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A Review Report on the Standardization of herbal medicines

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ABSTRACT: - In these article we are consider at the basic requirement of herbal medicine standardization and there process and use. Many types of regulatory body are mange and publish there article related to the process how to maintain their parameter. In this article mention there test perform for the check and balance physical, chemical, and biological activity. In it shown that the drug quality are responsible for the therapeutic effect and biological activity.

INTRODUCTION: -

Nature provides the basic resources for medicines, which have been used as medicines from ancient times to the present. People all over the world have a unique understanding of the natural resources on which they rely.

Including exceptional botanical knowledge. Traditional medicines meet the health needs of approximately 85% of the world's population. It is critical to maintain the safety, quality, and efficacy of the plant and its products in order to avoid serious health problems. Indian healthcare is characterised by medical pluralism, with ayurveda continuing to predominate over modern medicine, particularly for the treatment of a variety of chronic disease conditions. Traditional medicine, according to WHO, includes a wide range of health practises, approaches, knowledge, and beliefs. incorporating medicinal plants, animals, and/or minerals, spiritual therapies, manual techniques, and exercises applied singularly or in combination to maintain well being, as well as to treat, diagnose or prevent illness. According to their definitions, WHO has provided some terms related to herbal drugs. Herbal medicines include Herbs, herbal materials, herbal preparations, and finished herbal products are all examples of herbal products. Traditional herbal medicines in some countries may contain natural organic or inorganic active ingredients that are not of plant origin (for example, animal and mineral materials). Herbs are raw plant material such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes, or other plant parts that can be whole, fragmented, or powdered. Herbal materials include fresh juices, gums, fixed oils, essential oils, resins, and dry powders of herbs, in addition to herbs. In some countries, these materials may be processed using a variety of local methods, such as steaming, roasting, or stirbaking with honey, alcoholic beverages, or other ingredients. Herbal preparations serve as the foundation for finished herbal products and can include comminuted or powdered herbal materials, as well as extracts, tinctures, and fatty oils of herbal materials. Extraction, fractionation, purification, concentration, and other physical or biological processes are used to create them. Preparations made by steeping or heating herbal materials in alcoholic beverages are also included. beverages, honey, or other materials. Herbal preparations made from one or more herbs constitute finished herbal products. The term "mixture herbal product" can also be used when more than one herb is used. In addition to the active ingredients, finished herbal products and herbal mixtures may contain excipients. However, finished products or herbal mixtures containing chemically defined active substances, including synthetic compounds and/or isolated constituents from herbal materials, are not considered herbal. Herbal medicines are widely used in Traditional Medicine practises and therapies such as Chinese medicine, Ayurveda, Unani, Naturopathy, Osteopathy, and Homoeopathy. Plant-derived products have seen a surge in popularity in developed countries in recent years. These products are increasingly in demand as pharmaceuticals, nutraceuticals, and cosmetics. In India, there are approximately 6000 herbal

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manufacturers. Ayurvedic medicines are manufactured in quantities of over 4000 units. Because of a lack of infrastructure, skilled labour, reliable methods, and stringent regulatory laws, most of these manufacturers produce their products on a trial basis. It has become critical to develop reliable, specific, and sensitive quality control methods that use a combination of classical and modern instrumental methods of analysis in order to have a good coordination between the quality of raw materials, in process materials, and final products. Standardisation is an essential measurement for ensuring herbal drug quality control. The term "standardisation" refers to all measures taken during the manufacturing process and quality control that result in reproducible quality. It also includes the entire field of study, from plant development to clinical application. A drug is considered safe if it poses no known or potential risk to the user. There square measure three security classes that should be considered when determining the type of security requirements to be met.

The 3 classes of are:

1. Category 1: long safety
2. Category 2: Safe beneath such that conditions of use (such flavouring medicines ought to otherwise be coated by well-established documentation)
3. Category 3: flavouring healthful product of unsure safety (The safety knowledge needed for this category of healthful product square measure similar to those for brand new substances).

Standardization:- Plant-derived products have seen a surge in popularity in developed countries in recent years. These products are increasingly in demand as pharmaceuticals, nutraceuticals, and cosmetics4. It has become critical to develop reliable, specific, and sensitive quality control methods that use a combination of classical and modern instrumental methods of analysis in order to have a good coordination between the quality of raw materials, in process materials, and final products. Standardisation is an essential measurement for ensuring herbal drug quality control. 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 is known as standardisation of herbal medicines. It is the process of creating and approving technical standards. Experimentation and observations are used to develop specific standards, which lead to the process of prescribing a set of characteristics exhibited by specific medicines. As a result, standardisation is a tool in the quality control process.

According to the American Herbal Product Association, "standardisation refers to the body of information and control required to produce material of reasonable consistency." This is accomplished by reducing the inherent variation in natural product composition through quality assurance practises used in agricultural and manufacturing processes. The term "standardisation" refers to all measures taken during the manufacturing process and quality control that result in reproducible quality. It also includes the entire field of study, from plant development to clinical application. It also refers to adjusting the herbal drug preparation to a specific constituent or constituents by adding excipients or mixing herbal drugs or herbal drug preparations8. A drug's "evaluation" means confirming its identity, determining its quality and purity, and detecting the nature of its adulteration. Standardisation methods should consider all aspects that contribute to the quality of herbal drugs, such as sample identity, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, xenobiotic presence testing, microbial load testing, toxicity testing, and biological activity. The phytochemical profile is particularly important because it has a direct impact on the activity of herbal drugs. The fingerprint profiles serve as a guideline to the drug's phytochemical profile in ensuring quality, while quantification of the marker compound/s serves as an additional parameter in assessing sample quality.

NEED OF STANDARDIZATION:- Traditionally, vaidyas treated patients on an individual basis, preparing drugs based on the patient's needs. Almost all traditional medical systems have prioritised quality control from the start. Its Rishis, Vaidyas, and Hakims are subjected to scrutiny. Unlike in the past, when traditional practitioners prepared and tested the qualities of herbal medicines, the problem today is the economics of industrial scale production, shelf life, and long-distance distribution. This has necessitated the development of modern, objective

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standards for assessing the safety, quality, and efficacy of these medications. People are also becoming more aware of the potency and potential side effects. To gain public trust and integrate herbal products into today's health-care system, researchers, manufacturers, and regulatory agencies must employ rigorous scientific methodologies to ensure the quality and lot-to-lot consistency of traditional herbal products.11. Need for

The following summarises quality control and standardisation of herbal products:

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

The identity of the plants and seasonal variation (which affects the time of collection), ecotypic, genotypic, and chemotypic variations, drying and storage conditions, and the presence of xenobiotic13 are all important factors in the variation of herbal raw material. Environmental factors such as sunlight, rainfall, altitude, temperature, soil, and storage conditions, as well as different harvesting procedures, time and method of collection, manufacturing processes such as selecting, drying, purifying, and extracting, and genetic variability, can all cause significant variation in product quality and plant chemical concentrations. Environmental factors such as insect feeding and microbial infections can influence secondary metabolites and, as a result, the chemical composition of the plant. Also, different parts of the same plant (for example, roots, stems, and leaves) have varying concentrations of chemical constituents. In the meantime Variability in herbal medicines is also accounted for by diurnal variations (for example, paclitaxel and opium alkaloids) and seasonal changes. The therapeutic or toxic components of plants differ depending on the plant part used as well as the stage of ripeness.14. Products from different manufacturers differ significantly, and it is impossible to control all of the factors that influence the chemical composition of the plant. It is difficult to establish quality control parameters for plant-based drugs due to their complex nature and inherent variability, and modern analytical techniques are expected to help in this regard. Furthermore, the constituents allegedly responsible for the therapeutic effects are frequently unknown or only partially explained. The majority of herbal formulations, particularly traditional medicine's classical formulations, are polyherbal. Many preparations are either liquid or semisolid in nature. It is extremely difficult to establish quality control parameters for such formulations. Official standards are also unavailable. The unique processing methods used to manufacture these drugs transform the single drugs into a very complex mixture, making separation, identification, and analysis of the components extremely difficult. Herbal product standardisation can be divided into two categories: active constituents extracts, where biochemical principles are known and have therapeutic values, and marker extracts, where the active principle is unknown and a characteristic compound is used as a marker to assess the presence of other therapeutic biochemical compounds. Standardisation has limitations because it only considers isolated compounds, ignoring the herb's entire constituents, which may have synergistic or buffering activities to reduce side effects. Traditional medicine is standardising from the collection of raw materials to the most extreme clinical application. In traditional medicine, therapeutic efficacy is the sum of its chemical constituents.

As a result, quality and purity refer to the drug's overall profile rather than any of its characteristics. As a result, a multidimensional approach is required for the standardisation of traditional medicine. This multifaceted approach should address every detail of the drug, including the name, botanical source, geographical source, organoleptic, morphological, anatomical, physical, chemical, and biological activities. The World Health Organisation (WHO) emphasises the significance of qualitative and quantitative methods for characterising samples, quantifying biomarkers and/or chemical markers, and creating fingerprint profiles. If the primary active component is known, it is logical to quantify this compound. Botanical preparations should be standardised to active ingredients known to contribute to therapeutic efficacy. Where the active ingredients are unknown, a marker substance that is specific to the botanical could be chosen for analysis. References in pharmacopoeia establish the authenticity, quality, and purity of herbal drugs. These documents publish traditional and standardised herbal therapeutic uses.

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Provide a foundation for clinical practise. Monographs include a description of the herb, as well as botanical data, laboratory analysis, therapeutic indications, and drug interactions. The pharmacopoeia establishes (numerical value) structural, analytical, and physical standards for drugs.

CONVENTIONAL METHODS FOR STANDARDIZATION OF CRUDE DRUG:- Standardisation of herbal raw drugs includes raw plant drug passport data. It consists of a medico-botanical survey, identification, botanical authentication, and a macroscopic examination. Drug testing in accordance with approved procedures Fully pharmacognostical profile, identification by various chromatographic techniques, purity assessment by physico-chemical profile, strength assessment by active marker or assay estimation, and safety by heavy metal profiling, microbiological limit test analysis, aflatoxins analysis, pesticide residue analysis, and biological activity. Macroscopic identification of medicinal plant materials is based on sensory evaluation parameters such as shape, size, colour, texture, odour, and taste, whereas microscopy entails a comparative microscopic inspection of powdered herbal drug. Furthermore, the use of light and scanning electron microscopes (SEM) in herbal drug standardisation has increased the accuracy and capabilities of microscopy as a means of herbal crude material identification. Preliminary testing for the presence of different chemical groups, quantification of chemical groups of interest (e.g., total alkaloids, total phenolics, total triterpenic acids, total tannins), establishment of fingerprint profiles, multiple marker-based fingerprint profiles, and quantification of important chemical constituents are all part of the phytochemical evaluation for standardisation.

STANDARDIZATION OF HERBAL/ POLYHERBAL FORMULATION:- The herbal formulation in general can be standardised in order to formulate the medicament using raw materials collected from various locations and to compare the chemical efficacy of different batches of formulation. The preparations with the highest clinical efficacy will be chosen. All routine physical, chemical, and pharmacological parameters for all batches are checked in order to select the final finished product and validate the entire manufacturing process. As these are combinations of more than one herb to achieve the desired therapeutic effect, standardisation is an important aspect for maintaining and assessing the quality and safety of the polyherbal formulation. Standardisation reduces batch-to-batch variation and ensures the safety, efficacy, quality, and acceptability of polyherbal formulations.

The implementation of Good Manufacturing Practices is required for the standardisation of herbal formulation. Furthermore, the investigation of various parameters such as pharmacodynamics, pharmacokinetics, dosage, stability, self-life, toxicity evaluation, and chemical profiling of herbal formulations is deemed necessary. Heavy metal contaminations, as well as Good Agricultural Practises (GAP) in herbal drug standardisation, are critical.

Who Guidelines For Quality Standardized Herbal Formulations:- The chromatographic fingerprints (TLC, HPTLC, HPLC, and GC) should be used to standardise the bioactive extract based on active principles or major compounds. In general, all medicines, whether synthetic or natural, must meet the basic requirement of being both safe and effective. The term 'herbal drugs' refers to plants or plant parts that have been converted into phytopharmaceuticals through simple harvesting, drying, and storage processes.

1. **Quality control of crude drugs material, plant preparations and finished products.:-** Quality control refers to the processes involved in ensuring the quality and validity of a manufactured product. Quality control is generally based on three important pharmacopeial aspects

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

Identity can be determined through macro and microscopical examinations. In addition to identity tests, such as simple chemical tests, colour or precipitation tests, and chromatographic tests, are required. These chemical and chromatographic tests aid in batch-to-batch comparability, and the chromatogram can be used as a 'fingerprint' for the herbal ingredient by displaying the profile of some common plant constituents such as flavonoids, alkaloids, and terpenes. Criteria such as type of preparation, sensory properties, physical constants, adulteration, contaminants, moisture, ash content,

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and solvent residues must be checked to prove identity and purity. Voucher specimens are dependable sources of information. Disease outbreaks among plants can cause changes in the physical appearance of the plant and lead to incorrect identification. Purity is closely guarded.

It is associated with drug safety and addresses issues such as ash values, contaminants (for example, foreign matter in the form of other herbs), and heavy metals. Modern purity evaluation, however, includes microbial contamination, aflatoxins, radioactivity, and pesticide residues due to the use of improved analytical methods.

To determine the constant composition of herbal preparations, analytical methods such as photometric analysis, Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), and Gas chromatography (GC) can be used. Depending on whether the active principles of the preparation are known or unknown, different concepts such as "normalisation versus standardisation" must be applied to establish relevant uniformity criteria.

Because the active constituents of most herbal drugs are unknown, content or assay is the most difficult area of quality control to perform. Markers can be used at times. In all other cases, where no active constituents or markers for the herbal drug can be identified, the percentage extractable matter with a solvent may be used as a form of assay, as is common in pharmacopoeia. The determination of essential oils by steam distillation is a unique type of assay. When active constituents (e.g., sennosides in senna) or markers (e.g., alkydamides in Echinacea) are identified, a wide range of modern chemical analytical methods such as UV/VIS, TLC, HPLC, HPTLC, GC, mass spectrometry, or a combination of GC and MS (GC/MS) can be used.

2. **Stability assessment and shelf life.** Prolonged and apparently uneventful use of a substance usually provides evidence of its safety. In a few cases, however, investigation of the potential toxicity of naturally occurring substances widely used as ingredients in these preparations has revealed previously unknown potential for systematic toxicity, carcinogenicity, and teratogenicity. These findings must be communicated to regulatory authorities in a timely and reliable manner. They should also have the authority to respond quickly to such alerts, either by withdrawing or changing the licences of registered products containing suspect substances, or by rescheduling the substances to limit their use to medical prescription.

All procedures should be in accordance with good manufacturing practices.

Crude Plant Material

The botanical definition, including genus, species and authority, description, part of the plant, active and characteristics constituents should be specified and, if possible content limits should be defined. Foreign matter, impurities and microbial content should be defined or limited. Voucher specimens, representing each lot of plant material processed, should be authenticated by a qualified botanist and should be stored for at least a 10-year period. A lot number should be assigned and this should appear on the product label.

Plant Preparations

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

Finished Product

The manufacturing procedure and formula, including the amount of excipients, should be described in detail. A finished product specification should be defined to ensure consistent quality of the product. The finished product should comply with general requirements for particular dosage forms.

Stability

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

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3. **Safety assessment; documentation of safety based on experience or toxicological studies.**

Herbal medicines are generally considered safe due to their long history of use in various cultures. However, there have been reports of serious adverse events following the use of herbal products. Toxicity has been linked to contaminants and adulteration in many cases. Some of the plants used in herbal medicines, however, can be extremely toxic. If herbal medicines are not properly evaluated, there is a risk of adverse effects and drug-drug and drug-food interactions.

As a result, determining the safety of herbal products is the top priority in herbal research.

These are different approaches to determining the safety of herbal medicines. The toxic effects of herbal preparations can be attributed to the following factors: Manufacturing malpractice and contamination, as well as the inherent toxicity of plant constituents and ingredients.

To assess the toxic effects of plant constituents in herbal formulations, extensive phytochemical and pharmacological research is required. However, based on human experiences in various cultures, it is safe to assume that the use of toxic plant ingredients has already been largely eliminated, and recent reports of toxicity may be due to misidentification and overdosing of certain constituents. Another significant issue is the adulteration of botanical preparations. Many medicinal plants have become endangered or rare as a result of over-exploitation of certain plants, habitat loss, and forest fragmentation. These and other factors (such as the cost of raw materials) impede the availability of genuine drugs, encouraging plant adulteration through substitution with inferior commercial varieties, artificially manufactured substances, exhausted drugs, or cheaper plant or by another vegetative part37. According to several reports, many herbal products contain unknown pharmaceuticals and heavy metals38. It is possible to use pharmaceutical adulterants on purpose.

Agrochemicals are used to protect plants from raw plant material. Furthermore, many herbs' mechanisms of action,pharmacokinetics, and drug-drug interactions are still in their infancy. Simultaneously, an increasing number of reports of fatal or adverse effects of herbal preparations highlights the need for national regulation and registration of herbal medicines, as well as the establishment of safety monitoring. Clinicians should not prescribe or recommend herbal remedies that lack well-established efficacy as if they were medications proven effective through rigorous research.

Assessment Of Toxicity:- Toxicity investigation will also be required because the analysis alone is unlikely to reveal the contributions to toxicity itself. In assessing toxicity of an herbal medicine, the dose chosen is very important. Toxicity assessment involves one or more of the following techniques- In vivo techniques, in vitro techniques, cell line techniques, micro- array and other modern technique, standardization and techniques to adequately model toxicity.

4. **Assessment of efficacy by ethno- medical information and biological activity evaluations.**

Herbal medicines are fundamentally distinct from conventional pharmacological treatments, but there is currently no way to assess their efficacy other than through currently used conventional clinical trial methodologies, in which efficacy is conventionally measured by clinical, laboratory, or diagnostic outcomes: Clinical outcomes include parameters such as decreased morbidity, decreased pain or discomfort, increased appetite and weight gain, decreased blood pressure, decreased tumour size or extent, and increased quality of life. Laboratory/other diagnostic outcomes include parameters such as blood glucose reduction, haemoglobin status improvement, opacity reduction as measured by radiological or imaging techniques, and improvement in electrocardiogram (ECG) findings.

Implementation of a standardized approach for the herbal practitioners and collection of the prospective data necessarily creates an interventional design which, if planned properly, may closely resemble single-blind randomized trials. Even if it differs from double-blind randomized trials in the degree of rigor, the design may be the optimum, both biologically and economically, for rapid evaluation of herbal products. Standardization, however, may sometimes be incompatible with the existing legislative framework and caution is needed regarding the ethical implications of such studies. Although randomized clinical trials (with double blind trials as the gold standard) are relatively difficult to be implemented in the case of herbal medicine, they are not ruled out per se in assessing the efficacy of these products. Data from case

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series studies may provide sufficient scientific and ethical validity to conduct such trials, but acceptance of this protocol needs a paradigm change in the methodoly of drug evaluation as understood in conventional medicine.

Standardization and Quality control of herbal drugs involve wide array of scientific investigations, which include physical, chemical and biological evaluation employing various analytical method and tools.

a. **Physical Evaluation**- Physical constants are sometimes used to evaluate a specific drug. Water content, specific gravity, optical rotation, refractive power, melting point, viscosity, and solvent solubility are all examples. in addition to that, The following parameters are included: inclusions, total ash, acid-insoluble ash, water-soluble ash, swelling index, foam index, sequential extraction value, moisture content, viscosity, pH, disintegration time, friability, hardness, flowability, flocculation, and sedimentation. as well as the amount of alcohol. All of these physical properties can be used to identify and detect plant constituents.

Ash value: The residue left after burning herbal remedies is known as the ash value, which represents naturally occurring minerals. Used to assess the purity of raw materials. It can be analyzed by determining various ash values. In herbal medicine, higher ash represents impurities.
Method: 2-3 g of ground drug is burned in a tared silica dish at a temperature not exceeding 450 °C, cooled and weighed. Calculate the ash content based on the air-dried drug.

Sr.no.	Name of drug	Total ash (%w/w)
1.	Acacia Catechu	Not More Than 15%
2.	Rawulfia Surpentina	Not More Than 8%

table 01: example of ash value

Extractive Value: This method is used to determine the amount of chemical constituents of crude drugs extracted with different solvents such as water-soluble extract, alcohol-soluble extract, ether-soluble extract.

Sr.no.	Name of drug	Alcohol soluble extractive value(%W/W)
1.	Amla	Not More Than 40
2.	Ashoka	Not More Than 15
3.	Curcuma longa	Not More Than 8

table 2: examples of extractive value

Moisture Content: The amount of water contained in herbal medicines. It should be reduced to prevent drug degradation and estimation of the actual weight of the drug substance. The measurement method can be achieved by weighing an empty tarred porcelain bowl and pouring the appropriate amount of medicine into it. Place the porcelain mold in a convection oven at 105 °C for 5 hours until it reaches a constant weight.

Sr. no.	Drug	Moisture Content%	W/W
1.	Ajwain	Not More Than	10
2.	Ashwaghandha	Not More Than	12
3	Sunthi	Not More Than	12

table 3: example of moisture content

Refractive index : Refractive index gives an idea of purity. A ray of light bends as it travels from a thinner medium to a denser medium. This bending of light is called refraction. Therefore, the ratio of the speed of light in a vacuum to the speed of light in a substance is known as the refractive index of the second medium. It is considered an important tool for standardization as it is constant for liquids

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of a certain purity level. It is affected by the wavelength of incident light, temperature, and pressure.

Sr.no.	Drug	Refractive index
1.	Caraway Oil	1.4838 – 1.4858
2.	Clove oil	1.527 – 1.535

table 7: example of refractive index.

Determination of specific optical rotation:- It depends on a phenomenon called polarization. Polarization means that light rotates clockwise, called right-handed, and counterclockwise, called left-handed, when the plane of polarization passes through the liquid.

It can be calculated using the formula:
$D25 = 100 \times \varphi lc$
Where, φ = observed rotation in drug at -25°
D = D line of sodium light
l = length of polarimeter tube.
c = concentration of substance in % w/v.

Sr.no.	Drug	Angle Optical rotation
1.	Caraway oil	+75 - +85
2.	Clove oil	0 - +6
3.	Honey	+3 - -15

table 4: Specific optical rotation

Melting Point: Phytochemicals and herbal medicines have different melting points. It is relatively constant for phytochemicals and contains mixed chemicals for herbal medicines.

Sr.no.	Drug	Melting point
1.	Colophony	75-85
2.	Cocca butter	30-33
3.	Bees wax	62-65

Table 5: Example of Melting point

Biological Evaluation- Pharmacological activity of certain drugs has been applied to evaluate and standardize them. The assays on living animals and on their intact or isolated organs can indicate the strength of the drug or their preparations.

The pharmacological activity of specific drugs has been used to evaluate and standardize them. Assays on live animals and on intact or isolated organs can demonstrate efficacy of drugs or their formulations. These assays are known as biological assays or bioassays.

Determination of Bitterness Value:

Bitter medicinal plants are usually used therapeutically as appetite stimulants. Their bitter taste stimulates the gastrointestinal tract, especially the secretion of gastric juice. Bitter substances can be judged by taste. However, since most consist of two or more components with different bitterness, the total bitterness must first be measured by taste. The bitterness profile of a plant material is determined by comparing the threshold bitterness concentration of an extract of the material to that of a dilute solution of quinine hydrochloride. The bitterness value is expressed in units corresponding to the bitterness of a solution containing 1g of quinine hydrochloride in 2000ml. Safe drinking water should be used as a medium for extracting botanicals and to rinse the mouth after each tasting. Using distilled water will dull your taste buds quickly. Water hardness rarely has a significant effect on bitterness.

Determination of swelling index: The swelling index is the amount (mL) absorbed by swelling of 1 gram of plant material under certain conditions. That determination is based on the addition of water or swelling agents specified in the individual plant material test specifications. After repeating the shaking for 1 hour using a graduated cylinder with a glass stopper, it is allowed to stand for the required time. Then the volume of the mixture is read. It is easy to mix the whole grass ingredients with the bulking agent, but the chopped or powdered ingredients must be shaken vigorously at regular intervals to evenly distribute the ingredients in the bulking agent.

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Determination of foaming index: Many medicinal plant materials contain saponins, and stirring an aqueous decoction can cause a persistent foam. The foaming power of aqueous decoctions of plant materials and their extracts is measured as foam index.

Determination of Pesticide Residues: Pesticide residues are specific substances in food, agricultural products, or animal feed that result from the use of pesticides. Herbal medicines tend to contain pesticide residues resulting from agricultural practices such as spraying, soil behavior during cultivation, and addition of fumigant during storage. Various methods are used for the determination of pesticides by GC, MS, or GCMS. Some simple methods have also been published by WHO
and the European Pharmacopoeia generally have limit values for pesticide residues in medicines.

Chemical Evaluation- Chemical analysis of the drug is done to assess the potency of vegetable material in terms of its active principles. It covers screening, isolation, identification, and purification of the chemical components. It help to determine the identity of the drug substance and possible adulteration.Most pharmaceuticals contain specific chemical ingredients on which their pharmacological and biological activities depend. A qualitative chemical test to determine drug quality and purity.Identification,isolation, and purification of active chemical constituents rely on chemical evaluation methods. A preliminary survey of phytochemicals is also part of the chemical evaluation. Qualitative chemical tests for chemical evaluation of herbal medicines include saponification number and acid number.

Detection test for alkaloid:
- Dragaondroff reagent
- Mayer's reagent
- Wagner reagent
- Hager's test

Detection test for glycoside :
- Modified brontragger's test
- Legal test
- Froth test
- Foam test

Detection test for carbohydrates:
- Molish test
- Benedict's test
- Fehling's test

Detection of Tannis:
- Gelatin test
- Gold bitter skin test

Analytical Methods- It helps in determining identity, quality and relative potency**.**

Sample preparation is the most important step in the development of analytical methods for botanical and herbal preparations. The basic operation includes steps such as pre-washing, drying of plant materials or freezedrying, and grinding in order to obtain a homogeneous sample and, in many cases, to improve the kinetics of extraction of the plant material. constituents. Methods such as sonication, heating under reflux, Soxhlet extraction, and others are commonly used in pharmacopoeial monographs. Such methods, however, can be time-consuming, necessitate the use of a large amount of organic solvent, and may result in lower extraction efficiencies. To address this issue, new methods are constantly being sought. Because target compounds can be polar or nonpolar, and even thermally labile, the suitability of extraction methods must be considered. Newer sample preparation methods, such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), or pressurised liquid extraction (PLE), have been introduced to reduce or eliminate the use of organic solvents and improve extraction processes for the extraction of targeted constituents present in plant materials. Pharmacopeial monographs are the most practical approach to quality control of herbal medicines, and many of them exist (EMEA, 2005; WHO, 1998a,b, 1996a, 1998a, 1981). If pharmacopoeial monographs are not available, analytical method development and validation should be performed by the manufacturer. The best strategy is to strictly follow the pharmacopoeial definitions of identity, purity and potency or assays. Critical to meeting all monograph criteria is the need for appropriate analytical methods to determine identity, quality, and relative potency. A wealth of analytical methods is available there. However, among the analytical tools known to standardize monographs, chromatography is important, although it is often difficult to determine which one is the most suitablec.There are different analytical technique for the standardization of herbal drug like HPLC , HPTLC , TLC , GC , LC-MS,
IR,Thermal analysis, GC-MS.

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High Performance Liquid Chromatography (HPLC): Preparative and analytical HPLC is widely used in the following fields: Pharmaceutical industry for the isolation and purification of plant compounds. There are basically two types of HPLC primers. low pressure HPLC (usually under 5 bar) and high pressure HPLC (pressure >20 bar). The main goal is to isolate the plant and as with any analytical work, the goal is to obtain information about the sample. Preparative HPLC is closer to analytical HPLC than conventional PLC, with higher column efficiencies and faster solvent velocities so you can perform your most difficult separations faster. High Performance Liquid Chromatography (HPLC), also known as High Pressure Liquid Chromatography, is essentially a form of column chromatography that involves packing small particles (3-50µm) in a stationary phase with small bores (2-5mm). .), one end of which is connected to a source of pressurized liquid eluent (mobile phase). The three forms of high performance liquid chromatography are ion exchange, partition, and adsorption. In the research field of liquid chromatography, many new techniques have been developed recently to achieve better separations. These include capillary micellar electrokinetic chromatography (MECC), high-speed countercurrent chromatography (HSCCC), low-pressure size exclusion chromatography (SEC), reversed phase ion-pair HPLC (RPIPC-HPLC), and strong ion-exchange HPLC (SAX). Is included. -HPLC). They offer new opportunities to successfully isolate multiple specific extracts of multiple herbal drugs. On the other hand, the advantage of HPLC lies in its versatility for analyzing compounds contained in herbal medicines.

High Performance Thin Layer Liquid Chromatography (HPTLC):

High performance thin layer chromatography is an improved version of thin layer chromatography. High Performance Thin Layer Chromatography is planar chromatography in which the separation of sample components on high performance layers with detection and detection is performed using advanced workstations, increasing the efficiency of the separation process. HPTLC is appropriate for qualitative, quantitative and micro-preparative natural process. The HPTLC technique is wide utilized in the pharmaceutical business within the method of development, identification and detection of impurities in flavourer product and aids within the management of pesticides, phytotoxin content and quality of herbs and health foods. according Associate in Nursing HPTLC technique for the detection of withaferrin A and beta-sitosterol-diglucoside in four formulations of ashwagandha. antecedently HPTLC was accustomed discover, monitor and quantify bacosides A and B in Bacopa monaria which formula. it's wide according that multiple samples will be analyzed at the same time exploitation fewer mobile phases than HPLC. There are reports that mobile phases of pH scale or higher will be used. Used for HPTLC. Another advantage of HPTLC is that the recurrent identification (sampling) of chromatograms till they're identical or totally different. HPTLC has so been investigated for the synchronic use of multiple elements in multicomponent formulations. this system will be accustomed standardize totally different plant species and assess their stability. and consistency of their formulation from totally different manufacturers.

Steps concerned in HPTLC analysis:-

- choice of stationary section
- Selection of mobile section
- Sample preparation and application
- recording Development (separation)
- Investigation

Infrared Spectroscopy (IR):- IR spectroscopy was used to determine the functional groups present in the samples. Infrared absorption spectroscopy is the measurement of the wavelength and intensity of light absorption in the near-infrared range of a sample. Mid-infrared light has enough energy to excite nuclear vibrations to higher energy levels. The wavelengths of some IR absorption bands are characteristic of certain types of chemical bonds, and IR spectroscopy has shown the greatest utility in the qualitative analysis of organic molecules and metals. The IR method is widely used in the herbal medicine industry as a quality control tool for direct identification and quantification of herbs or extracts. Different powders of Panax Notoginseng were identified using FT-IR and 2D FT-IR and the combination of ATR-FTIR microscopy was directly applied to determine the complex composition of herbal powders.

Thin Layer Chromatography (TLC): TLC is a technique in which a solute is partitioned between two phases, a stationary phase acting by adsorption

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and a mobile phase in liquid form. The sorbent is a relatively thin, uniform layer of finely divided drug material applied to a glass, plastic, or metal foil/plate. glass plates are most commonly used. Separation can also be achieved based on a combination of partition/distribution and adsorption, depending on the particular support and its use with different solvents. It can be identified by observing spots on the sample plate with the same Rf value and size obtained for the unknown and reference samples. A visual comparison of spot size and intensity is usually used for semi-quantitative estimation. TLC has the advantage of having different ways to detect herbal medicines in the analysis. Also, TLC is fairly simple and can be used to analyze multiple samples. More than 30 sample spots can be interrogated for each plate. Useful qualitative and quantitative information can be obtained from the developed TLC plates using the CA MAG video storage system and the TLC-QA-UV method.

Gas Chromatograhy – Mass Spectroscopy : The Gas Chromatograph can be easily connected to various types of Rapid Scan Spectrometers. Capillary column flow rates are generally low, but sufficient for the column. The output can easily be fed directly into the MS ionization chamber. Among them, the simplest mass detector in GC is the ion trap detector. An efficient, rapid and accurate capillary gas chromatography method was used to determine organochlorine pesticide residues. SPE extracts were separated by capillary column using an electrochemical detector. The fractionation ratio was 1:2.2 using N 2 carrier gas at a flow rate of 1.4 mL/min. The injector temperature is 220 °C and the detector temperature is 330 °C. By comparison, the organochlorine pesticides gave good linearity. It has been used to identify numerous constituents present in natural and biological systems. The advantages of GC-MS are:

(1) With capillary columns, GC-MS generally has very good separation power and can produce high quality chemical fingerprints.

(2) Combining mass spectrometry with a corresponding mass spectrometry database,

The GC-MS provided relative qualitative and quantitative information about the composition of the studied herb, aiding further studies to elucidate relationships. Very helpful. Further research between the chemical constituents of herbal medicines and their pharmacology. Therefore, in our opinion, GC-MS should be the best tool for the analysis of volatile compounds in herbal medicines.

Liquid Chromatography – Mass Spectroscopy: LC-MS has become the method of choice at many stages of drug development. Recent advances include e-beam, thermal spray, and ion implantation, liquid secondary ion mass spectrometry, followed by laser mass spectrometry at 600 MHz, which offer the unique advantages of high detection sensitivity and specificity, allowing molecules to be Allows accurate identification of the proteins and peptides used. weights are enabled. Isotope patterns can be detected with this technique.

DNA Fingerprint Technique : DNA analysis has proven to be an important tool in the standardization of herbal medicines. This technique helps distinguish between phytochemically indistinguishable genuine drugs and alternatives or adulterations. DNA fingerprinting genomes have been reported to remain the same regardless of the plant part used, whereas phytochemical levels vary with the plant part used, physiology and environment.

Thermal Analysis of herbal drugs: Differential scanning using thermogyitravimetric analysis (TGA), differential thermal analysis (DTA) and calorimetry (DSC), physical or chemical changes in various products including herbal and pre-formulation or pharmaceutical research. Compatibility of excipients. Thermal analysis is a term encompassing a group of techniques used to monitor the physical or chemical properties of a substance or its reaction products as a function of time or temperature, the temperature of the sample being controlled programming exposed to the atmosphere below. The use of thermal analysis techniques is increasing in the field of drug development, where they are used for excipient characterization and formulation development. For compatibility testing, this analysis allows to observe the thermal degradation profile of drugs, excipients, and their mixtures. Factors such as drug protection, reactions, intermediate and final degradation products generated, and degradation kinetics can be assessed.

DNA FINGERPRINTING TECHNIQUE:- DNA analysis has been proved as an important tool in herbal drug standardization. This technique is useful for the identification of phytochemically

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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. 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. 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.DNA markers are helpful to identity cells, individuals or species as they can be used to produce normal, functioning proteins to replace defective ones. Moreover, these markers help in treatment of various diseases and help in distinguishing the genuine herb from adulterated drug. Cannabis sativa and Arabidopsis thaliana L. Heyne have been differentiated from their adulterated species by using ISSR markers.

Omics: A New Technique in Herbal Drug Standardization and Quantification: It is same as the DNA Fingerprinting Method The Omics study contains a large number of scores for each endpoint, giving you comprehensive and integrated knowledge while viewing different factors separately. Omic technology is primarily used to identify biomedical resources such as:

- Genomic technique in DNA sequencing
- Fingerprinting or DNA microarrays.

Omics technology is the genome, transcriptome (total number of genes converted into transcripts (i.e., mRNA molecules)), proteome (total proteins found in a given cell or tissue), metabolome (total metabolites and intermediate stages of a cell or tissue), interactome (position of molecules such as biologically active metabolites that interact with a particular protein), and phenomenon (the sum of all observable features of an organism) level.

ROLE OF GENETIC MARKERS IN THE STANDARDIZATION OF HERBAL DRUGS:- A genetic marker is a gene or DNA sequence that is associated with a specific gene or trait and has a known location on a chromosome. It is a variation that can occur as a result of a mutation or alteration in the There are genomic loci that can be observed. A genetic marker can be either a short DNA sequence, such as one encircling a single base-pair change (single nucleotide polymorphism SNP), or a long one, such as minisatellites. 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) are some common types of genetic markers. They can be further classified71. RAPD-based molecular markers have been found to be useful in distinguishing between different neem accessions collected from different geographical regions. Another important area where a lot of effort has been put in is germplasm analysis to study genetic diversity. Rice wheat, chickpea, pigeon pea, pearl millet, and other crops are being fingerprinted extensively. Sequence characterised amplified region (SCAR), AP-PCR, RAPD, and RFLP have all been used successfully to differentiate these plants and detect substitution by other closely related species. P. ginseng, for example, is frequently replaced by P. quinquefolius (American ginseng). RAPD markers have been used in the selection of micropropogated Piper longum plants for conservation.