IJPSR (2017), Volume 8, Issue 9 (Review Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 February, 2017; received in revised form, 28 June, 2017; accepted, 12 August, 2017; published 01 September, 2017

A COMPREHENSIVE REVIEW ON STANDARDIZATION OF HERBAL DRUGS

Rohit Kumar Bijauliya *, Shashi Alok, Dilip Kumar Chanchal and Mayank Kumar

Department of Pharmacognosy, Bundelkhand University, Jhansi - 284128, Uttar Pradesh, India.

Keywords:

Herbal Drugs, Standardization, Chromatographic Fingerprinting, DNA Fingerprinting

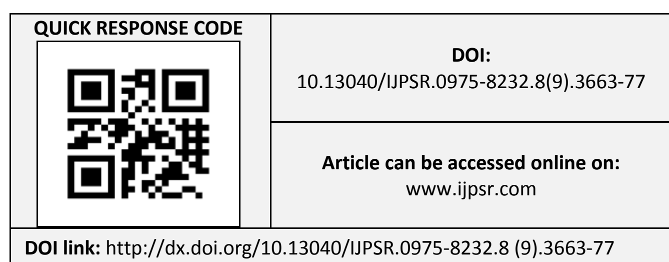
Correspondence to Author:
Rohit Kumar Bijauliya

Research Scholar,
Institute of Pharmacy,
Bundelkhand University,
Jhansi - 284128, Uttar Pradesh, India.

E-mail: rkpharma3791@gmail.com

ABSTRACT: In recent years more people throughout world are turning to use medicinal plant products in healthcare system. Worldwide need of alternative medicine has resulted in growth of natural product markets and interest in traditional systems of medicine. Proper integration of modern scientific techniques and traditional knowledge is important. There is a growing focus on the importance of medicinal plants in the traditional health care system (*viz.* Ayurveda, Unani, Homoeopathy, Yoga) in solving health care problems. Systematic approach and well-designed methodologies for the standardization of herbal raw materials and herbal formulations are developed. The stability testing of herbal products check the quality of herbal products which varies with the time under the influence of environmental factors, such as temperature, humidity, light, oxygen, moisture, other ingredient or excipient in the dosage form, particle size of drug, microbial contamination, trace metal contamination, leaching from the container, *etc.* and also provide statistics for the determination of shelf lives. Therefore evaluation of the parameters based upon chemical, physical, microbiological, therapeutic and toxicological studies can serve as an important tool in stability studies. Standardization of herbal drugs means confirmation of its identity, Quality and purity. The present overview covers the standardization parameters with their standards value of the some herbal drugs.

INTRODUCTION: India is a mother hub for development of Ayurveda, Unani, Siddha; Homoeopathy and other natural herbs based health science (Ayush). Ayush Pharmaceutical industry is having great potential and opportunities for development in future. Mainly in following herbal medicinal plants and their value added products well accepted in domestic and international market *e.g.* Ayurvedic medicines, Unani medicines, Siddha medicines, Homoeopathic medicines, herbal nutraceuticals, herbal cosmoceutical, herbal health drinks, dietary health supplements, medicinal plants / crude drugs, herbal extracts / concentrates, herbal veterinary medicines, health foods, Ayush health care management, Ayurvedic panchakarma centre and health spa. Standardization of drug means confirmation of its identity, quality and purity throughout all phases of its cycle *i.e.* shelf-life, storage, distribution and use by various parameters. As we all know in our Ayurvedic system of medicines drug standardisation of Ayurvedic formulation is a big challenge. Clear cut guidelines have not been developed so far. So it is necessary to promote ISM manufacturing industry people for drug standardization work. Ministry of Ayush, Government of India recently established Pharmacopoeial Commission of Indian medicines and Homoeopathy (PCIM and H) for setting up drug standard of ASU and H Medicines [1].



QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(9).3663-77

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (9).3663-77

World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical

markers and the fingerprint profiles. If a principle active component is known, it is most logical to quantitate this compound. Where active ingredients contributing to therapeutic efficacy are known botanical preparations should be standardized to these compounds. Where the active ingredients are not yet known a marker substance which should be specific for the botanical could be chosen for analytical purpose [2].

As commercialization of the herbal medicine has happened, assurance of safety, quality and efficacy of medicinal plants and herbal products has become an important issue. The herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the plants and seasonal variation (which has a bearing on the time of collection), the ecotypic, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotic [2].

Standardization as defined by American Herbal Product association: "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 [3].

Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs.

The fingerprint profiles serve as guideline to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker compounds would serve as an additional parameter in assessing the quality of the sample. Phytochemical standardization encompasses all possible / information generated with regard to the chemical constituents present in an herbal drug. Hence, the phytochemical evaluation for standardization purpose includes the following:

1. Preliminary testing for the presence of different chemical groups.
2. Quantification of chemical groups of interest (*e.g.*, total alkaloids, total phenolics, total triterpenic acids, total tannins). Establishment of fingerprint profiles.
3. Multiple marker-based fingerprint profiles.
4. Quantification of important chemical constituents [4].

2. Methods of Standardization: Phytotherapeutic agents are normally marketed as standardized preparations in the form of liquid, solid (powdered extract), or viscous preparations. They are prepared by maceration, percolation or distillation (volatile oils). Ethanol, water, or mixtures of ethanol and water are used for the production of fluid extracts. Solid or powered extracts are prepared by evaporation of the solvents used in the process of extraction of the raw material. Some phytotherapeutic agents are greatly concentrated in order to improve their therapeutic efficacy [5].

The identification, purity and quality of herbal drugs are determined by reference given in a pharmacopoeia. Pharmacopoeia prescribes like Analytical, physical and structural standards for the herbal drugs. The essential standards are given in pharmacopoeia shown in **Fig. 1**. A significant identification and examination of crude drugs is important in processes of herbal formulation because of more diversity and changes in their chemical nature or characters.

To reduce this problem all pharmacopeias have certain standards. Specific test for specific plant material are given below. Alkaloids content dragendorff test, Fat content Acid value Iodine value, saponification value molish test carbohyadrates Millon tests Amino acid Volatile oil Hemolytic activity Assay for Phosphate/ Aluminium/ Camphor /Potassium /Lead/ Iron/Gold/ Calcium [6, 7]. The Standardization of crude drug materials is done by authentication: Stage of collection, parts of the plant collected, identity like phytomorphology, microscopical and histological analysis (characteristic of cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes,

fibers, vessels *etc.*), Leaf constant: palisade ratio, vein islet number, vein termination, stomatal number, and stomatal index. Other histological test are trichomes, Stomata, quantitative microscopy, taxonomical identity, foreign matter, organoleptic evaluation, ash values and extractive values, moisture content determination, chromatographic and spectroscopic evaluation, heavy metal determination, pesticide residue, microbial contamination, radioactive contamination. The herbal formulation in general can be standardized schematically as to formulate the medicament using raw material collected from different localities and a comparative chemical efficacy of different batches of formulation are to be observed. The preparations with better clinical efficacy are to be selected. All the routine physical, chemical and pharmacological parameters are checked for all the batches in order to select the final finished product and to validate the whole manufacturing process.

FIG. 1: FLOW CHART ON STANDARDIZATION AND EVALUATION OF HERBAL DRUG

The stability parameters for the herbal formulations which include physical, chemical and microbiological parameters are as follow: Physical parameters include color, odor, appearance, clarity, viscosity, moisture content, pH, disintegration time, friability, hardness, flow ability, flocculation, sedimentation, settling rate and ash values. Chemical parameters include limit tests, chemical tests, chemical assays *etc.* Chromatographic analysis of herbals can be done using TLC, HPLC, HPTLC, GC, UV, GC-MS, fluorimetry *etc.*

Microbiological parameters include total viable content, total mold count, total enterobacterial and their count. Limiters can be utilized as a quantitative or semi quantitative tool to ascertain and control the amount of impurities like the reagents used during abstraction of various herbs, impurities coming directly from the manufacturing vessels and from the solvents *etc* [8].

3. Morphological or Organoleptic Evaluation: It includes the evaluation of herbal drugs by size, shape colour, odour, taste and particular characteristics like touch, texture *etc.* This is a technique of qualitative evaluation related to the study of morphological and sensory report of whole drugs. *eg*. Fractured surfaces in cascara, cinchona, and quillia bark and quassia wood are essential characteristics. Umbelliferous fruits have aromatic odour and liquorice have sweet taste are the example of this type of evaluation. Shape of drug may be conical (aconite), subcylindrical (podophyllum), cylindrical (sarsapilla), fusiform (jalap). Size represents thickness, length, breadth and diameter. Color represents external color which various from white to brownish black are essential diagnostic features. Taste which is a specific type of sensation feel by epithelial layer of tongue. Taste may be sweetish (saccharic), sour (acidic), salt like (saline) and bitter or tasteless [9 - 11].

4. Macroscopic and Microscopic Examination: Medicinal plant materials are categorized according to sensory, macroscopic and microscopic characteristics. An examination to determine these characteristics is the first step towards establishing the identity and the degree of purity of such materials, and should be carried out before any further tests are undertaken.

Wherever possible, authentic specimens of the material in question and samples of pharmacopoeial quality should be available to serve as a reference. Visual inspection provides the simplest and quickest means to establish identity, purity and, possibly, quality. If a sample is found to be significantly different, in terms of colour, consistency, odour or taste, from the specifications, it is considered as not fulfilling the requirements.

However, judgment must be exercised when considering odour and taste, owing to variability in assessment from person to person or by the same person at different times. Macroscopic identity of medicinal plant materials is based on shape, size, color, surface characteristics, texture, fracture characteristics and appearance of the cut surface. However, since these characteristics are judged subjectively and substitutes or adulterants may closely resemble the genuine material. It is often necessary to substantiate the findings by microscopy and/or physicochemical analysis.

Microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials; the specimen may have to be treated with chemical regents. An examination by microscopy alone cannot always provide complete identification, though, when used in association with other analytical methods, it can frequently supply invaluable supporting evidence.

Any additional useful information for preparation or analysis should also be included in the test procedures for individual plant materials, for example, the determination of vein islets and the palisade ratio [12].

It involves the detailed assessment of the herbal drugs and it is used to recognize the organized drugs on the basis of their known histological characters. It is regularly used for qualitative analysis of organized crude drugs in total and powder form with the help of microscope. The inner pseudoparenchyma cells are round or oval shape. They contain protein and fixed oil. Crude drugs are microscopically identified by taking thin TS (Transverse section), LS (Longitudinal Section) in a bark, wood and leaf. The various parameters included in microscopy are given bellow.

I. Stomata II Trichomes III Leaf Content IV Quantitative Microscopy

TABLE 1: SOME MICROSCOPIC IDENTIFICATION TEST ARE GIVEN BELOW [13 - 15]

Sr. no.	Name of Constituents	Procedure for test/Reagents	Result
1.	Starch, Hemicellulose	T.S. of Crude drug + 1 Drop of Iodine Solution	Blue color
2.	Mucilage	Ruthenium Red	pink color
3.	Lignin	T.S. of crude drug + 1 drop of phloroglucinol + 1 drop of HCl	Pink color

5. Physical Evaluation: Each monograph contains detailed botanical, macroscopic and microscopic descriptions with detailed illustrations and photographic images which provide visual documentation of accurately identified material. A microscopic analysis assures the identity of the material and as an initial screening test for impurities [16 - 17].

5.1 Determination of ash: The ash remaining following ignition of medicinal plant materials is determined by three different methods which measure total ash, acid-insoluble ash and water-soluble ash. The total ash method is designed to measure the total amount of material remaining after ignition. This includes both “physiological ash”, which is derived from the plant tissue itself, and “non- physiological” ash, which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

TABLE 2: SOME EXAMPLES OF DRUG WITH THEIR TOTAL ASH VALUE [9]

Sr no.	Drugs	Total Ash (%w/w)	Acid Insoluble ash (%w/w)
1.	Agar	-	1.00
2.	Bael	3.50	-
3.	Cannabis	15.00	5.00
4.	Ginger	6.00	1.7(water soluble ash)

5.2 Determination of extractable matter: This method determines amount of active constituents extracted with solvents from a given amount of medicinal plant material [12].

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

TABLE 3: SOME EXAMPLES WITH THEIR EXTRACTIVE VALUES [9, 15]

Sr no.	Drugs	Water Soluble extractives (%w/w)	Alcohol Soluble extractives (%w/w)	Ether Soluble extractives (%w/w)
1.	Aloe	NLT 25.00	NLT 10.00	-
2.	Ginger	NLT 10.00	NLT 4.50	-
3.	Capsicum	-	-	NLT 12.00
4.	Nutmeg	-	-	NLT 25.00

Note: NLT means Not Less Than

5.3 Determination of Foreign Matter: Herbal drugs should be prepared from the confirmed part of the plant. They should be totally free from insects or moulds, including visible and excreta contaminant such as stones, sand, harmful and poisonous foreign matter and chemical residues. Animal objects such as insects and invisible microbial contaminants, which produces toxins, as well as the potential contaminants of herbal medicines. Macroscopic evaluation can easily used to determine the presence of foreign matter, although microscopy is essential in certain special cases for example starch intentionally added to "dilute" the plant material [15, 18].

% 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 field

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

W = Weight in mg of lycopodium taken

M= weight in mg of the sample

P= number of characteristics particles per mg of the pure foreign matter

94,000= number of spores per mg of lycopodium [15, 19]

6. Chemical Evaluation: The most of drug contain definite chemical constituents to which their pharmacological and Biological activity depended. Qualitative chemical test used to identify drug quality and purity. The identification, isolation and purification of active chemical constituents is depends chemical methods of evaluation. Preliminary phytochemical investigation is also a part of chemical evaluation. Some Qualitative chemical test for chemical evaluation crude drug are Saponification value and acid value *etc.* [15, 20, 21].

TABLE 4: SOME IMPORTANT TEST USED IN CHEMICAL EVALUATION

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. bester value
3.	Resins	1. Sulphated Ash 2. Acid value
4.	Gums	1. Methoxy determination 2.Volatile acidity

6.1 Chromatographic Fingerprinting and Marker Compound Analysis: A chromatographic fingerprint of an Herbal Medicine (HM) is a chromatographic pattern of the extract of some common chemical components of pharmacologically active and or chemical characteristics. This chromatographic profile should be featured by the fundamental attributions of "integrity" and "fuzziness" or "sameness" and "differences" so as to chemically represent the HM investigated. It is suggested that with the help of chromatographic fingerprints obtained, the authentication and identification of

herbal medicines can be accurately conducted (integrity) even if the amount and/or concentration of the chemically characteristic constituents are not exactly the same for different samples of this HM (hence, "fuzziness") or, the chromatographic fingerprints could demonstrate both the "sameness" and "differences" between various samples successfully.

Thus, we should globally consider multiple constituents in the HM extracts, and not individually consider only one and/or two marker components for evaluating the quality of the HM products. However, in any HM and its extract, there are hundreds of unknown components and many of them are in low amount. Moreover, there usually exists variability within the same herbal materials. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the HM.

6.1.1 TLC: Thin layer chromatography is simply known as TLC. It is one of the most popular and simple chromatographic technique used of separation of compounds. In the phytochemical evaluation of herbal drugs, TLC is being employed extensively for the following reasons:

1. It enables rapid analysis 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.

Fingerprinting using HPLC and GLC is also carried out in specific cases In TLC fingerprinting, the data that can be recorded using ma high-performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (R_f) values, the color of the separated bands, their absorption spectra, λ_{max} and shoulder inflection/s of all the resolved bands.

All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the sample. The information so generated has a potential application in the identification of anauthentic drug, in excluding the adulterants and in maintaining the quality and consistency of the drug. HPLC fingerprinting includes recording of the chromatograms, retention time of individual peaks and the absorption spectra (recorded with a photodiode array detector) with different mobile phases. Similarly, GLC is used for generating the fingerprint profiles of volatile oils and fixed oils of herbal drugs.

Furthermore, the recent approaches of applying hyphenated chromatography and spectrometry such as High- Performance Liquid Chromatography-Diode Array Detection (HPLC–DAD), Gas Chromatography–Mass Spectroscopy (GC–MS), Capillary Electrophoresis - Diode Array Detection (CEDAD), High - Performance Liquid Chromatography-Mass Spectroscopy (HPLC–MS) and High-Performance Liquid Chromatography-Nuclear Magnetic Resonance Spectroscopy (HPLC–NMR) could provide the additional spectral information, which will be very helpful for the qualitative analysis and even for the on-line structural elucidation [22 - 23].

6.1.2 HPTLC: HPTLC technique is widely employed in pharmaceutical industry in process development, identification and detection of adulterants in herbal product and helps in identification of pesticide content, mycotoxins and in quality control of herbs and health foods [24]. It has been well reported that several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC [25]. It has also been reported that mobile phases of pH 8 and above can be used for HPTLC.

Another advantage of HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions. Consequently, HPTLC has been investigated for simultaneous assay of several components in a multi-component formulation [26]. With this technique, authentication of various species of plant possible, as well as the evaluation of stability and consistency of their preparations from different manufactures.

Various workers have developed HPTLC method for phytoconstituents in crude drugs or herbal formulations such as bergenin, catechine and gallic acid in *Bergenia cilliata* and *Bergenia lingulata* [27].

6.1.3 HPLC: Preparative and analytical HPLC are widely used in pharmaceutical industry for isolating and purification of herbal compounds.

There are basically two types of preparative HPLC: low pressure HPLC (typically under 5 bar) and high pressure HPLC (pressure >20 bar) [28]. The important parameters to be considered are resolution, sensitivity and fast analysis time in analytical HPLC whereas both the degree of solute purity as well as the amount of compound that can be produced per unit time *i.e.* throughput or recovery in preparative HPLC [29]. In preparative HPLC (pressure >20 bar), larger stainless steel columns and packing materials (particle size 10-30 µm are needed. The examples of normal phase silica columns are Kromasil 10 µm, Kromasil 16 µm, Chiralcel AS 20 µm whereas for reverse phase are Chromasil C18, Chromasil C8,YMC C18. The aim is to isolate or purify compounds, whereas in analytical work the goal is to get information about the sample.

This is very important in pharmaceutical industry of today because new products (Natural, Synthetic) have to be introduced to the market as quickly as possible. Having available such a powerful purification technique makes it possible to spend less time on the synthesis conditions [30 - 32].

6.1.4 Liquid Chromatography - Mass Spectroscopy: (LC-MS) LC-MS has become method of choice in many stages of drug development [33]. Recent advances includes electrospray, thermospray, and ionspray ionization techniques which offer unique advantages of high detection sensitivity and specificity, liquid secondary ion mass spectroscopy, later laser mass spectroscopy with 600 MHz offers accurate determination of molecular weight proteins, peptides. Isotopes pattern can be detected by this technique [30].

6.1.5 Liquid Chromatography- Nuclear Magnetic Resonance (LCNMR): LC-NMR improves speed and sensitivity of detection and found useful in the areas of pharmacokinetics, toxicity studies, drug metabolism and drug discovery process. The combination of chromatographic separation technique with NMR spectroscopy is one of the most powerful and time saving method for the separation and structural elucidation of unknown compound and mixtures, especially for the structure elucidation of light and oxygen sensitive substances. The online LC-NMR technique allows the continuous registration of time changes as they appear in the chromatographic run automated data acquisition and processing in LC-NMR improves speed and sensitivity of detection. The recent introduction of pulsed field gradient technique in high resolution NMR as well as three-dimensional technique improves 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 [34].

6.1.6 Gas Chromatography (GC-MS): GC equipment can be directly interfaced with rapid scan mass spectrometer of various types. GC and GC-MS are unanimously accepted methods for the analysis of volatile constituents of herbal medicines, due to their sensitivity, stability and high efficiency. Especially, the hyphenation with MS provides reliable information for the qualitative analysis of the complex constituent [35 - 36]. The flow rate from capillary column is generally low enough that the column output can be fed directly into ionization chamber of MS. The simplest mass detector in GC is the Ion Trap Detector (ITD).

In this instrument, ions are created from the eluted sample by electron impact or chemical ionization and stored in a radio frequency field; the trapped ions are then ejected from the storage area to an electron multiplier detector. The ejection is controlled so that scanning on the basis of mass-to-charge ratio is possible. The ions trap detector is remarkably compact and less expensive than quadrapole instruments. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system [37].

6.1.7 GC-FID: A number of detectors are used in gas chromatography. The most common are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Coupling capillary column gas chromatographs with Fourier Transform Infrared Spectrometer provides a potent means for separating and identifying the components of different mixtures (Sharma). Both are sensitive to a wide range of components, and both work over a wide range of concentrations. While TCDs are essentially universal and can be used to detect any component other than the carrier

gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. However, an FID cannot detect water. Both detectors are also quite robust. Since TCD is non-destructive, it can be operated in-series before an FID (destructive), thus providing complementary detection of the same analytes [38].

6.1.8 Supercritical Fluid Chromatography (SFC): Supercritical fluid chromatography is a hybrid of gas and liquid chromatography that combines some of the best features of each. SFC permits the separation and determination 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. (Matthew *et al*., 2006). These compounds are either nonvolatile or thermally labile so that GC procedures are inapplicable or contain no functional group that makes possible detection by the spectroscopic or electrochemical technique employed in LC [34].

6.2 DNA Fingerprinting: DNA analysis has been proved as an important tool in herbal drug standardization. This technique is useful for the identification of phytochemically indistinguishable genuine drug from substituted or adulterated drug. It has been reported that DNA fingerprint genome remain the same irrespective of the plant part used while the phytochemical content will vary with the plant part used, physiology and environment [39].

Deoxyribonucleic acid (DNA) is the fundamental building component of all living cells. Our characteristics, traits and physical features are determined by the specific arrangement of DNA base-pair sequences in the cell. It is this distinct arrangement of adenine, guanine, thymine and cytosine (called DNA nucleotides) that regulates the production of specific proteins and enzymes via the Central Dogma Theory. Central Dogma theory can be defined as the fundamental theory of molecular biology that genetic information flows from DNA to RNA to proteins [40]. This concept of fingerprinting has been increasingly applied in the past few decades to determine the ancestry of plants, animals and other microorganisms.

Genotypic characterization of plant species and strains is useful as most plants, though belonging to the same genus and species, may show considerable variation between strains. Additional motivation for using DNA fingerprinting on commercial herbal drugs is the availability of intact genomic DNA from plant samples after they are processed. Adulterants can be distinguished even in processed samples, enabling the authentication of the drug [41].

The other useful application of DNA fingerprinting is the availability of intact genomic DNA specificity in commercial herbal drugs which helps in distinguishing adulterants even in processed samples [42].

6.2.1 Genetic Marker: A genetic marker is a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait. It can be described as a variation, which may arise due to mutation or alteration in the genomic loci that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism SNP), or a long one, like mini satellites.

Some commonly used types of genetic markers are

- RFLP (or Restriction fragment length polymorphism)
- AFLP (or Amplified fragment length polymorphism)
- RAPD (or Random amplification of polymorphic DNA)
- VNTR (or Variable number tandem repeat)
- Micro satellite polymorphism
- SNP (or Single nucleotide polymorphism)
- STR (or Short tandem repeat)
- SFP (or Single feature polymorphism)

They can be further categorized as dominant or codominant. Dominant markers allow for analyzing many loci at one time, *e.g*. RAPD. A primer amplifying a dominant marker could amplify at many loci in one sample of DNA with one PCR reaction. Co-dominant markers analyze one locus at a time. A primer amplifying a co-dominant marker would yield one targeted product [43].

6.3 Radioactive Contamination: The microbial growth in herbal drugs is usually avoided by irradiation. Dangerous contamination, 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 radio nuclides resulting from major nuclear accidents. The nuclear accident in chernobyl and Fukushima may be serious and depend on the specific radionuclide, the stage of contamination and the quantity of the contaminant consumed. Examples of such radionuclides include long lived and short lived fission products, actinides and activation products. Therefore, at current no limits are proposed for radioactive contamination [44 - 47].

7. Biological Evaluation:

7.1 Determination of Bitterness Value: Medicinal plant materials that have a strong bitter taste are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice. Bitter substances can be determined by taste. However, since they are mostly composed of two or more constituents with various degrees of bitterness, it is first necessary to measure total bitterness by taste. The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride. The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1 gm of quinine hydrochloride in 2000ml. Safe drinking water should be used as a vehicle for the extraction of plant materials and for the mouth wash after each tasting. Taste buds dull quickly if distilled water is used. The hardness of water rarely has any significant influence on bitterness.

7.2 Determination of Haemolytic Activity: Many medicinal plant materials, especially those derived from the families Caryophyllaceae, Araliaceae, Sapinaceae, Primulaceae, and Dioscoreaceae contain saponins. The haemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of a reference material, saponin, which has a haemolytic activity of 1000 units per gm. A suspension of erythrocytes is mixed with equal volumes of a serial haemolysis and is determined after allowing the mixtures to stand for a given period of time. A similar test is carried out simultaneously with saponin.

7.3 Determination of Swelling Index: The swelling index is the volume in ml taken up by the swelling of 1 gm of plant material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material. Using a glass stoppered measuring cylinder, the material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture is then read. The mixing of whole plant material with the swelling agent is easy to achieve, but cut or pulverized material requires vigorous shaking at specified intervals to ensure even distribution of the material in the swelling agent.

7.4 Determination of Foaming Index: Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

7.5 Determination of Pesticide Residues: Limits for pesticide residues should be established following the recommendations of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) which have already been established for food and animal feed. These recommendations include the analytical methodology for the assessment of specific pesticide residues [12]. Pesticides residue are any particular substance in food, agriculture commodities or animal feed resulting from the use of a pesticides. Herbal drugs are prone to contain pesticide residue, which gather from agricultural practices, such as Spraying, behavior of soil during cultivation and addition of fumigants during storage. The Pesticides contain chlorine in the molecules, which can be determined by analysis of chlorine, insecticides containing phosphate can be detected by measuring total organic phosphorus. The various methods are used to measure pesticides by GC, MS, OR GCMS. Some simple methods are also published by the WHO and Europian pharmacopeia has in general limits for pesticides residue in medicine [48 - 50].

TABLE 5: THE LIST OF APPROVED PESTICIDES FOR SPICES AND THEIR MAXIMUM RESIDUE LIMITS (MRLs) (CODEX ALIMENTARIUS COMMISSION, 2005)

Sr. no.	Pesticides (CCPR number)	Group or sub group of spices	MRL(mg/kg)
1.	Acephate (095)	Entire group 028c	0.2
2.	Azinphos-methyl (002)	Entire group 028c	0.5
3.	Chlorpyrifos (017)	Seeds	5
		Fruits or berries	1
		Roots or rhizomes	1
4.	Chlorpyrifos-methyl (090)	Seeds	1
		Fruits or berries	0.3
		Roots or rhizomes	5
5.	Diazinon (22)	Seeds	5
		Fruits or berries	0.1
		Roots or rhizomes	0.5
6.	Dicofol (026)	Seeds	0.05
		Fruits or berries	0.1
		Roots or rhizomes	0.1

7.6 Determination of Arsenic and Heavy Metals: Contamination of medicinal plant materials with arsenic and heavy metals can bemattributed to many causes including environment pollution and traces of pesticides.

7.6.1 Method for Determination of Arsenic by Gutzeit Apparatus: The solution of the substances to be examined is prepared as specified and transferred to the wide mouthed bottle. To this add 1 g of potassium iodide (5 ml of 1M KI), 5 ml of stannated hydrochloric acid solution and 10 g of zinc. Immediately place the glass tube in position and immerse the bottle in a water-bath at a temperature such that a uniform evolution of gas is maintained.

The most suitable temperature for the test is about 40 °C. The reaction is allowed to continue for 40 minutes. After 40 minutes, the yellow stain produced on the HgCl paper compared with the standard stain produced by treating 1.0 ml of the arsenic standard solution (10 ppm As) diluted to 50 ml with water in the same manner. If the intensity of the yellow stain produced in the test solution is less than that of standard stain, the sample passes the limit test for arsenic and *vice-versa*. The stain produced on paper fades on keeping and therefore the stains should be compared immediately.

7.6.2 Method for Determination of Heavy Metal

Standard solution: Pipette 1.0 ml of standard lead solution (20 ppm Pb) into a Nessler cylinder labeled as “Standard” and dilute to 25 ml with water. Adjust the pH between 3.0 and 4.0 with dilute acetic acid or dilute ammonia solutions, dilute to 35 ml with water and mix.

Test solution: Take 25 ml of the solution prepared as directed in the individual monograph into a Nessler cylinder labeled as “Test” or dissolve the specified quantity of the substance in water to produce 25 ml. Adjust the pH between 3.0 and 4.0 with dilute acetic acid or dilute ammonia solution, dilute to 35 ml with water and mix.

Procedure: Add 10 ml of freshly prepared hydrogen sulphide to each of the Nessler cylinder containing test solution and standard solution respectively. Mix, dilute to 50 ml with water and allow to stand for 5 minutes. Compare the compare the colour by viewing vertically downwards over a white surface. The colour produced with the test solution in not more intense than that produced with the standard solution [12].

TABLE 6: EXAMPLES OF NATIONAL LIMITS FOR HEAVY METALS IN HERBAL MEDICINE AND PRODUCTS [51 - 53]

		Arsenic (As)	Lead (Pb)	Cadmium (Cd)	Chromium (Cr)	Mercury (Hz)	Copper (Cu)	Total Heavy metals as Lead
				For Herbal Medicine				
Canada	Raw herbal material	5ppm	10ppm	0.3ppm	2ppm	0.2ppm		
	Finished herbal products	0.01 mg/day	0.02 mg/day	0.006 mg/day	0.02 mg/day	0.02 mg/day		
China	Herbal materials	2ppm	10ppm	1ppm		0.5ppm		20ppm
Malaysia	Finished herbal	5mg/kg	10mg/kg			0.5mg/kg		

	products						
Korea	Herbal materials						30ppm
Singapore	Finished herbal products	5ppm	20ppm		0.5ppm	150ppm	
Thailand	Herbal material, finished herbal products	4ppm	10ppm	0.3ppm			
WHO recommendation (2)			10mg/kg	0.3mg/kg			
For Other Herbal Products							
National sanitation foundation draft proposal (Raw dietary supplement)		5ppm	10ppm	0.3ppm	2ppm		
National sanitation foundation draft proposal (finished dietary supplement)		0.01mg/kg	0.02mg/kg	0.006mg/kg	0.02mg/kg	0.02mg/kg	

7.7 Determination of Microorganism: Methods for decontamination are restricted. For example, the use of ethylene oxide has been forbidden within countries of the European Union. Treatment with ionizing irradiation is also for bidden or requires a special registration procedure in some countries. In addition, the presence of aflatoxins in plant material can be hazardous to health if absorbed even in very small amounts. They should therefore be determined after using a suitable clean up procedure *e.g.* liquid chromatography (LC). Aflatoxins are extracted from a ground sample with methanol- water (80 + 20, v/v), and after a single cleanup step on a mini column packed with basic aluminum oxide, they are quantitated by LC equipped with a C18 column, photochemical reactor, and fluorescence detector [12].

8. Stability Testing of Herbal Products:

8.1 Analytical Methods for Herbal Products: The analysis of herbal preparations is mostly done by running high performance liquid chromatography (HPLC) [54] or gas chromatography (GC) and thin layer chromatography (TLC) methods, quantitative determinations by UV visible spectroscopy or combinations of these. HPLC and GC methods can be used for identification and purity testing, as well as the detection of single compounds for assay, is possible during one analysis. LC and GC mass coupling [55] are the also tools for determination but, they are highly sophisticated and expensive methods.

8.2 Shelf-Life: The determination of shelf life of herbal medicinal drug products is same as chemically defined APIs, but special nature of herbal product should be taken into consideration. It is recommended that in case of a herbal medicinal product containing a natural product or a herbal drug preparation with constituents of known therapeutic activity, the variation in component during the proposed shelf-life should not exceed ± 5% [56, 57] of the initial assay value, unless justified to widen the range up to ±10 per cent or even higher. The low marker concentration in the finished product, justify the wider range.

Additionally, due to the influences of climate, harvesting and biological variance, the natural variation of the marker content needs to be taken into account. For example, the linearity of the method may be tested over a range of 40-160 per cent of the marker's expected content in the extract and/or product. During stability testing, a setting up of the limits to ±10 per cent is accepted for the finished product, by the justification of matrix effects (placebo), the lack of precision and selectivity (combination products) and the low analyte concentrations. Considering that the marker content cannot be defined to a specified level, the relative changes from the starting value are specified (95-105 per cent or 90-110 per cent 'from the initial value).

8.3 Challenges in Stability testing of Herbal Medicinal Product: Evaluating the stability [58] of HMPs presents a number of challenges when compared to chemically defined substances. In particular:

1. Active substances (herbal substances and/or herbal preparations) in HMPs consist of complex mixtures of constituents and in most cases the constituents responsible for the therapeutic effects are unknown.

2. The situation is further complicated when two or more herbal substances and/or herbal preparations are combined in a HMP. In many cases where combinations of herbal substances and/or herbal preparations are present in HMPs, they have similar constituents and this gives rise to even more analytical challenges.

3. In addition, many herbal substances/herbal preparations are known to be unstable. Taking into account these special features of HMPs, adequate quality concepts have been established. As part of a total control strategy for herbal substances, herbal preparations and HMPs, a set of test criteria including qualitative and quantitative parameters has been recognized as quality indicating. With regard to stability tests, chromatographic fingerprints as well as appropriate methods of assay *via* marker substances represent the fundamental part of this concept, laid down in shelf-life specifications. Notwithstanding the appropriateness of this approach, its realization is often associated with analytical problems and high costs.

In summary, HMPs have a number of characteristics that clearly differentiate them from chemically defined medicinal products and therefore specific stability guidance needs to be established, which covers particular aspects that existing specific herbal guidelines and general guidelines on stability do not address.

8.4 Mechanisms Involved in Change Product: Loss of activity, Change in concentration of active component [59], Alteration in bioavaibility, Loss of content uniformity, Loss of elegance, Formation of toxic degradation product, Loss of packaging integrity. Importance of Stability testing: It evaluates the efficacy of a drug. Stability studies [60] are used to develop suitable packaging information for quality, strength, purity & integrity of product during its shelf life. It is used for determination of the shelf life.

Stress Testing: Stress testing help to identify the degradation product, which can help to establish the degradation pathway. Stress tests are usually considered unnecessary for herbal drug & its preparation.

1. For herbal drugs and herbal drug preparations, a testing under accelerated or intermediate conditions may be omitted. This should apply to finished products as well, because it is known that most products fail at 30°C/65 percent relative humidity (RH) and at 40°C/75 per cent RH in particular. Herbal drug substances at only 25°C/60 percent RH, with no requirement for intermediate/ accelerated testing.

2. If intermediate conditions are tested, the three-month time-point is omitted (that is, 0, 6, 9 and 12 months). In some cases of combination products, it is hardly possible to provide the required two batches of each extract at the same time due to different harvesting times.

Selection of batches: Long term testing is to be provided with on at least two batches of the drug substance and three batches [61] of drug product. In some cases of combination products, it is hardly possible to provide the required two batches of each extract at the same time due to different harvesting times. This should be taken into consideration when planning the schedule for stability study.

8.5 Predictable Changes in Herbal Medicinal Product: Following predictable changes [62] may occurs in herbal medicinal product during storage and in shelf life determination: Hydrolysis, Oxidation, Racemization, Geometric isomerization, Temperature, Moisture and Light.

Hydrolysis: Reaction with water takes place results in degradation of product [63].

Oxidation: Due to addition of electro negative atom (o), Removal of electro positive atom, radicals formation results in decomposition of natural products.

Racemization: Racemization is the process in which one enantiomer of a compound, such as an L-amino acid, converts to the other enantiomer. The compound then alternates between each form while the ratio between the (+) and (–) groups approaches 1:1, at which point it becomes optically inactive.

Geometric Isomerization: Products can be change in trans or cis form. One form may be more therapeutically active.

Polymerization: There is combination of two or more identical molecule to form much larger & more complex molecule.

Temperature: The rate of most chemical increase with increase in temperature. So that "Tropical" area must be taken in consideration during preparation of the formula of the herbal substance.

Moisture: Moisture absorbed on to the surface of solid drug will often increase the rate of decomposition, if it is susceptible to the hydrolysis.

Light: Many type of chemical reaction induced by exposure to light of high energy. Autoxidation of volatile oil /fixed oil takes place and substance becomes coloured.

CONCLUSION: The Indian herbal industry is growing in a tremendous rate. More number of herbal products is arrived in the market. The safety and efficacy of herbal products are dependent upon the standardization of these herbal drugs. The traditional approach towards standardization is insufficient for current herbal market and hence there is need for more advanced techniques for standardization. The quality of herbal drugs is the sum of all factors which contribute directly or indirectly to the safety, effectiveness and acceptability of the product. Due to advancement in the chemical knowledge of crude drugs various methods like botanical, chemical, spectroscopic and biological methods are used for estimating active constituents present in the crude drugs. Standardization methods should take into consideration all aspects contributing to the quality of the herbal drugs.

The development of modern analytical tools in testing the various quality parameters for an effective quality control herbal product cannot be over emphasized. The assurance of the safety and efficacy of a herbal drug requires monitoring of the quality of the product from collection through processing to the finished packaged product.

It is recommended that various government agencies should follow a more universal approach to herbal quality by adopting the WHO guidelines and also developing monographs using the various quality parameters outlined above. This will strengthen the regulatory process and minimize quality breach.

ACKNOWLEDGEMENT: The authors thankful with our deepest core of heart to Dr. Shashi Alok (Assistant Professor), for his valuable guidance.

CONFLICTS OF INTEREST: Nil.

REFERENCES:

1. Information available on Web portal ministry of AYUSH, Government of India , www.indianmedicnes.nic.in
2. Dixit VK and Yadav NP: Recent approaches in herbal drug standardization. Integr Biol, 2008; 2 (3): 195-203.
3. Chanchal DK, Niranjan P, Alok S, Kulshreshtha S, Dongray A and Dwivedi S: A Brief Review on Medicinal Plant and Screening Method of Antilithiatic Avtivity. Int J Pharmacognosy 2016; 3(1): 1-9:.doi link: http://dx.doi.org/ 1013040/IJPSR.0975-8232.IJP.3(1).1-9.
4. Calixto JB and Barz J: Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents), Med Biol Res, 2000; 33: 179-189.
5. Schulz V, Hänsel R and Tyler VE: Rational Phytotherapy. A Physicians' Guide to Herbal Medicine. 3rd edn. Springer-Verlag, Berlin.1996.
6. Neeli Rose Ekka: Standardization Strategies for Herbal Drugs-An Overview Research J. Pharm. and Tech. 2008; 1(4): 310-312.
7. Swapnil G, Patil. Standard Tools for Evaluation of Herbal Drugs: An Overview, the Pharma Innovation - Journal. 2013; 2(9):60.
8. Yi-Zeng Liang, Peishan Xie and Kelvin Chan, Journal of Chromatography, December 2004:812(1-2): 53-70.
9. Kokate CK, Gokhale SB and Purohit AP: Pharmacognosy, Nirali Prakashan, Pune. 2010, I, II.
10. Ansari SH: Essentials of Pharmacognosy, Birla Publications Pvt Ltd, 2011.
11. Gupta MK and Sharma PK: Test Book of Pharmacognosy, Ayurvedic formulations, Pragati Prakashan Meerut Vol II, Ist edition. 2007.
12. Indian Pharmacopoeia 2010; I: 198-201.
13. Swapnil G, Patil. Standard Tools for Evaluation of Herbal Drugs: An Overview, The Pharma Innovation –Journal. 2013; 2(9):60.
14. WHO. Quality Control Methods for Medicinal Plant Materials. World Health. 1988.
15. Organization, Geneva, Archana Gautam. Identification, evaluation and standardization of herbal drugs: A review, Der Pharmacia Lettre, 2010; 2(6): 302-315.
16. The 2002 United States Pharmacopoeia and National Formulary, USP 25 NF 19/National Formulary 20, Rockville, MD, U. S. Pharmacopoeial Convention, Inc. 2002.
17. Pharmacopoeia of the People's Republic of China, English ed., The Pharmacopoeia Commission of PRC, Beijing. 2000.
18. WHO Guidelines on Good Agricultural and Collection Practices (GACP). World Health Organization, Geneva. 2003.

19. WHO. Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. World Health Organization, Geneva, 2004.
20. Choudhary N and Sekhon BS: An overview of advances in the standardization of herbal drugs, J Pharm Educ Res. 2011; 2(2).
21. Swapnil G and Patil: Standard Tools for Evaluation of Herbal Drugs: An Overview, The Pharma Innovation - Journal. 2013; 2(9):60.
22. Liang YZ, Xie P and Chan KJ: Quality control of herbal medicines, Chromatogr B, 2004; 812: 53–70.
23. Ong ES: Chemical assay of glycyrrhizin in medicinal plants by pressurized liquid extraction (PLE) with capillary zone electrophoresis (CZE). J Sep Sci, 2002; 25: 825-831.
24. Soni K and Naved T: HPTLC- Its applications in herbal drug industry. The Pharma Review 2010, 112-117.
25. Jianga Y, David B, Tu P and Barbin Y. Recent analytical approaches in quality control of traditional Chinese medicines-A review, Anal. Chim. Acta 2010; 657: 9–18.
26. Thoppil SO, Cardoza RM, and Amin PD: Stability indicating HPTLC determination of Trimetazidine as bulk drug and in pharmaceutical formulations, J. Pharm. Biomed. Anal, 20011; 25(1): 5-20.
27. Dhalwal K, Sindhe V.M, Biradar YS and Mahadik KR: Simultaneous quantification of bergenin, catechine, and gallic acid from *Bergenia ciliate* and *Bergenia lingulata* by using thin-layer chromatography, J. Food. Comp. Anal, 2008; 21: 496-500.
28. Chimezie A, Ibukun A, Teddy E and Francis O: HPLC analysis of nicotinamide, pyridoxine, riboflavin and thiamin in some selected food products in Nigeria. Afr J Pharm Pharmacol 2008; 2(2):29-36.
29. Rao Udaykumar B and Anna NP: Stability- indicating HPLC method for the determination of efavirenz in bulk drug and in pharmaceutical dosage form. Afr J Pharm Pharmacol 2009; 3(12):643-650.
30. Bhutani KK: Herbal medicines enigma and a challenge for science and guidelines for new initiatives. J Nat Prod 2003; 19(1): 3-8.
31. Brandt A, Schering AG and Kueppers S: Practical Aspects of Preparative HPLC in Pharmaceutical and Development Production. (www.lcgceurope.com), 2002; 2-5.
32. Marston A: Role of advances in chromatographic techniques in phytochemistry. Phytochem, 2002; 68: 2785-2797.
33. Mike Lee S and Edward Kerns H: LC/MS applications in drug development. Milestone Development Services, Pennington, New Jersey, 24 July 1999.
34. Patil PS and Rajani S: An advancement of analytical techniques in herbal research. J Adv Sci Res 2010; 1(1):8-14.
35. Guo FQ, Huang LF, Zhou SY, Zhang TM and Liang YZ: Comparison of the volatile compounds of Atractylodes medicinal plants by headspace solid-phase micro extraction-gas chromatography–mass spectrometry. Anal. Chim. Acta, 2006; 570: 73-78.
36. Teo CC, Tan SN, Yong JWH, Hewb CS and Ong ES: Evaluation of the extraction efficiency of thermally labile bioactive compounds in *Gastrodia elata* Blume by pressurized hot water extraction and microwave assisted extraction. J. Chromatogr. 2008; A 1182: 34– 40.
37. Sharma, Handbook of Thin Layer Chromatography. Chromatographic Science Series, Marcel Dekker, Inc, New York Press; 55: 353-387.
38. Patra KC, Pareta SK, Harwansh RK and Kumar KJ: Traditional Approaches towards Standardization of Herbal Medicines. Journal of Pharmaceutical Science and Technology 2010; 2(11):372-379.
39. Shikha S and Mishra N: Genetic markers - a cutting-edge technology in herbal drug research. J Chem Pharm Res 2009; 1:1-18.
40. Breithaupt H: Back to the roots EMBO Rep, 2003; 4(1): 10-12.
41. Cai ZH, Li P, Dong TT and Tsim KW: Molecular diversity of 5S-rRNA spacer domain in Fritillaria species revealed by PCR analysis., Planta Med 1999, 65: 360-64.
42. Mihalov JJ, Marderosian AD and Pierc JC: DNA identification of commercial ginseng samples, 2000.
43. Lazarowych NJ, Pekos P. The use of fingerprint and marker compounds for identification and standardization of botanical drugs. J Drug Inform 1998; 32: 497-512.
44. Gomez RL, Olsen HG, Lingaas F, Klungland H, Våge DI, Olsaker I, Talle SB, Aasland M and Lien S. The Use of Genetic Markers to Measure Genomic Response to Selection in Livestock. Genetics. 2002; 162: 1381-1388.
45. Derived intervention levels for radionuclides in food. Guidelines for application after wide spread radioactive contamination resulting from a major radiation accident. Geneva, World Health Organization, 1988.
46. Archana A and Khle BA, Sytanderdization of herbal drugs a overview, International Research Journal of Pharmacy. 2011; 2(12):56-60.
47. AOAC. Official Methods of Analysis of AOAC International, 18th edn. AOAC International, Gaithersburg, MD, 2005.
48. PAGM DS, Keller K, Hansel R and Chandler RF: Aristolochia species In: Adverse Effects of Herbal Drugs, Springer-Verlag, Heidelberg, 1992.
49. Patel P, Patel NM, Patel PM, WHO. Guidelines on Quality control of herbal medicine, IJRAP, 2011; 2(4):1148-1154.
50. Pesticides Residue in Food-Method of analysis and sampling codex Alimentarius. Part 1, 2nd edition Rome, Joint FAO/WHO Food Standards Programme, 2000; 2.
51. WHO. Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, 1998a.
52. Watson DG. Pharmaceutical Analysis. Churchill Livingstone, Edinburgh, 1999.
53. Sarmani SB, Abugssa I, Hamzah A, Yahya MD. Biol. Trace Elem Res. 1999; 71-72:365.
54. WHO. Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. World Health Organization, 2007.
55. Sachan AK, Sachan NK, Kumar S, Sachan A, Gangwar SS: European journal of scientific research, 2010; 46 (2): 194-203.
56. Physical and Physicochemical Methods, European Pharmacopoeia, 6th edition, Chapter 2.2
57. Nikhat S and Fazil M: International Journal of scientific research and management, 2013; 1(8): 415- 420.
58. Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances (CPMP/ICH/367/96).
59. Reflection paper on stability testing of herbal medicinal products and traditional herbal medicinal products (Ref.EMA/HMPC/3626/2009, adopted 21 October 2010).
60. Bajaj S, Singla D, Sakhuja N: Journal of Applied Pharmaceutical Science, 2012; 02(03): 129-138.
61. Sachan NK, Pushkar S, Sachan AK and Ghosh SK: Asian journal of chemistry, 2013; 25(11): 5930-5934.
62. Basar SU, Rani S and Zaman R: Journal of pharmaceutical and scientific innovation, 2013; 2(4): 1-8.
63. Gafner S and Bergeron C. Current pharmaceutical analysis, 2005; 1(2): 203-215.

How to cite this article:
Bijauliya RK, Alok S, Chanchal DK and Kumar M: A comprehensive review on standardization of herbal drugs. Int J Pharm Sci Res 2017; 8(9): 3663-77. doi: 10.13040/IJPSR.0975-8232.8(9).3663-77.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)