Review Article

www.ijrap.net

REVIEW ON STANDARDIZATION OF HERBAL CHURNA

Nitin V. Kokare[1]*, Kiran A. Wadkar[2], Manish S. Kondawar[1]
[1]Department of Quality Assurance, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India
[2]Department of Pharmacognosy and Phytochemistry, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India

Received on: 13/04/14 Revised on: 06/05/14 Accepted on: 24/05/14

*Corresponding author
Nitin V. Kokare, Department of Quality Assurance, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India
E-mail: nitinkokare111@gmail.com
DOI: 10.7897/2277-4343.05382

ABSTRACT
In the last some years, the use of herbal drugs has been increased all over the world due to their huge therapeutic effect and less adverse effects as compared to other medicines. The rising use of herbal drug by the human is forcing the driving force to evaluate the health claim of these agents and to develop standards of quality, purity, safety and efficacy of the drug. Mostly herbal drugs are effective but due to adulteration and lack of standardization, the effectiveness of the herbal drug is decreased. So there is need of development of standardization parameters. In the standardization of the herbal drug the physical, chemical, biological, analytical parameters are carried out. It assures the quality, purity and safety of herbal drug.
Keywords: Standardization, herbal churna, standardization parameter.

INTRODUCTION

Herbal medicine has become a popular form of healthcare. The consumption of plant-based medicines and other botanicals in the west has increased in recent years. For example, between 1990-2000, an increase of over 380 % in sales in the United States was recorded. A global sale of herbal products, including herbal medicines, is already over USD 1011 and is expected to exceed USD 1012 in the next 20 years at the present growth rate. Standardization of herbal formulation is essential in order to assess the quality drugs, based on the concentration of their active principles, physical, chemical, phyto-chemical, and standardization, *In-vitro* and *In-vivo* parameters[1]. The quality assessment of herbal formulation is of vital importance in order to justify their acceptability in modern system of medicine. Methods of standardization should take in to consideration all aspect that contribute to the quality of the herbal drugs, namely authentication 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[2]. The fingerprint serves as guideline to phytochemical profile of the drug in ensuring the quality, while quantification of marker compound would serve as an additional parameter in assessing the quality of sample. In evaluation of quality of the herbal formulation chromatographic methods are also used. The methods like an HPTLC, HPLC and LC-MS, GC-MS method for standardization of plant material with reference to marker compound are of utmost importance. For standardization of herbal formulation WHO sets guidelines for herbal formulation[3].

Parameters used for Standardization of Herbal Churna

- Physical parameters include colour, odour, appearance, fluorescence analysis, ash value, crude fiber, moisture content, extractive value, swelling index, foaming index and pH value, angle of repose and flow properties and bulk and tap density, determination of tannins[4].
- Chemical parameters include limit tests, chemical tests etc.
- Chromatographic analysis done using TLC, HPLC, HPTLC, GC, UV, GC-MS, fluorimetry etc.
- Microbial parameters and their count, detection of Aflatoxin[5].

Table 1: Types of evaluation

Methods	Evaluation parameter
Authentication	Parts of plants collection like leaf, flower, root, stolen Regional status, Family Biological source Chemical constituents
Morphology or organoleptic evaluation	Odour, Taste, Size, Shape Special feature
Microscopy evaluation	Leaf content, Trichomes Stomata, Quantitative microscopy
Chemical evaluation	Chemical test, Chemical assay Phytochemical assay
Physical evaluation	Moisture content, Viscosity Melting point, Solubility Optical rotation, Refractive index Ash values, Extractive value Volatile oil content Foreign matter etc.
Biological evaluation	Microbial content, Pesticide contamination

Authentication

In India, two governments organizations support for authentication of plants, first is central council for research in unani medicine (CCRUM) and central council for research in Ayurvedic medicine. To ensure the quality of herbal medicines, the government of India has notified Good Manufacturing Practices (GMP) under schedule 'T' of the drugs and cosmetics act 1940. The guidelines of Good Agricultural Practices (GAP) seek to lay down a cultivation programmed designed to ensure optimal yield in terms of both quality and quantity of any crop intended for health purposes. Some institutes for authentication of plants are cited below[5].

National institutes

National Institute of Homeopathy (NIH),
National Institute of Ayurveda (NIA),
National Institute of Unani medicine (NIUM),
National Institute of Naturopathy (NIN),
National Institute of Siddha (NIS),
Institute of Post-Graduate Training and Research in Ayurveda (IPGTRA),
Rashtriya Ayurved Vidyapeeth (RAV),
Morarji Desai National Institute of Yoga (MDNTY)

Name of institutes

Central Council for Research in Ayurveda and Siddha (CCRAS),
Central Council for Research in Unani Medicine (CCRUM),
Central Council for Research in Homeopathy (CCRH),
Central Council for Research in Yoga and Naturopathy (CCRYN),
Central council for Indian Medicine (CCIM),

Laboratories

Pharmacopoeial Laboratory for Indian Medicine (PLIM),
Homeopathy Pharmacopoeia Laboratory (HPL)

Evaluation Parameter of Herbal Drugs

Macroscopic Evaluation

In these methods, description, general condition of the drug like size, shape outer surface and inner surface are referred. A sensory or organoleptic character describes colour, odour, taste, consistency. The fractured surface in cinchona, quillia and cascara barks and quassia wood are important characteristics. Aromatic odour of umbelliferous fruits and sweet taste of liquorice are the example of this evaluation. The ovoid tears of gum acacia, ribbon shaped characterizes of tragacanth, disc shaped structure of nux- vomica and conical shape of aconite quills of cinchona are some of the diagnostic macroscopic characters[4,5].

Microscopic Evaluation

The inner pseudo parenchyma cells are oval or rounded and they contain fixed oil and protein. The whole tissue is devoid of cellulose and lignin. Various parameters includes in microscopy are[5]:

- Leaf content
- Trichomes
- Stomata

Determination of leaf content

This include parameter like stomatal number, stomatal index, vein islet number, vein termination number determined by standard methods[6-8]. For e.g. - Indian senna (*Cassia augustifolia*)

Table 2: Quantitative microscopic parameters

Parameter	No.
Vein islet no.	19.5 to 22.5
Stomatal index	17 to 20
Palisade ratio	7.5 (upper epidermis) 5.1 (lower epidermis)

Types of trichomes

Table 3: Types of trichomes

Types of trichomes	Sub type of trichomes	Examples of plants
Covering trichomes	Unicellular trichomes	*Nux vomica*, cannabis
	Multicelluar unbranched trichomes	
	Uniseriate	Datura
	Biseriate	*Calendula officinalis*
	Multiseriate	Male fern
	Multicellular branched trichomes	*Verbascum thapsus*
Glandular trichomes	Unicellular glandular trichomes	Vasaka
	Multicellular glandular trichomes	*Digitalis purpurea*
Hydathoda trichomes	-	*Piper betal*[6-8]

Stomatal number and stomatal index study

There are several types of stomata, distinguished by the forms and arrangements of the surrounding cells[6-8].

Table 4: Types of stomata

Anomocytic	Irregular-celled
Anisocytic	Unequal-celled
Diacytic	Cross-celled
Paracytic	Parallel-celled

Determination of Stomatal Index

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells including the stoma being counted as one cell. Place leaf fragment of about 5×5 mm in size in a test tube containing about 5 ml chloral hydrate[6-7]

$$\text{Stomatal index} = S/E+S \times 100$$

Where: S = Total number of stomata in a given area of leaf,
E = Number of epidermal cells (including trichomes) in the same area of leaf

Physical Evaluation

Determination of foreign matter

Drugs should be free from moulds, insects, animal, fecal matter and other contamination such as earth stones and extraneous matters[4] .

Determination of total ash

The residue remaining after incineration is the ash content of drugs, which simply represents inorganic salts,

naturally occurring in drugs or adhering added to it as form adulteration. It uses to detect the contamination and adulteration like sand or earth, unwanted part mixed in crude drug[5-7,9].

Type: Total ash, Physiological ash, Non-physiological ash, carbonated ash, Sulphated ash, Nitrate ash, Acid soluble and Water soluble ash.

Method

Total ash

Place about 2-4 g of grounded material (as mentioned in monograph of drug) in crucible and ignited it until it is white.

Acid insoluble ash

Residue obtained after extracting the total ash treated with hydrochloric acid (HCL). Use to detect the contamination from sand or soil. Boil ash with 25 ml of 2M HCL for 5 minutes, collect the residue on ashless filter paper, wash with hot water, ignite cool in desiccators and weigh.

Water soluble ash

Water soluble ash is that part of the total ash content which is soluble in water. It is good indicator of either previous extraction of the water soluble salt in the drug or incorrect preparation. Boil the total ash for 5 minutes in 25 ml distilled water. Collect the insoluble residue on ashless filter paper, wash with hot water, ignite and cool in desiccators and weigh. Subtract the weight of insoluble matter from the weight of total ash gives water soluble ash value[4,5].

Extractive Value Determination

Extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents in that particular solvent depends upon the nature of the drug and solvent used. Extractive value determined the amount of active constituents in a given amount of medicinal plant material when extracted with solvent. It is employed for that material for which no chemical or biological assay method exist. As mentioned in IP – 1996, BP – 1980, the determination of water soluble and alcohol soluble extractives is used as a means of evaluating crude drug which are not readily estimated by other means[5].

Determination of alcohol soluble extractive

5 g of the air dried drug, coarsely powdered drug need to be macerated with 100 ml of ethanol of specified strength in a closed flask for 24 h, shaking frequently during the first 6 hours and allowing standing for 18 h. Filter and evaporates 25 ml of the filtrate to dryness at 105^0C and weigh[4,5].

Determination of water soluble extractive

5 g of the air dried drug, coarsely powdered drug need to be macerated with 100 ml of water close flask for 24 h, shaking frequently during the first 6 h and allowing standing for 18 h. Filter and evaporates 25 ml of the filtrate to dryness at 105^0C and weigh[5,6].

Determination of Moisture Content

Moisture is expected component of crude drug, which must be eliminated as far as practicable. Drying of crude drug is important during collection of drug and is also important for preservation, preventing hydrolytic degradation of active constituents and for easy size reduction of crude drug. Excess moisture or insufficient drying is responsible for spoilage of drug due to growth of microbes. Therefore drying process should reduce the moisture content of drug below the critical level. Moisture content determination is used to check the total water content in given weight of crude drug. Excess moisture content in a crude drug sample suggests not only that the purchaser could be paying a high price due to unwanted water, but also that the drug has been incorrectly stored[5].

Method

Loss on drying, azeotropic distillation method and Karl Fischer method are used for moisture content determination in crude drug

Loss on drying

Required quantity of sample taken as mentioned in monograph of drug and dried in hot air oven at 105^0C to a constant weight. Difference in weight indicates the moisture content of drug[5].

Determination of pH

The pH value conventionally represents the acidity or alkalinity of an aqueous solution. In pharmacopoeial standards and limits of P^H have been provided for those pharmacopoeia substances in which pH as a measure of the hydrogen-ion activity is important from the standpoint of stability of physiological suitability. The determination is carried out at a temperature of 25^0C ± 2^0C, unless otherwise specified in the individual monograph[9,10].

Apparatus

The pH value of solution is determined potentiometrically by means of a glass electrode and a pH meter either of the digital or analogue type.

Refractive Index

When a ray passes through a one medium to another medium of different density, it is bent from original path. Thus, the ratio of velocity of light in vaccum to its velocity in a substance is termed as refractive index of the second medium. Depending upon purity, it's constant for a liquid and can be considered as one of its standardization parameter. Refractive index of a compound varies with the wavelength of the incident light, temperature and pressure[10]. Refractive indices of the following compound are for sodium light and a temperature 25^0C.

Table 5: Drugs refractive index

Herbal drugs	Refractive index
Arachis oil	1.4678-1.470
Caraway oil	1.4838-1.4858
Castor oil	1.4758-1.527
Clove oil	1.527-1.535

Volatile oil content

Pharmaceutical significance of aromatic drugs is due to their odorous principle that is volatile oil. Such crude drugs are standardized based on their volatile oil content.

Table 6: Volatile oil content

Drugs	Volatile oil content (% w/w)
Caraway not less than	2.5
Lemon peel not less than	2.5
Fennel not less than	1.4
Clove not less than	15
Dill not less than	2.5
Cardamom not less than	4.0

Pesticide residue

WHO and FAO (Food and Agricultural Organization) set limits of pesticides, which are usually present in the herbs. These pesticides are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, toxaphene and aldrin cause serious side effects in human beings if the crude drugs are mixed with these agents[5].

Determination of microbial content

Usually medicinal plants which contain and molds come from soil and atmosphere. Analysis of the limits of *E. coli* and molds clearly throws light towards the harvesting and production practices. The substance known as Aflatoxin produces serious side-effects if consumed along with the crude drugs.

Method

1 g of churna is dissolved in lactose broth and volume is adjusted to 100 ml with the same medium. About 10 ml of sample is transferred into 100 ml of Macconkey broth and incubated for 18-24 hours at 43-45°C. A subculture is prepared on a plate with Macconkey agar and incubated at 43-45°C for 18-24 hours. The growth of red, generally non-mucoid colonies of gram negative rods appearing as reddish zones indicates the presence of *E. coli* if not then it indicates the absence of *E. coli*[5,6].

Detection of Aflatoxin

Aflatoxin is a toxin from *Aspergillus flavus* and *Aspergillus parasiticus* having the chemical formula $C_{12}H_{12}O_6$ and may cause hepatica carcinoma in human being. The plant species may be contaminated with this toxin. The test for Aflatoxin as prescribed by WHO for the herbal drugs is designed to detect the presence of B1, B2, and G1, G2 which are the dangerous contaminants in any plant material of plant origin.

Method

Not less than 100 g of crude drug of plant origin is grinded to a moderately fine powder (sieve 355/180). 50 g of the powder material is mixed with 170 ml of methanol and 30 ml of water in a conical stopper flask and shaken vigorously using a mechanical device for 30 minutes. Through a medium porosity filter paper it is filtered. 100 ml of filtrate from the start of flow (filtrate - A) is collected. Otherwise first 50 ml of the filtrate is discarded and next 40 ml of the filtrate is to be collected (Filtrate - B). 100 ml filtrate transferred into another flask and 20 ml zinc acetate – aluminum chloride and 80 ml of water is added. It is stirred and allowed to stand for 5 minutes. 5 g of filter aids like diatomaceous earth are added, mixed and filtered through a medium porosity filter paper. First 50 ml of the filtrate need to be discarded and next 80 ml is to be collected (filtrate - C). Either filtrate B or C is transferred to a separating funnel and 40 ml of sodium chloride (100 g/ lit) and 25 ml of light petroleum ether are added and shaken for one minute. Allow the layers to separate and lower layer is transferred to a second separating funnel. It is extracted twice with 25 ml of dichloromethane and shaken for 1 minute. The layers are allowed to separate out and combined each of the lower layers in a 125 ml conical flask. Several boiling chips are added and are evaporated to dryness on a water bath. The residue obtained used for TLC. To the residue obtained above add 0.2 ml mixture of chloroform and acetonitrile (98:2) and close properly and shake vigorously until the residue is dissolved. The chromatographic detail as follows[5]

- Stationary phase – Silica Gel-G
- Mobile Phase – chloroform: acetone: 2-propanol (85:10:5)
- Std: - 2.5, 5, 7.5 and 10 µl Aflatoxin
- Visualization: - UV light at 365 nm. [Blue spot for Aflatoxin]

Radioactive Contamination

Microbial growth in herbals is usually avoided by irradiation. This process may sterilize the plant material but the radioactivity hazard should be taken into account. The radioactivity of the plant samples should be checked according to the guidelines of International Atomic Energy (IAE), Vienna and that of WHO[5,6].

Melting Point

In case of pure chemicals or phytochemicals, melting points are very sharp and constant. Since the crude drugs from animal or plant origin contain the mixed chemicals, they are described with certain range of melting point[6,10].

Table 7: Melting point range for few crude drugs

Drugs	Melting Point (°C)
Colophony	75-85
Kokum butter	39-42
Coca butter	30-33
Bees wax	52-65
Wool fat	34-44

Chromatography

- Thin layer chromatography
- High performance thin layer chromatography
- High performance liquid chromatography
- Ultra-violet spectroscopy

Thin Layer Chromatography (TLC)
Thin layer chromatography is particularly valuable for the qualitative determination. TLC is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of liquid. The adsorbent is relatively thin uniform layer of dry finely powdered material, applied to a glass, plate, are the most communally used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption depending on the particular types of support its preparation and its use with different solvent system[17]. E.g. Pippali churna

Stationary Phase: Silica gel 60 F254 of 0.2 mm
Mobile Phase: Toluene: ethyl acetate: formic acid (5: 15: 0.5)

High Performance Thin Layer Chromatography (HPTLC)
A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of curcumin both as a bulk drug and in formulations was developed and validated[16,17].

Curcumin determination by HPTLC
Method details
Mobile phase: Chloroform: methanol (9.25:0.75 v/v)
Stationary phase: Aluminum plates precoated with silica gel 60F- 254

High Performance Liquid Chromatography
Haritaki churna
HPLC system use
Waters chromatographic system consisting Waters 2695 separation module (quaternary pump) equipped with an auto injector and Waters 2998 photodiode array detector[13].
Column use: Thermo Scientific BDS HYPERSIL Phenyl reversed phase column (100 mm × 4.6 mm, 3 µm).
Mobile phase: 0.02 % triethyl amine aqueous pH 3.0 with ortho-phosphoric acid and acetonitrile.
Flow rate: The flow rate was 1.0 ml/min and aliquots of 10 µl were injected.

Ultra- violet Spectroscopy
Sitopaladi churna
Instrument use
UV- spectrophotometric
Std. Piperine solution preparation: Piperine (100 mg) was dissolved in methanol and volume was made up to 100 ml with methanol in volumetric flask. Two ml of this solution was diluted with methanol up to 100 ml in volumetric flask to give 20 µg/ml piperine solutions.

Preparation of piperine extract of Sitopaladi churna
Reflux the powder Sitopaladi churna (1 g) with 60 ml ethanol for 1 hour. Filter the extract and reflux the marc left with 40 ml of methanol for another 1 h, filter and combine the filtrate. Concentrate the methanol extract under vaccum till the semisolid mass is obtained. Dissolve the residue in 75 ml methanol and filter through sintered glass funnel (G-2) by vaccum filtration assembly. The filtrate was centrifuged at 2000 rpm for 20 minutes, the supernatant was collected in 100 ml volumetric flask and volume was made with methanol[15].

CONCLUSION
For the quality assured herbal product, the standardization is required. In standardization, the above mentioned parameters i.e. authenticity, biological parameter, chemical parameter, physical parameter and analytical profiling gives the quality assured herbal product. HPTLC tool is mostly used for identification of the compound. Other instrument like IR spectroscopy, UV-VIS spectroscopy, HPLC, Mass spectroscopy and NMR spectroscopy are also used in standardization.

REFERENCES

1. Mosihuzzaman M. protocols on safety, efficacy, standardization and documentation of herbal medicine, International Union of pure and Applied Chemistry 2008; 80(10): 2195-2230.
2. Rasheed A, Reddy S. A review on standardization of herbal formulation, International J of Phytotherapy 2012; 2(2): 74-88.
3. Nikam P, Jadhav A. Future trends in standardization of herbal drugs, J of Applied Pharmaceutical Sci 2012; 2(6): 38-44.
4. Gautam A, Sharma P. Identification, evaluation and standardization of herbal drugs: A Review, Scholars Research Library 2010; 22(6): 302-315.
5. Borhade P, Khandelwal K. Review on standardization of churn, World Journal of Pharmacy and Pharmaceutical Sciences 2012; 1(4): 1260-1274.
6. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 45th ed., Nirali Prakashan, Pune; 2006. p. 06.01-06.46.
7. Trease and Evans. Text Book of Pharmacognosy. 15, 126.
8. Khandelwal KR, Practical Pharmacognosy. 1sted, Nirali Publications, Pune; 1995. p. 88-90.
9. Mukherjee PK. Quality Control of Herbal Drug. 1sted, Business Horizons Pharmaceutical publishers, India, New Delhi; 2005. p. 185-215.
10. More HN, Hajare AA. Practical Physical Pharmacy. 3rded, Career Publication, Nasik; 2010. p. 126-131.
11. Yadav NP, Dixit VK. Recent approaches in herbal drug standardization. International Journal of Integrative Biology 2008; 2(3): 195.
12. Meena AK, Rao MM, Panda P, Yadav A, Singh U. Standardization of Ayurvedic Polyherbal Formulation, Pancasama Churna. International Journal of Pharmacognosy and Phytochemical Research 2010; 2(1): 11-14.
13. Mahajan AD, Pai NR, Development and Validation of HPLC Method for Quantification of Phyto constituents in Haritaki Churna. International Journal of Chem. Tech Research 2011; 3(1): 329-336.
14. Panda RK, Dash NC. Standardization of Pippali churna-an Ayurvedic Medicine. International Journal of Pharma World Research 2011; 2(3): 1-8.
15. Gupta V, Jain UK. Quantitative analysis of piperine in Ayurvedic formulation by UV Spectrophotometry. International Journal of Pharma Sciences and Research 2011; 2(2): 58-61.
16. Ansari MJ. Stability-indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations, Journal of Pharmaceutical and Biomedical Analysis 2005; 39: 132-138. http://dx.doi.org/10.1016/j.jpba.2005.03.021
17. Bansal Y, Bansal G. Analytical methods for standardization of *Aegle marmelos*: A review, J Pharm Educ Res 2011; 2(2).

Cite this article as:
Nitin V. Kokare, Kiran A. Wadkar, Manish S. Kondawar. Review on standardization of Herbal churna. Int. J. Res. Ayurveda Pharm. 2014;5(3):397-401 http://dx.doi.org/10.7897/2277-4343.05382

Source of support: Nil, Conflict of interest: None Declared