International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 869-882 www.ijprajournal.com ISSN: 2456-4494

Standardization Techniques of Herbal Medicines

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Date of Submission: 10-05-2024 Date of Acceptance: 20-05-2024

ABSTRACT: Herb is a plant or part of a plant valued for its medicinal, aromatic, or savoury qualities.They are eaten, swallowed, drunk, inhaled, or applied topically to the skin Herbal products often contain a variety of naturally occurring biochemicals from plants, many of which contribute to the plant's medicinal benefits. Chemicals known to have medicinal benefits are referred to as "active ingredients" or "active principles" and their presence depends on a number of factors including the plant species, the time and season of harvest, the type of soil, the way the herb is prepared, etc.

KEYWORDS: chromatography, crude drug, extraction, standardization, botanical product, pharmacogenetics, toxicology

I. INTRODUCTION

According to WHO, standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquityHerbal drug:

Finished label products that contain active ingredients such as aerial and underground parts of plant or other plants material

The term "herbal drugs" denoted by means of plant or part of plants that have been converted into phytopharmaceuticals by simply means of processes involving collection or harvesting, drying and storingHerbal drugs are of two types:

i. Single/ crude drug
ii. Multiple herbal formulations

Single /crude drugs:

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

Multiple herbal formulations:

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

A. ADVANTAGES OF HERBAL MEDICINES-

a)Herbal medicines are very cheap in comparison to the conventional form of medication. It's something which every pocket can afford, unlike other forms of medication which can create a big hole in your wallet.

b)Herbal medicines can be consumed without the aid of any kind of prescription. They can be found very easily from a local drug store.

c)Herbal medicines are known to be more productive in comparison to other forms of medication in curing certain conditions. Unless mixed with other chemical components, they are known to be all natural.

d)One of the greatest benefits associated with herbal medicine is the non-existence of side effects. Also, they tend to offer long lasting benefits in terms of overall wellness

B.DISADVANTAGES OF HERBAL MEDICINES

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a)The government does not approve of any kind of herbal medication. It's usually consumed upon the person's own risk, and when it comes to branded herbal supplements, one can't expect any kind of quality assurance.
b)Admits all the advantages and disadvantages, there is no denying to the fact that the merits of herbal medicines overpower the demerits. It's always advisable to seek help from a good practitioner of herbal medicines to make the most of it.
c)Herbal medicines are known to be ineffective against serious ailments. Herbal medication cannot cure a broken hand, nor is it able to deal with heart attack related issues as effectively as a conventional doctor.

Concept and scope

Generally, all medicines, whether they are synthetic or of plant origin, should fulfil the basic requirements of being safe and effective (EMEA, 2005; WHO, 2002c, 1998c, 1996, 1991a,b, 1990, 1988).Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine.

Presently it is very difficult to identify the presences of all the ingredients as claimed in a formulation. Hence the first important task is to evolve such parameter by which the presence of the entire ingredient can be identified, various chromatographic and spectrophotometric methods and evaluation of physicochemical properties can be tried to evolve pattern for identifying the presence of different ingredient.

II. NEEDS AND OBJECTIVES

1.The lack of quality standards has resulted in mild to serious adverse effects ranging from hepatic toxicity to death. Hence, herbal ingredients require tools for determining identity, purity and quality and tools have to be technically sufficient, rapid and cost effective with GMP requirements.
2.World health organization has set specific guidelines for the assessment of safety, efficacy and quality of herbal medicines. Standardization of herbal drug is not an easy task as numerous factors influence the bio efficacy, reproducible therapeutic effect.
3.In order to obtain quality oriented herbal product care should be taken right from the proper identification of plants, season, area of collection, their extraction and purification and rationalizing the combination in case of polyherbal drugs.
4.In almost all the traditional system of medicine, the quality control aspect has been considered from its inspection of its Rishis, Vaidya's and Hakims. Unlike in olden times where traditional practitioners prepared and tested the qualities of herbal medicines, the problem faced today are these of economics of industrial scale production, shelf life and distribution to long distances

III. STANDARDIZATION OF HERBAL MEDICINES:

The herbal raw material prone to a lot of variation in phytoconstituents due to several factors different places of collection (indigenous and naturalized plants), time and season of collection, different environmental conditions, (primary causes like light, moisture, temperature, oxygen etc. secondary causes as involvement of living organisms like bacteria, Molds, mites, nematodes, worms, insects etc.), genotypic and variation, presence of xenobiotic (foreign chemical substances there is a need of quality control tests for crude drug or medicine to ensure quality .In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection, their extraction and purification process.

METHOD OF STANDARDIZATION

1.Authenticated raw material is the basic starting point in developing a botanical product. In addition, each step of harvest, storage, processing and formulation may dramatically alter the quality and consistency of final product. Therefore, methods to ensure quality control in manufacturing and storage are requisite tools to ensure optimal efficacy and safety of these products. Furthermore, such controls are critical for the evaluation of pharmacological, toxicological or clinical studies involving botanical products. Authentication is especially useful in cases of drugs that are frequently substituted or adulterated with other varieties which are morphologically and chemically indistinguishable. Several herbal drugs in the market still cannot be identified or authenticated based on their morphological or histological characteristics. Use of

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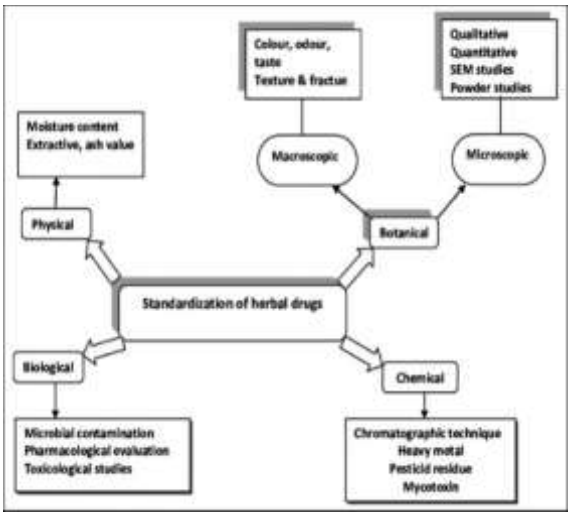
Volume 9, Issue 3 May-June 2024, pp: 869-882 www.ijprajournal.com ISSN: 2456-4494

wrong drugs may be ineffective or it may worsen the condition.

2.According to WHO (1996a and b, 1992), standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion.



A)PHYSICAL -

1. Foreign organic matter: This involves removal of matter other than source plant to get the drug in pure form.

2. Ash values: These are criteria to judge the identity and purity of crude drug – Total ash, sulphated ash, water soluble ash and acid insoluble ash etc.

3. Moisture content: Checking moisture content helps reduce errors in the estimation of the actual weight of drug material. Low moisture suggests better stability against degradation of product.

4. Extractive values: These are indicative weights of the extractable chemical constituents of crude drug under different solvents environment.

a) Crude fibre: This helps to determine the woody material component, and it is a criterion for judging purity.

b) Qualitative chemical evaluation: This covers identification and characterization of crude drug with respect to phytochemical constituent. It employs different analytical technique to detect and isolate the active constituents.

c)Chromatographic examination: Include identification of crude drug based on the use of major chemical constituents as markers.

d)Quantitative chemical evaluation: To estimate the amount of the major classes of constituents.

e) Toxicological studies: This helps to determine the pesticide residues, potentially toxic elements, safety studies in animals like LD50 and Microbial assay to establish the absence or presence of potentially harmful microorganisms. The processes mentioned above involves wide array of scientific investigations, which include physical, chemical and biological evaluation employing various analytical methods and tools. The specific aims of such investigation in assuring herbal quality are as varied as the processes employed.

f) Organoleptic or macroscopic evaluation: Organic evaluation of drugs by means of organs of sense (skin, eye, tongue, nose, and ear) or microscopic evaluation which include evaluation of drugs by colour, Odor, taste, size, shape, and special feature, like touch, texture, etc. it is the technique of qualitative evaluation based on the study of morphological and sensory profile of whole drugs. The fractured surfaces in cinchona, quillaia, and cascara barks and quassia wood are important characteristics. Aromatic Odor of umbelliferous fruits and sweet taste of liquorices are the examples of this type of evaluation where Odor of drugs depends upon the type and quality of odorous principles (volatile oils) present.

g) microscopicevaluation: It involves detailed examination of the drugs and it can be used to identify the organized drugs by their known histological characters. It is mostly used for qualitative evaluation of organized crude drugs in entire and power forms with help of microscopic. Using microscope detecting various cellular tissues, trichomes, stomata, starch granules, calcium oxalate crystals and aleuronic grains are some of important parameters which play important role in identification of certain crude drugs standardization Starch and hemicelluloses is identified by blue colour with iodine solution.

h) Chemical evaluation: Most of drugs have definite chemical constituents to which their biological or pharmacological activity is attributed. Qualitative chemical test is used to identify certain drug or to test their purity. Isolation, purification, identification of active constituents is based on chemical methods of evaluation.

i) Physical evaluation: Physical constants are sometimes taken into consideration to evaluate certain drugs. These include moisture content,

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Volume 9, Issue 3 May-June 2024, pp: 869-882 www.ijprajournal.com ISSN: 2456-4494

specific gravity, optical rotation, refractive, melting point, viscosity and solubility in different solvents. All these physical properties are useful in identification and detecting of constituents present in plants.
j) biological evaluation: Some drugs have specific biological and pharmacological activity which is utilized for their evaluation. Actually, this activity is due to specific type of constituents present in the plant extract. For evaluation the experiments were carried out on both intact and isolation organs of living animals. With the help of bioassays, strength of drug in its preparation can be evaluated

B. BOTANICAL –
1. Qualitative aspects of microscopic studies
I.Stomata
There are several types of stomata, distinguished by the forms and arrangement of the Surrounding cells, e.g.
(a)Anomocytic (Ranunculaceous) irregular celled: Digitalis.
(b)Anisoytic (Cruciferous) unequal – celled: Datura.
(c)Diacytic (Caryophyllaceous) cross – celled: Mentha.
(d)Parasitic (Rubiaceous) parallel celled: Senna

II.Trichomes
Trichomes are divided and subdivided as follows
(i)Covering trichome
(a)Unicellular trichomes: Nux vomica, Cannabis sativa.
(b)Uniseriate multicellular unbranched trichomes: Datura.
(c)Biseriate multicellular unbranched trichomes: Calendulaofficinalis
(d)Multiseriate multicellular unbranched trichomes: Male fern.
(e)Multicellular branched trichomes: VerbascumThapsus.

(ii)Glandular Trichomes
(a)Unicellular glandular trichomes: Justiciaadhatoda.
(b)Multicellular glandular trichomes: Digitalispurpurea.
(iii)Hydathode Trichomes: Piper beetle.

Quantitative aspects of microscopic studies
i.Palisade ratio
It is defined as average number of palisade cells beneath each epidermal cell. E.g. Atropa belladonna (6-10), Digitalis lanata (2.5-6.5).

ii.Stomatal No
It is defined as average number of stomata per square millimetre area of epidermis.
e.g.: Atropa belladonna: 6.0 to 14-37.5 (Upper Surface), 62.5 to 93-174 (lower Surface).

iii.Stomata index
It is the percentage which the number of stomata forms to the total number of epidermal cells. It is calculated by,
S.I.=S ×100 / (E+S).
Were,
S.I. = Stomatal Index; S= Number of stomata per unit area;
E= Number of Epidermal cells in the same unit area.
e.g.
Atropa belladonna: -2.3-3.9 to 10.5 (Upper Surface), 20.2 to 21.7- 23.0 (Lower Surface).

a. Vein islet number
It is defined as average number of Vein Islet per square millimetre of the leaf surface midway between midrib and the margin.
I.Digitalis lanata —2.0-8.0.
ii. Digitalis Purpurea —2.0-5.5.

b.Vein termination number
It is defined as average number of Vein terminations per square millimetre of the leaf surface midway between midrib and the margin.
ii.Atropa belladonna — 6.3-10.3.
iii.Atropa acuminata —1.4-3.5

Physicochemical parameter
1.Physicochemical test
The values of Physicochemical parameters of the individual drugs or the proprietary medicines can be compared with the standard values of pharmacopoeia and standardized. These tests are actually the Pharmacopeia standards for authenticity, quality and purity of herbal drugs and are as follows. Mostly used physicochemical methods are as follows.

2.Solubility
The presence of adulterant in a drug could be indicated by solubility studies identify by various solvents. e.g., pure soluble in carbon disulphide.

I. Alcohol
5 gm of powdered material along with 100 ml of alcohol are shaken well occasionally for the first 6 hours and kept undisturbed for 18 hours. The liquefied extract thus obtained was concentrated in a

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vacuum oven and the percentage was calculated with the weight of the drug powder taken.

ii.Water
The procedure adopted for solubility percentage of alcohol is used with chloroform water instead of alcohol to get the water solubility.

iii. Viscosity
Viscosity of a liquid is constant at a given temperature and is an index of its composition. So, it can be used as a means of liquid drugs standardization.[11]

3.Determination of moisture /Loss on drying procedure
•Weigh about 1.5g of the powdered drug into a weighed flat and thin Porcelain dish.
•Dry it in the oven at 100°C or105°C.
•Cool in desiccators and watch the loss in weight is usually recorded as moisture.

Note

A very useful form of dish for the determination of moisture and of ash is a thin flat porcelain dish. If a platinum dish available it may be used. The burning of the powder should proceed slowly and the material must not be allowed to catch fire or to give off smoke as dense fumes.

4.Refractive index

When a ray passes from a one medium to another of different density, it is bent from original path. Thus, the ratio of velocity of light in vacuum 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 consider as one of its standardizations. Refractive index of a compound varies with the wave length of the incident light, temperature and pressure.

Crude drugs	Refractive index
Arachishypogaea	1.467-1.470
Carumcarvi	1.4838-1.4858
Ricinuscommunis	1.475-1.527
syzygiumaromaticum	1.527-1.535

5.Volatile oil content

Pharmaceutical significance of aromatic drugs is due to their odorous principal that is volatile oils such crude drugs are standardized on basis of their volatile content

Aromatic drugs (w/w)	Volatile content
Carumcurvi	2.5
Citrus +limon	2.5
Syzyguimaromaticum	15
Foeniculum vulgar	1.4
Elettaria cardamomum	4.0

6.Melting point
pure chemicals or phytochemicals possess very sharp and constant melting point. Since the crude drugs from animal or plant origin contain the mixed chemicals, they are described with certain range of melting point.

Crude drug	description	Melting point
Colophony	Translucent brittle substance from pine	75-85
Kokum butter	Oil of kokum tree	39-42
Cocoa butter	Fat of Theobroma cocoa	30-33
Wool fat	Extracted from wool	34-44

7.Ash determination

After ignition the remaining material is known as ash. There are two types of ash one is the ash derived from plant tissues (physiological ash) and the other one is residue of extraneous matter adhering to plant surface (non-physiological ash)Ash is determined by following three methods.

a. Total ash
 Weight of residue obtained after ignition.
 About 2gm of powdered drug was weighed accurately into a tarred silica crucible. Incinerated at 450°C in a muffle furnace until free from carbon.
 The crucible was cooled and weighed.
 Percentage of total ash was calculated with reference to air- dried substance.
Determination of total ash value formula.
total ash value of the sample = 100 (z-x) % /y.
X= weight of empty dish.
Y= weight of the drug taken.
Z= weight of the dish + ash (after complete incineration).

b. Acid insoluble ash
Weight of residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the

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remaining insoluble matter. The method of analysis is as follows.
 Ash obtained from the total ash was boiled with 25ml of 2N HCl for a few minutes. Filtered through an ash less filter paper.
 The filter paper was transferred into a tarred silica crucible.
 Incinerated at450°C in a muffle furnace until free from carbon.
 The crucible was cooled and weighed.
 Percentage of acid insoluble ash was calculated with reference to air-dried substance.

c.Water soluble ash
Difference in the weight between the total ash and the residue after treatment of total ash with water the method is as follows
 Ash obtained from the total ash was boiled with 25 ml of distilled water for a few minutes and filtered through an ash less filter paper.
 The filter paper was transferred into a tarredsilica crucible.
 Incinerated at 450°C in a muffle furnace until free from carbon.
 The crucible was cooled and weighed.
 Percentage of water-soluble ash was calculated with reference to air-dried substance

8.Extractive value
Extractive value determines the number of active constituents extracted with solvent from a given amount of medicinal plant. It gives an idea about the nature of the chemical constituents
Total ash value of the sample = 100 (z-x) % /y.
X= weight of empty dish.
Y= weight of the drug taken.
Z= weight of the dish + ash (after complete incineration).

Acid insoluble ash
 Weight of residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. The method of analysis is as follows.
 Ash obtained from the total ash was boiled with 25ml of 2N HCl for a few minutes. Filtered through an ash less filter paper.
 The filter paper was transferred into a tarred silica crucible.
 Incinerated at 450°C in a muffle furnace until free from carbon.
 The crucible was cooled and weighed.
Percentage of acid insoluble ash was calculated with reference to air-dried substance

Determination of alcohol soluble extractive value
 About 5gms of air dried coarse powdered drug was weighed and macerated with 100ml of 90% alcohol in a closed flask for 24 hours, shaking frequently during the first 6 hrs. and these allowed standing for 18 hrs.
 Thereafter it was filtered rapidly taking precautions against loss of the solvent.
 25 ml of the filtrate was evaporated todryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed.
 The %age of the alcohol soluble extractive values was calculated with reference to the air-dried drug.

Chemical test	Reagents used	Results
Alkaloids		
Mayer	Potassium mercuric iodide solution	Creamy precipitate
Wagner	Iodine potassium solution	Brown precipitate
Hager	Saturated solution of picric acid	Yellow color
Dragendroff	Potassium bismuth iodide solution	Reddish brown precipitate
Amino acid		
Millons test	Millions reagent	White precipitate

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Determination of water-soluble extractive value

 About 5gm of air-dried powdered drug was taken & macerated with 100 ml of chloroform water in a closed flask for 24 hrs. Shaking frequently during the first 6 hrs. and then allowed to stand for 18 hrs.
 Thereafter, it was filtered rapidly taking precautions against loss of the solvent.
 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed.
 The percentage of the water-soluble extractive value was calculated with reference to the air-dried-up

9.CHEMICAL TESTS

The aims of these tests are as under.

 Preliminary (primary) testing for different chemical(functional)groups
 Quantification (% age) of chemical groups of interest (e.g. total alkaloids,
phenolics, triterpenes, tannins) or establishment of fingerprints.
 Multiple marker-based finger prints profiles (use of different marker compounds which indicate the % presence of more than one chemical group Qualitative chemical test such as acid value, saponification value etc. Some of these are useful in evaluation of resins (acid value, spatterdash), balsams (acid values, saponification values and ester values). volatile oils (acetyl and ester values) and gums (methoxy determination and volatile acidity).

Table no.- 4 – chemical tests

Ninhydrin	Ninhydrin solution	Violet color
Folin	Folin phenol reagent	Blue color
Pauly	Sulphanillic acid, sodium nitrite and sodium carbonate	Cherry red color
Carbohydrates		
Molisch	Alcoholic alpha naphthol+ sulphuric acid	Purple to violet color changes
Barfoed	Barfoed reagent	Red color
Selivanoff	Selivanoffs reagent	Rose color
Tests for pentoses	Hydrochloric acids phloroglucinol	Red color
Anthraquinone glycosides		
Borntrager	Borntrager reagent	Pink ammonical layer

Some important preliminary tests with their obtaining results and regents used.

Tannis		
Ferric chloride	Ferric chloride	Blue color
Flavonoids		
Alkaline reagent	10%sodium hydroxide solution	Intense yellow color
Ammonium hydroxide	10% ammonium hydro oxide	Yellow fluorescence
zinc	Zinc dust and conc. HCl	Red color

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Quantification of important chemical constituents (extraction / isolation, separation / fractionation, purification, identification of active compounds by using different chromatographic and spectroscopic techniques like.

1)Chemometric and Spectral methods

Initially the use of infrared (IR) spectroscopic method is restricted only for structural elucidation of isolated compounds from the herbal matrices. It is also found useful in phytochemical studies as a finger printing device for comparing a natural with synthetic sample. With the advance of computer technology, chemo metric method has become a leading tool among the scientific communities towards faster analysis and shorter product development time. Among others, an unsupervised pattern recognition technique such as Principal

Component Analysis (PCA) is the most often used method for handling multivariate data without prior knowledge about the study samples.

While the supervised classification procedure using Soft Independent Modeling of Class Analogy(SIMCA) based on making a PCA model to assign unknown samples into the predefine class model has also been applied to the analysis of infrared spectra. A study using FTIR transmission spectroscopy, associated with the appropriate chemo metric methods (PCA and SIMCA) was done to classify (well known as Java tea for treating infections of urinary tract, bladder and kidney based on its geographical origin and varieties from the obtained characteristics infrared spectrum. Chemo metric analysis of spectra is rapid and simple since no chemical treatment of samples is required.

2)Biological evaluation / Biological assays

Pharmacological activity of crude drugs has been applied to evaluate and standardize them. The assays on living animal and on their intact or isolated organs can indicate the strength of the drug or their preparations. Some drugs have specific biological and pharmacological activity which is utilized for their evaluation. Actually, this activity is due to specific type of constituents present in the plant extract. For evaluation the experiments were carried out on both intact and isolated organs of living animals. With the help of bioassays, strength of drug in its preparation can also be evaluated.

Pharmacological parameter

1. Determination of bitterness value

The bitterness properties of plant material are determined by comparing the threshold bitter concentration of an extract of the material with that of dilute solution of quinine hydrochloride. The bitterness value is expressed in units equivalent to the bitterness of solution containing 1gm of quinine chloride in 2000ml.The bitterness of plant material measures by taste. Bitter taste employed therapeutically. Bitterness stimulates secretions in the gastrointestinal tract especially of gastric juice.

2. Determination of swelling index

1gm of plant material dipped in water or swelling agent in glass stoppered measuring cylinder, the material is shaken repeatedly for 1hr and then allowed to stand for a required period of time. The volume of the mixture (ml) taken up by the swelling of plant material is then read. The mixing of whole plant material with the swelling agent is easier than pulverized or reduced plant material as it requires vigorous shaking at specific interval to ensure even distribution of material in swelling agent.

3. Determination of Foaming Index

Many plant materials cause persistent foam (due to the presence of saponins) when an aqueous decoction is shaken. The foaming ability is measured in terms of foaming index.

4. Hemolytic activity

The hemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of reference material, saponins.

5. Antibiotic activity

Some bacteria such as Salmonella typhi, Staphylococcus aureus and E. coli are used to determine the antiseptic value (the degree of antiseptic activity e.g. phenol co-efficient of certain drugs). The activity of antibiotics is also determined by using Klebsiella pneumonia, Micrococcus flatus, Sarcinaluteaetc. Living bacteria, yeast and molds are used to evaluate certain vitamins. Microbiological assays by cylinder plate method and turbid metric method are used in evaluation.

6. Antifertility activity

Antifertility drugs include contraceptives and abortificients. Contraceptive drugs are used to prevent pregnancy and abortifacient to terminate pregnancy. Female rats are used for antifertility activity i.e. measure the pregnancy rate (anti

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ovulation and anti-implantation) and male rats are used for aspermatogenic activity and spermicidal activity of herbal drugs.

7. Hypoglycemic activity

Rabbits, rats or mice are used to test hypoglycemic activity of plant extract. Radio-immunoassay (RIA) or Enzyme linked immunosorbent assay (ELISA) are done for measurement of insulin levels.

8. Neuropharmacological activity

Testing the herbal drugs with effects on central and autonomic nervous system. CNS acting drugs like cocaine, morphine (Papaver somniferous), cannabinol (Cannabis sativa) are tested using rodents. For testing the herbal drugs for their effects on ANS guinea pig ileum for antispasmodic activity, rabbit jejunum for adrenergic activity, rat phrenic nerve-diaphragm for muscle relaxant activity, frog rectus for skeletal muscles activity.

Toxicological parameter

a). Determination of pesticide residues

The food and agriculture organization of the United Nations (FAO) and WHO established limits of pesticide residue for safe consumption of food and animal feed. These pesticides are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, cause serious side effects in human beings, if the crude drugs are mixed with these agents.

b). Determination of microorganisms and aflatoxins

Microbial parameters, like total viable content of pathogenic bacteria like enter other gram-positive bacteria and presence of aflatoxins determine for safe herbal drug consumption.

Limits given in official books can be utilized as a quantitative or semi quantitative tool to control the number of purities coming from different steps of preparation, storage and preservation.

Chromatography Techniques:

I. TLC (Thin layer chromatography):

TLC was the most common, versatile methods of choice for herbal analysis before instrumental chromatography methods like gas chromatography and HPLC were established. Even now a day's TLC is still frequently used for the analysis of herbal medicines since various pharmacopeias such as Indian herbal pharmacopeia, Ayurvedic pharmacopeias, American herbal pharmacopeias, and Chinese drugs monographs. Rather TLC is used as an easier method of initial screening with a semi-qualitative evaluation together with other chromatography techniques as there is relative less change in the simple TLC separation of herbal medicines the with instrumental chromatography.

TLC is a technique in which solute undergoes distribution between two phases a stationary phase acting through adsorption and mobile phase in the form of liquid. The adsorbent is relatively thin, uniform layer of drug finely powdered material apply to glass, plastic, metal sheet/plate. Glass plates are the mostly commonly used. Separation may also be achieved on the basis of partition /a combination of partition and adsorptions depending upon the particular support its use with different solvent. Identification can be affected by observation of spots of identical Rf value and equal magnitude obtained, respectively with an unknown and a reference sample chromatography on the sample plate. A visual comparison of the size and the intensity of spots usually serve for semi-quantitative estimation. TLC has advantages of many folded possibilities of detecting in analysis herbal medicines. In addition, TLC is rather simple and can be employed for multiple sample analysis. For each plate more than 30 spots of sample can be studied. CA MAG video stored system and TLC QA-UV methods it's is possible to get useful qualitative and quantitative information from the developed TLC plates. For example, the four sample of cordyceps sinensis from that joint product of China and Japan co-operation has more valuable medicinal effect compared to other as they contain the most effective component "cordycepin" more over with the help of imagine analysis and digitized technique developed in computer science, evaluation of similarities between different samples is also possible.

TLC is being employed extensively for the following reasons:

* It enables rapid analysis of herbal extracts with minimum sample clean-up requirement.
* It provides qualitative and semi-quantitative information of the resolved compounds.

II.Gas Chromatography:

Gas chromatography also known as gas liquid chromatography, It is a technique for separation of mixtures of mixtures into components by a process which depends on the redistribution of the components between a stationary phase or the support material in the form of a liquid, solid or

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combination of both and a gaseous mobile phase. It is well-known that many pharmacologically active components in herbal medicines are volatile chemical compounds. Thus, the analysis of volatile compounds by gas chromatography is very important in the analysis of herbal medicines. The GC analysis of the volatile oils has a number of advantages. Firstly, the GC of the volatile oil gives a reasonable "finger print" which can be used to identify the plant. The composition and relative concentration of the organic compounds in the volatile oil are the characteristic of the particular plant and the presence of impurities in the volatile oil can be readily detectedfor this, it is necessary to use tedious sample work-up which may include derivatization. Therefore, the liquid chromatography becomes another necessary tool for us to apply the comprehensive analysis of herbal medicines

The first fully automated on-line GC-IR system was developed by Scott et al. Each eluted solute was adsorbed in a cooled packed tube, and then thermally regenerated into an infrared vapor cell. Subsequent to the IR spectrum being obtained, a small sample of the vapor was drawn from the IR cell into a low-resolution mass spectrometer and the mass spectrum was also be taken. As some of the bioactive constituents of herbal medicines are volatile, GC analysis can often be used for authentication and quality control. The high selectivity of capillary columns enables separation of many volatile compounds simultaneously within comparatively short times.

III. High-Performance Liquid Chromatography (HPLC):

High performance liquid chromatography is also known as high pressure liquid chromatography in which the stationary phases consist of small particle (3-50μm) packing contained in a column with a distribution bore (2-5μm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). The three forms of high-performance liquid chromatography most often used are ion exchange, partition and adsorption. HPLC is a popular method for the analysis of herbal medicines. Because it is easy to learn and use and is not limited by the volatile or stability of the sample compound. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Reversed-phase (RP) columns may be most popular columns used in the analytical separation of herbal medicines It is necessary to notice that the optimal separation condition for the HPLC involves many factors, such as the different compositions of the mobile phase, their pH adjustment, pump pressures, etc.

Thus, a good experiment design for the optimal separation seems in general necessary. In order to obtain better separation, some new techniques have been recently developed in research field of liquid chromatography.

IV.Chromatographic fingerprinting:

Chromatographic fingerprinting is the most powerful approach for the quality control of herbal medicines. Chromatographic fingerprint of Herbal Medicine is a chromatographic pattern produced from extract of some common chemical components which may be pharmacologically active or have some 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 herbal medicines investigated. This suggest that chromatographic fingerprint can successfully demonstrate both sameness and differences between various samples and the authentication and identification of herbal medicines can be accurately conducted even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples of herbal medicine.

Chromatographic fingerprinting can be carried out using techniques such as thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC) and other hyphenated techniques.

SOME ANCIENT METHODS FOR STANDARDISATION:

 Collection time: Rutu, i.e. some drugs are seasonal as well as some parts of herb should collect in specific Rutu.
 Desha: Availability of some drugs in specific area e.g. Himalaya, so geographical is important one.
 Nakshtra:Collection of some drugs should be done on specific Nakshtra. Indications for using dry drugs & wet drugs.

FACTORS AFFECTING ON HERBAL DRUG STANDARDIZATION:

a) Microscopic Evaluation:

Quality control of herbal drugs has traditionally been based on the appearance and today microscopic evaluation is indispensable in the initial identification of herbs, as well as, in

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identifying small fragments of crude or powdered herbs, and detection of foreign matter and adulterants. A primary visual evaluation, which seldom needs more than a simple magnifying lens, can be used to ensure that the plant is of the required species, and that the right part of the plant is being used. They should be entirely free from molds or insects, including excreta and visible contaminant such as sand and stones, poisonous and harmful foreign matter and chemical residues. Animal matters such as insects and "invisible" microbial contaminants, which can produce toxins, are also among the potential contaminants of herbal medicines.

b) MacroscopicExamination:

It can easily be employed to determine the presence of foreign matter, although, microscopy is indispensable in certain special cases (for example, starch deliberately added to "dilute" the plant material). Furthermore, when foreign matter consists, for example, of a chemical residue.

c)Heavy Metals:

Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such as mercury, lead, copper, cadmium, and arsenic in herbal remedies can be attributed to many causes, including environmental pollution, and can pose clinically relevant dangers for the health of the user and should therefore be limited. The potential intake of the toxic metal can be estimated on the basis of the level of its presence in the product and the recommended or estimated dosage of the product. This potential exposure can then be put into a toxicological perspective by comparison with the so-called Provisional Tolerable Weekly Intake values (PTWI) for toxic metals, which have been established by the Food and Agriculture Organization of the World Health Organization (FAO-WHO).

d)Microbial contaminants and aflatoxins:

Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi, and viruses. Inevitably, this microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations. Risk assessment of the microbial load of medicinal plants has therefore become an important subject in the establishment of modern Hazard Analysis and Critical Control Point (HACCP) schemes. Herbal drugs normally carry a number of bacteria and molds, often originating in the soil. Poor methods of harvesting, cleaning, drying, handling, and storage may also cause additional contamination, as may be the case with Escherichia coli or Salmonella spp. while a large range of bacteria and fungi are from naturally occurring microflora, aerobic spore-forming bacteria that frequently predominate.

e) Radioactive contamination:

Dangerous contamination, however, may be the consequence of nuclear accident. The WHO, in close cooperation with several other international organizations, has developed guidelines in the event of a wide spread contamination by radionuclide resulting from major nuclear accidents. These publications emphasize that the health risk, in general, due to radioactive contamination from naturally occurring radio nuclides is not a real concern, but those arising from major nuclear accidents such as the nuclear accident in Chernobyl and Fukushima may be serious and depend on the specific radionuclide, the level of contamination, and the quantity of the contaminant consumed. Taking into account the quantity of herbal medicine normally consumed by an individual, is unlikely to be a health risk. Therefore, at present, no limits are proposed for radioactive contamination. Validation: The validation of herbal products is a major public health concern both in developed and resource poor countries, where fakers selling adulterated herbal medicines are common. In this regard, there is no control by the government agencies, despite the existence of certain guidelines in some individual countries and those outlined by the WHO.

RECENT APPROACHES FOR STANDARDIZATION IN HERBAL MEDICINE-

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

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characteristic constituents are not exactly the same for different samples of this HM

2. 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.

3.SUPERCRITICALFLUID 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).

4. 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 (Shikha et al,2009). 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 (Breithaupt, 2003).

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)

A) ROLE OF GENETIC MARKER IN HERBAL DRUG TECHNOLOGY:

a) Genetic variation/genotyping:

It has been well documented that geographical conditions affect the active constituents of the medicinal plant and hence their activity profiles. Many researchers have studied geographical variation at the genetic level. Estimates of genetic diversity are also important in designing crop improvement programmes for management of germ plasm and evolving conservation strategies. RAPD-based molecular markers have been found to be useful in differentiating different accessions of neem collected from different geographical regions (Khanuja, 2002). Germplasm analysis to study genetic diversity is another important area in which a lot of efforts have been put in. Fingerprinting of crops like rice wheat, chickpea, pigeon pea, pearl milletetc is being carried out extensively (Khanuja, 2002; Ramakrishna et al., 1994) Authentication of medicinal plants DNA-based techniques have been widely used for authentication of plant species of medicinal importance.

b) Medicinal Plant Breeding –

ISSR–PCR has been found to be an efficient and reliable technique for the identification of zygotic plantlets in citrusinterploidy crosses. Molecular markers have been used as a tool to verify sexual and apomictic offspring of intraspecific crosses in Hypericonperforatum, a well-known anthelminthic and diuretic. An attempt has been made towards marker-assisted selection of fertile clones of garlic with the help of RAPD markers. RAPD markers have been successively used for selection of micropropagated plants of Piper longum for conservation.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 869-882 www.ijprajournal.com ISSN: 2456-4494

IV. RESULTS AND DISCUSSION

In field of drug research there is large scope for Ayurvedic Researchers, as India is the major country and can play the lead role in production of standardized, therapeutically effective Ayurveda formulation. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques. These guidelines for the assessment of herbal medicines are intended to facilitate the work of regulatory authorities, scientific bodies and industry in the development, assessment and registration of such products. The advancement of analytical techniques will serve as a rapid and specific tool in the herbal research, there- by, allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf- life of herbal drugs. The effective regulation and control of herbal medicines moving in international commerce also requires close liaison between nation- al institutions that are able to keep under regular review

V. SUMMARY AND CONCLUSION

SUMMARY

India can emerge as the major country and play the lead role in production of standardized, therapeutically effective herbal formulation. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization such as UV-visible, TLC, HPLC, HPTLC, GC-MS, spectrofluorimetric and other methods. 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.

COCLUSION

Plant materials are used throughout the developed and developing world as home remedies, in overate-counter drug products, and as raw material for the pharmaceutical industry, and they represent a substantial proportion of the global drug market. 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. Now a day the field of herbal drugs and formulation is very fast and there is still lot to explore on the subject of standardization of these. So, while developing an herbal formulation it is must to have all the related knowledge of that particular drug including all its organoleptic characters to phytoconstituents to pharmacological action to its standardization in respect to various parameters via various techniques.

The Indian medicinal industry is developing in a tremendous change. With the tremendous increase in traditional herbal treatment many companies regarding the safety, quality and efficacy of herbal drug have been observed. There are need for more advanced techniques of standardization. The advance analytical techniques will serve as a rapid and specific tool in the herbal medicine research, the manufacturers to set quality standards and specifications to find marketing approval from regulatory authorities for therapeutic efficacy, shelf- life and safety of herbal medicine. The national health organization should ensure that all herbal pharmaceutical drug product subject to their control.Quality control of herbal product have not only to establish reasonable analytical methods for analyzing the active ingredient in herbal medicines, but different factors should be affected such as pesticides residue, toxins content, the heavy metals contamination, good agricultural practice (GAP), good manufacturing practice (GMP), etc. There is the need for development of techniques which includes both traditional methods of evaluation and standardization.

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