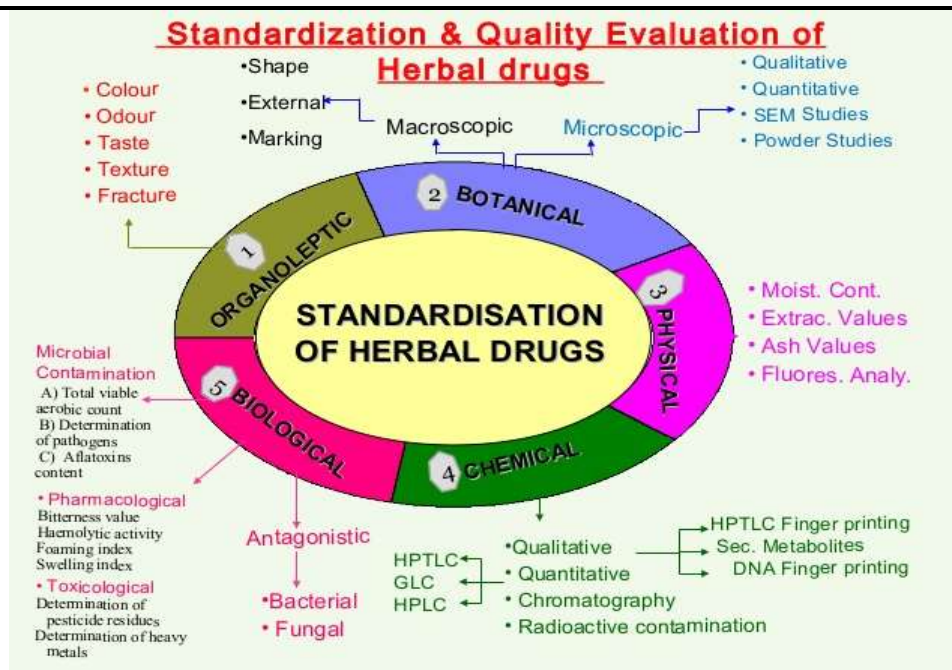
Phyto therapeutic agents which are normally marketed as a standardized preparation in the form of liquid, solid (powdered extract), or the viscous preparations. They are prepared by maceration, percolation as well as distillation (volatile oils). Ethanol, water or mixtures of ethanol & water are used for the production of fluid extracts. Solid particle or powered extracts of drug which are prepared by evaporation of the solvents which is used in the process of extraction of the raw material. Some Phyto therapeutic agents which are greatly concentrated in order to improve their therapeutic efficacy.<sup>86</sup>

A significant identification & examination of crude drugs which is important in the processes of herbal formulation because of more diversity & changes in their chemical nature or characteristics.

▪ To reduce the problem all pharmacopoeias, have certain standards. Specific test for specific plant material is given below.

1. Alkaloids content -dragendorff test
2. Iodine value, saponification value- molish test
3. carbohydrates - Millon tests

Amino acid Volatile oil Hemolytic activity Assay for Phosphate/ Aluminum/ Camphor /Potassium /Lead/ Calcium.<sup>87-88</sup>



### 8.3. CONVECTIONAL METHOD FOR STANDARDIZATION OF HERBAL DRUG FORMULATION

#### 8.3.1. THIN LAYER CHROMATOGRAPHY (TLC)

TLC is one of the most popular and simple chromatographic technique used of separation of compounds. In the phytochemical evaluation of herbal drugs, TLC is the common fingerprint technique for herbal analysis. The herbal compounds can easily identify by TLC.<sup>89</sup> In this technique, the authentication of various species, evaluation of stability and consistency of their preparations from different manufacturers TLC is being employed extensively for the following reasons:

1. It helps rapid estimation of herbal extracts with minimum sample clean-up requirement,
2. It provides qualitative and semi quantitative information of the resolved compounds.
3. It enables the quantification of chemical constituents<sup>90</sup>

#### 8.3.2. GAS CHROMATOGRAPHY

Gas chromatography (GC) is also known as gas liquid chromatography (GLC). It is a technique for separation of mixtures into components. The redistribution of the components has been done in this process between a stationary phase or support material in the form of a liquid, solid or combination of both and a gaseous mobile phase.

It is well-known that in herbal medicines many pharmacologically active components are volatile chemical compounds.

Thus, the analysis of volatile compounds by gas chromatography is one of the method important in the analysis of herbal medicines.

There are number of advantages of the GC analysis of the volatile oils.

Firstly, a reasonable “fingerprint” is given by the GC of the volatile oil, which can be used to identification of the plant. For identification of the particular plant, the composition and relative concentration of the organic compounds in the volatile oil are characteristic. Along with composition and relative concentration, presence of impurities in the volatile oil can be readily detected. Secondly, the extraction of the volatile oil is relatively straightforward components can be identified using GC-MS analysis.<sup>91</sup>

#### 8.3.3. SUPER CRITICAL FLUID CHROMATOGRAPHY (SFC)

Supercritical fluid chromatography is a mixture of gas and liquid chromatography. This combines some of the best features of each. SFC enables the separation and estimation of a group of compounds that are not conveniently handled by either gas or liquid chromatography. SFC has been applied to a wide variety of materials including natural products, drugs, food and pesticide.<sup>92</sup>

These compounds are either non-volatile or thermally labile. So that GC procedures which contain no functional group that makes possible detection by the spectroscopic as well as electrochemical technique employed in LC.<sup>93</sup>

#### 8.3.4. ELECTROPHORETIC METHODS

There are different types of electrophoretic methods. Mostly used techniques are capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE) and capillary isoelectric focusing (CIEF). Capillary Electrophoresis is most promising method for the separation and analysis of active ingredients in herbal medicines, since it needs only small amounts of standards and can analyze samples rapidly with very good separation ability. Also, in the herbal medicines it is a good tool for producing the chemical fingerprints. Since it is having similar technical characteristics of liquid chromatography. Currently, several studies dealing with herbal medicines have been reported and two kinds of medicinal compounds, i.e. alkaloids and flavonoids, have been studied.<sup>94-95</sup>

#### 8.3.5. HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

HPTLC is the common fingerprint technique. The compounds which is having low or moderate polarities are mainly used to analyze. In pharmaceutical industry for process development, identification and detection of adulterants, substituent in the herbal products HPTLC technique is widely used. It also helps in the identification of pesticide content, mycotoxins and in quality control of herb and health products.<sup>96</sup> HPTLC technique was reported for simultaneous estimation of gallic acid, Rutin, Quercetin in terminalia chebula.<sup>97</sup>

Quantitative evaluation of Syzygium Jambolanum was done in terms of stability, repeatability, accuracy and Phyto constituents such as glycoside (jamboline), tannin, ellagic acid and gallic acid by HPTLC.<sup>98</sup> It was also used for detection, monitoring and quantification of bacoside A & B in Bacopa monnieri and its formulation.<sup>99</sup> Also HPTLC method for Phyto constituents in crude drugs or herbal



formulations such as bergenin, catechine and gallic acid in *Bergenia cillata* and *Bergenia lingulata*.<sup>100</sup> Chandanasava known to be effective in karsya (malnutrition) was standardized by organoleptic study, physico-chemical analysis, TLC and HPTLC.<sup>101</sup>

### 8.3.6. INFRARED SPECTROSCOPY

For rapid determination of active components, species, geographic origin, special medicinal formula, on-line quality control, identification of counterfeit and discrimination of geographical origins of Chinese herbal medicines, Near-infrared spectroscopy technique has been used.<sup>102-104</sup>

Two-dimensional near-infrared (NIR) correlation Spectroscopy was applied to the discrimination of *Fructus lycii* (a traditional Chinese medicinal herb) of four different geographic regions.<sup>105</sup> To identify and discriminate herbal medicines for quality control in the fingerprint region 400–2000 cm<sup>-1</sup>, FTIR along with the statistical method principal component analysis (PCA) was applied. For herbal medicines, the ratio of the areas of any two marked characteristic peaks was found to be nearly consistent for the same plant from different regions, thereby, an additional discrimination method. PCA clusters herbal medicines into different groups, clearly showing that IR method can adequately discriminate different herbal medicines using FTIR data.<sup>106</sup>

## 8.4. NEWER METHODS FOR STANDARDIZATION OF HERBAL DRUGS:

### 8.4.1. DNA FINGERPRINTING TECHNIQUE:

In herbal drug standardization, DNA analysis has been proved as an important tool. For the identification of phytochemically indistinguishable genuine drug from the substituted and adulterated drug, this technique is currently used. DNA fingerprinting is well established and highly precious method for standardization of herbal drug because, DNA fingerprint of genome remain the same irrespective of the plant part used while the phytochemical content will vary with different plant part used.<sup>107</sup>

The fundamental living components of all living cells is Deoxyribonucleic acid (DNA).

The specific arrangement of DNA base-pair sequences in the cell determine the characteristics, traits and physical features. Via the Central Dogma Theory, distinct arrangement of adenine, guanine, thymine and cytosine (called DNA nucleotides) it is this arrangement that regulates the production of specific proteins and enzymes Theory. Central Dogma theory can be defined as the fundamental theory of molecular biology that genetic information flows from DNA to RNA to proteins.<sup>108</sup>

Though most of the plants belonging to the same genus and species, may show considerable variations between strains, genotypic characterization of plant species and strains is useful.

The availability of intact genomic DNA specificity in commercial herbal drugs is other important application of DNA fingerprinting, which helps in distinguishing adulterants even in processed samples. Adulterants can be distinguished even in processed samples, enabling the authentication of the drug.<sup>109</sup>

One of the unique and inexpensive popular technique of DNA finger printing is Inter-Simple Sequence Repeat (ISSR) a PCR-based application which include the characterization of genetic fingerprinting, gene tagging, estimation of clonal variation, phylogenetic analysis, detection of genomic instability, and assessment of hybridization.<sup>110</sup> By using ISSR markers, *Cannabis sativa* and *Arabidopsis thaliana* L. Heyne have been distinguished from their adulterated species.<sup>111</sup>

### 8.4.2. CHEMO METRIC METHODS

Chemo metrics is a statistical approach to analyze instrument data, it is often resulting in a faster and more precise evaluation of composition of a product or even physical or sensory properties. Chemometrics is designed to recognize patterns in virtually any type of multidimensional analytical data. Chemometrics can be used to speed methods development and make routine the use of statistical models for data analysis. To facilitate the data processing, software named Computer Aided Similarity Evaluation (CASE) has been developed.

All programs of chemo metric algorithms for CASE are coded in METLAB5.3 based on windows. With this software, investigation of data loading, removing, cutting, smoothing, compressing, similarity comparison background and retention time shift correction, normalization, peak identification and spectral matching, variation assessment of common peaks/regions, sample classification and other data processes associated with the chromatographic fingerprint can be done.<sup>112-113</sup>

### 8.4.3. METABOLOMIC TECHNIQUES

Metabolomics is an advanced emerging field of 'omics' research. This technique is concerned with characterizing large numbers of metabolites using NMR, chromatography and mass spectrometry. It is commonly used in biomarker identification and the metabolic profiling of cells, tissues or organisms. The data processing challenges in this technique are quite unique and often require specialized (or expensive) data analysis software. Metabolomics has been used for identification of active phytoconstituents from herbal medicine.<sup>114</sup>

Metabolomic approach was employed to identify the chemical constituents in *Sophora flavescens*, which were further analyzed for their effect on Pregane X receptor activation and Cytochrome P3A regulation. The greater potential of metabolomics has been reported in the development of active secondary metabolites from medicinal plants as novel or improved Phyto therapeutic agents.<sup>115-116</sup> The recent studies showed that NMR-based metabolomics approach combined with orthogonal projections to latent structure-discriminant analysis identified the purity of an herbal medicine.<sup>117</sup>

### 8.4.4. X-RAY POWDER DIFFRACTOMETRY (X-RPD)

To identify minerals, crystalline materials and metallic based herbal formulations this technique is used. The tin based herbal drug Vanga Parpam was estimated by XRD and the intense sharp diffraction peaks clearly confirmed the presence of high crystallinity in Vanga Parpam.<sup>118</sup> XRD analysis of metallic based Indian traditionally medicine Ras-sindoor indicated the presence of mercury sulphide which is represented by sharp peak<sup>119</sup>

X-ray powder diffractometry data confirmed the formation of phospholipid complex with emodin, naringenin, quercetin, gallic acid.<sup>120-121</sup>

### 8.4.5. MASS SPECTROSCOPY

Electrospray, thermos pray, and ionspray ionization techniques are the different techniques used in MS. This offer unique advantages of high detection sensitivity and specificity; liquid secondary ion mass spectroscopy are the recent advances. Later accurate determination of molecular weight proteins, peptides been done by laser mass spectroscopy with 600 MHz offers.

Isotopes pattern can be detected by this technique.<sup>122</sup> LC-MS has become method of choice in many stages of drug development<sup>123</sup>

Chemical standardization of an aqueous extract of the mixture of the 20 herbs provided 20 chemical compounds serving as reference markers using LC-MS.<sup>124</sup>

Further, LC-MS analysis of aminoglycosides evaluated that these drugs are highly soluble in water, exhibited low plasma protein binding, and were more than 90% excreted through the kidney. Furthermore, this technique also helps in analysis of aminoglycosides in plasma samples with ion pairing chromatography.<sup>125</sup>

#### 8.4.6. THERMAL ANALYSIS

Thermogravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have been Used to study different physical or chemical changes in various products including herbal drugs and also used to study preformulation or drug excipient compatibility.<sup>126</sup>TGA may be operated under sub ambient conditions to analyses alcoholic content in various herbal formulations such as asavas and arista.<sup>127</sup>TGA and DTA Analytical method are used to determine mercury based Indian traditional metallic herbal drug Ras-sindoor indicated the presence of mercury sulphide based on a sharp peak at 354oC which corresponded to melting temperature of mercury sulphide.<sup>128</sup> It is also used in determination of metals present in Bhasma.<sup>129</sup> DSC thermograms data confirmed the formation of phospholipid complex with emodin (an anthraquinone).<sup>130-131</sup>

#### 8.4.7. DIFFERENTIAL PULSE POLAROGRAPHY (DPP)

DPP can be used to study trace amounts of chemicals with very small detection limits. Some heavy metals, including lead, cadmium, zinc, copper and iron were successfully identified and determined in chamomile and calendulea flowers by DPP.<sup>132-133</sup> Accumulation of heavy metals, namely Pb, Cd, Cu and Zn was estimated in marketed as well as genuine samples of important herbal drugs of India viz., *Alpinia galanga*, *Artemesia parviflora*, *Butea monosperma*, *Curcuma amada*, *Euphorbia prostrata*, , *Malaxis accuminata* etc. The concentration of Pb and Cd was found beyond the WHO permissible limits in most samples.<sup>134</sup> Trace amounts of selenium in Chinese herbal medicines<sup>135</sup> and flavonoids in small amount of medicinal herb samples were determined byDPP.<sup>136</sup> A DPP method has been for the determination of total hypericin in Phyto therapeutic preparations in various buffer systems over the pH range 3.5–10.0.<sup>137</sup>

#### 8.4.8. NUCLEAR MAGNETIC RESONANCE

The recent introduction of pulsed field gradient technique in high resolution NMR as well as three-dimensional technique showed the improve application in structure elucidation and molecular weight information. These new hyphenated techniques are useful in the areas of pharmacokinetics, toxicity studies, drug metabolism and drug discovery process.<sup>138</sup>

The separation and structural elucidation of unknown compound and mixtures, especially for the structure elucidation of light and oxygen sensitive substances the combination of chromatographic separation technique with NMR spectroscopy is used. This combined technique is the most powerful and time saving method.<sup>139</sup>

### IX. CONCLUSION:

The Indian herbal drug industries are growing in a tremendous rate. In the last few years, plant derived preparations are increasingly being sought out as medicinal products, nutraceuticals and cosmetics and are currently easily available in health food shops and pharmacies. Standardization of these herbal drugs depends upon the safety and efficacy of herbal products. As per the current traditional approach towards standardization of herbal drug is insufficient for current herbal market and hence there is need for more advanced techniques for standardization. There are basically two techniques used for standardization these are chromatographic fingerprinting and DNA fingerprinting. The chromatographic fingerprinting is one of the conventional techniques which is based on the chromatographic separation and identification of marker compound from other constituents. For these purpose TLC, HPTLC, HPLC, LC-MS, LC-NMR, GC-MS, GC-FID and SFC methods are mainly used. The other conventional method used is DNA fingerprinting.

DNA fingerprinting is well established and highly precious method for standardization of herbal drug because, DNA fingerprint of genome remain the same irrespective of the plant part used while the phytochemical content will vary with different plant part used.

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