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Herbal Drug Standardization A Systemic review

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Abstract : *The demand for herbal medicines and herbal products is increasing worldwide. Therefore, maintaining their quality, safety, and effectiveness has become very important. The quality of herbal materials can be affected by various factors, such as environmental conditions, geographical location, physical and chemical properties, and improper storage. Adulteration is also a major problem that can reduce the quality and reliability of herbal products.*

To ensure the quality of herbal medicines, different methods are used, including physical, chemical, and phytochemical tests, along with modern analytical techniques. These methods help identify the active compounds and evaluate the purity and quality of herbal materials. Nowadays, herbal formulations are widely used for the treatment and management of various health conditions.

Standardization plays an important role in herbal medicine manufacturing because it helps maintain consistent quality, chemical composition, and biological activity from batch to batch. It also supports the development of safe and effective herbal products. Following the guidelines recommended by the World Health Organization (WHO) is essential for evaluating the quality, safety, and efficacy of herbal medicines and promoting their global acceptance. This review focuses on various methods used for the extraction, identification, characterization, and standardization of herbal nanomedicines.

Keywords: *herbal medicines*.

I. INTRODUCTION

Nowadays, plant-based products are becoming increasingly popular in the fields of medicine, nutraceuticals, and cosmetics. Herbal products are easily available in pharmacies and health food stores, where people use them for self-medication or on the recommendation of traditional healthcare practitioners.

Quality is one of the most important factors in healthcare, especially when it comes to medicines. All medicines must meet proper quality standards because they directly affect human health and safety. Synthetic medicines are manufactured under strict rules and regulations. Before reaching the market, they must undergo several quality tests to ensure their safety, effectiveness, and purity.

Herbal medicines are prepared from different parts of plants, such as leaves, roots, stems, flowers, seeds, and fruits. These plant materials generally undergo processes such as collection, drying, and storage before being used in herbal formulations. Herbal products also include plant-derived substances such as essential oils, fatty oils, resins, and gums.

Quality control is an important step in ensuring the purity, safety, and effectiveness of herbal medicines. Various modern analytical techniques are used to examine both raw herbal materials and finished products. Some commonly used methods include High-Performance Liquid

Chromatography (HPLC), Gas Chromatography (GC), and Mass Spectrometry (MS). High-

Performance Thin-Layer Chromatography (HPTLC) is also widely used because it is simple, reliable, and effective. It helps in identifying, authenticating, and checking the **quality** of herbal drugs through fingerprint analysis.

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STANDARDIZATION OF HERBAL DRUG

Standardization is an important process that helps maintain the quality, purity, safety, and effectiveness of herbal medicines. It ensures that every batch of a herbal product contains a
consistent amount of active ingredients and provides similar therapeutic effects. Before a herbal medicine is considered scientifically reliable, it must be properly identified, authenticated, and characterized. This helps maintain consistency during the manufacturing process.

Herbal medicines may sometimes cause harmful effects due to incorrect identification, contamination, impurities, or interactions with other medicines. These problems can lead to adverse reactions and toxicity. Therefore, proper quality testing and standardization are necessary to ensure the safety of herbal products.

The therapeutic effects of herbal medicines mainly depend on the phytochemical compounds present in the plants. These compounds are responsible for their different pharmacological
activities. However, identifying and measuring these active constituents accurately is a major
challenge for researchers. Modern analytical techniques are used to identify, quantify, and study important marker compounds and bioactive ingredients present in herbal preparations.

QUANTITATIVE ANALYSIS AND MARKER COMPOUND

Quantitative analysis is an important method used to determine the amount of one or more chemical compounds present in herbal medicines. It helps researchers measure the concentration of important phytochemical constituents and maintain the quality of herbal products.

Marker compounds are specific chemical substances present in medicinal plants that are used to identify, authenticate, and evaluate the quality of herbal drugs. These markers may or may not have direct therapeutic effects. They are mainly used to check the presence and quantity of important chemical constituents in herbal formulations.

Most marker compounds are naturally occurring secondary metabolites, such as terpenes, steroids, alkaloids, flavonoids, and glycosides. These compounds contain different chemical groups, including alcohol, carbonyl, ester, acid, and amide groups. They are useful for identifying and e HIGH valuating individual medicinal plants and their crude drugs.

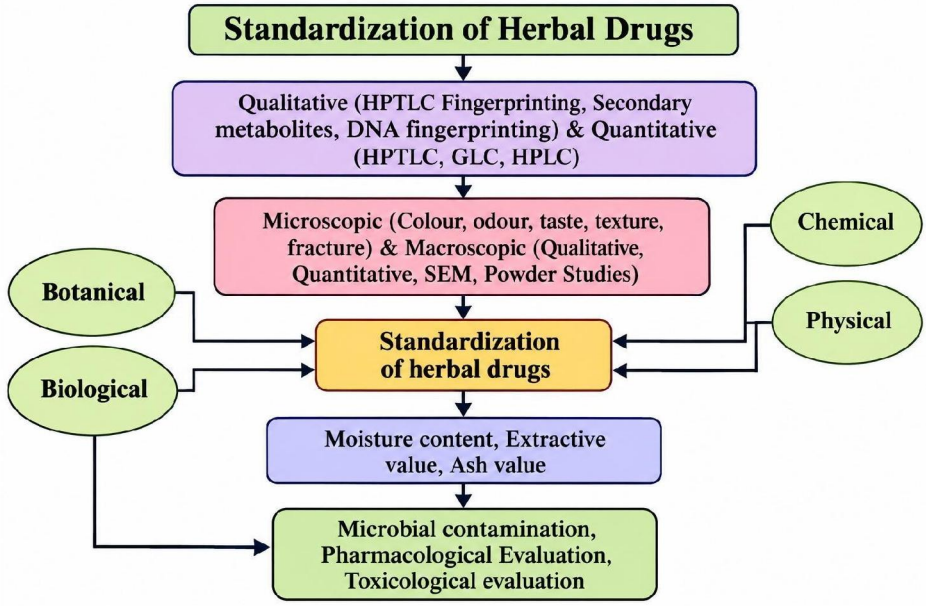


Figure 1: Block diagram of different evaluation parameter for standardization of herbal drugs.

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ANALYTICAL TECHNIQUES USED IN HERBAL DRUG IDENTIFICATION AND QUANTIFICATION

1. Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is one of the oldest and most commonly used techniques for the identification and quality testing of herbal medicines. Before the development of advanced techniques such as Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC), TLC was widely used for the analysis of medicinal plants. Even today, it is an important method mentioned in various pharmacopoeias, including the Indian Herbal Pharmacopoeia, Chinese Pharmacopoeia, American Herbal Pharmacopoeia, and Ayurvedic Pharmacopoeia.TLC is a simple, economical, and reliable technique used for the initial screening of herbal drugs. It helps separate different chemical compounds present in plant extracts and provides information about their chemical composition. It is also useful for the preliminary identification and semi-quantitative analysis of herbal samples.

In this technique, the herbal extract and a reference sample are applied to a TLC plate. After developing the plate with a suitable solvent system, different compounds appear as separate spots. The identification of a compound can be done by comparing the colour, size, intensity, and retention factor (Rf) value of the sample spots with those of the reference standard. The approximate quantity of a compound can also be estimated by comparing the size and intensity of the spotsOne of the major advantages of TLC is that several herbal samples can be analysed simultaneously on a single plate. More than 30 samples can be examined on one plate, depending on its size and arrangement. Different detection methods, including ultraviolet (UV) light and chemical spraying reagents, can be used to identify and analyse the separated compounds.

High-Performance Thin-Layer Chromatography (HPTLC) provides more detailed information about herbal samples. An HPTLC scanner can record important details such as chromatograms, Rf values, colours of separated bands, absorption spectra, and other characteristics of individual compounds. These results help create a characteristic fingerprint profile of herbal medicines.TLC and HPTLC fingerprinting are useful for identifying genuine herbal drugs, detecting adulteration, and maintaining the quality and consistency of herbal products.

Example: TLC fingerprinting has been used to estimate the amount of piperine in the methanolic extract of Sitopaladi Churna. In this study, a Silica Gel G plate was used with a mobile phase containing toluene, ethyl acetate, and formic acid in the ratio of 5:3.5:0.5 (v/v/v). Piperine showed an Rf value of 0.69 and was detected at a wavelength of 342 nm. This demonstrates the usefulness of TLC fingerprinting in the identification and quantitative estimation of important phytochemical compounds in herbal formulations.

2. HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC)

High-Performance Thin-Layer Chromatography (HPTLC) is an advanced analytical technique widely used in the pharmaceutical industry for the quality testing and standardization of herbal medicines. It is useful for identifying adulterants, detecting pesticides and mycotoxins, and evaluating the purity and quality of herbal and health food products.

One of the major advantages of HPTLC is that it requires a smaller amount of mobile phase compared to High-Performance Liquid Chromatography (HPLC). It also allows several samples to be analysed simultaneously, which saves time and reduces the cost of analysis. HPTLC can also work with mobile phases having a pH of 8 or above.

Another important feature of HPTLC is that the same chromatogram can be scanned repeatedly under similar or different conditions. This helps researchers obtain additional information about the chemical compounds present in herbal samples.

HPTLC is particularly useful for analysing multiple chemical compounds in herbal formulations containing several ingredients. It also helps in identifying different plant species and checking the quality, uniformity, and stability of herbal preparations obtained from different sources.

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High-Performance Liquid Chromatography (HPLC) is an important analytical technique used in the pharmaceutical industry for the separation, identification, isolation, and purification of chemical compounds present in herbal medicines.HPLC is mainly divided into two types: preparative HPLC and analytical HPLC. Preparative HPLC is used to separate and collect larger quantities of specific compounds, whereas analytical HPLC is mainly used to identify and measure the compounds present in a sample.

Preparative HPLC can be classified into low-pressure HPLC (usually below 5 bar) and highpressure HPLC (above 20 bar). High-pressure preparative HPLC generally uses larger stainlesssteel columns with packing materials having particle sizes of approximately 10–30 µm.In analytical HPLC, important factors include good separation of compounds, high sensitivity, and shorter analysis time. Different types of columns are selected according to the nature of the compounds being analysed. For normal-phase chromatography, columns such as Kromasil 10 µm, Kromasil 16 µm, and Chiralcel AS 20 µm are used. For reverse-phase chromatography, columns such as Kromasil C18, Kromasil C8, and YMC C18 are commonly used.

HPLC plays an important role in modern pharmaceutical research because it provides accurate and reliable information about the chemical composition of samples. It also helps research rsisolate and purify important compounds more efficiently, reducing the time required for pharmaceutical research and product development.

LIQUID CHROMATOGRAPHY NUCLEAR MAGNETIC RESONANCE (LCNMR)

Liquid Chromatography–Nuclear Magnetic Resonance (LC-NMR) is an advanced analytical technique that combines liquid chromatography with Nuclear Magnetic Resonance (NMR) spectroscopy. It is mainly used for separating, identifying, and determining the chemical structures of unknown compounds present in complex mixtures.This technique is useful in several areas of pharmaceutical research, including pharmacokinetics, toxicological studies, drug metabolism, and drug development. It helps researchers analyse chemical compounds with improved speed and sensitivity.

One of the major advantages of LC-NMR is that it can help identify the structures of unknown compounds, including substances that are sensitive to light and oxygen. The combination of chromatographic separation and NMR spectroscopy provides valuable information about the chemical structure of individual compounds.Automated data collection and processing make LCNMR analysis faster and more efficient. Online LC-NMR techniques also allow continuous monitoring of changes occurring during the chromatographic process.

Recent developments in three-dimensional NMR techniques and pulsed-field gradient methods have further improved the ability to study molecular structures and obtain molecular weight-related information.Overall, LC-NMR is a valuable analytical tool in pharmaceutical research. It supports the identification of unknown compounds and contributes to drug discovery, drug metabolism studies, toxicological evaluation, and the development of new pharmaceutical products.

GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)

Gas Chromatography Mass Spectrometry (GC-MS) is an advanced analytical technique widely used for the identification and analysis of volatile compounds present in herbal medicines. It combines Gas Chromatography (GC), which separates different chemical compounds, with Mass Spectrometry (MS), which helps identify them based on their massto-charge ratio.GC and GC-MS are commonly used in herbal drug analysis because of their high sensitivity, reliability, stability, and accuracy. The combination of GC with MS provides useful information about the chemical composition of complex herbal extracts and helps in the identification of different phytochemical constituents.In this technique, the separated compounds from the GC capillary column directly enter the ionization chamber of the mass spectrometer. Since the flow rate of the capillary column is relatively low, it is suitable for direct connection with the mass spectrometer.An Ion Trap Detector (ITD) is one of the detectors used in GC-MS. In this system, ions are produced from the separated compounds through electron impact or chemical ionization. These ions are trapped inside

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a radiofrequency field and are later released according to their mass-to-charge ratio. The released ions are detected by an electron multiplier detector, which helps generate a mass spectrum for compound identification.

Compared with quadrupole mass analysers, ion trap detectors are generally smaller in size and less expensive. GC-MS has been successfully used to identify various chemical constituents present in natural products, medicinal plants, and biological samples.Overall, GC-MS is an important analytical tool for the identification and characterization of volatile phytochemicals present in herbal medicines.

DNA FINGERPRINTING

DNA fingerprinting is an important molecular technique used for the identification and standardization of herbal medicines. It helps distinguish genuine medicinal plants from substituted, adulterated, or falsely labelled herbal materials, even when their phytochemical compositions are very similar.The chemical composition of medicinal plants may change depending on factors such as the plant part used, environmental conditions, and physiological characteristics. However, the DNA fingerprint generally remains the same in different parts of the same plant. Therefore, DNA analysis provides a reliable method for identifying and authenticating medicinal plants.

DNA (Deoxyribonucleic Acid) is the basic genetic material present in living cells. It contains genetic information that determines the characteristics and properties of an organism. DNA is made up of four main nitrogenous bases: adenine (A), guanine (G), thymine (T), and cytosine (C). The specific arrangement of these bases determines the genetic information of an organism.According to the Central Dogma of molecular biology, genetic information flows from DNA to RNA and then to proteins. This process plays an important role in controlling the formation of proteins and enzymes in living organisms.

DNA fingerprinting has become increasingly useful for identifying and classifying different plants, animals, and microorganisms. It is particularly important in herbal medicine because different varieties of plants belonging to the same genus and species may show genetic differences. Therefore, genetic identification helps distinguish between closely related plant species and varieties.Another important application of DNA fingerprinting is the authentication of commercially available herbal medicines. Even in processed herbal materials, genetic analysis may help detect unwanted plant substitutes and adulterants when sufficient intact DNA is available.Thus, DNA fingerprinting is a valuable technique for the identification, authentication, and quality control of herbal medicines. It helps maintain the purity and authenticity of herbal raw materials and reduces the chances of adulteration in herbal products.

Role of Genetic Markers in Herbal Drug Technology

Genetic Variation and Genotyping

Medicinal plants grown in different geographical regions may contain different amounts of active chemical compounds. These differences can affect their medicinal properties. Genetic marker techniques help researchers identify and study these variations. They are also useful in improving agricultural practices, conserving plant resources, and selecting better plant varieties. For example, RAPD markers are used to identify different neem varieties collected from different geographical regions. Genetic studies have also been carried out on crops such as rice, wheat, chickpea, pigeon pea, and pearl millet.

Authentication of Medicinal Plants

DNA-based techniques are widely used to identify and confirm the correct species of medicinal plants. They are particularly useful when two or more plants look similar or have similar chemical properties. These techniques help prevent the use of incorrect or substitute plant materials. For example, RAPD markers have been used to distinguish the dried fruits of Lycium barbarum from closely related species. They have also been used to identify three medicinal plants in the traditional Chinese herbal formulation Yu-Ping-FengSan.

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Detection of Adulteration and Substitution

Genetic marker techniques help identify adulteration and the replacement of genuine medicinal plants with other similar species. Different methods, such as SCAR, AP-PCR, RAPD, and RFLP, are used to detect these unwanted substitutions. For example, Panax quinquefolius is sometimes used as a substitute for Panax ginseng. DNA-based methods help distinguish between these plants and ensure the authenticity of herbal ingredients.

Kon

Medicinal Plant Breeding

Genetic markers are useful in developing and selecting improved varieties of medicinal plants. Techniques such as ISSR-PCR and RAPD help researchers identify plants with desirable genetic characteristics. These methods have been used in citrus plants, Hypericum perforatum, garlic, and Piper longum. They also help identify suitable plants for breeding, propagation, and conservation.

Quality Control and Standardization of Medicinal Plant Materials

Quality control and standardization are important to maintain the purity, safety, and effectiveness of herbal medicines. Different varieties of the same plant may contain different chemical compounds, which can influence their therapeutic effects. DNA markers help identify the correct plant variety and maintain the quality of medicinal raw materials. They are reliable because the genetic makeup of a plant generally remains unchanged by ageing, physiological conditions, and environmental factors. DNA can also be extracted from both fresh and dried plant materials. Different molecular techniques, including hybridization, PCR, and DNA sequencing, are used to study genetic variations and support the quality control of medicinal plants.

II. CONCLUSION

The herbal products market in India is growing rapidly, and many new herbal products are becoming available. Therefore, proper standardization of herbal medicines is important to ensure their safety, quality, and effectiveness. Traditional methods of standardization are not always sufficient to meet the needs of the modern herbal industry. Hence, advanced techniques are required to improve the quality and reliability of herbal products.

The quality of herbal medicines depends on several factors that may directly or indirectly affect their safety, effectiveness, and acceptability. Various methods, such as botanical, chemical, spectroscopic, and biological techniques, are used to identify and measure the active constituents present in medicinal plants. Modern analytical instruments also play an important role in the testing and quality control of herbal medicines .

Overall, quality monitoring should be carried out at every stage, starting from the collection of raw materials to processing, manufacturing, packaging, and storage. Proper standardization and quality control help ensure that herbal medicines are safe, effective, and of consistent quality for consumers.

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