International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

# Advanced Methods for Standardizing Herbal Medicines: A Detailed Review

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**ABSTRACT**

The term "herbal drugs" denotes plants or plant parts that have been converted into Phyto pharmaceuticals by means of simple processes involving harvesting, drying, and storage. A practical addition to the definition is also to include other crude products derived from plants, which no longer show any organic structure, such as essential oils, fatty oils, resins, and gums. There is increasing awareness and general acceptability of the use of herbal drugs in today's medical practice. Although, most of these applications are unorthodox, it is however a known fact that over 80% of the world population depends on herbal medicines and products for healthy living. This rise in the use of herbal product has also given rise to various forms of abuse and adulteration of the products leading to consumers and manufacturers disappointment and in some instances fatal consequences. The challenge is innumerable and enormous, making the global herbal market unsafe. Evaluation of herbal drug is an important tool in the formulation of high-quality herbal products. This review seeks to enlighten stakeholders in herbal medicine on the need to establish quality parameters with the help of advanced analytical tools and well-defined standardization methods in ensuring the safety of the global herbal market. The processes of good quality assurance and standardization of herbal medicines and products using various spectroscopic, chromatographic and electrophoresis methods were also discussed. In fact, the research field of quality control of herbal medicines is really interdisciplinary research. It needs crossover of chemistry, pharmacology, medicine and even statistics to provide a platform for the quality control of traditional herbal medicines and further to discover the novel therapeutics composed of multiple chemical compounds Medicinal plants are an important source for creating medicines. Medicinal plants and herbal treatments play a major role in the drug industry. As people have started to notice more side effects from man-made drugs, many medicines today are made from natural herbs. But herbal medicines have some problems because they don't have clear standards. The big issue is that there are no fixed rules for the materials used, how they are processed, the final product, the way it's given, and there are no clear rules for checking quality. herbal treatments are commonly used for many health problems. Scientists find it hard to create reliable ways

to check the chemicals in these herbs, including measuring the active parts and other main ingredients. Having clear standards is very important to make sure herbal medicines work the same every time, have the same chemical makeup, and meet quality rules during production.

**Keywords:** Herbal drugs, Standardization, Quality control, Chromatography.

## • INTRODUCTION

To ensure the quality of herbal medicines, factors like the concentration of chemical elements, physical properties, chemical makeup, photochemical content, standardization processes, and both in-vitro and in-vivo parameters are all considered. Evaluating the quality of herbal formulations is essential to make them accepted in modern medical systems [1]. One of the biggest problems in the herbal industry is the absence of strong quality control standards for herbal products. To address this, the Ayush department of the Indian government launched a central plan aimed at setting up standard operating procedures for manufacturing processes, which will help in creating pharmacopeial standards for Ayurvedic medicines. It is important to study the medicinal plants of India, but this can only happen if herbal products are analyzed using advanced standardization techniques. Traditional and herbal remedies are preferred because they are cost-effective, safe, and trusted by people. The World Health Organization (WHO) supports the use of these remedies in natural healthcare programs. The WHO has also emphasized the need to use modern methods to ensure quality control of medicinal plant products through various resolutions.[2] The phrase "herbal drugs" refers to various plants or parts of plants that have been transformed into Phyto pharmaceuticals through basic methods including collection, dehydration, and preservation. Herbs consist of unrefined plant components like leaves, blossoms, fruits, seeds, stalks, wood, bark, roots, rhizomes, or other sections of plants, which can be captured whole, in pieces, or in powdered form. Besides herbs, herbal substances encompass fresh juices, gums, oils, essential oils, resins, and dried herbal powders. In certain nations, these substances might undergo different local treatments, such as steaming, roasting, or stirring with honey, alcoholic drinks, or other ingredients.[3] Quality Assurance and Regulation of Herbal Products – Definition and Range As noted by WHO (1996a and b, 1992), the regulation and quality assurance of herbal items entails the examination of the physical and chemical properties of raw substances, addressing elements like the choice and management of raw materials, evaluation of safety, effectiveness, and durability of the final product, recording of safety measures and risks derived from experience, supplying product details to consumers, and marketing of the product. [4]

## • AIM AND SCOPE OF PRESENT WORK

The aim of the present work is to understand and review how the quality and standardization of herbal drugs are studied and maintained. This article focuses on exploring different scientific methods used to check the purity, safety, and effectiveness of herbal medicines. The main purpose is to highlight why standardization is important for ensuring that every herbal product is genuine, free from contamination, and provides the desired therapeutic effect.

## • HERBAL MEDICINES

The term "herb" refers to any plant or any part of a plant that is prized for its medicinal, fragrant, or culinary properties. Herbs can be seen as organic factories that produce a wide range of chemical substances. Unrefined extracts or plant pieces that contain a variety of substances, many of which have synergis-

tic interactions, make up herbal remedies and medicines. Herbal medicine, also known as herbalism, is the practice of employing herbs or herbal goods for their therapeutic or medicinal benefits. The majority of these originate from leaves, roots, stems, seeds, and blooms, but they may also be found elsewhere throughout the plant. They can be applied topically, inhaled, sipped, consumed, or swallowed. Many herbal therapies are made up of a variety of naturally occurring bio-chemicals taken from plants, many of which have therapeutic properties. The phrase "active ingredients" or "active principles" refers to substances recognized for their therapeutic benefits, and their accessibility is contingent upon a variety of factors, such as the plant species, the timing and season of the harvest, the soil quality, the manner in which the herb is processed, and others.[5] Herbs include crude plant materials, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. Herbal preparations are the basis for finished herbal products, may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials.[6]

## ➢ STANDARDIZATION OF HERBAL DRUGS

In the last few years, there has been a significant interest in plant-based products among developed nations. These products are becoming more popular as remedies, dietary supplements, and beauty items.[7] To achieve effective alignment regarding the quality of raw materials, materials in progress, and end products, it has become crucial to establish trustworthy, targeted, and responsive quality assurance techniques that utilize both traditional and contemporary analytical instruments. Establishing standards is a vital metric for guaranteeing the quality assurance of herbal medications.[8] The standardization of herbal treatments involves establishing a collection of criteria or intrinsic properties, clear qualitative and quantitative values that provide a guarantee of quality, efficacy, and consistent parameters reproducibility and safety. The method involves creating and agreeing on technical standards. Particular The procedure of prescribing a standard is based on experimentation and observations. a collection of qualities displayed by the specific medications. As result, standardization is a tool for quality control method. [9] As per the American Herbal Product Association, "Standardization involves the collection of data and control needed to create a product that is fairly uniform. This is achieved by minimizing the inherent variability through the application of quality assurance techniques to modify the composition of natural items sourced from farming and production processes. "[10] safety evaluation, and biological efficacy. Among these factors, the phytochemical profile is particularly crucial due to its direct influence on the effectiveness of herbal preparations. The fingerprint profiles act as a reference for the drug's phytochemical profile, ensuring quality assurance, while the measurement of marker compounds can serve as an extra criterion for evaluating the quality of the sample.[11]

### ○ NEED OF STANDARDIZATION

The approaches to standardization ought to encompass all factors that impact the quality of herbal medications, including the accurate identification of the specimen, sensory examination, pharmacogenetic assessment, assessment of volatile substances, quantitative analysis (such as ash and extractive values), phytochemical assessment, checks for the existence of foreign compounds, testing for microbial content, In ancient times, Vaidya's would treat each patient personally and create medications tailored to the patient's needs. In nearly all traditional medical practices, quality control has been addressed through the

oversight of Rishis, Vaidya's, and Hakims. Unlike in historical periods when traditional healers would formulate and assess the quality of herbal remedies, contemporary challenges include the economics of large-scale manufacturing, product longevity, and distribution over extensive distances. These issues have led to the necessity for the establishment of modern and objective criteria to assess the safety, quality, and effectiveness of these remedies. Individuals are increasingly recognizing both the benefits and the potential adverse effects. To earn public confidence and integrate herbal products into today's healthcare system, it is essential for researchers, manufacturers, and regulatory bodies to implement stringent scientific approaches to guarantee the quality and consistency between batches of traditional herbal products.[12]

- **Need of Quality control and standardization of herbal products are as follows:-**

- When conventional medicines were created, the approach to technology and the idea of standardization were significantly different.
- Over the last millennium, the ongoing process of evolution might have altered the characteristics of plant materials.
- The commercialization has made it difficult to obtain authentic raw materials
- The qualities of botanical substances may have altered as a result of time and environmental influences.[13] Standardization of plant-based products can be classified into two main types. The first type is an extract of active constituents, where the biochemical compounds are identified and possess healing properties. The second type is a marker extract, in which the active substance is unidentified, and a specific compound is utilized as a marker to determine the presence of other therapeutic biochemical substances.[14]

- **STANDARDIZATION OF HERBALOR POLYHERBAL FORMULATION**

The herbal mixture can generally be standardized to create the medication utilizing raw ingredients sourced from various regions while comparing the chemical effectiveness of different formulation batches. The preparations demonstrating superior clinical effectiveness will be chosen. All typical physical, chemical, and pharmacological parameters are evaluated for each batch to determine the ultimate finished product and to confirm the entire manufacturing process. Standardization plays a crucial role in preserving and evaluating the safety and quality of the polyherbal formulation, as these are blends of multiple herbs aimed at achieving the desired therapeutic outcomes. Standardization helps reduce variability between batches and ensures the safety, effectiveness, quality, and acceptance of the polyherbal formulations. [15,16] Standardizing herbal formulations necessitates the adoption of Good Manufacturing Practices. Moreover, examining different factors like pharmacodynamics, pharmacokinetics, dosage, stability, shelf-life, toxicity assessment, and chemical profiling of the herbal products is deemed crucial. Contamination by heavy metals and the adherence to Good Agricultural Practices in the standardization of herbal medicines is also significant. [17,18]

- **WHO GUIDELINES FOR QUALITY STANDARDIZED HERBAL FORMULATIONS**

1. Oversight of raw herbal substances, botanical formulations, and end products.
2. Evaluation of durability and expiration period.
3. Evaluation of safety; recording safety information derived from experience or toxicological research.
4. Evaluation of effectiveness through traditional medical knowledge and assessments of biological activity.

**1) Quality Control of Herbal Drugs**

Quality control denotes the procedures that ensure the quality and validity of a product that has been manufactured. Typically, quality control relies on three key pharmacopeial elements –

a. Identity or authenticity - it must include a single herb

b. Purity – it should be free from any contaminants aside from the herb

c. Assay or Content - the active ingredients must fall within specified limits.

- **Identity or authenticity**

Identity can be established through both macro and microscopic analyses. Alongside these identity assessments, it is also essential to conduct identity tests that involve basic chemical evaluations, such as color changes or precipitation reactions, as well as chromatographic assessments. These chemical and chromatographic evaluations aid in ensuring comparability from one batch to another, and the chromatogram can act as a distinctive identifier for the herbal component by illustrating the characteristics of certain common plant compounds like flavonoids, alkaloids, and terpenes. To validate identity and purity, various criteria must be examined, including the preparation type, sensory characteristics, physical constants, the presence of adulterants and contaminants, moisture content, ash levels, and residues from solvents.[19] Purity is intricately associated with the secure utilization of medications and concerns aspects like ash content, impurities (such as extraneous materials resembling other plants), and the presence of heavy metals. Nevertheless, with advancements in analytical techniques, contemporary purity assessments also take into account factors like microbial contamination, aflatoxins, radioactivity, and pesticide residues. Various analytical techniques, including photometric analysis, Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), High-Performance Thin Layer Chromatography (HPTLC), and Gas Chromatography (GC), can be utilized to ascertain the consistent makeup of herbal formulations. Depending on whether the active compounds in the formulation are identifiable or not, various approaches such as “normalization and standardization” must be adopted to set appropriate standards for consistency. The aspect of content or assay represents the most challenging segment of quality assurance, as the active ingredients in most herbal remedies remain unidentified. Occasionally, markers are applicable. In situations where neither active components nor markers can be established for the herbal remedy, the proportion of extractable compounds using a solvent may be employed as a form of assay, a method frequently encountered in pharmacopoeias. [20,21]

- **Stability Assessment and Shelf Life**

Extended and seemingly uneventful utilization of a substance typically serves as an indication of its safety. Nevertheless, in certain cases, examinations into the potential hazards of naturally sourced materials commonly utilized as components in these products have uncovered previously unrecognized risks for systemic toxicity, cancer development, and teratogenic effects. Regulatory bodies must be swiftly and reliably updated regarding these discoveries. Furthermore, they should possess the power to act quickly in response to these notifications, whether by suspending or modifying the licenses of approved products that include questionable ingredients, or by reclassifying the substances to restrict their use to medical prescriptions.

- **Assessment of Quality**

**All processes must comply with established manufacturing standards.**

- Crude Plant Material The botanical classification, encompassing genus, species, and authority, description, part of the plant, along with active and defining constituents should be outlined, and wherever feasible, content limits should be established.

- Representative sample specimens from each batch of the plant material must be verified by a qualified botanist and retained for at least ten years. A batch number ought to be assigned and displayed on the product's label.

**Plant Preparations**

The manufacturing process should be elaborated in detail. If additional substances are included during production to adjust the plant preparation to a specific concentration of active or defining constituents, or for any other reason, these substances should be detailed in the manufacturing guidelines. A method for the identification and, if feasible, analysis of the plant preparation should be included. If identifying an active ingredient proves impossible, it should be adequate to recognize a characteristic component or combination of components to guarantee the preparation's quality remains consistent.

**Finished Product**

The manufacturing method and formulation, including the quantities of excipients, should be described thoroughly. A specification for the finished product must be established to ensure its quality remains consistent. The completed product should meet the standard requirements corresponding to specific dosage forms. Stability The product's physical and chemical stability within the container intended for marketing should undergo testing under specified storage conditions, and its shelf-life should be determined.

**Safety Assessment :-**

Herbal remedies are often perceived as reliable due to their extensive application in numerous societies. Nonetheless, there are documented incidents of severe negative reactions following the use of herbal products. In many situations, the harmful effects have been linked to impurities and fraudulent practices. Additionally, certain botanicals used in these remedies can possess significant toxicity. Overall, herbal treatments may pose a risk for side effects as well as interactions with other medications and food if they are not evaluated correctly. Consequently, the evaluation of the safety of herbal products is the foremost concern in herbal studies. There are several methods for assessing the safety of herbal remedies. The harmful impacts of herbal formulations can primarily be ascribed to the following factors: the natural toxicity of plant elements and ingredients along with manufacturing negligence and contamination.[22]

**Evaluation of Effectiveness**

Herbal remedies fundamentally differ from standard pharmaceutical treatments; currently, the only method available for evaluating their effectiveness relies on conventional clinical trial techniques. Clinical results encompass aspects like enhanced morbidity, alleviation of pain or discomfort, increased appetite and weight, lowered blood pressure, decreased tumor dimensions or severity, and better overall quality of life. Although it may lack the thoroughness of double-blind randomized studies, this design could be the most effective, both from a biological standpoint and a cost perspective, for quick assessments of herbal products. While randomized clinical trials, particularly double-blind trials as the preferred standard, present challenges for herbal medicine. Information gathered from case series studies may offer enough scientific and ethical foundation to support the execution of these trials; however, adopting this approach necessitates a transformation in the conventional understanding of drug evaluation methodologies. [23,24]

## GUIDLINESS FOR STANDARDIZATION OF HERBAL EXTRACTS

**Table 1 Parameters for standardization of herbs**

| ○ **Parameter** | ○ **Specifications** |
|---|---|
| ○ Authentication | ○ Identification of plant types using their Latin names entails various steps, |

| | |
|---|---|
| | including taxonomic classifications, and studies that are both macroscopic and microscopic. |
| ○ Physical parameters | ○ Physical tests include organoleptic evaluation, viscosity, moisture content, pH, disintegration time, hardness, ash value etc. |
| ○ Chromatographic and spectroscopic ○ evaluation | ○ Sophisticated modern techniques of standardization such as UV, FTIR, HPTLC, HPLC, GCMS, LCMS, NMR |
| ○ Microbiological ○ parameters | ○ Microbial contamination can be measured according to validated or pharmacopeia methodology. Example: E. coli and molds, total enterobacterial, aflatoxin |
| ○ Pesticide residue ○ analysis | ○ Standard limits of pesticides as per WHO and FAO (Food and Agricultural Organization. Example: DDT, BHC, toxaphene and aldrin |
| ○ Heavy metal analysis | ○ Standard limits of pesticides as per WHO and FAO (Food and Agricultural Organization. Example: DDT, BHC, toxaphene and aldrin |

- **METHODOLOGY**
- **CHROMATOGRAPHY METHOD TLC:-**

Before the advent of instrumental analysis, TLC was the most favored and versatile approach for examining herbs. Established chromatography methods include HPLC and gas chromatography. TLC continues to be utilized today and remains popular among various pharmacopeia's, such as those related to Indian herbal medicine, American herbal pharmacopeia's, Chinese medicinal texts, and Ayurvedic pharmacopeias. Instead, TLC serves as a basic preliminary screening approach coupled with a semi-qualitative evaluation. Other chromatography techniques can be more complex as the straightforward separation provided by TLC experiences relatively minor changes. This method facilitates the examination of herbal medicines that utilize instrumental chromatography techniques. In TLC, a solute is distributed across two phases, with one being stationary. It employs a liquid mobile phase and processes involving adsorption. The adsorbent layer remains consistently thin. Herbal preparations that are finely ground can be applied to metal plates, glass, or plastic. Glass plates are the most commonly used surface for this purpose. Moreover, separation can also be achieved through partition techniques or a blend of partitioning and adsorption. The choice of solvent relies on the specific support material in use. Identification is possible by comparing spots that have the same size and Rf value, which are produced on the sample plate from unknown and reference samples respectively. A photograph can be taken. For semi-quantitative analysis, the sizes and intensities of the spots are generally assessed in relation to one another. TLC offers numerous advantageous possibilities for recognizing herbal treatments. It is also relatively straightforward, making it appropriate for the analysis of several samples. Each plate can accommodate over thirty sample spots. Useful qualitative information can be gathered through the CA MAG video storage system and TLC QA-UV techniques, which yield quantitative data from developed TLC plates. For example, four samples of cordyceps sinensis from a joint China-Japan product exhibit the most potent active component, resulting in superior medicinal properties compared to other samples. The compound cordycepin can be analyzed more effectively using computer-generated digital methods and imaging analysis. [56-58]

- **ELECTROPHORETIC METHOD**:

Capillary electrophoresis emerged in the early 1980s as an effective technique for analysis and separa

tion and has experienced significant growth. It provides a proficient means to evaluate the purity and complexity of a sample and is capable of managing almost any type of charged components, from basic inorganic ions to DNA molecules. Consequently, there has been a clear rise in the use of electrophoretic techniques, particularly capillary electrophoresis, for the analysis of herbal products over the past several decades. The rapid advancements in capillary electrophoresis since its inception have closely followed the developments in liquid chromatography. The predominant methods utilized include capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), and capillary isoelectric focusing (CIEF). Capillary electrophoresis shows great potential in the separation and evaluation of active substances found in herbal medicines. [27]

- **GAS CHORMATOGRAPHY:**

Gas chromatography, often referred to as gas liquid chromatography, is a method used to separate mixtures into individual components based on their redistribution between a stationary phase, which may be a liquid, solid, or a combination thereof, and a gas phase that moves. It is commonly understood that many active constituents found in herbal remedies are volatile compounds. Consequently, utilizing gas chromatography for the examination of these volatile substances is crucial when studying herbal medicines. The analysis of volatile oils via gas chromatography offers several benefits. To begin with, this technique produces a distinctive "fingerprint" that aids in identifying the specific plant. The makeup and relative amounts of organic compounds present in the volatile oil are defining features of that plant, allowing for the easy detection of any impurities in the oil. Therefore, liquid chromatography becomes an essential method for us to conduct a thorough analysis of herbal medicines.[28] The recognition and measurement of chemical components in the poly herbal oil formulation (Megni) were conducted using gas chromatography to assess Eugenol, utilizing a DB-5 fused silica capillary column with helium serving as the carrier gas. The retention time recorded was 8. 63 minutes (Fig. 4)

- **High-Performance Liquid Chromatography (HPLC):**

High-performance liquid chromatography is also referred to as high-pressure liquid chromatography, and it involves a stationary phase made up of small particles ranging from 3 to 50 micrometers, packed within a narrow column with a diameter of 2 to 5 micrometers. One end of this column connects to a source that supplies a pressurized liquid eluent, known as the mobile phase. The three main types of high-performance liquid chromatography typically utilized are ion exchange, partition, and adsorption. HPLC is widely used for analyzing herbal medicines due to its user-friendly nature and its capability to handle samples regardless of volatility or stability. Generally, HPLC can be employed to examine nearly all compounds present in herbal medicines. Reversed-phase (RP) columns are among the most commonly utilized columns for the analytical separation of herbal medicines. This novel detector enables direct HPLC analysis of various pharmacologically active constituents in herbal medicines, as the response of the ELDS is influenced solely by the dimensions, shape, and quantity of the herbal medicine's fingerprints. Additionally, performing qualitative analysis or determining the structures of chemical components in herbal preparations through simple HPLC is not feasible; this requires the use of advanced techniques with coupled HPLC methods, such as HPLC-IR, HPLC-MS, and HPLC-NMR, for the evaluation of herbal medicines. [59,60]

- **EVALUATION PARAMETRES**

**1. PHYSICAL EVALUATION:**

The assessment of physical characteristics is an essential element in the standardization of raw medicin-

es. This process aids in the assessment of unrefined drugs concerning factors such as moisture level, thickness, melting temperature, acidity, and so forth. [29] Importance - Physical assessment serves as a vital instrument for establishing the quality, amount, and cleanliness of raw drugs. [29] Foreign organic matter refers to any components of the crude drug that are not specified in its definition or description. The allowable threshold for foreign organic matter is outlined in the monograph, and exceeding this limit suggests a decline in the quality of the drug has occurred. Evaluating the physical properties of substances is essential for the standardization of crude drugs. This approach facilitates the assessment of crude drugs concerning factors like moisture content, viscosity, melting point, and PH. Physical evaluation serves as a vital method for assessing the quality, quantity, and purity of crude drugs.

**2. Procedure:**

Take a sample of the drug weighing between 100 to 500 grams for examination. Distribute it into a thin layer. Foreign organic matter can be identified through visual inspection or with the aid of a 6x magnifying lens. Collect and weigh the identified foreign matter.

The formula for calculating the percentage of foreign organic matter is: n × W × 94,100 × 100 / S × M × P

**In this equation:**

N= represents the count of chart particles observed in 25 fields.

S =signifies the number of spores counted in the same 25 fields.

W= denotes the weight in milligrams of lycopodium used.

M= indicates the weight in milligrams of the sample, which has been dried at 105 degrees Celsius.

P =reflects the number of characteristic particles per milligram of the clean foreign matter.

94,000 is the spore count per milligram of lycopodium. [30]

- **LOSS ON DRYING (LOD):**

LOD refers to the decrease in weight expressed as a weight-to-weight ratio and can be determined by executing the following method. The percentage of chemical constituents in crude drugs is reported on an air-dried basis. Significance-This test determines water and volatile content in the crude drug.[31]

**Table 2: Examples of Drugs with Foreign matter [32]**

| Name Of Drug | F.O.M Limit |
|---|---|
| Rauwolfia Serpentina | Not More Than 2% |
| Acacia Catechu (Bark) | Not More Than 2% |
| Emblica Officinalis | Not More Than 3.0% |
| Curcuma Longa | Not More Than 2.0% |
| Commiphora Wightii | Not More Than 5.0% |

**Table 3: Crude drugs with moisture content analysis [32]**

| Name Of Drug | Moisture Content (%w/w) |
|---|---|
| Rauwolfia Serpentine | Not More Than 12% |
| Acacia | Not More Than 15% |

**3. METHOD:**

Place approximately 10 grams of the drug into a pre-weighed evaporating dish.

Once the specified quantity of the drug is in the dish, dry it at a temperature of 105º for a duration of 5 hours, then measure its weight. Proceed with the drying and weighing process at hourly intervals until the variation between two consecutive weightings is no greater than 0.25 percent. A constant weight is achieved when two successive weighing, after a drying period of 30 minutes followed by a cooling period of 30 minutes in a desiccator, show a difference of no more than 0. 01 grams.[33]

- **ASH VALUE:**

The leftover material after burning is the ash component of the medication.

**4. Importance -** The ash measurement is a crucial factor in validating the acceptability and quality of drugs that have been gathered or maintained improperly. High levels of ash signify the presence of impurities, replacement, or adulteration in raw drugs.[37] The form of adulteration. Ash value is determinant of identity or purity of drug. The ash value is determined by three methods- total ash, acid insoluble ash, water soluble ash.

**Table 4: Examples of Drugs with their Total Ash content**

| Name Of the Drugs | Total Ash (%w/w) |
|---|---|
| Acacia Catechu (Bark) | Not More Than 15 % |
| Rauwolfia Serpentine | Not More Than 8 % |
| Centella Asiatica | Not More Than 2.0% |
| Coriandrum Sativum | Not More Than 6.0% |
| Menth X Piperita | Not More Than 14% |

**Table 5: Examples of crude drugs with Acid insoluble ash content**

| Name Of the Drugs | Acid insoluble ash (%w/w) |
|---|---|
| Agar | Not More Than1.0 |
| Amla | Not More Than 2.0 |
| Bael | Not More Than1.0% |
| Andrographis Paniculata | Not More Than 6.0% |

**Table 6: Examples of crude drugs with Water soluble ash content**

| Name of Drugs | Water Soluble Ash (%w/w) |
|---|---|
| Ginger | Not More Than 1.7 |
| Curcuma Longa | Not Less Than 12% |
| Amla | Not More Than 40% |

**Table 7: Determination of Alcohol Soluble Extractive**

| Drugs | Alcohol Soluble Extractive(%w/w) |
|---|---|
| Amla | Not Less Than 40.0 |
| Ashoka | Not Less Than 15.0 |
| Plantago Ovate | Not Less Than 2.0 |
| Curcuma Longa | Not Less Than 8.0 |

## 5. REFRACTIVE INDEX

Refractive index provides insight into the level of purity. When light travels from a less dense medium to a denser one, it changes direction, and this change is referred to as refraction. [35]

## 6. CALCULATED BY USING THIS 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 percent w/v.

**Table 8: Examples of drugs showing refractive Index**

| Drug | Refractive Index |
|---|---|
| Caraway oil | 1.4838-1.4858 |
| Clove oil | 1.527-1.535 |

### ○ DETERMINATION OF SPECIFIC OPTICAL ROTATION

It is depended on phenomenon of polarization. Polarization means when plane of polarized light passes through liquid the, light gets rotated its clockwise rotation called dextro and anticlockwise rotation called Levo rotator. [36]

**It can be calculated by using 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 PERCENT W/V.

### ○ DETERMINATION OF ph :-

The PH Level can be described as the negative logarithm of the concentration of hydrogen ions based on 10. The pH value is measured using a glass electrode along with an appropriate pH meter. The PH levels for the majority of the extracts vary from 5 to 7 and can be regarded as a quality indicator.

E.g. Andrographis paniculata - 7.33

### ○ VOLATILE OIL CONTENT

The fragrant and unstable primary component of drugs is referred to as volatile oils, and such raw materials are regulated according to their volatile composition.[38]

**Table 9: Examples of drugs showing Volatile oil content**

| Drug | Volatile Oil Content |
|---|---|
| Clove | Not Less Than 15 |
| Fennel | Not Less Than 1.4 |
| Pudina | Not Less Than 1.2 |
| Tulsi | Not Less Than 0.4 |

| | |
|---|---|
| Dhania | Not Less Than 0.3 |

- **PESTICIDE RESIDUE**

World Health Organization and Food and Agricultural Organization establish maximum levels for pesticide remnants, which are commonly found in herbs. These residues are integrated with the herbs during their growing phase. Pesticides like DDT, BHC, toxaphene, and aldrin can lead to significant adverse effects, including poisoning in humans if these raw plant materials are contaminated with such chemicals. [38]

**Table 10: Examples of drugs with Pesticide residue:**

| Drug | Maximum Limit of Aldrin And Dieldrin |
|---|---|
| Fructose Ammi Majoris | Not more than 0.05mg/kg |
| Folium azadirtachti | Not more than 0.05mg/kg |

- **Microbial Contamination**

In therapeutic plants, primary origins of bacterial and fungal contamination are the soil and air. The assessment of E. coli and fungal thresholds is somewhat influenced by the methods of harvesting and production. The compound referred to as aflatoxins can lead to significant adverse reactions when ingested with raw medicinal products; therefore, it must be entirely eliminated or not be present at all.[39]

- **Radioactive Contamination**

Radiation sterilization is beneficial for preventing microbial development in herbal products. While this method can effectively sterilise the botanical materials, it is essential to consider the risks associated with radiation sterilization. The radioactivity levels of the plant samples must be monitored following the protocols set by the International Atomic Energy Agency in Vienna, as well as those established by the World Health Organization. [40]

- **Viscosity**

The viscosity of a liquid remains unchanged for that specific liquid at a defined temperature and serves as an indicator of its makeup, which is why it is a crucial instrument for the standardization of liquid medications. the importance lies in its ability to provide insight into the drug's composition and its stability. [39]

**Table 11: Examples Of Drugs with Their Kinematic Viscosities**

| DRUG | KINEMATIC VISCCOSITY |
|---|---|
| Liquid Paraffin | Not Less Than N64 Centistokes |
| Pyroxylin | 1100-2450 Centistokes |

| | |
|---|---|

- **MELTING POINTS: -**

The melting temperature for uncontaminated substances or plant compounds remains unchanged; however, raw materials sourced from plants or animals consist of various substances, which is the reason they are characterized by a specific melting point range.[34]

**Importance-**This is a criterion used to evaluate the quality and consistency of natural substances. [33]

**Table 12: Examples of drugs with their respective Melting Point. [34]**

| Drugs | Melting Point |
|---|---|
| Colophony | 75-85°C |
| Kokum Butter | 39-42°C |

- **MICROSCOPICAL EVALUATION**

Significance: This approach enables thorough drug examination and serves as a tool for standard drug identification. It is regarded as an essential component for the qualitative assessment of organized crude medicines.[33]

**Stomatal Number**

It is average number of stomata per square mm of the epidermis of the leaf.

e.g. Digitalis purpuria: 25-50

**Stomatal index**

The Stomatal index is the percentage of the number of stomata formed by the total number of epi dermal cells each stoma being counted as one cell.

Stomatal index= S / E+S×100

Where: S= Total number of stomata in a given area of leaf

E= Number of epidermal cells in the same area of leaf.

e.g. Digitalis purpuria: 1.3-3.5

**Vein islet number:**

The vein-islet number is average number of vein islet per square mm of leaf surface midway between midrib and margin. Various species of drugs are distinguished by vein-islet number. E.g. Alex andrian senna and Indian senna are distinguished because of their difference in vein islet numbers which are 27 and 22 respectively.

**Vein islet number of various drugs:**

- Datura metal 19-22
- Datura stramonium 12-16
- Datura fastuosa 18-24
- Cannabis sativa 20-30
- Bacopa monniera 6-13
- Azadirachta indica 10-18

**Vein termination number**

It is defined as the no. of veinlet termination per sq. mm of the leaf surface midway between midrib and margin.

**Trichomes [41]**

The elongated outgrowth of leaf called as tri chomes and they are also known as plant hairs.

**TYPES OF TRICHOMES**

**1. Covering trichomes**

a. Unicellular trichomes e.g. Nuxvomica, cannabis

b. Multicellular-unbranched trichomes

(i) Uniseriate- e.g. Datura

**(ii) Biseriate –e.g. Calendula officinalis**

**(iii)Multiseriate- e.g. Male fern**

c. Multicellular branched trichomes- e.g. Verba's cum Thapsus Detection of alkaloids

**2.Glandular trichome**

a. Unicellular glandular trichome- e.g. Vasaka

b. Multicellular glandular trichome- e.g. Digitalis purpurea

3.Hydathode trichome – e.g. Piper betal

**CHEMICAL EVALUATION (PRE PHYTO CHEMICAL SCREENING)**

Numerous natural remedies contain specific chemical components, and their biological or pharmacological effects rely on these components. Analyzing the chemical composition of these remedies is beneficial for recognizing particular drugs or assessing their quality.[61]

**Table13: Detection of Phyto-constituents.**

| **Detection Of Alkaloids**<br>**Extracts Were Dissolved Individually in Dilute Hydrochloric Acid & Filtered** | | | | |
|---|---|---|---|---|
| **Sr. No** | **Name of Test** | **Procedure** | **Observation** | **Inference** |
| 1. | Mayers test | Filtrates + Mayer's reagent (potassium mercuric iodide) | yellow precipitate | Presence of alkaloids |
| 2. | Wagners test | Filtrates + Wagners reagent Iodine in potassium Bismuth iodide) | brown/reddish precipitate | Presence of alkaloids. |
| 3. | Dragendroff's test | Filtrates + Dandruff's reagent (Solution of Potassium Bismuth Iodide) | red precipitate | Presence of alkaloids |
| 4. | Hager's test | Filtrates + Hager's reagent (Saturated picric acid solution) | yellow precipitate | Presence of alkaloids |

**SPECTROSCOPICAL ANALYSIS:**

**Ultra Violet Spectroscopy**

UV spectroscopy is important technique in the analysis of herbal as well as synthetic drugs. It gives idea about purity of the substance. Its detection capability depends on Beer-Lamberts Law that is absorbance is directly proportional to the concentration and path length.[43]

**Infrared spectroscopy: [46]**

Importance -It is a method of analysis used for identification of functional groups. Plant materials can have their IR spectra measured in an IR spectrophotometer that records automatically. The solid sample is sampled using the practice of pondering.

- Some important IR Frequencies
- Amines -3300-3500 cm-1
- Alkanes-2940-2860 cm-1
- Carboxylic acid-3520 cm-1
- Cynide-2225 cm-1
- Hydroxyl- 3400-3500 cm-1.

**Mass Spectrometry:**

It gives idea about molecular weight and molecular formula. The idea of molecular weight is generated from molecular ion peak. The index of hydrogen deficiency is useful for prediction of validated molecular formula and no of unsaturation. [46]

**NMR Spectroscopy**

It is spectroscopic technique which gives idea of no and types of protons present in particular structure of compound. [46]

**Chromatographic evaluation: -**

**Thin Layer Chromatography**

TLC serves as a crucial instrument for the separation of compounds. This technique is commonly utilized in chromatography. It operates on the principle of adsorption. In this approach, the stationary phase consists of a finely powdered solid, which is spread as a thin layer on a support plate, while the mobile phase is a liquid that moves across the plate's surface through capillary action. Typical adsorbent substances employed include silica gel, alumina, and kieselguhr. [47]

**Table 14: Examples of drugs with their solvent system**

| Drug | Adsorbent | Solvent System |
|---|---|---|
| Rauwolfia Alkaloids | Silica Gel 60 F 254 | Ethyl acetate: Methanol: Water (100:13.5:10) |
| Colchicum Alkaloids | Silica Gel 60 F 254 | Ethyl acetate: Methanol: Water (100:13.5:10) |
| Foeniculum Valorie | Silica Gel 60 | Toluene: Ethyl acetate (93:7) |
| Tribulus Terrestris | Silica Gel G | Toluene: Ethyl acetate (8:2) |

**HPTLC**

HPTLC a layer thickness of 100-150micron is used to achieve separation. HPTLC uses open layers of adsorbents on plates or foils to separate component of samples.[45]

**Importance of HPTLC: -** Recognition and detection of contaminants in herbal products and it also plays a crucial role in determining pesticide levels, mycotoxins, and in maintaining the quality of herbs and health foods. [48

**Examples:**

**i. 18 beta-glycyrrhizin acid**

- Stationary Phase: Silica gel
- Mobile Phase: Acetate: Menthol: Ammonia (10:3:1)
- Ethyl Quantification: UV absorbance (260nm). [33]

**ii. Panaxadiol and Panaxatriol**

- Stationary Phase: Silica
- Mobile Phase: Chloroform: Ether (1:1) ace
- Detection: Spraying with 10% Sulphuric acid in methanol, heating at 1050C for 10 min.
- Quantification: UV absorbance (544nm and 52nm). [33]

**iii. Flavanol Glycosides**:

- Stationary Phase: Silica gel Mobile
- Phase: Chloroform: Benzene: Ethanol: Acetic acid: Water (11:4:2: 1:2)
- Detection: Spraying with 8% AlCl3 in ethanol.
- Quantification: UV absorbance 370nm. [33]

**iv. Carvone:**

- Stationary Phase: Silica gel
- Mobile Phase: Chloroform: acetone (100:2)
- Detection: By dipping in anisaldehyde sulphuric acid reagent, heating at 800 for about 10 min.
- Quantification: UV absorbance 410nm.[33]

**v. Aloin:**

- Stationary Phase: Silica gel
- Mobile Phase: Ethyl acetate: formic acid: Water (17: 2: 3)
- Quantification: UV absorbance 350 nm. [17]

**vi. Andrographis Paniculata**

- Pre-coated plates: TLC Silica gel 60 F, 0.25mm, 108 x 20cm.
- Prewashing: methanol Mobile phase: Chloroform: methanol (8:1)
- Preconditioning: Chloroform: methanol (8:1)
- Development and drying: Automatic development chamber.[45]

**HPTLC chromatogram of Vasicine [50]**

- Pre-coated plates: Silica gel 60 F 254
- Stationary Phase: Silica gel 60F 254
- Mobile Phase: Ethyl acetate: Diethyl amine (88:12)
- Quantification: UV absorbance 236 nm.
- Rf Value: 0.24

HPLC serves as an effective method for the separation and refinement of plant-based substances. It can be categorized into two forms: low pressure HPLC (approximately 5 bar) and high pressure HPLC

(greater than 20bar). [51] This technique holds significant value in the pharmaceutical sector as it provides an efficient means of purification while requiring less time under synthesis conditions.[52]

**Liquid Chromatography-Mass Spectroscopy (LC-MS)**

This method provides excellent accuracy, sensitivity, and specificity for detection. LC-MS offers precise measurement of proteins and peptides with high molecular weights. [54]

**Gas Liquid Chromatography & GC-MS:**

Importance: Gas chromatography serves as a crucial method for identifying volatile compounds.

Gas-liquid chromatography distinguishes these volatile compounds by allowing a gas to flow over a stationary phase. The principle behind separation in gas-liquid chromatography relies on the distribution of the sample between the film of liquid that coats an inert solid. Nitrogen and helium are the most frequently utilized gases in gas chromatography. The strengths of these techniques include their exceptional sensitivity, consistent performance, and high efficiency. Particularly, when combined with mass spectrometry, they yield dependable data for the qualitative assessment of intricate components.[55]

**Evaluation of Anethole in fennel oil**

Sample for testing: Fennel oil
Stationary phase used: FFAP
Gas carrier: Helium
Volume of sample: 0. 1 micro liter

**Evaluation of Eugenol in clove oil**

Sample for testing: Clove oil
Stationary phase used: FFAP
Gas carrier: Helium
Volume of sample: 0. 20 microliters

## CONCLUSIONS:

Ensuring quality and standardization in herbal drug technology requires a multi-dimensional approach that seamlessly integrates traditional knowledge with modern scientific rigor, focusing on the three preferred characteristics of authenticity, purity, and assay. Authenticity acts as the primary validation of the material's true identity through morphology, microscopy, and DNA analysis, while purity and assay protocols are essential for confirming freedom from contaminants and assessing therapeutic potential through both chemical and biological profiling. A critical component of this modernization is chemo profiling, specifically fingerprinting, which establishes a distinct chemical signature for plant extracts to ensure batch-to-batch consistency (Bhutani, 2000). However, reliance on conventional standards, such as those in the *Ayurvedic Pharmacopoeia of India*, often proves inadequate for modern industrial applications because raw materials frequently arrive at production facilities in processed forms that preclude effective microscopic assessment. Consequently, to avoid the discrepancies and lack of reproducibility associated with unstandardized methods, the contemporary landscape must move beyond simple botanical evaluation to embrace a comprehensive suite of physical, chemical, analytical, and chromatographic evaluations that can accurately assess phytochemical composition.

## Discussion:

Global reliance on herbal medicines highlights an urgent need for robust, science-driven standardization

systems that ensure safety, efficacy, and reproducibility. Traditional evaluation methods—primarily morphological, organoleptic, and microscopic assessments—have served historically as essential tools for confirming the authenticity of crude drugs. However, in contemporary herbal industries, these methods alone are insufficient because raw materials often arrive in powdered or processed forms where the original anatomical features are no longer visible. As a result, dependence on classical pharmacopeial standards becomes inadequate, making the modernization of quality assessment unavoidable. A major advancement in modern standardization is the adoption of chemo-profiling, particularly chromatographic and spectroscopic fingerprinting. These techniques provide a chemical signature of plant extracts, enabling reliable identification of botanical sources and ensuring batch-to-batch consistency even when active constituents are unknown. As Bhutani (2000) emphasized, fingerprinting helps overcome variability caused by geographical, environmental, and processing factors. High-Performance Thin Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and advanced hyphenated systems such as LC-MS and GC-MS allow detailed mapping of phytochemical profiles, contributing to more objective and reproducible evaluation criteria. Alongside authenticity, purity assessment remains essential to ensure consumer safety. Modern methods extend beyond physical parameters such as ash values and moisture content to include sensitive detection of heavy metals, pesticide residues, mycotoxins, microbial contaminants, and solvent residues. Techniques like atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and molecular diagnostic tools such as PCR-based assays significantly enhance detection specificity. These tools address the growing concerns about adulteration—intentional or accidental—which has increasingly compromised herbal drug quality in commercial markets. Equally important is assay development, which aims to quantify bioactive or marker compounds to assess therapeutic potential. In many herbal drugs, the active constituents remain unidentified, making conventional assays difficult. In such cases, normalization techniques such as total extractive value, biological activity assays, and metabolite quantification serve as surrogate indicators of efficacy. Metabolomics, chemometrics, and multivariate statistical tools now support these processes by enabling analysis of complex phytochemical matrices and establishing correlations between chemical profiles and pharmacological activities. The integration of DNA barcoding and molecular authentication techniques has revolutionized identity verification. These methods eliminate ambiguities associated with morphological similarity among species and provide rapid, highly specific identification, even in processed materials. When combined with chromatographic and spectroscopic fingerprints, molecular tools offer a multidimensional authentication platform far superior to traditional methods alone. Overall, the discussion underscores the necessity of shifting from basic botanical evaluation toward a comprehensive, interdisciplinary framework that incorporates advanced analytical, chromatographic, molecular, and chemometric tools. Such an integrated approach not only enhances confidence in the quality of herbal medicines but also supports regulatory harmonization and global acceptance. As herbal products continue to gain prominence in healthcare, scientifically sound standardization practices will remain the cornerstone of ensuring safety, therapeutic effectiveness, and commercial sustainability.

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