Traditional And Modern Approaches for Standardization of Herbal Drug

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Abstract:

Standardized procedures have to be established in order to guarantee the efficacy, safety, and quality of herbal medications due to their growing popularity. This essay examines both conventional and contemporary methods for standardizing herbal medications. The significance of indigenous knowledge and cultural practices in herbal medicine is emphasized by traditional approaches, which frequently rely on historical information, empirical practices, and qualitative assessments. Modern methods, on the other hand, use cutting-edge scientific tools like mass spectrometry, chromatographic procedures, and molecular markers to quantitatively evaluate the phytochemical composition of herbal items. This dual viewpoint emphasizes the necessity of an integrated framework that blends contemporary scientific rigor with the knowledge of ancient traditions. This study intends to offer insights into creating thorough standards for the standardization of herbal medications, guaranteeing their safe and efficient use in modern medicine, by analyzing the advantages and disadvantages of each strategy.

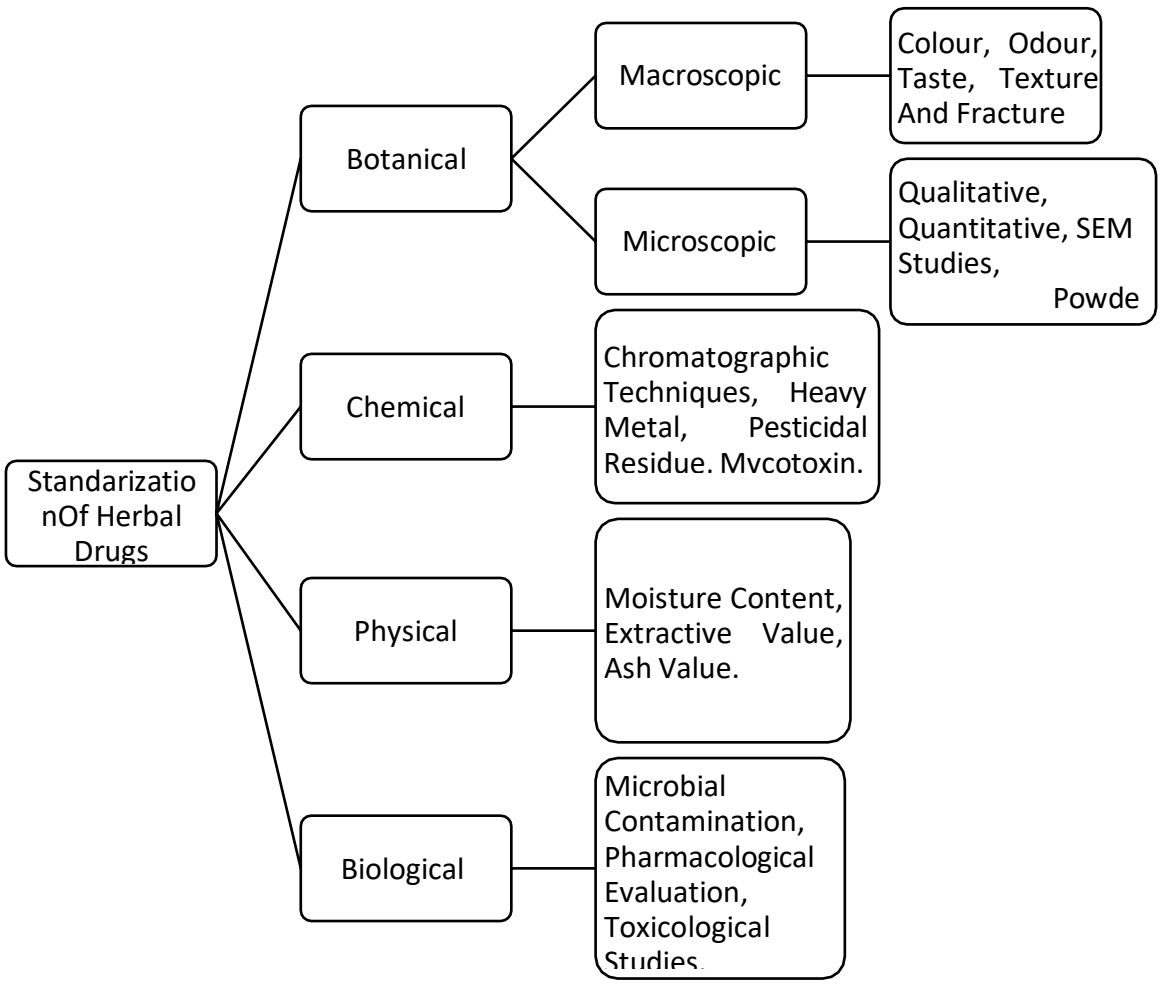
Keywords: Herbal medicine, Standardization, Traditional methods, Modern techniques, Indigenous knowledge.

INTRODUCTION:

India has a long history of using many forms of traditional medicine, including naturopathy, Ayurveda, Siddha, Unani, and homeopathy. For many decades, traditional medicine has been thriving in this nation [1]. Although there are currently over 20,000 known medicinal plant species in India [2], Approximately 800 plant species are utilised by more than 500 traditional communities to treat a variety of disorders [3]. 70 percent of Indians ,according to the WHO, heavily rely on traditional and alternative medicine for their medical needs [4]. Since the beginning of human history, herbal remedies have served as the cornerstone of healthcare around the globe and are still in widespread use today. Every nation in the world continues to acknowledge the medicinal, pharmacological, and economic benefits of herbs. Drug development and pharmacological research benefit greatly from the uses of medicinal plants. Additionally, medicinal plants can be used as models for pharmacologically active chemicals or as lead materials for drug synthesis. Strict regulation of the use and export of medicinal plants and their active ingredients is necessary, as is international coordination and cooperation for their conservation in order to guarantee their continued availability [5[].Since ancient times, medicinal plants have been utilised to cure a variety of ailments. Modern medicine has advanced steadily in recent decades, and plants have long played a significant role in traditional medical systems that have been utilized for ages to cure illness. Herbal remedies include whole parts of plants, algae, and fungus in their untreated country, normally in dried state but sometimes fresh. [6]. Herbal medicines, often known as plant materials or herbalism, are used to cure wounds or diseases by using complete plants or plant components [7]. Herbal treatment is the application of therapeutic herbs to avoid or cure diseases or to encourage recovery and wellness.[8]. These are drugs or formulations made from a plant or plants for any of these purposes.The earliest known medical treatment is herbal medicine. Herbal medications are essential components of several traditional medical systems, including neuropathic, homeopathic, and Ayurvedic [9]. Herbal medications are defined the World Health Organization (WHO) as full, labeled medications contain active substances, aerial or secretive plant parts, or other plant material or mixtures. According to WHO estimates, 80% of people worldwide presently receive their major medical care from herbal remedies. The number of herbal drug producers has rapidly increased as a result of the usage of herbal remedies due to the toxicity and adverse effects of allopathic medicines. . People without prescriptions have been using natural medications more and more over the past few decades. Throughout the millennia of their use, herbal medicines have included extracts from seeds, leaves, stems, bark, roots, and flowers. As analgesic, sedative, antidepressant, antianxiety, antispasmodic, antibacterial, antidiabetic, antifertility, antiaging, antiarthritic, Herbal remedies have become widely accepted as effective treatments for cirrhosis, asthma, acne, impotence, menopause, migraine, gallstones, chronic fatigue, Alzheimer's disease, and memory-enhancing qualities. They also have anti-inflammatory, anti-HIV, vasodilatory, and hepatoprotective qualities. [10]

STANDARDIZATION OF HERBAL DRUG:

A technique of standardization guarantees a certain quantity, purity, and therapeutic impact of substances in every dosage. [11] If medicines tested has not been verified and described to guarantee Considering the production process's repeatability, the herbal product cannot be considered scientifically valid. Additionally, a variety of detrimental and lethal side effects have been reported recently, such as direct toxic effects, allergic reactions, contaminant effects, and interactions with herbal drugs.6. The phytochemical components of a herbal preparation determine its therapeutic efficacy. One of the biggest challenges facing scientists is the development of trustworthy analytical methods for profiling the phytochemical content, such as quantitative analyses of bioactive and a marker molecules and other crucial components. Given the aforementioned, standardization is a crucial first step in creating a consistent chemical profile, a consistent biological activity, or even just a quality assurance program for the manufacturing and production of herbal drugs. [12] Both pharmaceutical firms and public health depend on the ability to identify adulterants from genuine medicinal plants and authenticate herbal drugs in order to ensure the reproducible quality of herbal medicine.



Knowledge gained

IMPORTANCE OF STANDARDIZATION IN CURRENT SCENARIO:

When quality control is taken into consideration, herbal compositions reveal the amount of issues. This is due to the characteristics of the herbal constituents and the various secondary metabolites they contain. Furthermore, the chemical composition of the herbal product varies due to both internal and external influences, including growing, harvesting, storing, and drying techniques. Interest in the development of herbal product quality and standardization stems from two key factors. First, the use of medical plants, including traditional remedies, dietary supplements, phytomedicines, and ingredients for food and drink. Natural secondary products continue to be a significant source for the development of novel drugsA product's quality is the result of combining all the various factors that significantly affect it. Changeable characteristics for herbal medications include things like the herb's origin, botanical identification, purity, potency, stability, and marker compound compositionFurthermore, the quality of the herbal products is directly evaluated using good manufacturing practices (GMP) and good agricultural practices (GAP). It is a challenging task to sustain the herbal product commerce while maintaining its quality. To overcome this obstacle, herbal products must be standardized and their quality assessed in compliance with international standards. Standardizing natural products is difficult since they are made from a variety of plant parts and have a wide range of compositionsIt is crucial to properly regulate the starting material in order to guarantee repeatable quality in herbal goods. [13]

TECHNIQUES IN HERBAL DRUG:

1.High Performance Liquid Chromatography (HPLC):

Because HPLC is simple to use and understandIt is an extensively utilised method for the analysis of herbal medicines. Moreover, it is not limited by the volatility or stability of the sample compound. Generally speaking, HPLC can be used to analyse almost any component present in herbal remedies. The spectrum of applications for HPLC is significantly greater than that of GC. Most organic chemicals can be detected using HPLC, which is outfitted with various

mobile phases and detectors. The pharmaceutical industry makes extensive use of both preparative and analytical HPLC to separate and purify herbal components. HPLC Low pressure (usually lesser than 5 bar) or high pressure HPLC (pressure great than 20 bar) are the two main categorieses of preparative HPLC. Fast analysis, sensitivity, and resolution are crucial Resolution and sensitivity are important considerations. time in analytical HPLC, but in preparative HPLC, both the solute purity level and the amount of chemical that can be produced per unit of time, or throughput or recovery, are important considerations. Larger stainless-steel columns and packing materials with particle sizes between 10 and 30 μm are required for preparative HPLC (pressure >20 bar) [14]. The most widely used columns are reversed phase columns. utilized to separate herbal medications analytically. Some new techniques, such as Micellarelectrokinetic capillary chromatographyTo improve separation, researchers in the field of liquid chromatography are developing techniques such as High Speed Counter Current Chromatography, Low Pressure Size Exclusion Chromatography, Reversed Phase Ion-pairing HPLC, and Strong Anion Exchange HPLC. One of the primary benefits of HPLC is the ability to perform hyphenation using a variety of detectors, including a diode array detector for herbal fingerprinting [16], an ultraviolet detector for UV absorbing compounds [15], a chemo-luminescence detector for non-UV absorbing compounds [17], an NMR detector for metabolomic profiling [18], or mass spectrometry for identifying the separate compounds [19].

2 .High Performance Thin Layer Chromatography (HPTLC):

The most popular fingerprints technique for herbal analysis of TLC. TLC of the resins made it simple to identify four types of herbal remedies.In addition to evaluating the stability and uniformity of their preparations from diverse manufacturers, this technique allows for the verification of distinct species of Radix Puerariae and Ginseng. Di et al. used automated multiple development to create a fingerprint of fungal polysaccharide acid hydrolyzates, whereas HPTLC fingerprints are often used to analyze components with low or moderate polarity. The HPTLC technology is used extensively in the pharmaceutical sector for process development, adulterant identification or detection in herbal products, pesticide content identification, mycotoxin detection, and quality control of herbs or health foods. The simultaneous measurement of beta-

sitosterol-dglucoside or withaferin An in four ashwagandha formulations was reported using the HPTLC technique. HPTLC was used to quantitatively assess Syzygium jambolanum's phytoconstituents, including glycoside (jamboline), tannin, ellagic acid, and gallic acid, as well as its stability, repeatability, and accuracy. Bacosides A and B in Bacopa monnieria and its formulations were detected, tracked, and quantified using HPTLC.By utilizing HPTLC to estimate the amount of cannabinoids in a urine sample, Cannabis stavia was standardized. Withaferine A, a component of Withania somnifera in herbal extract and polyherbal formulations, was estimated using HPTLC. Swetiamarin has been quantitatively estimated using the HPTLC method in a number of commercially available polyherbal preparations as well as in small, large, and fresh fruits of the E. littorale species. Organoleptic research, physico-chemical analysis, TLC, and HPTLC were used to standardize Chandanasava, which is reported to be beneficial in treating karsya (malnutrition). The chemical changes brought about by decocting and the chemical consistency of conventional and dispensed granule decoctions were assessed using ultra-performance liquid chromatography (UPLC). The functioning mechanism of traditional Chinese medicine (TCMs) and additional control over their intrinsic quality are made possible by the combination of chromatographic fingerprinting and metabolomics. Furthermore, the thorough investigation of chromatographic fingerprinting in conjunction with multivariate analytic techniques created in the fields of bioinformatics and chemometrics reinforced TCMs' intrinsic quality and further controlled and strengthened its operating mechanisms in a comprehensive way. [20–26]

3.Ultra-High Performance Liquid Chromatography (UHPLC):

UHPLC has been showing promise as a practical method for herbal product quality control in recent years. By making hardware changes to the traditional HPLC equipment, UHPLC raises the bar for liquid chromatographic analysis and can tolerate pressures of up to 8000 psi. By employing solid phase particles smaller than 2 mm in diameter, UHPLC enables high resolution separations that are superior to HPLC analysis in terms of sensitivity and resolution. Shorter column sizes result in shorter analytical times with minimal solvent use, and smaller particle sizes increase separation efficiency [27]. UHPLC tests revealed an up to eight-fold reduction in

analytical time without information loss when compared to HPLC. In comparison to traditional HPLC analysis, the results demonstrated a significant improvement in selectivity in addition to a reduction in analysis time. [28]

4.Liquid Chromatography- Mass Spectroscopy (LCMS):

In a number of drug development stages, LC-MS has become the method of choice.Using LC-MS, 20 chemical components acting as reference markers were produced by chemically standardizing an aqueous extract of the mixture of the 20 herbs. Additionally, aminoglycosides' LC-MS study revealed that they were more than 90% eliminated by the kidney, had little plasma protein binding, and were highly soluble in water. Additionally, this method aids in the ion pairing chromatography analysis of aminoglycosides in plasma samples. Aristolochic acid I and II in herbal remedies were analyzed using two HPLC techniques: one with mass spectrometry (LC/MS) and another with a photodiode array detector (LC/UV). Positive-ion electrospray ionisation MS and an acetate buffer-acetonitrile solvent system were employed for the LC/MS method, whereas a Cosmosil 5C18-MS column with a gradient solvent system composed of phosphate buffer-acetonitrile and a UV detector (390 nm) was utilised for the LC/UV approach. At m/z 359, m/z 324, m/z 298, and m/z 296 for aristolochic acid I, and at m/z 329, m/z 294, and m/z 268 for aristolochic acid II, the distinctive fragment ions were chosen. [29–31]

5.Liquid Chromatography-Nuclear Magnetic Resonance Spectrometry (LC-NMR)

Liquid chromatography-nuclear magnetic resonance spectrometry is the method that combines HPLC with NMR. This method is frequently used to analyze complicated mixtures that contain synthetic polymers, natural compounds, and unknown contaminants. Pharmacokinetics, toxicity studies, drug metabolism, and the drug discovery process are among the fields that find usage for LC-NMR since it increases detection speed and sensitivity. One of the most effective and efficient methods for the separation and structural elucidation of unknown compounds and mixtures is the combination of chromatographic separation technology and NMR spectroscopy, particularly for the structure elucidation of substances that are sensitive to light and oxygen. The automated data collecting and processing in LCNMR enhances detection speed and sensitivity, and the online LCNMR technique permits the continuous recording of temporal changes as they occur in the chromatographic run. The recent introduction of the pulsed field gradient technique in high resolution NMR and three-dimensional technology improves molecular weight information and structure elucidation. These innovative hyphenated techniques have potential applications in the domains of pharmacokinetics, toxicity research, drug metabolism, and the drug discovery process.[32]

6.Gas Chromatography (GC) And Gas Chromatography-Mass Spectroscopy(GC- MS):

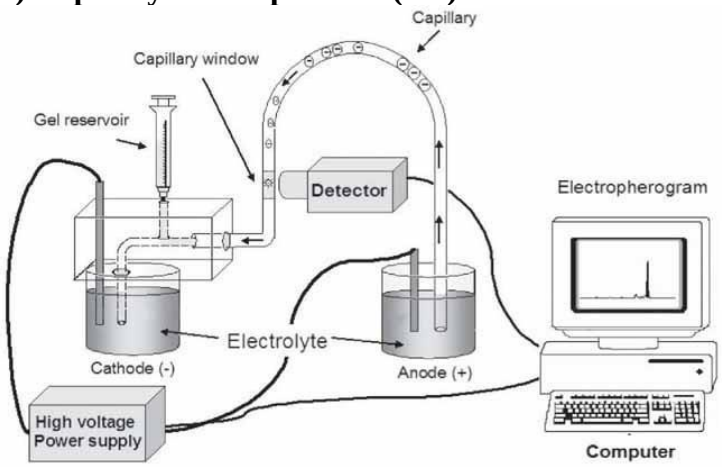
Numerous components found in biological and ecological systems have been identified using GC-MS equipment. The GCMS method was used to identify and quantify the chemical elements found in the polyherbal oil formulation (Megni), which contains nine substances, namely Myristica fragrans, Eucalyptus globulus, Gaultheria procumbens, and Mentha piperita. The volatile components in Rhioxma Curcumae Aeruginosae, a traditional Chinese medicine (TCM), were analyzed using a headspace solid-phase microextraction method. A total of 35 volatile chemicals were discovered and separated. The determination of organochlorine pesticide residues in Scutellaria baicalensis was accomplished using a capillary gas chromatography approach that was efficient, quick, and precise. Fructus arctii, Anemarrhena asphodeloides, Angelica dahurica, Arisaema erubescens, Platycodon grandiflorum, Belamcanda chinensis, and Salvia miltiorrhiza. Using an electrochemical detector, the SPE extract was separated using a 30

m x 0.25 mm i.d. x 0.25 microm capillary column. The split ratio was 1:2.2 when using the carrier gas N2 (99.999%) at a flow rate of 1.4 mL/min. The injector had a temperature of 220 degrees Celsius, whereas the detector had 330 degrees. From 100 degrees Celsius to 190 degrees Celsius (hold for 1 minute), the column's temperature was increased by 20 degrees Celsius each minute, and then by 4 degrees Celsius per minute to 235 degrees Celsius, which was held for 7 minutes. For thirteen organochlorine, excellent linearities were obtained.

7 . Super Critical Fluid Chro matography (SFC):

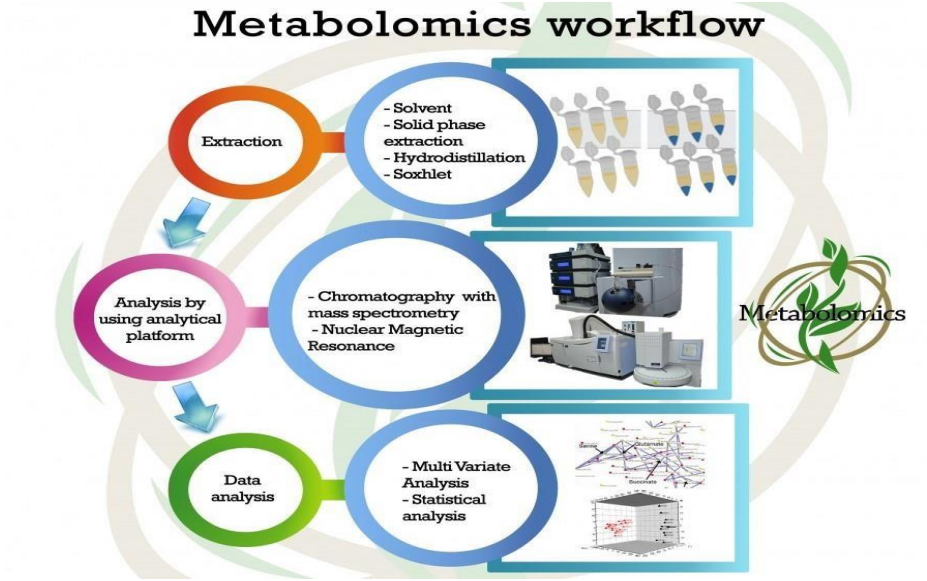
Combining some of the best aspects of gas and liquid chromatography, supercritical fluid chromatography is a hybrid. A set of substances that are difficult for gas or liquid chromatography to handle can be separated and determined using SFC. Natural goods, medications, food, and pesticides are just a few of the things to which SFC has been applied. These compounds are either nonvolatile or thermally labile, making GC procedures ineffective, or they lack a functional group that would allow identification by the spectroscopic or electrochemical techniques employed in LC. [36]

8) Capillary Electrophoresis (CE):



The significance of CE for herbal medicine quality control was assessed by researchers. Numerous CE studies on herbal remedies have been published, and two types of medicinal compounds—alkaloids and flavonoids—have been thoroughly investigated. In order to assess the specificity, sensitivity, and precision of a single plant medication, the CE approach was developed; the outcomes were consistent with those of the HPLC method. Additionally, compared to HPLC, the CE method's analytical time was doubled, and its solvent usage was more than 100 times lower. A distinctive Flos carthami fingerprint created with CE helped with multiple research items at once: recognizing the unprocessed herb, assisting in the differentiation of the adulterant or substitute, and further evaluating the variations.When Radix scutellariae's CE and HPLC fingerprints were compared, the analysis time for the CE fingerprint decreased from 40 to 12 minutes, but the number of observed peaks also decreased from 14 to 11. Although the hyphenated CE instruments, including CE-diode array detection, CE-MS, and CE-NMR, have been used, there have been reports of certain repeatability issues with CE hyphenations.[37]

9) Metabolomic Techniques:



The most recent branch of functional genomics, known as "Metabolomics," which focuses on the biochemical makeup of cells and tissues, enters the world of genomic sequencing. Metabolomics is the comprehensive quantitative and qualitative analysis of each metabolite present in a certain cell, tissue, or organism. A biological organism's whole collection of metabolites, which are mostly the result of its gene expression, is represented by its metabolome. An individual organism's life history, encompassing age and environmental variables including soil type, moisture content, temperature, and stressors, is represented by its metabolomes. In the postgenome age, "metabolomics," a recently developing field in natural product research, is the study that entails the in-depth examination of these metabolomes. Targeted (a particular group of metabolites) and

untargeted (all metabolites) metabolite analysis are the two main methods in Metabolomics analysis. In contrast to a full metabolome analysis, targeted metabolite analysis, also referred to as metabolite profiling, targets a subset of metabolites in a sample using a particular set of analytical techniques or hyphenated analytical techniques, such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS)Among the contemporary analytical methods used in metabolite analysis armory are nuclear magnetic resonance (NMR), Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR), and Fourier transform ion cyclotron mass spectrometry (FTMS). At the moment, MS is the most extensively used metabolomics technology. GC-MS is a widely used MS technology for metabolite profiling of plant extracts or human bodily fluids. To find spectral patterns within a variety of data sets, the untargeted method—also referred to as the chemometric—combines multivariate statistical studies with spectral profiling. This technique has some very particular data processing issues that frequently call for specialist (or costly) data analysis software. Many metabolomics databases have lately been made publically available. These databases are based on chemical and spectral data or chemical and biological and biochemical data. Future developments in computational metabolomics will be aided by databases such as the BioMagResBank (BMRB), the Madison Metabolomics Consortium Database (MMCD), MassBank.jp for high-field mass spectral data, the GolmMetabolome Database (GMD), and BiGG, which offer adequate spectral, chemical, and biological insight into metabolic profiles. Future advancements in metabolomics will depend on the optimization of chromatographic and spectral methods of analysis with improved sensitivity instruments, with higher resolution capabilities, and greater mass accuracy. It is anticipated that a thorough integration of data from proteomics, metabolomics, and genomes would yield strong scientific justifications for the creation of contemporary phytomedicines and their validation. The discovery of active phyto-constituents in herbal medicine has been done using metabolomics. The chemical components of Sophoraflavescens were identified using a metabolomic technique, and their effects on cytochrome P3A regulation and Pregane X receptor activation were then examined. The production of active secondary metabolites from medicinal plants as new or enhanced phytotherapeutic drugs has been documented as one of metabolomics' larger potentials. Recent research shown that the purity of a herbal remedy could be determined by combining an orthogonal projection to latent structure with an NMR-based metabolomics technique. [38, 39]

10 .Thermal Analysis of Herbal Drugs:

Preformulation or drug excipient compatibility, as well as an physical and chemical change in a variety of goods, including herbal medications, have been studied usingdifferential scanning calorimetry (DSC), differential thermal analysis (DTA), and thermogravimetric analysis (TGA). TGA can be used in subambient environments to analyse the ethanol in herbal remedies such asavas and arista. The existence of mercury sulfide was shown by TGA and DTA analysis of the mercury-based Indian traditional metallic herbal medication Ras-sindoor. This was based on a steep peak at 354 °C, which was mercury sulfide's melting point. The optimised extraction from the distillation showed that the volatile oil in dried ginger was a part of the volatile oil-beta-cyclodextrin inclusion complex using DTA. Data from DSC thermograms verified that a phospholipid complex had formed.[40]

11 X-Ray Powder Diffractometry (X-RPD): Minerals, crystalline compounds, and herbal

compositions with a metallic foundation are identified using this method. XRD was used to assess the tin-based herbal medication Vanga Parpam, and the crisp, intense diffraction peaks amply demonstrated the drug's great crystallinity. Ras-sindoor, a metallic-based Indian traditional medicine, contained mercury sulphide, as shown by strong peak139 in the XRD analysis. Data from X-ray powder diffractometry verified that a phospholipid complex containing gallic acid, emodin, naringenin, and quercetin had formed. [41]

12 **Differential Pulse Polarography (DPP):** Chemical traces with detection limits of 10-8M can be investigated using differential pulse polarography. DPP was successful in identifying and determining the presence of several heavy metals in calendulea and chamomile flowers, including Pb, Cd, Zn, Cu, and Fe. Heavy metal accumulation, specifically Pb, Cd, Cu, and Zn, was assessed in the market together with authentic samples of significant Indian herbal medications, such as Alpiniagalanga, Artemesiaparviflora,

13 . Infrared Spectroscopy

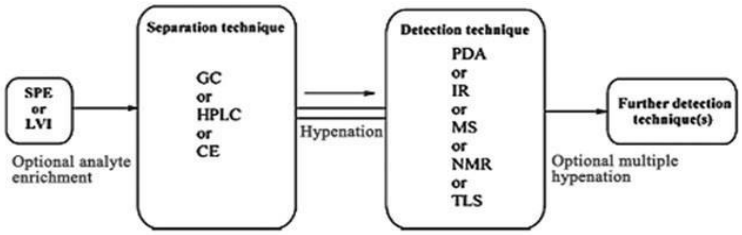
For quality control, FTIR and the statistical technique principal component analysis (PCA) were used to identify and distinguish herbal medications in the 400–2000 cm-1 fingerprint range. An additional way of discrimination for herbal medicines was discovered: the ratio of the areas of any two marked distinctive peaks was almost constant for the same plant from different places. By grouping herbal medicines into several categories, PCA demonstrates that the IR technique can effectively distinguish between various herbal medicines using FTIR data. Rapid identification of active ingredients, species, geographic origin, unique medicinal formula, online quality control, counterfeit detection, and geographical origin discrimination of Chinese herbal medicines have all been accomplished with the use of near-infrared spectroscopy. Fructus discrimination was done using two-dimensional near-infrared (NIR) correlation spectroscopy.

14. Hydrophilic Interaction Chromatography (HILIC):

HILIC has drawn interest in herbal fingerprinting due to its high hydrophilic compound separation quality. The aqueous solution used to extract many of the polar chemicals found in herbal remedies may be better separated utilizing HILIC. As an alternative to normal-phase liquid chromatography, HILIC was introduced. Aqueous mobile phases and polar compounds on polar stationary phases can be separated thanks to HILIC. Its

foundation is the idea of partitioning between a relatively hydrophobic mobile phase, which typically contains 5–40% water inorganic solvent, and a water-enriched layer in the hydrophilic stationary phase. Because this method uses water and polar organic solvents as the mobile phase, it is more environmentally friendly than normal-phase liquid chromatography. Furthermore, the polar chemicals dissolve better in the HILIC mobile phase. Since HILIC is a relatively new technology, there aren't many published studies that analyze herbal items

15 **Hyphenation Of Chromatography and Spectroscopy Techniques:** Chromatographic Different detection methods, including infrared spectroscopy (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS), can be combined with separation techniques. Compared to traditional methods, these hyphenated techniques offer greater sensitivity by providing information on the structure of the compound present in the chromatogram. [46]



16 **Gas Chromatography-Flame Ionization Detector (GC-FID):** In gas chromatography, a variety of detectors are employed. The most often used ones are the thermal conductivity detector (TCD) and the flame ionization detector (FID). A powerful way to separate and identify the constituents of various mixes is to combine capillary column gas chromatographs with a Fourier Transform Infrared Spectrometer (Sharma). Both are sensitive to different components and function throughout a wide range of concentrations.Even though TCDs are nearly ubiquitous and can detect any component other than the carrier gas (as long as their thermal conductivities differ from the carrier gas's at detector temperature), FIDs are more sensitive to hydrocarbons than TCDs. However, a FID cannot detect water. Both detectors are also quite dependable.

TCD can be used in series with a destructive FID to provide complementary detection of the same analytes because it is nondestructive Water cannot be detected by a FID, though. Additionally, both detectors are quite reliable. TCD can be used in series with a destructive FID to provide complementary detection of the same analytes because it is nondestructive [47].

17 **.Gas Chromatography Fourier Transform Infrared spectrometry (GC-FTIR):** A powerful way to separate and identify the constituents of various mixes is to combine capillary column gas chromatographs with Fourier Transform Infrared Spectrometers. [48]

18 **.Chemometrics:** Chemometrics is used to resolve the mixture into linear components, enhance experimental procedures and glean important insights from chromatographic data. Chemometrics can be used to determine the quality of herbal remedies.
In herbal drug standardization, a number of chemometric techniques are used to address the chromatographic fingerprint, including principal component analysis (PCA), linear discriminate analysis (LDA), spectral correlative chromatography (SCC), information theory (IT), local least square (LLS), heuristic evolving latent projections (HELP), and orthogonal projection analysis (OPA). This is due to the complexity of the chromatographic fingerprint, as well as the irreproducibility of chromatographic and spectral instruments and experimental conditions.Variation determination of common peaks/regions and similarity comparison using the similarity index and linear correlation coefficient are the fundamental tenets of this methodology. Software called Computer Aided Similarity Evaluation (CASE) has been created to make data processing easier. All of the CASE chemometric algorithm applications are written in METLAB5.3 using Windows. This software can be used to examine chromatographic fingerprint data loading, removal, cutting, smoothing, compressing, background and retention time shift correction, normalization, peak identification and spectral matching, variation determination of common peaks/regions, similarity comparison, overly of sample classification, and other data processes.[49, 50]

19 **.DNA Markers Fingerprinting:** Many plant species of therapeutic significance have been authenticated using DNA-based methods. For species or varieties that are frequently mixed or substituted with others that have the same morphology and/or phytochemistry, this is

especially useful. DNA molecular markers offer several benefits over traditional phenotypic markers because each species' genetic composition is unique and unaffected by age, physiological state, or environmental factors. They are also trustworthy for revealing polymorphisms. Since DNA may be extracted from both fresh and dried organic tissue of the botanical material, detection is not limited by the physical form of the sample for evaluation. The specificity of that system's genotype allows one to link a certain DNA profile to a specific organism. Therefore, a variety of DNA marker-based techniques can be applied to medicinal plant species classification and adulteration detection. Several authors have previously reviewed DNA profiling for authentication and their patents. DNA polymorphism is assessed using a variety of DNA-based molecular approaches. PCR, sequencing, and hybridization are the three types of procedures that fall under this category. [51]

- **Hybridization-Based Methods:** Variable numbers tandem repeats and restriction fragment of length polymorphism are examples of hybridization-based techniques. Labeled probes are hybridized to filters that contain DNA, such as random genomic clones, cDNA clones, and probes for micro satellite and minisatellite sequences.

  broken down by restriction enzymes. When bands are present or absent during hybridization, polymorphisms are identified.[52]

- **Polymerase Chain Reaction (PCR)-Based Methods:** With the uses of specified or arbitrary oligonucleotide primers or the thermostable DNA polymerase enzyme, specific DNA sequences or loci are amplified in vitro to create markers based on polymerase chain reactions. DNA amplification fingerprinting (DAF), arbitrarily primed PCR (AP-PCR), and random amplified polymorphic DNA (RAPD) are PCR-based methods that employ random primers. In inter simple sequence repeats (ISSRs) polymorphism, a specialised primer-based polymorphism detection technique, the DNA between two opposing SSRs of the same type is amplified using a terminally anchored primer specific to a particular SSR. Polymorphism occurs when one genome is missing from one of the SSRs or has an insertion or deletion that changes the distance between the repeats. A new method based on the identification of genomic restriction fragments by PCR amplification is called amplified fragment length polymorphism (AFLP). After attaching adaptors to the ends of restriction fragments, adaptorhomologous primers are used for amplification. AFLP can be applied to DNAs of any complexity or origin and can identify hundreds of distinct loci. [53]

- **Sequencing-Based Markers:** Another reliable method for determining species is DNA sequencing. It is possible to directly evaluate variations brought about by transversion, insertion, or deletion and to gather data on a specific locus. At the single nucleotide level, genetic variation is widespread.Such single nucleotide polymorphisms, which typically rely on how closely related the organisms being compared are, can be effectively found by direct sequencing. Analysis of the varied internal transcribed spacer (ITS) sequences of ribosomal DNA (rDNA) is one of the other sequencing-based techniques. Numerous angiosperm families have found the ITS region of 18s–26s rDNA to be a valuable

sequence for phylogenetic analysis. Depending on the relationship, different taxonomic levels within families have varying amounts of ITS sequence variation appropriate for phylogenetic analysis. As diagnostic tools for authentication, several researchers have also sequenced additional DNA sections, such as the spacer region of 5s rDNA and the trnK of chloroplasts. [54]

- **Conclusion :** The standardization of herbal drugs is crucial for ensuring their safety, efficacy, and quality inan increasingly competitive market. This study highlights the complementary roles of traditional and modern approaches in achieving effective standardization. Traditional methods, grounded in centuries of empirical knowledge, provide valuable insights into the cultural significance and therapeutic potential of herbal remedies. Conversely, modern scientific techniques enhance precision and reliability, enabling quantitative analysis of active compounds and rigorous quality control. An integrated framework that harmonizes these approaches can foster a more comprehensive understanding of herbal medicines, facilitating their acceptance within the broader medical community. Future research should focus on developing standardized protocols that respect traditional practices while leveraging modern technology, ultimately leading to safer and more effective herbal therapies. This collaborationbetween tradition and innovation can pave the way for improved regulatory standards and greater consumer confidence in herbal products.

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