

STANDARDISATION AND EVALUATION METHODS FOR TRADITIONAL MEDICINAL PLANTS

Abstract

This chapter provides an insightful exploration into the crucial aspects of standardization and evaluation methods for traditional medicinal plants. Traditional medicine, rooted in centuries of cultural and empirical practices, has gained renewed attention in contemporary healthcare. The chapter delves into the significance of standardization as a pivotal process in ensuring the quality, safety, and efficacy of medicinal plant products. It discusses various parameters and methodologies employed for the standardization of botanicals, including phytochemical analysis, marker compound identification, and quality control measures. Furthermore, the chapter examines the diverse evaluation methods utilized to assess the bioactivity, toxicity, and pharmacological properties of traditional medicinal plants. The integration of modern scientific techniques with traditional knowledge is emphasized to establish a bridge between heritage practices and contemporary healthcare standards. Through a comprehensive exploration of standardization and evaluation, this chapter aims to contribute to the advancement of the field, fostering a deeper understanding of the potential of traditional medicinal plants in modern therapeutic applications.

Keywords: Standardization, phytochemical analysis, quality, safety, and efficacy of medicinal plant products.

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I. INTRODUCTION

Traditional herbs have been explored for treating ailments of human beings for a long time. The popularity of herbs globally has been increasing due to their safety profile. Natural herbs and their products have also gained popularity worldwide nowadays due to their diverse applications such as alternative medicines, nutraceuticals, cosmetic purposes and medical devices (Ekor, 2014). Despite enormous applications traditional herbs and their extracts are not as prevalent as popular medicine due to several hurdles. The herbs and extracts face the challenges of regulatory aspects that need to be fulfilled (Jaiswal & Williams, 2017). The second challenge is quality, apart from appropriate analytical methods for the identification and testing of herbs and their extracts (Muyumba et al., 2021). The development of the method for determining of content of natural products is a rigorous task. They required a lot of time and manpower for the estimation of content, which would increase the testing budget of natural products. However, the testing cost can be reduced by alternative methods and modern instrumental techniques. The selection of a robust method of analysis would play a crucial task in this regard (Muyumba et al., 2021). Some herbs and herbal extracts may pose challenges or issues where they come under narcotics. They must go through the complex procedures of customs officers (Muyumba et al., 2021). This book enlightens the different methods of identification and testing of natural herbs and their products using modern instrumental methods of analysis. Moreover, it also focused on quality control, stability testing, and safety efficacy (Alhazmi & Albratty, 2023).

II. PHARMACOGNOSTIC PARAMETERS OF HERBAL DRUGS

The pharmacognostic parameters such as Organoleptic, Microscopic, Physical, Chemical, and Biological parameters are taken into consideration here.

1. Organoleptic Parameters/ Evaluation

Organoleptic evaluation is a method of studying drugs that involves assessing their characteristics using our senses. This analysis includes evaluating factors such as colour, taste, odour, size, shape, and other special features like touch and texture. Often, just by looking at a plant or extract, we can identify it. The acacia gum is usually substituted with Talka gum. The colours of different shades can help identify the adulterant in this case. The slight brown shade indicates talka gum whereas acacia gum can be identified by its white-to-yellow color (Selvam, 2015).

2. Microscopical Parameters/Evaluation

Microscopic evaluation is a critical component in identifying herbs. It can aid in detecting small portions of crude or grounded herbs and spotting adulterants such as insects, mould, animal faeces, fungi, etc. Additionally, it can help to identify the plant by examining its characteristic tissue features. Each plant has a unique tissue structure that can be observed by studying tissue arrangement, cell walls, and configuration when correctly mounted in stains, reagents, and media. The characteristic features of cell walls, starch grains, cell contents, calcium oxalate crystals, fibres, trichomes, and vessels have been thoroughly studied (Ichim et al., 2020).

3. Chemical Parameters/evaluation

Chemical evaluation involves various methods, including qualitative and chemical assays quantitative chemical tests, and instrumental analysis. The methods of evaluation comprise the isolation where active constituents are separated. Another process includes purification and identification of chief constituents are important processes of chemical evaluation. There are two types of chemical tests for evaluation: i) Qualitative chemical tests that help identify different phytoconstituents such as alkaloids, tannins, and glycosides and ii) Quantitative chemical tests that determine the acid value (resins, balsams), ester value (balsams, volatile oils), saponification value (balsams), and acetyl value (volatile oils), among others.

Chemical groups of active constituents are analysed using various chromatographic and spectroscopic methods. Chromatographic techniques such as paper chromatography, gas chromatography, thin-layer chromatography, high-performance liquid chromatography, and high-performance thin-layer chromatography are employed for this purpose. In addition, spectroscopic techniques such as ultraviolet and visible spectroscopy, mass spectroscopy, infrared spectroscopy, and nuclear magnetic resonance spectroscopy are also used (Alhazmi & Albratty, 2023).

4. Physical Parameters/Evaluation

In plant analysis, physical methods determine solubility, optical rotation, specific gravity, refractive index, viscosity, melting point, degree of fibre elasticity, water content, and other properties of the herbs and their constituents. (Sarkar et al., 2022).

- **Solubility:** When examining oils and oleoresins, it is important to consider drug-specific behaviours towards solvents.
- **Optical Rotation:** Certain types of crystalline solids and samples that contain an excess of one enantiomer of a chiral molecule can hinder the plane of polarized light by altering its orientation. This is referred to as optical rotation and any substance with this property is said to be optically active. The enantiomers are the compounds that can be identified by their ability to rotate the plane of polarized light in clockwise/right-hand side directions, which is alternatively termed dextrorotatory (d) or (+). Another counterpart of enantiomer is laevorotatory where it propagates the plane of polarized light in an anticlockwise direction and it can be termed as laevorotatory enantiomer. The degree of orientation may be varied and can be correlated to the quality. An example of such optical rotation is eucalyptus oil, which can rotate the light from 0° to 10°. The honey and Chenopodium oil also possess optical rotation properties having angles of rotations +3° to -15° and -30° to -80° respectively (Muyumba et al., 2021).
- **Refractive Index:** The refractive index (RI) is another important parameter to check the quality of herbal-derived products. It is related to the speed of light which is measured by passing through the material and space. More specifically it can be estimated by dividing the speed of light passing through material by the speed of light in a vacuum space. The difference of diffractions of light is the basis in this case. The

frequency of the light differs with colour variations that affect the diffractions of light. Herbal extracts and oil such oils can be tested for refractive index to check the quality as well as to distinguish the quality of the herbal drug products. The refractive index is expressed in the ranges. The castor oils have RI values ranging from 1.4758 to 1.527, whereas, clove oil has RI values in between the ranges of 1.527 to 1.535 (WHO, 1998).

- **Specific Gravity:** Specific gravity (SG) is alternatively termed as relative density. SG is computed by dividing the mass of an equal volume of distilled water at 4°C (39°F) by the mass of a liquid or solid. The SG varied with different materials that will be used as quality control tools to distinguish the products. some examples of specific gravity of drugs are cottonseed oil, which has a value between 0.88-0.93, coconut oil with a value of 0.925, and castor oil with a value of 0.95 (Muyumba et al., 2021)..
- **Viscosity:** Viscosity is the measure of a fluid's resistance to flow. This resistance opposes the movement of any solid object through the fluid, as well as the movement of the fluid itself when it passes stationary obstacles. The viscosity of a liquid remains constant at a particular temperature and serves as an indicator of its composition (Muyumba et al., 2021).
- **Melting Point:** The melting point is a simple quality control tool for herbal product testing. The melting point is termed as the transformation of solid to liquid at elevated temperature. The extracted and isolated plant constituent possesses a specific and consistent melting point range. The melting point ranges are varied for crude drugs due to the presence of mixed components. Examples of the plant materials that possess ranges of melting points are 62-65 °C and 34-44°C for beeswax and wool fat respectively etc (Muyumba et al., 2021).
- **Moisture Content:** It is crucial to control the moisture content of crude drugs. The presence of moisture may decompose the herbal product or alter its chemical nature easily. The estimation of moisture content can be carried out by heating the material in the dryer till it reaches the equilibrium weight. The heating can be carried out at approximately 100°C. If the crude is stored under controlled humidity conditions it increases the shelf life as well as maintains the quality of the products. Digitalis and Ergot are examples of crude drugs where moisture content plays an important role in their stability. The control moisture content levels recommended are 5 and 8 % w/w, for Digitalis and Ergot etc (Heindl & Müller, 2007).
- **Ultraviolet Light:** Some drugs have the property of fluorescing when their portion of crude drugs or powder after getting exposed to ultraviolet radiation. This characteristic can help identify these drugs. In some cases, it can be challenging to distinguish between different types of rhubarb such as rhapsodic, Indian, and Chinese rhubarb, especially in powdered form. However, when examined under ultraviolet light, the fluorescence properties of each type are distinct enough to enable easy differentiation.

- **Ash Value:** Ash value estimation is a useful tool to determine the quality of crude drugs. It helps to identify low-grade products, excess sandy or earthy matter, and exhausted drugs. Total ash, acid-insoluble ash, water-soluble ash, and sulphated ash are different parameters that are used to assess the quality of crude drugs. However, it's important to note that the highest temperature for incineration is 450°C, which may lead to the volatilization and loss of alkali chlorides (Ajazuddin & Saraf, 2010).
- **Extractive Values:** When analyzing the chemical constituents of crude drugs, various solvents are used to obtain an approximate estimation of their components. The screening of solvents depends on the type of constituents being analysed. For crude drugs that contain hydrophilic constituents such as glycosides, mucilage, tannins etc., a water-soluble extractive is used; for crude drugs that contain tannins, glycosides, resins, etc., an alcohol-soluble extractive is used; and for crude drugs containing fat and volatile components, an ether-soluble extractive can be used (Ahmad et al., 2013).
- **Foreign Organic Matter:** The crude drug with foreign organic matter may also alter the quality of the herbal drug products. Foreign matters specifically include things such as insects, animal excreta, earth matter etc. There are certain limits to foreign organic matter, which is present in plant crude drugs. Garlic and saffron are examples of crude drugs where per cent of foreign matters are restricted to 2. Another example of crude drugs is satavari, where a 1 % limit of foreign organic matter is permissible (Balekundri & Mannur, 2020).

5. Biological Parameters/ Evaluation

The biological activities must be evaluated in addition to the physicochemical parameters. The biological activities include potency, toxicity and pharmacological activity. Pharmacological assays need to be performed on animal models. To assess the safety and efficacy of the crude drugs healthy animal models can be utilized. Animal models such as mice and rats can be tested. Examples of biological activities such as hepatoprotective activity, anti-inflammatory and hypoglycaemic activity of plant crude drugs and extracts can be tested on animal models. Other examples of biological activities include the antimicrobial activity of crude drugs using microbial strain. The antimicrobial assays are performed to check the antimicrobial properties of crude drugs.(Sarkar et al., 2022)(Barba-Ostria et al., 2022).

6. Quantitative Microscopy

Quantitative microscopic evaluation is one of the tools to determine the quality of crude drugs. Various parameters can be tested in microscopic examination including size and length of starch grains. The trichomes, stomatal numbers, and vein islet numbers are also the parameters used to study the pharmacognostic features of leafy crude drug samples. The important parameters of quantitative microscopy include stomatal index, vein islet number, vein islet termination number, palisade ratio etc (Rathour et al., 2022).

III. MICROBIAL CONTAMINANTS

Herbal drugs are derived from different parts of plants and may contain various types of microbial contamination, such as bacteria, fungi, protozoa, and viruses. The level of contamination depends on various factors, including the environment, the quality of the herbal products, and the preparation methods. Generally, herbal drugs carry many bacteria and molds that are often present in the soil, due to poor methods of harvesting, cleaning, drying, handling, and storage. Therefore, risk assessment of microbial load in herbal products is a crucial subject. Identification of microbial contamination and reduction of microbial contamination through technological processes is very important.

The technological process of raw material has many stages and generally acts against microbial growth, but the complete elimination of microbial contamination depends on the initial and work conditions utilized. The main aim of this work was to verify the microbial contamination, like extracting solution and spray dried extract to evaluate the reduction of contamination after the decoction and the spray dry. The analysis of the product was performed by the total count method and MPN coli form (de Sousa Lima et al., 2020).

IV. ANALYTICAL TECHNIQUES OF STANDARDIZATION OF HERBAL PRODUCTS

Analytical techniques refer to methods used to determine the chemical or physical properties of a substance, element or mixture. These techniques range from simple identification to advanced methods that utilize highly specialized instruments. Standardization of herbal products is the process of establishing a set of standards, parameters, and definitive qualitative and quantitative values that guarantee quality, efficacy, safety, and reproducibility (Balekundri & Mannur, 2020) (Amruta Balekundri 2020).

To assess the quality control of herbal products, different techniques such as LC-MS (liquid chromatography-mass spectrometry), HPTLC (high-performance thin-layer chromatography), SFC (supercritical fluid chromatography), thermal analysis, and GC-MS (gas chromatography-mass spectrometry) are employed for quantitative estimation (Balekundri & Mannur, 2020).

The LC-MS technique significantly enhances detection sensitivity and provides valuable information on structure characterization, molecular mass, fragmentation, retention time and analytical compound separation. LC-MS is a technique that can be used to characterize the structure of molecules, determine their molecular mass, provide information on fragmentation, and retention time, and detect a broad range of analytical compounds with high separation. This combined technique can be used to identify, quantify and perform quality control of raw plant material extracts and marketed products such as herbs (Lynch, 2017).

HPTLC is a widely used technique for the quality control of herbal plants. It is commonly used for both identification and quality control purposes. This technique involves comparing the reference and sample simultaneously. It is a useful tool for obtaining peak profiles and their intensities from the HPTLC fingerprint images. These profiles provide both qualitative and quantitative results that can be compared to reference standards. The HPTLC

technique is widely used for identifying adulteration and substitutes, conducting pharmacognostic and phytochemical tests, and analyzing physicochemical properties. HPTLC allows for the creation of chromatograms, fingerprints, digital images, and visual detection of microliter concentrations of samples and standards. Thanks to its ability to apply samples and standards simultaneously on the same plate, HPTLC makes it easier to conduct comparative studies of herbal drugs and formulations (Cobzac et al., 2021).

The SFC method uses compressed carbon dioxide with a small amount of organic solvents as the mobile phase. This unique combination gives the SFC method its name as an alternative chromatography. Compared to liquid chromatographic techniques, the SFC method requires fewer organic solvents and has a lower viscosity of the mobile phase. This results in less pressure drop, making it a more efficient and cost-effective technique. The SFC technique enables the analysis of a wide range of compounds such as lipids, flavonoids, phenolics, alkaloids, saponins, carbohydrates, fat-soluble vitamins, polar and non-polar compounds, and many others. It is a faster and more eco-friendly method compared to other techniques, as it requires less solvent and a shorter analysis time. The technique also allows for multi-residue analysis and has an unconventional sample preparation method. Due to its selective capabilities, SFC is commonly used in large-scale industries (Cobzac et al., 2021).

The thermal techniques TGA and DTA offer thermal stability, mass and enthalpy determination, high sensitivity, reproducibility, and rapid response to variations in results. Thermal analysis is a useful method to characterize herbal extracts and herbal drug products. It helps to control the quality of raw materials, determine purity, thermal stability, and compatibility of substances, and analyze drugs qualitatively and quantitatively. Thermal techniques like TGA and DTA evaluate different parameters, such as reaction order (n), activation energy (Ea), frequency factor (A), and degradation constant, to characterize herbal extracts. These techniques also provide information on absolute water content, crystal water content, and thermal degradation (Saadatkah et al., 2020).

GC-MS is a technique used for chemical analysis that combines two methods: GC and MS. GC separates different components of chemical mixtures, while MS analyzes the components separated by GC. This technique is primarily used to analyze thermo-stable volatile compounds and their derivatives. GC-MS is commonly used for qualitative and quantitative analysis of volatile oil determination, multiple components determination, and drug metabolites determination. Gas chromatography (GC) uses matrix-matched calibration standards to adjust for the matrix effect. However, the GC-mass spectrometry (GC-MS) technique cannot be used for thermolabile compounds. For non-volatile components, they need to be derivatized first before analysis can be carried out (Saadatkah et al., 2020).

The comparison of HPTLC and GC chromatographic techniques is shown in Table No. 1.

Table 1: Comparison of Chromatographic Techniques

Parameters	GC	HPTLC
Stationary phase	Liquid / Solid	Paper
Mobile phase	Pure inert gas	Solvent mixtures
Pressure	Controlled pressure	--

Sample	One-at-one run	Many at a single run
Results	Peaks	Peaks and visuals by bands
System	Closed	Open
Time	2 – 60 min	1 – 30 min
Resolution	High to very high	Moderate to high
Temperature	Increasing	Constant

V. STABILITY ASSESSMENT AND SHELF LIFE

Stability assessment is very necessary for prolonged use of the products. To check the stability of substances physical, chemical parameters and microbiological parameters are also considered.

1. Physical Parameters

Physical parameters of products include different parameters such as appearance, colour, odour, pH, moisture contents, hardness, friability, flow property, ash values etc.

2. Chemical Parameters

Different chemical parameters are considered to check product stability, including limit tests, extractive value, and chemical assays. Chemical analysis can be performed using TLC, HPTLC, HPLC, UV, Fluorimeter, GCMS, and other methods (Kim et al., 2019).

VI. SAFETY ASSESSMENT

Herbal medicines are generally considered safe for long-term use, and there are typically no serious adverse effects after administration. However, the toxicity of medicinal plants may occur due to a mixture of active compounds, interactions with other herbs and drugs, contaminants, adulterants, or their inherent toxicity. Therefore, the plants used in herbal medicine can produce toxicity, and there is a risk of adverse drug effects and drug interactions if the given herbal medicine is not properly assessed (Tsimidou & Boskou, 2003).

The assessment of herbal products is a priority in herbal research, and there are several approaches to evaluate their safety. Firstly, it is essential to determine the active chemical constituents of plants used in herbal medicine and the inherent toxicity of the plant constituents and contamination. Evaluation of the toxic effects of plant constituents of herbal formulation requires detailed phytochemical and pharmacological studies and ensures safe administration to humans (Tsimidou & Boskou, 2003).

VII. ASSESSMENT OF TOXICITY

It is important to investigate the toxicity of medicinal plants as they can contain adulterants and contaminants that can be pharmacologically active and cause unexpected toxicity in herbal products. Ayurvedic medicines have been known to cause lead poisoning in children due to their contamination with heavy metals like arsenic and mercury. The process

of toxicity assessment involves various techniques such as in-vivo techniques, in-vitro techniques, micro-array techniques, cell line techniques, and other modern standardization techniques. By using these techniques, we can assess the potential toxicity of medicinal plants to ensure their safety for human consumption(Saad et al., 2006).

VIII. CONCLUSION

In conclusion, the exploration of standardization and evaluation methods for traditional medicinal plants holds immense significance in bridging the gap between traditional wisdom and modern scientific practices. By establishing robust standards, we not only ensure the safety and efficacy of these medicinal plants but also pave the way for their integration into mainstream healthcare. This chapter underscores the importance of a systematic approach to assess and validate traditional remedies, fostering a harmonious coexistence of ancient healing practices and contemporary medical standards. Embracing a balanced perspective, we can harness the therapeutic potential of traditional medicinal plants while upholding the principles of quality, consistency, and evidence-based practice.

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