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Dhiman Nandini
Consultant (Medicinal Plant), State Medicinal Plants Board, Haryana, India

Shyam Radhay
Senior Consultant, Directorate of AYUSH, Haryana, India

Dua Chandan
Nodal Officer, State Medicinal Plants Board, Haryana, India

Corresponding Author:
Shyam Radhay
Senior Consultant, Directorate of AYUSH, Haryana, India

Evolution of medicinal plant evaluation: Bridging traditional ethanobotanical with modern phytochemical and analytical approaches

Dhiman Nandini, Shyam Radhay and Dua Chandan

Abstract
For centuries, medicinal plants have played a pivotal role in healthcare, offering natural solutions to a variety of ailments. The primary bioactive constituents of these plants, including tannins, alkaloids, saponins, and flavonoids, vary across species and contribute to their therapeutic potential. Historically, the medicinal value of these plants was determined based on observable features such as color, shape, texture, and taste. However, these methods often lacked precision, leading to inconsistencies in efficacy and safety. With the advancement of phytochemistry and analytical techniques, the evaluation of medicinal plants has evolved significantly, prioritizing the identification and quantification of bioactive compounds as essential indicators of quality. The introduction of modern extraction techniques and analytical tests-such as Million's, Fehling's, Shinoda's, and Liebermann's-has facilitated accurate phytochemical profiling, ensuring standardization, potency, and reliability. These advancements have enhanced the credibility of plant-based medicines, fostering their incorporation into contemporary healthcare and regulatory systems. Nevertheless, challenges persist, including variations in phytochemical composition due to environmental factors, the complexity of plant extracts, and the need for global regulatory uniformity. This literature review traces the transformation of medicinal plant evaluation from traditional morphological observations to advanced phytochemical analyses, highlighting its implications for therapeutic applications, regulations, and future research directions in herbal medicine.

Keywords: Phytochemicals, medicinal plants, quality assurance, standardization

Introduction
Medicinal plants have served as a cornerstone of human healthcare for generations, underpinning traditional healing practices such as Ayurveda, Unani, and Chinese medicine [1]. Ancient civilizations-including the Sumerians, Egyptians, Greeks, and Chinese-recognized the healing attributes of these plants and incorporated them into their medicinal frameworks [2]. Notably, Hippocrates documented approximately 400 plant species with therapeutic value, underscoring their historical significance [3].

The medicinal properties of plants stem from their diverse phytochemical compositions, including alkaloids, glycosides, flavonoids, phenolics, saponins, terpenes, and tannins [4]. These bioactive constituents exhibit various pharmacological effects, such as anti-inflammatory, antioxidant, immunomodulatory, and anticancer activities [5]. Traditional systems have long relied on plants like *Chlorophytum borivilianum* (Safed Musli), *Terminalia chebula* (Haritaki), *Azadirachta indica* (Neem), *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Guduchi), and *Centella asiatica* (Brahmi) for their wide-ranging health benefits [6]. However, scientific validation of their efficacy remained limited until modern analytical advancements facilitated more rigorous investigations [7].

In recent years, the growing preference for medicinal plants has been driven by their therapeutic potential and lower risk of adverse effects compared to synthetic drugs [8]. This renewed interest has propelled phytochemical research, emphasizing the accurate identification, quantification, and standardization of bioactive compounds [9]. Advanced analytical techniques-such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), and Nuclear Magnetic Resonance (NMR)-have revolutionized phytochemical analysis, ensuring safety, efficacy, and uniformity in herbal medicine formulations [10].

Despite these advancements, several challenges hinder the seamless transition from traditional

ethnobotanical knowledge to modern phytochemical assessments [3]. Issues such as overharvesting, habitat destruction, variability in chemical composition, and the diminishing transmission of traditional knowledge pose significant obstacles to standardizing herbal medicines [5]. Additionally, concerns regarding bioavailability and quality control persist [9]. Therefore, bridging traditional wisdom with contemporary scientific research is essential for validating therapeutic claims, enhancing safety and efficacy, and exploring new drug candidates derived from plant-based compounds [7].

This review explores the transformation of medicinal plant research from traditional ethnobotanical knowledge to advanced phytochemical methodologies. It examines historical insights, phytochemical diversity, analytical advancements, and case studies of key medicinal plants. Furthermore, it discusses existing challenges and future perspectives, advocating for sustainable practices, enhanced agro-technologies, and continued scientific exploration. By integrating traditional knowledge with modern science, this study seeks to contribute to the expanding field of herbal medicine and phytochemical research [11].

Aim & objective

- To investigate the historical significance and ethnobotanical foundations of medicinal plants.
- To analyze scientific advancements in phytochemistry and modern techniques used in medicinal plant evaluation.

Methodology

This research adopts a qualitative literature review methodology, exploring the evolution of medicinal plant evaluation from traditional morphological assessments to advanced phytochemical-based quality control. The study synthesizes information from primary research articles, review papers, and other academic sources. Literature searches were conducted using databases such as PubMed, Google Scholar, ScienceDirect, Web of Science, and Scopus. The keywords used included "medicinal plants," "phytochemical analysis," "traditional medicine," "quality control," "bioactive compounds," and "analytical techniques".

The selection criteria focused on English-language studies that examined both historical and modern evaluation methods of medicinal plants, phytochemical profiling, and standardization protocols. Studies that lacked empirical data or relied solely on anecdotal evidence were excluded. The collected literature was systematically analyzed, categorizing findings into traditional evaluation methods, contemporary phytochemical techniques, regulatory considerations, and prospective developments in herbal medicine research. This approach ensures a balanced and comprehensive review of evolving medicinal plant evaluation practices.

Review of literature

Medicinal plants have been a vital component of healthcare, offering natural solutions for health conditions. The main phytochemicals that are effective for various diseases present in medicinal plants are tannins, alkaloids, saponins, and flavonoids, among others, which vary among plants [11]. Traditionally, their therapeutic use was based on morphological characteristics such as shape, colour, texture, and taste. While effective in early medicine, these qualitative assessments lacked precision, often leading to inconsistencies in efficacy and safety [12]. Their relevance persists today due to the resurgence of interest in natural remedies, challenges associated with synthetic drugs, and the growing role of medicinal plants in personalized medicine [13]. Understanding their evolutionary journey not only bridges traditional knowledge with modern science but also offers insights into sustainable healthcare solutions. These systems utilized the therapeutic potential of plants based on their phytochemical composition. The effectiveness of medicinal plants and their mild side effects, compared to synthetic drugs, have sparked renewed interest in recent years. [14]

Traditional Methods vs. Modern Analytical Techniques

A) Traditional Methods

In ancient medical practices, the healing properties of plants were primarily identified through their physical attributes such as shape, color, texture, and taste. Various civilizations, including the Egyptians, Chinese, and practitioners of Ayurveda and Unani medicine, relied on these observable traits to determine a plant's medicinal potential. For instance, the Ebers Papyrus from ancient Egypt documented numerous plant-based remedies, while the Chinese text *Shennong Bencao Jing* detailed the use of botanicals like ginseng and licorice for treating various ailments [15]. Similarly, Hippocrates recorded the therapeutic properties of approximately 400 plant species, laying the groundwork for early Western pharmacology [16].

Traditional medicine utilized plants in their natural forms-such as infusions, powders, and tinctures-to manage diverse health conditions. The effectiveness of these remedies was believed to arise from the combined action of multiple compounds within the plant [17]. Knowledge of medicinal plants was passed down through generations based on experience rather than scientific validation. However, despite their benefits, these methods lacked precision and consistency. The efficacy and safety of plant-based treatments were influenced by factors such as proper plant identification, harvesting practices, and preparation techniques, leading to variations in therapeutic results [18].

With advancements in phytochemistry and analytical science, modern approaches have significantly improved the evaluation of medicinal plants. Techniques such as phytochemical profiling and chemical testing-including Million's, Fehling's, Shinoda's, and Liebermann's tests-have enabled the identification and quantification of bioactive compounds. These innovations have enhanced standardization, potency, and reproducibility, strengthening the scientific credibility of herbal medicine [19]. As a result, plant-based treatments are now better integrated into modern healthcare and regulatory systems [20].

B) Modern Methods

With the advancement of phytochemical research, there has been a fundamental shift in how medicinal plants are used. Instead of relying on whole-plant formulations, scientific studies have shown that specific bioactive compounds are primarily responsible for therapeutic effects. This understanding has led to the development of plant-based extracts with precise concentrations of active ingredients, offering several advantages over traditional herbal preparations [21].

Standardized extracts ensure consistency in potency and efficacy, which is particularly vital in clinical settings where accurate dosing is critical for both safety and therapeutic outcomes. Unlike crude plant formulations that contain a mix of compounds with varying biological activities, pure extracts

enable more targeted and predictable treatment effects [22]. For example, the medicinal plant *Withania somnifera* (Ashwagandha) has been extensively studied for its neuroprotective and anti-inflammatory properties. Research has shown that isolating its bioactive compounds, such as withanolides and alkaloids, enhances its therapeutic potential [23]. Likewise, *Azadirachta indica* (Neem) exhibits antibacterial, antifungal, and immunomodulatory properties, primarily due to active compounds such as nimbidin and azadirachtin [24]. By isolating and purifying these bioactive components, researchers can ensure greater consistency and effectiveness in medicinal applications.

Modern Analysis confirmed or contradicted back of some medicinal plants to traditional efficacy claims. [Refrences from: 25-44]

Sr No.	Botanical Name (Common Name)	Key Chemical compounds that link plants to traditional knowledge system & modern phytochemical studies.	Therapeutic Value	Economic Value
1.	*Withania somnifera* (Ashwagandha)	Withanolides, alkaloids, sitoindosides	Adaptogen, anti-stress, neuroprotective, immunomodulator	High export value; used in nutraceuticals and Ayurveda
2.	*Andrographis paniculata* (Kalmegh)	Andrographolide, flavonoids	Hepatoprotective, antipyretic, anti-inflammatory	Cultivated for immune supplements; high pharma demand
3.	*Azadirachta indica* (Neem)	Azadirachtin, nimbin, nimbolide	Antibacterial, antifungal, antidiabetic	Used in pesticides, skincare, pharmaceuticals; major export item
4.	*Aloe barbadensis miller* (Aloe vera)	Aloin, anthraquinones, polysaccharides	Wound healing, skin rejuvenation, laxative	Global cosmetic and wellness market; highly cultivated
5.	*Ocimum sanctum* (Tulsi)	Eugenol, ursolic acid, rosmarinic acid	Antioxidant, adaptogen, anti-inflammatory	High commercial demand in tea, supplements, cosmetics
6.	*Piper longum* (Pippali)	Piperine, alkaloids	Respiratory tonic, bioenhancer, digestive aid	Key Ayurvedic ingredient; growing trade interest
7.	*Crocus sativus* (Saffron)	Crocin, safranal, picrocrocin	Antidepressant, antioxidant, memory enhancer	One of world's costliest botanicals; high pharma use
8.	*Commiphora wightii* (Guggul)	Guggulsterones, diterpenes	Hypolipidemic, thyroid stimulant, anti-arthritic	Used in cholesterol-lowering drugs; wild-harvested and farmed
9.	*Butea monosperma* (Palash)	Butin, tannins, flavonoids	Astringent, anti-inflammatory, anthelmintic	Cultivated and wild-harvested; used in colorant and Ayurveda
10.	*Achyranthes aspera* (Apamarg)	Saponins, ecdysterone, alkaloids	Diuretic, wound healing, antifertility	Low-cost herb used in rural & traditional systems
11.	*Senna alata* (Candle Bush)	Anthraquinones (emodin), flavonoids	Antifungal (skin), laxative, anti-inflammatory	Cultivated in gardens, used in soaps & ointments
12.	*Catharanthus roseus* (Sadabahar)	Vincristine, vinblastine, ajmalicine	Anticancer, anti-diabetic, anti-hypertensive	Source of chemotherapy drugs; highly valuable crop
13.	*Bauhinia purpurea* (Kachnar)	Flavonoids, tannins, β-sitosterol	Anti-diabetic, hepato protective, anti-microbial	Cultivated; used in decoctions, local markets
14.	*Clerodendrum indicum* (Bharangi)	Clerodane diterpenes, flavonoids	Antioxidant, anti-inflammatory, antidiabetic	Used in traditional medicine; niche commercial use
15.	*Costus speciosus* (Keu)	Diosgenin, saponins, costunolide	Antidiabetic, anti-inflammatory, diuretic	Used for steroid precursors; moderate cultivation
16.	*Senna auriculata* (Avaram Senna)	Kaempferol, anthraquinones	Antidiabetic, hepatoprotective, skin care	Grown in South India; used in herbal teas
17.	*Dendrophthoe falcata* (Mistletoe)	Flavonoids, triterpenoids, tannins	Wound healing, anticancer, antioxidant	Harvested in tribal belts; limited commercial use
18.	*Calotropis gigantea* (Ak)	Cardenolides, calotropin, uscharin	Analgesic, anti-inflammatory, wound healing	Used in local medicine, pesticide production
19.	*Clerodendrum infortunatum* (Hill glory bower)	Clerodin, alkaloids, flavonoids	Anticancer, hepato protective, analgesic	Cultivated on small scale; used in herbal pastes
20.	*Piper betle* (Betel Leaf)	Chavicol, eugenol, allylpyrocatechol	Antioxidant, antimicrobial, carminative	Widely cultivated; significant in cultural and pharma markets

Phytochemical Composition and Classification

- **Alkaloids:** Nitrogen-containing compounds known for their anaesthetic, antimicrobial, and central nervous system (CNS) stimulant effects. Examples include morphine and caffeine.
- **Glycosides:** Bioactive molecules composed of a sugar unit linked to another compound, exhibiting cardiac, laxative, and anticancer properties.
- **Flavonoids:** Polyphenolic compounds recognized for their strong antioxidant and anti-inflammatory benefits.
- **Phenolics:** Naturally occurring plant pigments that possess antioxidant properties and may contribute to cancer prevention.
- **Saponins:** Bioactive agents with cholesterol-lowering effects and immune-boosting potential.
- **Terpenes & Essential Oils:** Aromatic plant compounds with antimicrobial and anti-inflammatory characteristics.
- **Tannins:** Astringent phenolic compounds used for their antiseptic properties and role in wound healing.

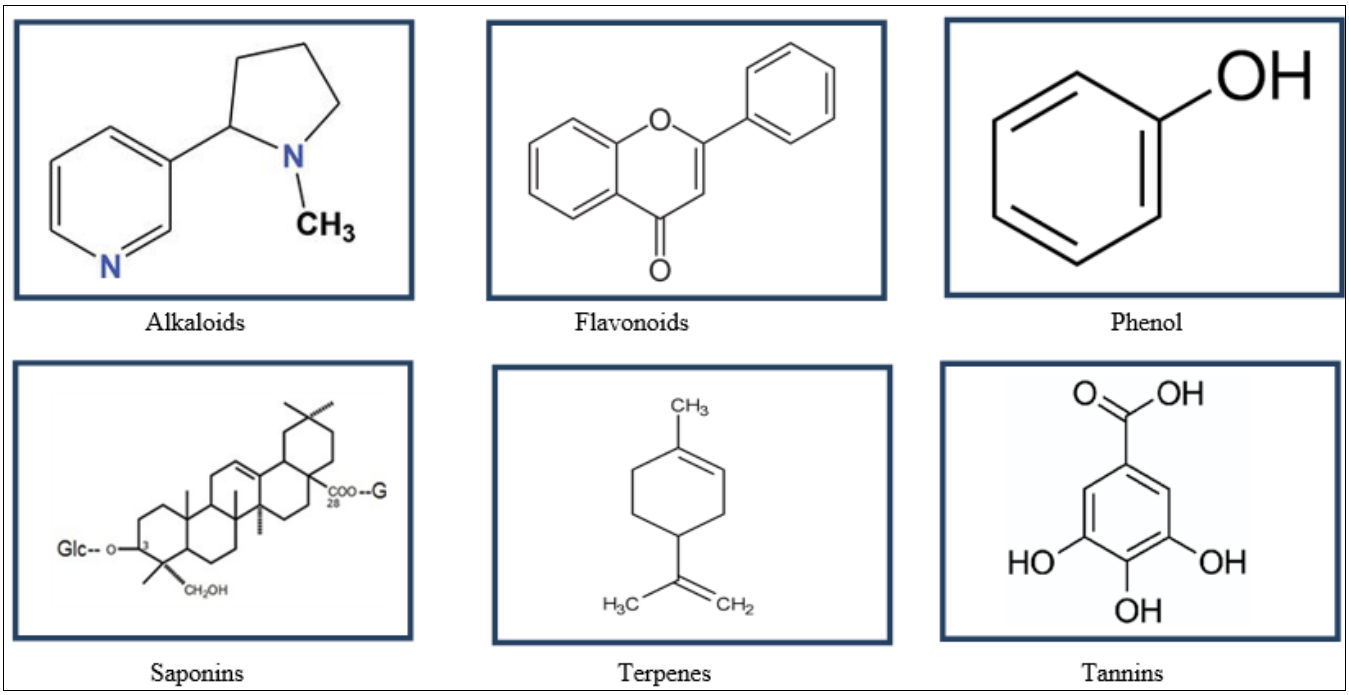


Fig 1: chemical structure of phytochemical present in medicinal plants. (Source:Google)

Advanced Analytical Techniques and Qualitative Tests for Phytochemical Analysis

Modern pharmacological research utilizes sophisticated analytical techniques to accurately identify, quantify, and standardize phytochemicals, ensuring the safety, efficacy, and uniformity of herbal medicines [45]. However, challenges such as high costs, limited availability, and sensitivity constraints need to be addressed to enhance their application in medicinal plant studies. These advanced techniques are often supplemented by qualitative tests, which assist in the preliminary identification of different phytochemical groups. Some commonly used tests include

- **Shinoda Test:** Detects flavonoids through a color change when magnesium and hydrochloric acid are introduced [46].
- **Wagner's Test:** Identifies alkaloids by forming a reddish-brown precipitate in the presence of iodine and potassium iodide solution [47].
- **Salkowski Test:** Confirms the presence of steroids and terpenoids by producing a red color upon reaction with chloroform and concentrated sulfuric acid [48].
- **Foam Test:** Determines the presence of saponins based on the formation of stable froth when shaken with water [49].
- **Borntrager's Test:** Detects anthraquinones, which exhibit a pink or red coloration when treated with ammonia [50].

In addition to qualitative tests, advanced analytical techniques play a crucial role in phytochemical analysis and identifying active compounds in plant materials:

1. Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique used for separating, identifying, and quantifying chemical compounds in complex mixtures. It combines two separate techniques: Gas Chromatography (GC) and Mass Spectrometry (MS).

Principle: GC-MS combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. In the gas chromatographic part, a sample is vaporized and transported by an inert carrier gas through a column containing a stationary phase. Compounds separate based on their boiling points and interactions with the stationary phase, resulting in different retention times [51]. Once separated, the compounds enter the mass spectrometer where they are ionized, typically by electron impact. This ionization process breaks the molecules into fragments, which are then detected and recorded based on their mass-to-charge (m/z) ratios. Each compound yields a unique mass spectrum, acting as a molecular fingerprint that can be compared against spectral libraries for identification [52].

Process Sample Preparation

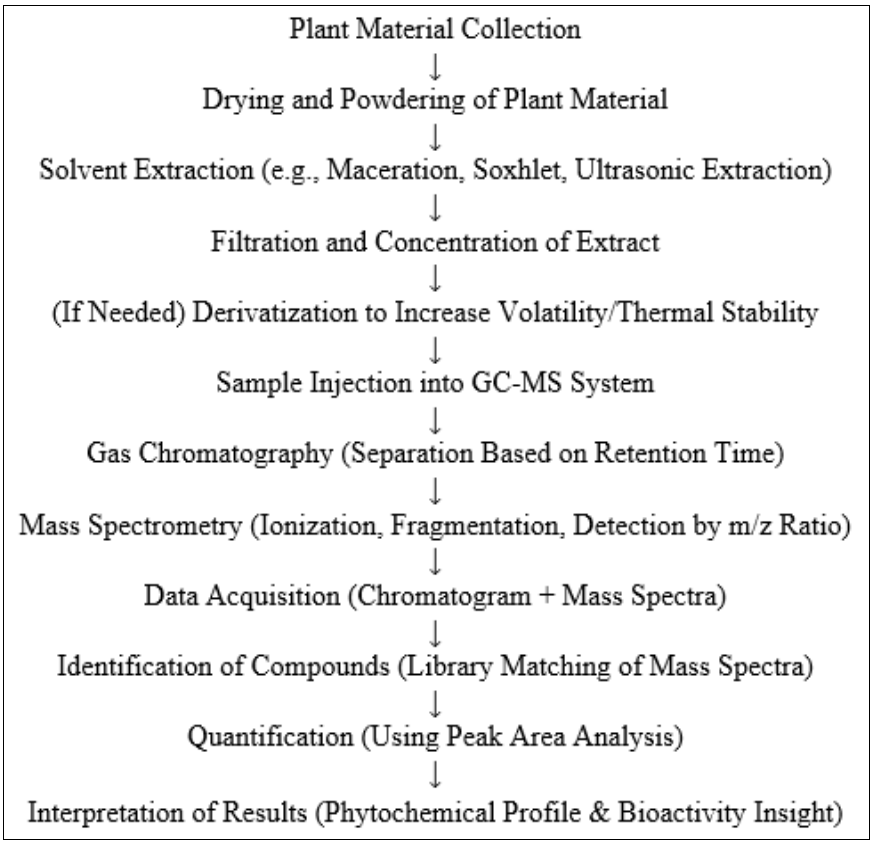
For phytochemical analysis, the sample-often a plant extract or essential oil-is first prepared using solvent extraction techniques such as maceration, Solet extraction, or ultrasonic-assisted extraction. The extract is concentrated, filtered, and occasionally derivatives to enhance volatility and thermal stability [53]. A small aliquot of the prepared sample is injected into the GC inlet, where it vaporizes and enters the column. As the analytes elute from the column, they pass into the mass spectrometer for detection. The resulting chromatograms and spectra are analysed to determine the chemical composition and structure of the sample's components [54].

GC-MS for Active Phytochemical Identification

GC-MS plays a critical role in the identification and quantification of active phytochemicals in plant materials. These compounds include alkaloids, terpenoids, flavonoids, and phenolic acids, many of which exhibit antioxidant, anti-inflammatory, antimicrobial, or anticancer properties [55]. The technique allows for the precise separation and detection of these compounds, even when they are present in trace concentrations. This is particularly valuable for discovering novel phytochemicals with potential therapeutic benefits. Furthermore, the ability to analyse fragmentation patterns facilitates the structural elucidation of unknown compounds [56]. Quantification is achieved through analysis of peak areas in the chromatogram, enabling researchers to determine the

relative abundance of each compound in the extract. GC-MS also facilitates comparative studies across different plant species or extraction methods to identify those with higher concentrations of bioactive molecules [57]

Phytochemical Analysis Process via GC-MS [58]



2. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique based on the interaction of infrared (IR) radiation with matter. The fundamental principle involves the absorption of IR radiation by chemical bonds within molecules, leading to molecular vibrations such as stretching, bending, and twisting [59]. Each type of bond and functional group within a molecule absorbs IR radiation at a characteristic frequency, producing a unique spectrum that serves as a molecular fingerprint. This spectral data provides qualitative information about the functional groups present in a compound and, when compared with reference spectra, can aid in compound identification [60].

FTIR Process

The FTIR process involves the passage of a broad-spectrum IR beam through a sample. This beam is modulated by an interferometer to produce an interferogram, a composite signal containing data from all IR frequencies. A Fourier Transform is then applied to convert the time-domain signal into a frequency-domain spectrum, revealing the absorbance or transmittance peaks corresponding to molecular vibrations [61]. Sample preparation varies depending on the nature of the material and can include techniques such as potassium bromide (KBr) pellet pressing, thin-film deposition, or Attenuated Total Reflectance (ATR), which enables direct analysis of solid and liquid samples with minimal preparation [62].

Identification of Active Compounds Using FTIR: FTIR spectroscopy is extensively used in the identification and characterization of bioactive compounds in complex matrices, such as plant extracts and traditional medicinal formulations. Functional groups such as hydroxyl (-OH), carbonyl (C=O), amine ($-NH_2$), and aromatic rings exhibit distinct absorption bands in the IR region, allowing researchers to detect major classes of phytochemicals like flavonoids, tannins, alkaloids, and terpenoids [63]. By comparing the obtained spectra with standard reference spectra, FTIR enables the rapid screening and tentative identification of active components [64]. In some cases, semi-quantitative analysis can also be conducted based on the intensity of characteristic peaks [65].

Connecting FTIR Findings to Traditional Efficacy Claims

Traditional medicinal systems, including Ayurveda, Traditional Chinese Medicine (TCM), and various indigenous practices, often attribute therapeutic benefits to specific herbs or natural ingredients. While these claims are rooted in empirical use, FTIR offers a scientific basis for validating such claims by identifying functional groups and bioactive compounds linked to known pharmacological activities [66]. For example, phenolic compounds detected through FTIR in herbal formulations used for inflammation are associated with antioxidant and anti-inflammatory properties, lending scientific support to traditional applications [67]. Moreover, FTIR aids in quality control and standardization of traditional remedies, ensuring batch-to-batch consistency and authenticity [68].

Phytochemical Analysis Process [69]

1. Sample Collection
(Selection of plant material (leaves, roots, bark, etc.) based on traditional use or ethnobotanical knowledge.)

2. Sample Preparation
Washing, drying (shade or oven), and grinding into fine powder for consistent extraction.

3. Extraction
Using solvents (e.g., ethanol, methanol, water) to extract phytochemicals via maceration, Soxhlet, or ultrasound-assisted methods.

4. Preliminary Phytochemical Screening"
Qualitative tests to detect major phytochemical groups like alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids.

5. Fractionation and Isolation
Separation of crude extract into individual components using chromatography (TLC, column, HPLC).

6. Characterization of Compounds
Structural identification using spectroscopic techniques such as FTIR, NMR, MS, and UV-Vis.

7. Bioactivity Testing
Evaluation of pharmacological effects (e.g., antioxidant, antimicrobial, anti-inflammatory) using in vitro or in vivo assays.

8. Correlation with Traditional Claims
Linking identified active compounds and bioactivities to traditional therapeutic uses.

9. Standardization and Quality Control
Establishing consistent concentration of bioactive compounds and setting quality benchmarks for formulations.

10. Documentation and Publication
Reporting results in scientific literature and sharing data with regulatory bodies.

3. Inductively Coupled Plasma (ICP) and **Mass Spectrometry (MS)**

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a powerful analytical technique primarily used to measure trace elements and isotopic compositions in various samples. Its high sensitivity and ability to detect low concentrations of elements make it invaluable in diverse fields such as environmental analysis, biochemistry, materials science, and pharmacology [70].

Principle of ICP-MS: ICP-MS combines two distinct techniques: Inductively Coupled Plasma (ICP) **and** Mass Spectrometry (MS)**.** Inductively Coupled Plasma (ICP): The sample is introduced into the plasma, which is an electrically neutral, high-temperature gas created by ionizing argon. The plasma reaches temperatures around 10,000 K, which is sufficient to break down molecules and atomize elements in the sample. After atomization, the ionized particles are directed into the mass spectrometer. In the mass spectrometer, the ions are separated based on their mass-to-charge ratio (m/z). A detector then measures the abundance of each ion, providing information about the composition of the sample [71].

Process of ICP-MS

1. **Sample Introduction:** The sample is usually introduced into the system as a liquid, although solid samples may be converted into a liquid form using techniques like laser ablation or digestion [72].
2. **Ionization in Plasma:** The liquid sample is nebulized into a fine aerosol and introduced into the inductively coupled plasma. The high temperature of the plasma atomizes the sample and ionizes the atoms, converting them into positively charged ions [73].
3. **Mass Spectrometer (MS) Detection:** The ionized particles are then directed into the mass spectrometer. The mass spectrometer separates the ions based on their mass-to-charge ratio (m/z) and sends them to the detector [74].
4. **Signal Detection:** The detector counts the number of ions of each mass, generating a spectrum. The intensity of each peak corresponds to the concentration of the element or isotope in the sample [75].
5. **Quantification and Identification:** The resulting data is compared against calibration standards to quantify the concentration of elements in the sample. The elemental composition or isotopic profile of the sample is then determined [76].

Identify Active Compounds by ICP-MS: ICP-MS is particularly useful in identifying and quantifying trace elements and heavy metals in biological and pharmaceutical samples, including potential active pharmaceutical ingredients (APIs) or toxic substances.

- **Elemental Composition of Active Compounds:** Many biologically active compounds contain metal elements such as magnesium, calcium, or zinc, or are metalloproteins or metal-containing enzymes. ICP-MS can detect and quantify these metals at very low concentrations, providing crucial information about the elemental makeup of the active compounds [77].
- **Trace Contaminants and Purity:** In drug development,

it's essential to identify any trace contaminants or impurities in the formulation of active compounds. ICP-MS offers high sensitivity for identifying even low levels of trace metals, ensuring the purity and safety of the pharmaceutical products [78].

- **Isotopic Analysis:** ICP-MS can also measure isotopic ratios, which is useful in distinguishing between isotopic forms of elements in active compounds. This can help confirm the presence of specific isotopes in compounds synthesized with a particular isotopic signature or aid in identifying the source of a compound [79].
- **Pharmacokinetics and Bioavailability:** ICP-MS is used in pharmacokinetic studies to track the concentration of metal-based drugs in biological fluids, such as blood or plasma, over time. This helps in understanding the absorption, distribution, metabolism, and excretion (ADME) of metal-containing active pharmaceutical ingredients [80].

Connecting ICP-MS to Traditional Efficacy Claims: Efficacy claims for pharmaceutical products are traditionally substantiated through clinical trials, bioassays, and pharmacological studies. ICP-MS enhances this process in several ways

- **Verification of Active Ingredient Content:** By accurately quantifying the elemental composition of the active ingredients, ICP-MS provides direct evidence supporting the claims about the potency and quality of the active compound. For example, if a drug is claimed to contain a certain concentration of a metal (e.g., calcium or magnesium), ICP-MS can verify the actual concentration [81].
- **Toxicological Safety:** Efficacy claims often include safety profiles, and ICP-MS plays a critical role in detecting toxic metals or impurities that could compromise safety. Ensuring that the drug formulation is free from harmful metal contamination is crucial in supporting safety-related claims [82].
- **Support for Dosage and Formulation Development:** Accurate determination of trace elements can help optimize the dosage and formulation of drugs. By ensuring that the formulation is within the therapeutic range and free from harmful contaminants, ICP-MS can contribute to developing safe and effective drug products, thus validating efficacy claims [83].
- **Regulatory Compliance:** Regulatory bodies such as the FDA or EMA require rigorous testing to substantiate efficacy and safety claims for pharmaceutical products. The data obtained from ICP-MS can be used in regulatory submissions to demonstrate that the active compounds in a drug meet quality standards and are free from harmful impurities, supporting approval processes [69].

Inductively Coupled Plasma (ICP) and **Mass Spectrometry (MS)**. [84]

1. **Sample Collection**
Collection of plant material from relevant sources

2. **Sample Preparation**
Includes drying, grinding, and acid digestion to release elements

3. **Filtration**
Removal of particulates after digestion.

4. **Introduction into ICP System**
Liquid sample is nebulized and introduced into the plasma torch.

5. **Atomization and Ionization**
High-temperature plasma breaks down sample into atoms and ions.

6. **Ion Transfer to Mass Spectrometer**
Ions pass through interface into MS vacuum system.

7. **Mass Analysis**
Separation of ions based on mass-to-charge ratio (m/z) in the MS analyser.

8. **Data Processing and Interpretation**
Quantification and identification of elements; comparison with standards.

4. X-Ray Diffraction (XRD)

X-ray Diffraction (XRD) is a non-destructive analytical technique used to determine the crystallographic structure, chemical composition, and physical properties of materials. It is particularly effective for identifying crystalline phases and quantifying the structural properties of active compounds in both natural and synthetic samples [85].

Principle and Process: XRD operates on the principle of constructive interference of monochromatic X-rays with the periodic atomic planes in a crystalline material. When X-rays are directed at a crystalline substance, they are diffracted in specific directions. This diffraction follows Bragg's Law**:**

nλ=2dsin⁡θn\lambda = 2d\sin\thetanλ=2dsinθ

Where

- nnn is an integer (order of reflection),
- λ\lambdaλ is the wavelength of the X-rays,
- ddd is the distance between atomic planes in the crystal,
- θ\thetaθ is the angle of incidence.

The resulting diffraction pattern, composed of peaks at specific angles and intensities, is characteristic of the atomic arrangement within the crystal. By comparing the experimental pattern with standard reference patterns from databases such as the ICDD (International Centre for Diffraction Data), the compound can be precisely identified [86].

Application in Identifying Active Compounds: In natural product research and pharmaceutical sciences, XRD is employed to

- Identify and confirm the crystalline forms of isolated compounds such as alkaloids, flavonoids, and terpenoids.
- Differentiate between polymorphs, which significantly affect solubility, bioavailability, and pharmacokinetics [87].
- Detect impurities and assess the purity and consistency of extracts and formulations.
- Support structural elucidation when integrated with complementary techniques like NMR and FTIR spectroscopy [88].

XRD is particularly valuable in analyzing purified active components from complex botanical extracts, enabling the verification of crystal structure, composition, and phase purity of bioactive molecules [89].

Connection to Traditional Efficacy Claims: Traditional medicine systems, including Ayurveda, Traditional Chinese Medicine (TCM), and indigenous ethnomedicine, are rooted in centuries of empirical knowledge. However, modern validation of therapeutic efficacy requires the identification and characterization of bioactive compounds. XRD provides a robust scientific bridge between traditional knowledge and modern pharmacology by

- Authenticating traditional formulations through detailed structural verification of active components [90].
- Standardizing herbal products to ensure reproducibility and batch-to-batch consistency in terms of crystallinity and purity [91].
- Supporting the validation of traditional efficacy claims through integration with bioassays and pharmacological testing, thus enhancing the credibility and regulatory acceptance of traditional medicines [92].

Phytochemical Analysis Process with Integration of X-Ray Diffraction (XRD) [93]

1. Plant Material Collection
Harvesting and authentication of plant materials
↓
2. Drying and Grinding
Shade drying or oven drying, followed by grinding into powder.
↓
3. Extraction (e.g., Methanol, Ethanol, Water)
Solvent-based extraction using methanol, ethanol, water, etc.
↓
4. Preliminary Phytochemical Screening
Tests for alkaloids, flavonoids, tannins, etc.
↓
5. Fractionation & Isolation
Techniques: TLC, Column Chromatography, HPLC
↓
6. Purification of Active Compounds
↓
7. Structural Characterization
NMR, MS, FTIR
XRD (if compound is crystalline)
Determines crystal structure and phase purity
↓
8. Bioactivity Assays
Antioxidant, Antimicrobial, Anti-inflammatory
↓
9. Correlation with Traditional Efficacy
Match bioactive profile with ethnomedicinal claims
↓
10. Standardization & Quality Control
Confirm reproducibility using XRD and other techniques

5. High-Resolution Mass Spectrometry (HRMS)

High-Resolution Mass Spectrometry (HRMS) has emerged as a cornerstone analytical technique in natural product research and pharmacognosy. It enables precise characterization of complex mixtures, including those derived from traditional medicinal preparations. The integration of HRMS with traditional knowledge systems facilitates the scientific validation of ethnopharmacological efficacy claims through molecular-level investigation of bioactive constituents [94].

Scientific Principle of HRMS

HRMS operates on the fundamental principle of mass spectrometry, which involves ionizing chemical compounds to generate charged molecules or fragments, and then measuring their mass-to-charge (m/z) ratios. What distinguishes HRMS from conventional mass spectrometry is its superior resolving power and mass accuracy. Instruments such as Orbitraps and time-of-flight (TOF) analyzers can measure masses with errors typically below 1-5 ppm,

allowing for the exact determination of elemental compositions and the discrimination of compounds with nearly identical masses [95].

Analytical Process: The analytical workflow of HRMS typically comprises the following steps:

- **Sample Preparation:** Crude extracts from plant or other natural sources are processed using solvent extraction, filtration, and concentration techniques [96].
- **Chromatographic Separation:** Coupled with Ultra-High-Performance Liquid Chromatography (UHPLC), HRMS facilitates the separation of complex mixtures prior to mass analysis [97].
- **Ionization:** Electrospray Ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI) are commonly used to convert analytes into gas-phase ions [98].
- **Mass Detection and Data Acquisition:** The high-resolution analyzer detects ions with high accuracy, producing full-scan spectra or tandem MS (MS/MS) data for structural elucidation [99].
- **Data Interpretation:** Advanced software tools and databases such as GNPS, METLIN, and mzCloud are used for dereplication, structural annotation, and molecular networking [100].

Identification of Active Compounds: HRMS enables the identification of active compounds in complex natural product matrices by

- **Accurate Mass Determination:** Permits the assignment of molecular formulas to unknown compounds with high precision [101].
- **Isotopic Pattern Matching:** Assists in validating proposed elemental compositions and supports compound confirmation [102].
- **Fragmentation Analysis (MS/MS):** Provides structural insights, enabling the differentiation of isomers and localization of functional groups [103].
- **Untargeted Metabolomics:** Facilitates the comparison of treated versus control samples to identify biomarkers or pharmacologically active molecules [104].

Connection to Traditional Efficacy Claims: The application of HRMS in studying traditional medicines bridges the gap between empirical efficacy and modern scientific validation. Through accurate identification and characterization of bioactive compounds

- **Correlation with Bioactivity:** Identified compounds can be linked to specific pharmacological activities observed in traditional use [105].
- **Mechanistic Understanding:** Structural elucidation allows for further in vitro and in vivo assays to uncover mechanisms of action [106].
- **Standardization and Quality Control:** Enables the development of chemical markers for standardizing herbal formulations [107].
- **Discovery of Novel Leads:** Traditional knowledge often leads to the discovery of novel scaffolds and secondary metabolites, which HRMS can characterize rapidly and accurately [108].

Phytochemical Analysis Process Using High-Resolution Mass Spectrometry (HRMS): [109]

1. Sample Collection and Preparation

Collection of plant material (e.g., leaves, roots, seeds, flowers) - Drying and grinding of plant material - Extraction of phytochemicals using solvents (e.g., methanol, ethanol, hexane)

2. Extract Purification (Optional)

- Filtration or centrifugation to remove insoluble materials - Concentration of extract (e.g., rotary evaporation)

3. Sample Analysis via HRMS

- **Ionization:** Electrospray ionization (ESI), Atmospheric pressure chemical ionization (APCI), or Matrix-assisted laser desorption ionization (MALDI) - **Mass Spectrum Collection:** Analysis based on mass-to-charge ratio (m/z) - **High-Resolution Mass Spectrometry:** Accurate mass measurement to determine molecular formulas and elemental compositions

4. Data Interpretation

Peak Identification: Identifying compounds based on m/z values and fragmentation patterns - **Isotope Pattern Analysis:** Resolving isotopic information - **Comparison with Databases:** Databases like METLIN, ChemSpider for identification

5. Quantification and Qualitative Analysis

- **Quantitative Analysis:** Determining concentrations of specific compounds (e.g., using calibration curves) - **Qualitative Profiling:** Profiling different phytochemicals (e.g., flavonoids, alkaloids, terpenes)

6. Reporting Results

- Documentation of molecular identities of identified compounds - Statistical analysis (e.g., Principal Component Analysis for sample comparison)

7. Further Studies (Optional)

Isolation of compounds for structure elucidation (e.g., Nuclear Magnetic Resonance, NMR) - Bioactivity testing or pharmacological assessment

Benefits of Pure Extracts for Disease Treatment

The use of pure extracts in modern medicine offers several advantages over traditional whole plant preparations.

- **Consistency and Standardization:** Pure extracts contain a high concentration of the active compound(s), ensuring consistent potency across various batches and treatments. This is particularly important in clinical settings, where precise dosing is necessary for effective therapeutic outcomes [110].
- **Reduced Side Effects:** Whole plant formulations often contain numerous compounds, some of which may produce unintended or harmful effects. Isolating the bioactive compounds responsible for therapeutic benefits allows for more focused treatments, reducing the likelihood of side effects [111].
- **Improved Bioavailability:** By isolating and purifying active compounds, their bioavailability-meaning the body's ability to absorb and metabolize them-is often enhanced. This can lead to better therapeutic results with smaller doses [112].
- **Targeted Therapeutic Action:** Pure extracts enable more precise treatments. For example, using a purified flavonoid extract to combat inflammation or a saponin extract to reduce cholesterol offers a more targeted approach compared to the broader effects of whole plant treatments [113].
- **Scientific Validation and Drug Development:** Isolating specific bioactive compounds from medicinal plants has opened the door for plant-based pharmaceutical development. With advances in phytochemical analysis, researchers can now investigate new treatments based on these compounds, leading to potential drug discoveries [114].

Many medicinal plants have been extensively researched for their phytochemical properties and pharmacological benefits. Ongoing studies continue to validate traditional uses while revealing new therapeutic potentials. For example, research has demonstrated that *Withania somnifera* (Ashwagandha) offers neuroprotective effects through its modulation of neurotransmitter systems [115]. *Chlorophytum borivilianum* (Safed Musli) is known for its aphrodisiac, anti-diabetic, and immunomodulatory properties, linked to its saponin and carbohydrate content [116]. *Terminalia chebula* (Haritaki) shows antioxidant, anti-inflammatory, and antimicrobial activities due to its chebulic acid, gallic acid, and tannins [117]. *Azadirachta indica* (Neem) is celebrated for its antibacterial, antifungal, and immunomodulatory properties, attributed to compounds like Nimbidin and Azadirachtin [118]. *Tinospora cordifolia* (Guduchi) is known for its immunomodulatory and hepatoprotective effects, linked to its glycosides and diterpenoid lactones [119].

Challenges and Future Directions

Despite significant advancements in analytical methods, herbal medicine still faces various challenges, including phytochemical variability influenced by environmental factors [120], the complexity of multi-component plant extracts [121], and the need for global regulatory alignment [122]. The shift from traditional morphological evaluations to modern phytochemical-based quality control has greatly impacted therapeutic applications and regulatory systems [123]. Overcoming these challenges requires ongoing research and international collaboration to develop standardized regulatory frameworks.

Future research should focus on improving the standardization of phytochemicals, understanding the intricate interactions within plant extracts, and ensuring that global safety and efficacy standards are met [124]. Ethical concerns, such as bioprospecting and intellectual property rights related to traditional knowledge, must also be addressed [125]. The application of omics technologies, including genomics and metabolomics, will play a crucial role in deepening our understanding and promoting the sustainable use of medicinal plants [126].

Additionally, issues related to standardization, quality control, and bioavailability continue to pose challenges in the development of herbal medicines. Addressing these concerns will require advancements in agricultural technologies and sustainable practices to preserve traditional knowledge. Rigorous scientific validation and the use of advanced analytical techniques are essential for ensuring the safety and effectiveness of plant-based treatments, while also unlocking new opportunities for drug discovery [127].

Discussion

Medicinal plants have been integral to traditional healthcare systems, with numerous cultures relying on them to treat a wide range of health issues. The therapeutic effects of these plants are largely attributed to their diverse phytochemical content, which includes substances like tannins, alkaloids, saponins, and flavonoids. In traditional practices, plants were utilized based on visible characteristics such as shape, color, and taste, alongside accumulated experiential knowledge passed through generations. While this knowledge was often effective, it lacked the scientific rigor and precision necessary to ensure consistency and safety in the long run.

Although these traditional uses had some degree of success, the lack of standardized practices often led to variations in the strength of plant-based remedies, and at times, safety issues arose due to improper dosages or incorrect plant identification. However, in recent years, there has been a renewed interest in medicinal plants, driven by the growing awareness of the limitations of synthetic pharmaceuticals. While synthetic drugs are widely used, they often come with undesirable side effects and the potential for addiction, prompting many to explore natural alternatives.

In response to this shift, modern science has turned its attention to the phytochemical components of medicinal plants, using advanced technologies such as chromatography, mass spectrometry, and other analytical methods. These techniques have provided a more detailed understanding of the active compounds in plants and have helped validate many traditional remedies scientifically. This progress has paved the way for more accurate and effective applications in medicine.

One of the major innovations in modern phytotherapy is the development of advanced extraction methods that enhance the purity and potency of plant-based treatments. Traditional techniques like decoction or infusion often resulted in extracts with inconsistent concentrations of active ingredients, which limited their reliability. However, modern methods such as solvent extraction, supercritical fluid extraction (SFE), and cold-press extraction have significantly improved the consistency and quality of plant extracts. These methods allow for the extraction of specific bioactive compounds, ensuring that the plant's therapeutic properties are preserved and delivered in a standardized form. For instance, supercritical fluid extraction utilizes carbon dioxide in a supercritical state to extract essential oils and other

compounds without leaving harmful residues, offering a cleaner and more concentrated extract.

Moreover, these modern extraction techniques allow for more precise dosage standardization, minimizing the risk of side effects and enhancing the safety of plant-based treatments. Rigorous quality control has made it possible to produce reproducible results, offering patients more predictable and reliable therapeutic outcomes. These advancements are vital in bridging the gap between traditional plant-based medicine and the modern need for scientifically validated, safe treatments. Additionally, with the rise of personalized medicine, the ability to precisely extract and analyze plant compounds has opened the door to tailored treatments. Such treatments can be customized based on an individual's genetic makeup, health conditions, and other factors, thereby maximizing their therapeutic effects.

Conclusion

The progression of medicinal plants from traditional ethnobotanical practices to contemporary phytochemical analysis highlights the fusion of ancestral wisdom with modern scientific inquiry. The historical use of plants in healing systems provided the groundwork for investigating their therapeutic potential. The advent of advanced analytical techniques has transformed phytochemical research, ensuring that herbal medicines are safe, effective, and consistent. Ongoing research and innovation in this area are vital for identifying new drug candidates, optimizing the therapeutic benefits of medicinal plants, and safeguarding traditional knowledge for future generations.

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