Journal of Pharmaceutical Advanced Research
(An International Multidisciplinary Peer Review Open Access monthly Journal)
Available online at: www.jparonline.com

REVIEW ARTICLE JPAR 2024

Advancements in Phytochemical Analysis; Techniques and Applications: A systemic review

Rutvi Shah*, Salvi Shah

Neotech Institute of Pharmacy, Neotech Campus, Harni Virod Road, Vadodara-390001, India.

Received: 15.10.2024 Revised: 05.11.2024 Accepted: 18.11.2024 Published: 30.11.2024

ABSTRACT: Phytochemical profiling is a sophisticated analytical process critical for identifying and quantifying chemical constituents in plant-based materials. This technique is fundamental to developing herbal drugs, ensuring their quality, safety, and efficacy. Historically, phytochemical analysis relied on traditional methods like Thin Layer Chromatography (TLC) and Ultraviolet (UV) spectroscopy. While these methods provided some chemical insight, they had notable limitations. TLC, for instance, offered low sensitivity and selectivity, often yielding only qualitative data. UV spectroscopy, although useful for identifying certain compounds, was limited in its ability to deliver detailed quantitative or structural information. The evolution of modern instrumental methods has transformed phytochemical profiling, providing unprecedented precision and accuracy. 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) spectroscopy now enable detailed chemical analysis. HPLC facilitates the separation and quantification of individual compounds in complex mixtures. GC-MS offers in-depth molecular weight and structural information, while LC-MS combines the separation capabilities of liquid chromatography with the detailed analysis of mass spectrometry. NMR spectroscopy provides comprehensive structural insights into chemical compounds. These advancements have enabled the discovery of novel phytochemicals, comprehensive profiling of complex chemical matrices and the development of standardized herbal formulations. Understanding the pharmacokinetics (absorption, distribution, metabolism, and excretion) and pharmacodynamics (biological effects and mechanisms) of phytochemicals is also crucial. This knowledge optimizes drug delivery, improves therapeutic outcomes, and links chemical composition with biological activity. It further assists in identifying potential drug targets and refining the efficacy and safety profiles of herbal drugs.

Corresponding author:
Ms. Shah Rutvi
Dept. of Pharmacognosy
Neotech Institute of Pharmacy,
Neotech Campus, Harni Virod Road,
Vadodara-390001, India.
Tel: +91-9998282411
E. Mail ID: rutvishah1661@gmail.com

Keywords: Phytochemical analysis, HPLC, GC-MS, LC-MS, NMR, pharmacokinetics, pharmacodynamics, drug discovery, quality control.

INTRODUCTION:

The term "herb" originates from the Latin word "herba" and refers broadly to plants encompassing leaves, stems, flowers, and seeds that find various applications in human life, including culinary, medicinal, aromatic, and perfumery uses. These plants are valued not only for their aesthetic qualities but also for their diverse chemical compositions, which include phytochemicals. Phytochemicals are naturally occurring compounds within plants that possess biologically active properties.

They play crucial roles in human health, acting as medicinal ingredients and essential nutrients. Beyond their nutritional value, phytochemicals serve as protective agents for plants, helping them defend against diseases, environmental stresses like pollution and UV radiation, as well as pathogenic attacks [i]. Phytochemicals such as *Curcuma longa* contains curcuminoids, which shows anti-inflammatory activity. Berberine present in *Berberis aristate* shows good wound healing properties. Moreover, phytochemicals contribute significantly to the sensory characteristics of plants, influencing their colour, aroma, and flavour. Recent research has underscored their multifaceted benefits for human health when consumed regularly in the diet. These compounds have been shown to exhibit antioxidant activity, enhance antimicrobial effects, modulate detoxification enzymes, stimulate the immune system, reduce platelet aggregation, influence hormone metabolism, and even demonstrate potential anticancer properties [2]. The levels and types of phytochemicals present in plants vary based on factors such as plant variety and environmental conditions in which they are grown. Scientists have identified and classified over 4,500 phytochemicals to date, categorizing them according to their specific protective functions and unique physical and chemical characteristics. This diversity underscores the rich potential of phytochemicals in supporting human health and well-being through their myriad biological activities. Phytochemical profiling - Phytochemical profiling refers to the systematic analysis and identification of bioactive compounds present in plants (phytochemicals). These compounds include a wide range of chemical substances such as Alkaloids, Flavonoids, Phenolics, and Terpenoids, which contribute to the medicinal properties and biological activities of the plant [3].

Principle:

Phytochemicals are naturally occurring compounds found in plants that contribute to their color, flavor, and disease resistance. They are not essential nutrients like vitamins and minerals but are increasingly recognized for their potential health benefits. Phytochemicals exhibit diverse chemical structures and biological activities, including Antioxidant, Anti-inflammatory, Antimicrobial, and Anticancer properties. Examples include flavonoids (e.g., quercetin, catechins), phenolic acids (e.g., Rosmarinus acid, caffeic acid), alkaloids (e.g., caffeine, nicotine), and terpenoids (e.g., carotenoids, essential oils) [4]. The diversity of phytochemicals enables plants to interact with The diversity of phytochemicals enables plants to interact with their environment and defend against pathogens, while also offering potential therapeutic applications in human health and disease prevention their environment and defend against pathogens, while also offering potential therapeutic applications in human health and disease prevention [5]. Phytoconstituents, also known as plant constituents, encompass a vast array of chemical compounds found naturally in plants. These constituents contribute to the unique properties and characteristics of different plant species. The diversity of phytoconstituents can be categorized broadly into several classes, each with its own chemical structures and biological activities [6,7].

- Alkaloids: Nitrogen-containing compounds with potent physiological effects, like caffeine, nicotine, morphine, and quinine.
- Flavonoids: Polyphenolic compounds with antioxidant and anti-inflammatory properties, found in onions, apples (quercetin), green tea (catechins), and berries (anthocyanins).
- Phenolic Acid: Known for antioxidant and antimicrobial properties, found in coffee (caffeic acid), rosemary, oregano (rosmarinic acid), and tea, fruits (gallic acid).
- Terpenoids: Derived from isoprene units, including essential oils, carotenoids (colors in fruits/vegetables), and sterols (e.g., beta-sitosterol in plants and supplements).
- Glycosides: Comprising a sugar molecule and non-sugar molecule, with diverse actions like cardiac effects (digitalis glycosides) and antimicrobial properties (anthraquinone glycosides).
- Lignans: Polyphenolic compounds with antioxidant and phytoestrogenic properties, found in flaxseeds (secoisolariciresinol diglucoside) and various seeds/grains (enterodiol, enterolactone).
- Saponins: Glycosides with foaming characteristics and diverse biological activities, such as antimicrobial, antifungal, and anticancer effects (e.g., ginsenosides from ginseng, glycyrrhizin from licorice).
- Tannins: Polyphenolic compounds known for astringent taste, antioxidant, and antimicrobial properties, found in tea, wine, grapes, and berries [8].
- These phytoconstituents contribute to the medicinal, nutritional, and sensory qualities of plants, making

them valuable in traditional medicine and modern pharmaceuticals. Their diverse biological activities underscore their potential in health promotion and disease prevention.

Significance of phytochemical profiling in Herbal Medicine:

Phytochemical profiling is a comprehensive analytical approach used to identify and quantify the bioactive chemical compounds present in plants. Understanding the medicinal properties of plants is crucial for several significant reasons. (A) Identification of Bioactive Compounds: Plants produce a vast array of phytochemicals, including alkaloids, flavonoids, terpenoids, phenolics, and others. Each of these compounds may have specific biological activities and potential health benefits. By profiling the phytochemical composition of a plant, researchers can identify compounds present quantities. This information forms the basis for understanding the plant's potential medicinal properties. For example, St. John's Wort (*Hypericum perforatum*) contains hypericin and hyperforin, which are believed to contribute to its antidepressant effects. (B) Determination of Therapeutic Potential: Different phytochemicals exhibit diverse biological activities, such as antioxidant, anti-inflammatory, antimicrobial, antiviral, and anticancer [9]. Phytochemical profiling allows researchers to correlate these activities with specific compounds present in plants. This correlation helps in determining the potential therapeutic benefits of a plant for various health conditions. For instance, the phytochemical profiling of green tea (*Camellia sinensis*) has identified catechins such as epigallocatechin gallate (EGCG), which possess potent antioxidant properties. These compounds have been linked to numerous health benefits, including cardiovascular health and cancer prevention [10]. (C) Optimization of Extraction Methods: Effective extraction of bioactive compounds from plants is crucial for utilizing their medicinal properties in herbal medicines, dietary supplements, and functional foods. Phytochemical profiling provides insights into which compounds are desirable and they can be best extracted from plant material. For example, the phytochemical profile of ginseng (*Panax ginseng*) has guided researchers in optimizing extraction methods to obtain ginsenosides, which are the active compounds responsible for its adaptogenic and immune-modulating effects. (D) Quality Control and Standardization: Phytochemical profiling serves as a basis for quality control in the production of herbal medicines and dietary supplements. Standardized profile of bioactive compounds is established to ensure consistency in product potency and efficacy. For example, Echinacea (*Echinacea purpurea*) is known for its immunomodulatory effects due to its alkyl amides and polysaccharides. Phytochemical profiling helps standardizing echinacea preparations to relay on immunesupporting benefits [11]. (E) Drug Discovery and Development: Many pharmaceutical drugs are derived from plant compounds or are inspired by them. Phytochemical profiling plays a crucial role in drug discovery by identifying novel bioactive compounds with therapeutic potential.

These compounds can serve as leads for developing new drugs or as inspiration for designing synthetic analogs with improved efficacy and safety profiles. For instance, the phytochemical profiling of the Madagascar periwinkle (*Catharanthus roseus*) led to the discovery of vinblastine and vincristine, which are potent anticancer drugs used in chemotherapy [12].

Turmeric is another example of a plant whose medicinal properties have been extensively studied through phytochemical profiling. It contains curcuminoids, primarily curcumin, which impart its characteristic yellow color and numerous health benefits. Phytochemical profiling has revealed that curcumin exhibits antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Curcumin is proven for therapeutic applications in conditions such as Arthritis, Cardiovascular diseases, Alzheimer's disease. By understanding the phytochemical composition of turmeric, researchers can elucidate how curcumin exerts these effects at a molecular level, paving the way for the development of curcumin-based therapeutics and dietary supplements. Phytochemical profiling is indispensable for unlocking the medicinal potential of plants [13]. It integrates traditional knowledge with modern scientific methods, providing a foundation for evidence-based herbal medicine, nutraceuticals, and pharmaceutical development [14].

Applications of Phytochemical Profiling:

Quality Control and Standardization **[6]:**

- Standardization: Key markers can be utilized for standardization of phytochemicals present in herbal drug, quality and quantity can be analysed for single and combination of herbal drugs.

- Quality Control: Regular profiling monitors the quality of raw materials, intermediate products, and finished goods, minimizing the risk of substandard products.
- Batch-to-Batch Uniformity: Consistent phytochemical profiles ensure equal potency towards each batch whether it delivers the same therapeutic benefits [15].
- Efficacy Prediction: Correlating phytochemical markers with clinical outcomes helps predict the efficacy of herbal products.
- Safety Assessment: Monitoring potentially toxic compounds prevents adverse effects and ensures product safety.

***Phytochemical profiling*:**

a) Qualitative Analysis: This involves identifying the presence of different phytochemical classes (alkaloids, flavonoids, terpenoids, etc.) using colorimetric, chromatographic, or spectroscopic techniques [16].

b) Quantitative Analysis: Determining the concentration of specific phytochemicals using techniques like HPLC, GC-MS, or LC-MS/MS [17].

***Bioactivity Screening*:**

- Antioxidant Activity: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical cation decolorization assay, FRAP (Ferric Reducing Antioxidant Power) assay, ORAC (Oxygen Radical Absorbance Capacity) assay
- Anti-inflammatory Activity: Inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, Measurement of inflammatory mediators (e.g., cytokines, prostaglandins), In vivo models of inflammation (e.g., carrageenan-induced paw edema) [18].
- Antimicrobial Activity: Disk diffusion method, Broth microdilution method, Minimum inhibitory concentration (MIC) determination.
- ***Correlation Analysis:***

- Statistical Methods: Correlation coefficients (Pearson, Spearman) can be used to assess the relationship between phytochemical content and bioactivity.
- Multivariate Analysis: Techniques like principal component analysis (PCA) and partial least squares (PLS) can help identify complex relationships between multiple phytochemicals and biological activities.
- Structure-Activity Relationship (SAR) Studies: By comparing the structures of bioactive compounds, it can be possible to identify structural features responsible for the observed activities/therapeutic activity [19].

ANALYTICAL TECHNIQUES IN PHYTOCHEMICAL PROFILING:

Modern pharmacognosy finds plant materials crucial for therapy and the discovery of new natural substances with healing potential. These materials can be utilized directly, as formulations, or as isolated compounds from plant tissues. Pharmacopeial standards are established for plant materials, medicinal formulations, and isolated compounds to ensure their therapeutic efficacy through the study of biologically active constituents. Methods employed in studying plant materials include macroscopic (for botanical identification and purity), microscopic (involving histochemical analyses), biological (microbiological and biomolecular investigations), and analytical methods.

Analytical investigations aim to categorize the type of phytoconstituent, quantitatively assess active compounds, isolate substances from plant tissues, conduct physicochemical characterization, and analyse the structural properties of isolated compounds. However, it is important to note that not all medicinal herbs are included in pharmacopoeias. Traditional method such as TLC fingerprinting, UV- spectroscopy, cold compression method and hot maceration methods was performed for phytochemical characterization.

TLC (Thin layer chromatography):

Thin-layer chromatography (TLC) fingerprinting is a technique used in pharmacognosy and analytical chemistry to analyse the composition of plant extracts or herbal medicines TLC was performed to extract/separate the components of a mixture based on their differential migration rates through a thin layer of adsorbent material (silica gel or alumina) coated on a plate [20]. Different components are separated as per their polarity, creating distinct separation patterns [21].

***Application*:**

- Quality Control: TLC fingerprinting is used to establish a unique chromatographic profile (fingerprint) for a particular plant material or herbal extract.
- Identification: It helps identify and authenticate plant species by comparing the fingerprint with reference standards or databases.

- Quantification: TLC can semi-quantitatively determine the concentration of active compounds by comparing spot intensities or areas [22].
- A sample is spotted onto a TLC plate and placed in a solvent chamber, where capillary action draws the solvent up the plate.
- Different components of sample interact with the adsorbent and solvent, leading to separation as per the polarity.
- After development, the plate is visualized under UV light or treated with specific reagents to detect spots corresponding to different compounds [23].

***Advantages*:**

- Rapid: Provides quick results compared to other chromatographic techniques.
- Cost-effective: Requires relatively simple equipment and consumables.
- Versatility: Can analyse a wide range of compounds including alkaloids, flavonoids, and essential oils [24].

Limitations:

- Sensitivity: May not detect compounds present in very low concentrations.
- Quantitative accuracy: Semi-quantitative; Quantity of phytochemical are difficult to analyse.
- Subjectivity: Interpretation of results can vary depending on operator skill and conditions.

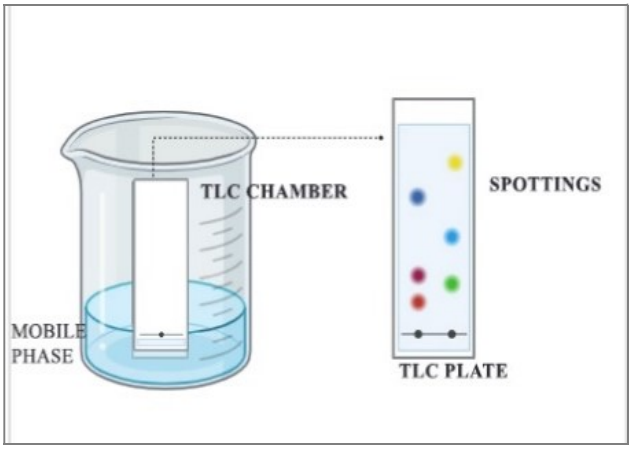

Fig 1. Separation of mixture of compound through TLC fingerprinting.

UV-Spectroscopy:

UV spectroscopy, also known as ultraviolet-visible spectroscopy (UV spectroscopy), is a powerful analytical technique used to study the absorption of ultraviolet and visible light by molecules.

Principle:

UV spectroscopy measures the absorbance of light in the UV and visible regions (typically 190 to 800 nm) by molecules in a sample [25]. When a molecule absorbs light, electrons are excited from their ground state to higher energy levels. The amount of light absorbed at each wavelength are characteristic of the molecule's structure and concentration. UV spectrophotometers consist of a light source (commonly a deuterium or tungsten lamp), a monochromator or prism to select specific wavelengths, a sample holder (cuvette or cell), and a detector (photomultiplier tube or photodiode array) [26].

The sample is placed in the path of light, absorbance of light passing through the sample is measured related to a reference.

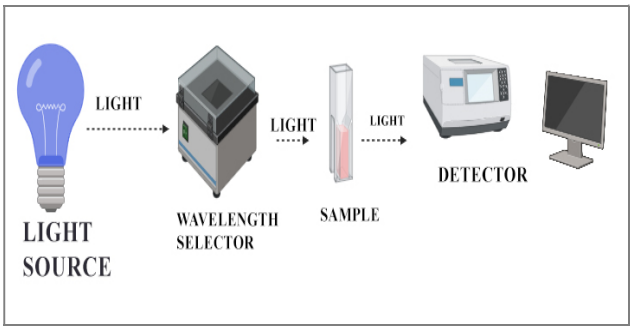

Fig 2. UV spectroscopy.

Applications:

Quantitative Analysis: UV spectroscopy is widely used for quantitative analysis of substances in pharmaceuticals, environmental samples, and biological fluids. It is used because absorbance is proportional to concentration according to Beer's Law. Helps identify compounds based on their unique absorption spectra. Each compound has characteristic absorption maxima that can be used for identification.

Purity Testing: Measures the purity of substances by comparing their spectra to known standards.

Kinetics Studies: Monitors reactions over time by observing changes in absorbance [27].

Advantages:

- Speed: Provides rapid analysis compared too many other techniques.
- Simplicity: Relatively simple instrumentation and sample preparation [28].
- Versatility: Can analyse a wide range of substances, from small molecules to large biomolecules like proteins.

Limitations:

- Specificity: Absorption spectra may overlap, limiting specificity in complex mixtures.
- Sensitivity: May not detect compounds present at very low concentrations without proper dilution or concentration.
- Solvent Interference: Absorption characteristics can be influenced by solvent used, requiring careful selection [29].

MODERN ANALYTICAL TECHNIQUES:

Phytochemical screening has benefited immensely from modern analytical techniques, which offer enhanced sensitivity, specificity, and efficiency compared to traditional methods.

Techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR) spectroscopy, and Liquid Chromatography-Mass Spectrometry (LC-MS) provide detailed chemical profiles and enable the identification and quantification of bioactive compounds in plant extracts [30].

These advancements not only facilitate the discovery of novel phytochemicals but also support their potential application in pharmaceuticals, health supplements, and other industries requiring natural product research and development.

HPLC:

High Performance Liquid Chromatography (HPLC) is an analytical technique extensively used throughout the pharmaceutical industry to provide information on the composition of drug-related samples.

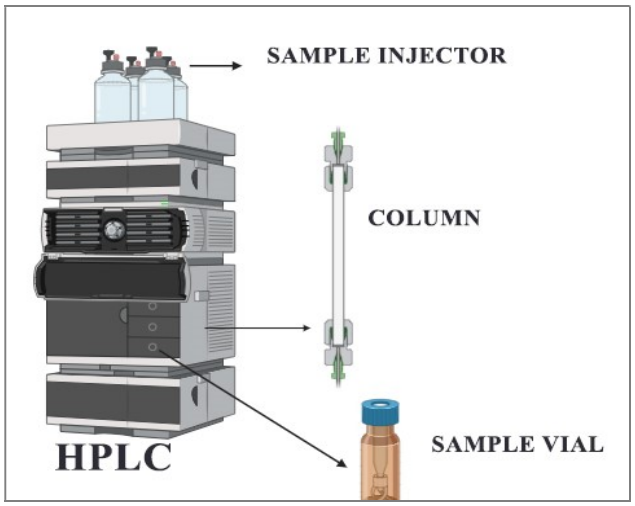

Fig 3. HPLC working system, single gradients HPLC and Gradient HPLC.

The information obtained can be qualitative, indicating which compounds are present in the sample, or quantitative, providing the actual amounts of compounds in the sample. HPLC is utilized at all stages of drug creation and is routinely used during drug manufacture [31]. The aim of the analysis depends on both the nature of the sample and the stage of development. HPLC is a chromatographic technique mentioned in Fig 3.

Principle:

High Performance Liquid Chromatography (HPLC) separates mixture components based on their differential interactions with a stationary phase:

Stationary Phase: Solid material in the column; can be non-polar (reversed phase HPLC) or polar (normal phase HPLC), chosen based on sample properties.

Mobile Phase: Liquid solvent carrying the sample through the column; polarity balanced with stationary phase (non-polar for reversed phase, polar for normal phase) [32].

Sample Injection: Introduction of the sample into the mobile phase stream for transport into the column.

Separation: Components interact differently with the stationary phase; stronger interactions- slow elution, weaker interactions lead to faster elution, resulting in separation over time.

Detection: Components exiting the column pass through a detector (e.g., mass spectrometer, UV-visible, fluorescence) and generates a chromatogram showing response v/s. time graph.

Data Analysis: Chromatogram peaks represent distinct components; peak area correlates with component amount, aiding identification and quantification [33].

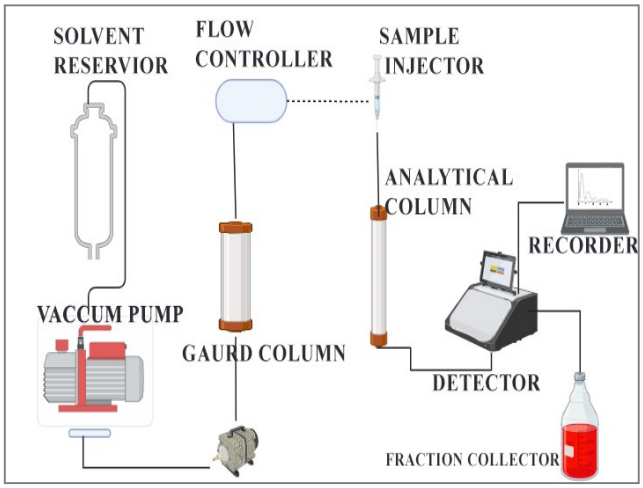

Fig 4. Parts of HPLC Instrument.

Types of HPLC:

Normal Phase HPLC: Uses polar stationary phase, non-polar mobile phase; for hydrophilic compounds.

Reversed Phase HPLC: Non-polar stationary phase, polar mobile phase; versatile, suitable for various compounds.

Ion Exchange HPLC: Charged stationary phase attracts ions in mobile phase; separates ions and polar molecules.

Size Exclusion HPLC (SEC): Separates by molecular size, larger molecules elute first.

Affinity HPLC: Stationary phase interacts selectively (e.g., antigen-antibody); used for purifying specific molecules like proteins.

Chiral HPLC: Separates chiral compounds (enantiomers) using chiral stationary phase. Each type has specific applications based on compound properties and separation goals [34].

Advantages of HPLC:

- High Resolution and Efficiency: HPLC provides excellent resolution, enabling precise separation and quantification of compounds in complex mixtures.
- High Sensitivity: The technique is highly sensitive, capable of detecting and quantifying analytes at low concentrations, crucial for applications in pharmaceutical and environmental analyses [35].
- Versatility: HPLC can analyse a wide range of compounds, including non-volatile, thermally labile, and high molecular weight substances.
- Speed: Modern HPLC systems offer fast analysis times, beneficial for high-throughput screening and industrial applications.
- Accuracy and Precision: HPLC ensures high accuracy and precision in quantitative analysis, essential for regulatory compliance and quality control in industries such as pharmaceuticals.
- Automation and Reproducibility: Automated HPLC systems reduce human error and enhance reproducibility across different analyses and operators.
- Simplified Sample Preparation: Minimal sample preparation requirements save time, reduce the risk of contamination, and utilize minimal amount of sample [36].
- Multiple Detection Options: HPLC systems can be equipped with various detectors (e.g., UV, fluorescence, mass spectrometry), providing flexibility in detection methods based on specific study needs.

Applications of HPLC in phytochemistry:

- Identification and Quantification of active Compounds: HPLC identifies and quantifies alkaloids, flavonoids, terpenoids, phenolic acids, and glycosides in plant extracts, crucial for evaluating their therapeutic potential.
- Herbal Product Quality Control: Ensures consistency, purity, and potency of herbal products by identifying marker components that meet regulatory standards.
- Fingerprinting and Standardization: Creates chemical fingerprints of plant extracts for batch consistency and standardization of herbal medicines.
- Bioactive Compound Isolation: Separates bioactive phytochemicals for structural elucidation and development of medicinal compounds.
- Metabolite Profiling and Metabolomics: Profiles secondary metabolites to understand plant metabolic processes and responses to environmental factors.
- Analysis of Dietary Supplements: Validates phytochemical content, checks for contamination, and confirms label claims for dietary supplement safety and efficacy.
- Evaluation of Antioxidant Activity: Quantifies phytochemicals with antioxidant properties to assess plant extract antioxidant potential for health applications.
- Researching Plant Responses to Stress: herbs respond towards biotic and abiotic stresses by analyzing changes in phytochemical profiles, enhancing crop production and resilience.

GC-MS:

Gas Chromatography-Mass Spectrometry (GC-MS) represents a powerful analytical technique renowned for its ability to identify and quantify volatile and semi-volatile substances. This method integrates the strengths of both gas chromatography (GC) and mass spectrometry (MS), making it indispensable across various scientific and industrial sectors, including pharmaceuticals, environmental analysis, food and flavour chemistry, and forensic science.

Gas Chromatography (GC):

In GC-MS, the process begins with gas chromatography, which isolates components from a complex mixture:

- Sample Injection: The sample, typically in liquid or gaseous form, is introduced into the GC system.
- Vaporization: Upon injection, the sample undergoes vaporization in the injection port and is carried by an inert gas (mobile phase) through a long, narrow capillary column coated with a stationary phase [37].
- Separation: As the vaporized components traverse the column, they interact differently with the stationary phase based on their volatility and polarity, leading to elution at distinct retention times.

***Mass Spectrometry (MS)*:**

Following separation by GC, constituents proceed to the mass spectrometer for further analysis. Steps for mass spectrometry analysis are followed:

- Ionization: Typically employing electron ionization (EI), the molecules exiting the GC column are bombarded with high-energy electrons to generate positively charged ions.
- Fragmentation: Ionization also induces fragmentation of molecules into smaller ions, each possessing a unique mass-to-charge ratio (m/z).
- Mass Analysis: These ions are then separated according to their m/z ratios using a mass analyser, such as anion merge, or time-of-flight (TOF) analyser.
- Detection: The resulting mass spectrum records the abundance of ions, providing a distinct fingerprint for each molecule [38].

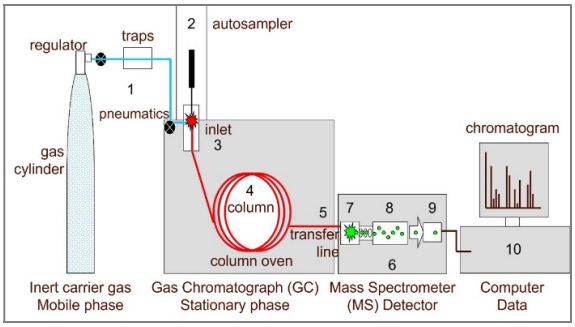

Fig 5. Procedure for GC-MS [39].

***Applications*:**

GC-MS finds wide application due to its precision, sensitivity, and versatility:

- Environmental Analysis: Detecting impurities and pollutants in soil, water, and air samples.
- Forensic Science: Identifying drugs, poisons, and explosives in criminal investigations.
- Food and Flavour Industry: Analyse food additives, contaminants, and flavour compounds.
- Pharmaceuticals: Assessing metabolites, identifying active ingredients, and ensuring purity [40].

***Advantages*:**

- High Specificity and Sensitivity: Capable of identifying chemicals even at trace levels.
- Comprehensive Analysis: Provides quantitative and qualitative insights into sample composition.
- Robustness and Reliability: Generates consistent and accurate results, essential for routine analysis [41].
- GC-MS stands as the gold standard for analysing volatile and semi-volatile substances, offering unparalleled separation capabilities and detailed molecular information. Its integration of GC and MS techniques expands the scope of applications and enhances analytical capabilities in research and industrial settings alike. Researchers and professionals benefit greatly from its ability to tackle complex analytical challenges with precision and reliability [42].

LC-MS:

Liquid Chromatography-Mass Spectrometry (LC-MS) is a sophisticated analytical technique that excels in identifying and characterizing complex mixtures of phytochemicals. By combining the separation power of liquid chromatography (LC) with the detection capabilities of mass spectrometry (MS) [43], LC-MS plays a crucial role in natural product research, pharmacology, and food science.

***Liquid Chromatography (LC)*:**

- LC is responsible for partitioning components within a mixture through several stages:
- Sample introduction: Introducing the liquid sample into the LC system.
- Separation: The sample undergoes separation by passing through a column filled with a stationary phase. Components separate based on their interactions with this phase, dictated by their chemical characteristics [44].

***Mass Spectrometry (MS)*:**

Following separation by LC, MS identifies and characterizes components through distinct steps:

- Ionization: Compounds are ionized using methods such as electrospray ionization (ESI) or air pressure chemical ionization (APCI).

- Fragmentation: Ionized molecules may fragment into smaller pieces at specific mass-to-charge ratio (m/z) [45].
- Mass Analysis: Fragments are analysed using instruments like quadrupoles, ion traps, or time-of-flight (TOF) analysers, sorting them according to their m/z ratio.
- Detection: The separated ions are detected, generating a mass spectrum.
- Data Analysis: The mass spectrum provides detailed information on the molecular weight and structure of substances. Sophisticated databases and tools facilitate the identification and structural characterization of phytochemicals.

In essence, LC-MS integrates LC's separation combined with MS's detection and analysis, enabling comprehensive analysis of complex mixtures in various scientific fields.

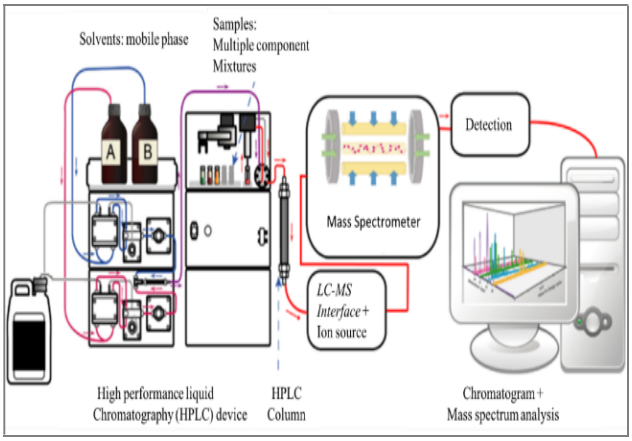

Fig 6. LC-MS instrument [46].

Applications in Phytochemical Analysis:

LC-MS is especially useful for researching phytochemicals since it can:

- Determine unknown substances: Structural elucidation can be determined by examining fragmentation patterns; LC-MS can ascertain the molecular structure of unidentified phytochemicals.
- Database Matching: For identification, mass spectra can be compared to huge phytochemical databases. Define Complicated Combinations: LC Has High Selectivity and Sensitivity-In complicated plant extracts, MS is able to identify low-abundance chemicals.
- Broad Spectrum of Analytes: It can analyse phenolic compounds, alkaloids, flavonoids, terpenes, and other phytochemicals [47].
- Analysis of Quantitative Data: Accurate Quantification: Phytochemicals may be precisely quantified using LC-MS, which is important for pharmacokinetic and bioavailability research.
- Profile of Metabolite - Metabolomics: By profiling plant metabolites using LC-MS, one can gain insight into biochemical pathways and the influence of environmental variables on the composition of phytochemicals [48].

Advantages:

- Versatility: Able to analyse a broad spectrum of molecules with different molecular weights and polarity.
- High Resolution: Offers precise and in-depth mass measurements to aid in the clarification of structural details.
- Sensitivity: Able to find minute concentrations of substances in intricate combinations. LC-MS, or liquid chromatography-mass spectrometry, is an effective method for classifying and detecting intricate phytochemical combinations. It is essential for natural product research as well as numerous pharmacological and food science applications due to its broad spectrum of natural component separation, identification, and quantification capabilities [49].

NMR:

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical method extensively used to elucidate the structural composition of phytochemicals. NMR provides comprehensive descriptions of the environment, kinetics, and molecular structure of organic molecules [50]. It stands as a crucial tool in natural product research, facilitating the identification and characterization of complex phytochemical structures. Radio frequency radiation and an external magnetic field interact with atomic nuclei:

- Magnetic Field: Some nuclei, such ~1H and ~13C, align themselves either with or against the field when exposed to a strong magnetic field.
- Magnetic Field: Some nuclei, such ~1H and ~13C, align themselves either with or against the field when exposed to a strong magnetic field.
- Radiofrequency Pulse: a radiofrequency radiation pulse disturbs the alignment of these nuclei. Relaxation: Radiofrequency signals are released by the nuclei as they realign, and these signals are picked up and converted into NMR spectra.

***Key Features of NMR Spectroscopy*:**

- Chemical Shift (δ): A nucleus's resonance frequency is dependent on its surrounding chemical environment. Chemical shifts reveal details on the functional groups and kinds of atoms surrounding the nucleus.
- Spin-Spin Coupling (J-Coupling): NMR signals divide due to interactions between nearby nuclei. This gives details on the quantity and connectivity of nearby nuclei [51].
- Integration: Relative amounts of various groups can be ascertained because the area under an NMR signal is proportionate to the number of nuclei that contribute to that signal. NMR spectroscopy plays a pivotal role in determining the structural characteristics of phytochemicals, leveraging its capability to provide detailed molecular insights [52].

***Application*:**

- Establishing Molecular Structure: a) Proton Nuclear Magnetic Resonance (~1H NMR) Offers precise details on hydrogen atoms' environment, interactions, and conditions within the molecule. b) Carbon-13 NMR (^13C NMR) Provides comprehensive information about the carbon framework of the molecule. c) 2D NMR Techniques (e.g., COSY, HSQC, HMBC): Facilitate the identification of atom connectivity, even across multiple bonds.
- Identification of Functional Groups: a) Chemical Shifts and Coupling Patterns: Enable the identification of specific functional groups (e.g., hydroxyl, carbonyl, alkene, aromatic) based on their distinct chemical shifts and coupling patterns. b) 2D NMR: Assists in pinpointing the presence and location of functional groups within molecules [53].
- Stereochemistry: a) Nuclear Overhauser Effect Spectroscopy (NOESY): Provides insights into stereochemistry and 3D structure by indicating spatial proximity between nuclei. b) ROESY (Rotating-frame Overhauser Effect Spectroscopy): Useful for examining spatial arrangements of atoms and interactions between closely positioned nuclei [54].
- Complex Mixture Analysis: a) Metabolomics: Enables comprehensive profiling of intricate phytochemical blends in plant extracts, offering insights into their metabolic composition. b) De-replication: Facilitates rapid identification of known compounds within complex mixtures, expediting the search for novel phytochemicals. NMR spectroscopy thus serves as a critical tool in phytochemical research, providing essential molecular information essential for structural elucidation and characterization [55].

***Advantages*:**

- Non-Destructive: The sample is not destroyed during NMR analysis, enabling additional testing.

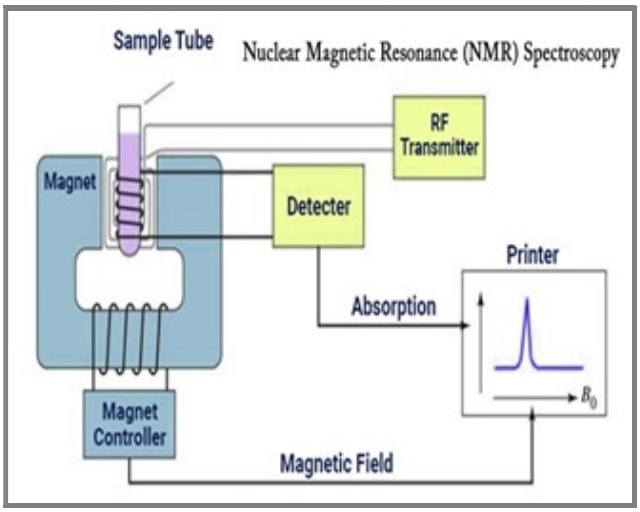

Fig 7. NMR instrument.

- Detailed Structural Information: Offers thorough understanding of the stereochemistry, connectivity, and functional groups of the molecule.
- Quantitative: Enables the relative quantities of constituents within a sample to be determined. NMR spectroscopy, or nuclear magnetic resonance, is a crucial technique for determining the structural makeup of phytochemicals. It is extremely useful in the study of natural products due to its capacity to offer comprehensive details regarding stereochemistry, functional groups, and molecular structure [56].

Pharmacokinetic and Pharmacodynamic study of Phytochemical Profiling:

***Pharmacokinetic study of Phytochemical Profiling*:**

A pharmacokinetic study is a scientific investigation that examines the absorption, distribution, metabolism, and excretion (ADME) of a drug within a biological system. It aims to quantitatively describe the time course and Advancements in Phytochemical Analysis: Techniques and non identified, including how it is absorbed into systemic circulation, distributed to tissues and organs, metabolized into other substances, and ultimately eliminated from the body through various routes such as

urine and faces. Pharmacokinetic studies provide essential data to understand the drug's bioavailability, half-life, clearance rates, and interactions with physiological processes, which are crucial for determining appropriate dosage regimens, predicting drug interactions, and optimizing therapeutic outcomes. Phytochemicals are bioactive compounds found in plants that have potential therapeutic benefits for humans. Phytochemicals eliminates several interesting correlations and applications:

- Absorption: Phytochemicals can enhance the absorption of drugs or nutrients by altering the permeability of cell membranes in the gastrointestinal tract. For example, piperine found in black pepper can enhance the absorption of certain drugs or phytochemicals themselves.
- Distribution: Phytochemicals can affect the distribution of drugs within the body by altering the binding properties of proteins such as albumin or by competing for binding sites on transport proteins. This can influence the concentration of drugs in tissues and organs.
- Metabolism: Phytochemicals can interact with drug-metabolizing enzymes such as cytochrome P450 (CYP) enzymes in the liver, potentially inhibiting or inducing their activity. This can lead to changes in the metabolism of co-administered drugs, affecting their bioavailability and therapeutic efficacy.
- Excretion: Phytochemicals may influence the excretion of drugs or other compounds by affecting renal clearance or bile secretion. For instance, certain flavonoids can inhibit the activity of transporters involved in renal excretion, thereby altering the elimination kinetics of drugs for example sennoside A and sennoside B present in *Cassia senna* [57].
- Drug Interactions: Understanding the pharmacokinetic interactions between phytochemicals and drugs is crucial for predicting potential drug interactions. These interactions can either enhance or diminish the therapeutic effects of drugs or lead to adverse effects.
- Clinical Relevance: Incorporating phytochemicals into pharmacokinetic studies can provide insights into their pharmacokinetic profiles and help establish safe and effective dosing regimens. This is particularly important in the context of herbal medicine and the use of botanical supplements, where understanding how phytochemicals interact with conventional drugs can guide clinical practice and improve patient safety.

***Pharmacodynamic Effects of Phytochemicals on Physiological Process*:**

Pharmacodynamic effects of phytochemicals on physiological processes refer to the mechanisms by which biologically active compounds derived from plants (phytochemicals) interact with the body to influence various physiological functions. These effects can be diverse and affect different systems within the body, including:

- Enzyme Inhibition or Activation: Phytochemicals may alter enzymatic activity, affecting processes such as metabolism or signalling pathways crucial for cellular function. For example, curcumin: Found in turmeric, curcumin inhibits enzymes involved in inflammation pathways, such as cyclooxygenase (COX) and lipoxygenase (LOX).
- Antioxidant Activity: Many phytochemicals possess antioxidant properties, scavenging free radicals and reducing oxidative stress, which is implicated in various chronic diseases. For example, resveratrol found in red grapes and wine, resveratrol exhibits antioxidant properties that protect cells from oxidative stress and may contribute to cardiovascular health.
- Hormonal Modulation: Certain phytochemicals can mimic or block hormone receptors, influencing hormone levels and their physiological effects. For example, isoflavones found in soybeans, isoflavones such as genistein and daidzein could bind to estrogen receptors and exert weak estrogenic or anti-estrogenic effects, potentially affecting hormone-related conditions.
- Anti-inflammatory Effects: Phytochemicals may inhibit inflammatory pathways, reducing inflammation markers and contributing to overall health and disease prevention. For example, quercetin found in onions, apples, and berries, quercetin inhibits inflammatory pathways by reducing the production of inflammatory cytokines and enzymes.
- Neurological Effects: Some phytochemicals have been shown to influence neurotransmitter function or protect against neurodegenerative diseases. For example, *Ginkgo biloba* contains flavonoids and terpenoids that improve blood flow to the brain,

potentially enhancing cognitive function and protecting against neurodegenerative diseases.

- Cardiovascular Effects: Phytochemicals like flavonoids can affect vascular function, potentially lowering blood pressure or reducing the risk of cardiovascular diseases. For example, omega-3 fatty acids: Found in fatty fish like salmon, omega-3 fatty acids such as EPA and DHA reduce triglyceride levels, lower blood pressure, and decrease the risk of cardiovascular events.
- Immune Modulation: Compounds like polysaccharides and flavonoids can enhance immune responses or modulate immune cell activity. For example, echinacea contains polysaccharides and caffeic acid derivatives that enhance immune function by stimulating white blood cell activity and production of cytokines.

Case studies:

Phytochemical profiling provides a comprehensive analysis of the diverse phytoconstituents present in herbal drugs. Subsequent analytical and therapeutic studies then demonstrate how specific phytoconstituents contribute to therapeutic effects in various disease conditions. Phytochemical profiling has significantly advanced understanding of medicinal plants by identifying and characterizing bioactive compounds responsible for their therapeutic effects. Here are few specific research examples where phytochemical profiling has played a crucial role:

- Turmeric (*Curcuma longa*): Studies have shown curcumin's anti-inflammatory, antioxidant, and anticancer properties, leading to its exploration in various therapeutic applications [58].
- *Ginkgo biloba*: Phytochemical analysis of Ginkgo biloba extracts has identified flavonoids and terpenoids as the main bioactive constituents. These compounds have been studied for their neuroprotective effects, potentially beneficial in treating cognitive disorders like Alzheimer's disease [59].
- Cannabis (*Cannabis sativa*): The discovery and profiling of cannabinoids such as THC (tetrahydrocannabinol) and CBD (cannabidiol) have been investigated for their analgesic, anti-inflammatory, and neuroprotective effects, influencing the development of medical marijuana and cannabinoid-based therapies [60].
- Green tea (*Camellia sinensis*): Green tea's health benefits are attributed to its polyphenolic compounds, particularly catechins such as epigallocatechin gallate (EGCG). Phytochemical studies have linked EGCG to antioxidant, anticancer, and cardioprotective properties, contributing to its widespread consumption for health promotion [61].
- *Echinacea species*: Phytochemical profiling of Echinacea species has identified alkamides, polysaccharides, and flavonoids as active constituents. These compounds are associated with immune-stimulating effects, supporting the traditional use of Echinacea in treating colds and enhancing immune function.
- St. John's Wort (*Hypericum perforatum*): Research on St. John's Wort has focused on its phytochemical constituents, particularly hypericin and hyperforin. These compounds have been investigated for their antidepressant and mood-stabilizing effects, highlighting the plant's potential as a natural antidepressant [61].
- Garlic (*Allium sativum*): Including its cardiovascular benefits and antimicrobial effects, are attributed to sulphur-containing compounds such as allicin. Phytochemical studies have helped elucidate these bioactive components and their mechanisms of action.
- Data plays a crucial role in optimizing therapeutic outcomes and developing new herbal formulations. Here are several ways in which phytochemical data can be utilized effectively [61].
- Identifying Active Compounds: Phytochemical analysis helps identify the specific bioactive compounds present in medicinal plants. These compounds can then be isolated and studied for their individual pharmacological effects. Understanding which compounds are responsible for therapeutic activities allows researchers to focus on developing formulations that maximize these beneficial effects.
- Standardization of Herbal Extracts: By quantifying the levels of key phytochemicals, such as polyphenols, alkaloids, or terpenes, in herbal extracts, standardization becomes possible. Standardization ensures consistency in the composition and potency of herbal products, which is critical for achieving reliable therapeutic outcomes in clinical settings [62].

- Synergistic Effects: Phytochemical profiling helps identify potential synergistic interactions between different compounds within a plant. These interactions can enhance therapeutic efficacy or provide additional health benefits beyond those of individual compounds alone. Formulations can be designed to leverage these synergies for optimized therapeutic outcomes [60].
- Optimizing Extraction Methods: Different phytochemicals may require specific extraction methods to be effectively extracted from plant material. Understanding the composition of phytochemicals guides the selection of appropriate extraction techniques (e.g., solvent extraction, supercritical fluid extraction) that maximize the yield and preservation of bioactive compounds.
- Tailoring Formulations to Target Conditions: Phytochemical data can inform the formulation of herbal products tailored to specific health conditions. For example, if a phytochemical is found to have potent antioxidant properties, it could be included in formulations intended for combating oxidative stress-related disorders [61].
- Safety and Efficacy: Detailed phytochemical analysis contributes to the safety assessment of herbal products by identifying potential toxic compounds or interactions. It also aids in establishing the efficacy of herbal medicines through evidence-based research that correlates phytochemical composition with clinical outcomes.
- Innovation in Herbal Medicine: Phytochemical data can inspire innovation in herbal medicine by suggesting new combinations of compounds or novel applications for existing plants. For instance, discovering a lesser-known phytochemical with promising therapeutic properties may lead to the development of new herbal formulations or supplements [63].

Challenges and future directions [64,65]:

Phytochemical profiling faces several challenges and offers numerous future directions that are crucial for advancing our understanding and utilization of medicinal plants:

***Challenges*:**

- Complexity of Phytochemical Composition: Medicinal plants often contain a complex mixture of phytochemicals, making comprehensive profiling challenging. Identifying and quantifying all relevant compounds requires advanced analytical techniques and expertise.
- Variability: Phytochemical composition can vary significantly based on factors such as plant genetics, geographical location, growth conditions, and harvest time. This variability complicates standardization and consistency in herbal preparations [70].
- Analytical Techniques: The selection and optimization of analytical methods for phytochemical analysis pose challenges due to the diversity and sometimes-low concentrations of bioactive compounds in plants. Developing robust, sensitive, and specific analytical techniques is essential.
- Data Interpretation: Interpreting phytochemical data requires expertise in pharmacognosy, pharmacology, and biochemistry. Understanding the biological relevance and potential synergies among different compounds is critical but complex [71,72].
- Regulatory Requirements: Herbal products must meet regulatory standards for safety, efficacy, and quality. Establishing reliable correlations between phytochemical profiles and therapeutic outcomes is essential for regulatory approval [73].

Future Directions:

- Integration of Omics Technologies: Incorporating genomics, transcriptomics, proteomics, and metabolomics can provide a more holistic understanding of phytochemical biosynthesis, regulation, and function. This integrated approach can enhance precision in phytochemical profiling.
- Standardization and Quality Control: Developing standardized methods for phytochemical analysis and quality control protocols will improve consistency and reliability in herbal products. This includes establishing marker compounds for authentication and potency assessment.
- Bioinformatics and Data Mining: Leveraging bioinformatics tools and databases for phytochemical data analysis can facilitate the identification of novel bioactive compounds, predictive modelling of synergies, and exploration of structure-activity relationships.
- Ethnopharmacological Studies: Integrating traditional knowledge with modern scientific approaches can guide the selection and prioritization of medicinal plants for phytochemical profiling.

This approach can uncover new therapeutic potentials and validate traditional uses.

- Personalized Phytotherapy: Moving towards personalized medicine involves tailoring herbal formulations based on individual variations in phytochemical metabolism, genetics, and health status. This approach may optimize therapeutic efficacy and minimize adverse effects.
- Sustainability and Conservation: Promoting sustainable practices in harvesting and cultivation of medicinal plants ensures their availability for future research and healthcare needs. Conservation efforts should consider the impact of harvesting on phytochemical composition.
- Clinical Translation: Conducting rigorous clinical trials that correlate phytochemical profiles with therapeutic outcomes will strengthen the evidence base for herbal medicines. This step is crucial for integrating herbal treatments into mainstream healthcare.

CONCLUSION:

Phytochemical profiling is vital for the development of herbal drugs, serving as a cornerstone for ensuring their quality, safety, and efficacy. The evolution from traditional techniques such as TLC and UV spectroscopy to modern instrumental methods like HPLC, GC-MS, LC-MS, and NMR has significantly enhanced our ability to analyze and characterize plant-based materials with greater precision and accuracy. These advanced methods have overcome the limitations of earlier approaches, providing detailed insights into complex chemical profiles and enabling the identification of novel bioactive compounds.

The application of these sophisticated techniques has been instrumental in the standardization of herbal formulations and has paved the way for more effective and reliable drug discovery. They offer superior capabilities in establishing quality standards, detecting adulterations, and correlating chemical composition with biological activity. However, challenges persist in phytochemical profiling, including issues related to the sensitivity and selectivity of detection, the complexity of phytochemical mixtures, and the need for more comprehensive data on pharmacokinetics and pharmacodynamics. Future advancements in analytical technologies, along with improved methodologies for data interpretation, are crucial for addressing these challenges. Continued research and innovation are needed to refine these techniques and explore new therapeutic targets, ultimately enhancing the therapeutic value and safety of herbal medicines.

In conclusion, while significant progress has been achieved in phytochemical profiling, ongoing efforts are essential to overcome existing limitations and fully harness the potential of herbal drugs. By advancing analytical methodologies and addressing current challenges, we can further improve the efficacy and safety of herbal formulations, benefiting both the field of drug discovery and public health.

LIST OF ABBREVIATIONS:

TLC- Thin Layer Chromatography, UV- Ultraviolet spectroscopy, HPLC- High Performance Liquid Chromatography, GC-MS- Gas Chromatography-Mass Spectroscopy, LC-MS- Liquid Chromatography- Mass Spectroscopy, NMR- Nuclear Mass Resonance, EGCG- Epigallocatechin gallate, DPPH- 2,2-diphenyl-1-picrylhydrazyl, ABTS- 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), FRAP- Ferric Reducing Antioxidant Power, ORAC- Oxygen Radical Absorbance Capacity, COX- Inhibition of cyclooxygenase, LOX- lipoxygenase, MIC- Minimum inhibitory concentration, PCA- Principal component analysis, PLS- partial least squares, SAR- Structure-Activity Relationship, SEC- Size Exclusion HPLC, EI- Electron ionization, TOF- time-of-flight, ESI- electrospray ionization, APCI- Air pressure chemical ionization, J-Coupling- Spin-Spin Coupling, ~1H NMR- Proton Nuclear Magnetic Resonance, ^13C NMR- Carbon-13 NMR, COSY- Correlation Spectroscopy, HSQC- Heteronuclear Single Quantum Coherence, HMBC- Heteronuclear Multiple Bond Correlation, NOESY- Nuclear Overhauser Effect Spectroscopy, ROESY - Rotating-frame Overhauser Effect Spectroscopy, ADME- Absorption, Distribution, Metabolism, and Excretion, CYP- cytochrome P450, COX- Cyclooxygenase, LOX- lipoxygenase, EPA- Environmental Protection Agency, DHA- Docosahexaenoic Acid, THC- Tetrahydrocannabinol, CBD- Cannabidiol, EGCG- Epigallocatechin gallate.

ACKNOWLEDGEMENT:

We would like to thank Neotech Institute of Pharmacy, Neotech Campus for providing platform.

This study involves the plant but as it is review, we doesn't require any approval of consent from national,

international or legislation or we dint required any licence to study for this review.

REFERENCES:

1. Tambare P, Tamboli FA, More HN. Standardization of herbal drugs: an overview. Int Res J Pharm, 2011; 2(12): 56-60.
2. Rf- Koche DE, Shirsat RU, Kawale MA. An overerview of major classes of phytochemicals: their types and role in disease prevention. Hislopia J, 2016; 9(1/2): 1-1.
3. Campos-Vega R, Oomah BD. Chemistry and classification of phytochemicals. Handbook Plant Food Phytochem, 2013; 27: 5-48.
4. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem, 2014; 2(5): 115-119.
5. Roy M, Datta A, Roy M, Datta A. Fundamentals of phytochemicals. In: Cancer Genetics and Therapeutics: Focus on Phytochemicals. Germany: Springer; 2019. pp. 49-81.
6. Nafiu MO, Hamid AA, Muritala HF, Adeyemi SB. Preparation, standardization, and quality control of medicinal plants in Africa. Medicinal spices and vegetables from Africa. 2017 Jan 1:171-204.
7. Balekundri A, Mannur V. Quality control of the traditional herbs and herbal products: a review. Fut J Pharm Sci, 2020; 6: 1-9.
8. Mena P, Cirlini M, Tassotti M, Herrlinger KA, Dall'Asta C, Del Rio D. Phytochemical profiling of flavonoids, phenolic acids, terpenoids, and volatile fraction of a rosemary (*Rosmarinus officinalis* L.) extract. Molecules, 2016; 21(11): 1576.
9. Parveen S, Bukhari N, Nazir M, Qureshi WA, Yaqoob A, Shahid M. Phytochemical analysis, *in-vitro* biological activities and Pearson correlation of total polyphenolic content with antioxidant activities of *Ziziphus mauritiana* fruit pulp and seed during different ripening stages. South Afr J Botany, 2023; 157: 346-354.
10. Jiang L, Numonov S, Bobakulov K, Qureshi MN, Zhao H, Aisa HA. Phytochemical profiling and evaluation of pharmacological activities of Hypericum scabrum L. Molecules, 2015; 20(6): 11257-11271.
11. Barreira S, Moutinho C, Silva AM, Neves J, Seo EJ, Hegazy ME, *et al*. Phytochemical characterization and biological activities of green tea (*Camellia sinensis*) produced in the Azores, Portugal. Phytomed Plus, 2021; 1(1): 100001.
12. Abdelmotaleb MM, Elshikh HH, Abdel-Aziz MM, Elaasser MM, Yosri M. Evaluation of antibacterial efficacy and phytochemical analysis of *Echinacea purpurea* towards MDR strains with clinical origins. Al-Azhar Bull Sci, 2023; 34(2): 3.
13. Bansal Y, Mujib A, Mamgain J, Dewir YH, Rihan HZ. Phytochemical Composition and Detection of Novel Bioactives in Anther Callus of *Catharanthus roseus* L. Plants, 2023; 12(11): 2186.
14. Aggarwal BB, Surh YJ, Shishodia S, editors. The molecular targets and therapeutic uses of curcumin in health and disease. Germany: Springer Science & Business Media; 2007.
15. Eberhardt MV, Kobira K, Keck AS, Juvik JA, Jeffery EH. Correlation analyses of phytochemical composition, chemical, and cellular measures of antioxidant activity of broccoli (*Brassica oleracea* L. var. italica). J Agri Food Chem, 2005; 53(19): 7421-7431.
16. Al-Dhubiab BE. Pharmaceutical applications and phytochemical profile of *Cinnamomum burmannii*. Pharmacogn Rev, 2012; 6(12): 125.
17. Zhou Y, Chu M, Ahmadi F, Agar OT, Barrow CJ, Dunshea FR, *et al*. A comprehensive review on phytochemical profiling in mushrooms: occurrence, biological activities, applications and future prospective. Food Rev Int, 2024; 40(3): 924-951.
18. Bittencourt ML, Ribeiro PR, Franco RL, Hilhorst HW, de Castro RD, Fernandez LG. Metabolite profiling, antioxidant and antibacterial activities of *Brazilian propolis*: Use of correlation and multivariate analyses to identify potential bioactive compounds. Food Res Int, 2015; 76: 449-457.
19. Waksmundzka-Hajnos M, Sherma J, Kowalska T. Overview of the field of TLC in phytochemistry and the structure of the book. Boca Raton: CRC Press; 2008.
20. Balekundri A. Greenness Evaluation and Simultaneous High-Performance Thin-Layer Chromatography Method for Determination of Powder Mixture and Ayurvedic Formulation. Natl Acad Sci Lett, 2023; 3: 233-237.
21. Mehta P, Shah R, Lohidasan S, Mahadik KR. Pharmacokinetic profile of phytoconstituent (s) isolated from medicinal plants—a comprehensive review. J Trad Complement Med, 2015; 5(4): 207-227.
22. Jesionek W, Grzelak EM, Majer-Dziedzic B, Choma IM. Thin-layer chromatography—direct bioautography for the screening of antimicrobial properties of plant extracts. J Planar Chromat, 2013; 26(2): 109-113.
23. Mendoza CE. Thin-layer chromatography and enzyme inhibition techniques: Introduction. J Chromat A, 1973; 78(1): 29-40.
24. Patle TK, Shrivas K, Kurrey R, Upadhyay S, Jangde R, Chauhan R. Phytochemical screening and determination of phenolics and flavonoids in Dillenia pentagyna using UV–Vis and FTIR spectroscopy. Spectrochimica Acta Part A: Mol Biomol, Spectrosc, 2020; 242: 118717.

25. Picollo M, Aceto M, Vitorino T. UV-Vis spectroscopy. Phys Sci Rev, 2019; 4(4): 20180008.
26. Wilkes TC, McGonigle AJS, Willmott JR, Pering TD, Cook JM. Low-cost 3D printed 1 nm resolution smartphone sensor-based spectrometer: instrument design and application in ultraviolet spectroscopy. Optics Lett, 2016; 42(21): 4323-4326.
27. Rafi M, Meitary N, Septaningsih DA, Bintang M. Phytochemical profile and antioxidant activity of Guazuma ulmifolia leaves extracts using different solvent extraction. Indones J Pharm, 2020; 31(3): 171-180.
28. Céspedes CL, Valdez-Morales M, Avila JG, El-Hafidi M, Alarcón J, Paredes-López O. Phytochemical profile and the antioxidant activity of Chilean wild black-berry fruits, *Aristotelia chilensis* (Mol) Stuntz (*Elaeocarpaceae*). Food Chem, 2010; 119(3): 886-895.
29. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Germany: Springer Science & Business Media; 1998.
30. Rabel F, Sherma J. New TLC/HPTLC commercially prepared and laboratory prepared plates: A review. *J Liq Chromatogr Relat Technol***,** 2016; 39(8): 385-393.
31. Virus ED, Sobolevsky TG, Rodchenkov GM. Introduction of HPLC/orbitrap mass spectrometry as screening method for doping control. J Mass Spectrom, 2008; 43(7): 949-957.
32. Cappiello A, Palma P, Panayiotopoulos IA, Biemann K. Efficient introduction of HPLC fractions into a highperformance tandem mass spectrometer. Chromatographia. 1990; 30: 477-483.
33. Kagel RA, Farwell SO. Analysis of currently available analgesic tablets by modern liquid chromatography: an undergraduate laboratory introduction to HPLC. J Chem Educ, 1983; 60(2): 163.
34. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development. Wiley; 2010.
35. Lazarowych NJ, Pekos P. Use of fingerprinting and marker compounds for identification and standardization of botanical drugs: strategies for applying pharmaceutical HPLC analysis to herbal products. Drug Inf J, 1998; 32(2): 497-512.
36. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 7th ed. UP: Cengage Learning; 2017.
37. Kovac J, Berg R, Sutherland J. Gas chromatography–mass spectrometry. In: Mass Spectrometry: A Foundation Course. New York: CRC Press; 2006. pp. 151-176.
38. Lee MS, and Lee HY. Applications of Gas Chromatography-Mass Spectrometry in Environmental Analysis. In: Analytical Methods for the Determination of Trace Elements in Water. Germany: Springer; 2015. pp. 231-254.
39. Hubschmann HJ. Handbook of GC-MS: fundamentals and applications. England: John Wiley & Sons; 2015.
40. Falkovich AH, Rudich Y. Analysis of semivolatile organic compounds in atmospheric aerosols by direct sample introduction thermal desorption GC/MS. Environ Sci Technol, 2001; 35(11): 2326-2333.
41. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray Ionization for Mass Spectrometry of Large Biomolecules. Science, 1989; 246(4926): 64-71.
42. Yang Y, Zhang Z, Li S, Ye X, Li X, He K. Synergy effects of herb extracts: pharmacokinetics and pharmacodynamic basis. Fitoterapia, 2014; 92: 133-147.
43. Yisimayili Z, Chao Z. A review on phytochemicals, metabolic profiles and pharmacokinetics studies of the different parts (juice, seeds, peel, flowers, leaves and bark) of pomegranate (*Punica granatum* L.). Food Chem, 2022; 395: 133600.
44. Keller RA, Schueller T. LC-MS for Phytochemical Analysis: Applications and Advantages. J Chromatogr A, 2009; 1216(48): 8398-8412.
45. Rai M, Singh AV, Paudel N, Kanase A, Falletta E, Kerkar P, *et al*. Herbal concoction unveiled: a computational analysis of phytochemicals' pharmacokinetic and toxicological profiles using novel approach methodologies (NAMs). Curr Res Toxicol, 2023; 5: 100118.
46. Viant MR. NMR-based metabolomics: a powerful approach for characterizing the effects of environmental stressors on organism health. Phytochem Anal Int J Plant Chem Biochem Tech, 2021; 32(1): 50-58.
47. Quez S, Jayaprakasha GK, Gowda GAN, Patil BS. Rapid separation and quantitation of curcuminoids combining pseudo two-dimensional liquid flash chromatography and NMR spectroscopy. J Agri Chem, 2014; 62(22): 5144-5150.
48. Sanders JKM, Hunter BK. Modern NMR spectroscopy: a guide for chemists. New Delhi: Oxford University Press; 1988.
49. Gronenborn AM, Driscoll PC, Wingfield PT, Bax A. Overcoming the overlap problem in the assignment of proton NMR spectra of larger proteins by use of three-dimensional heteronuclear proton-nitrogen-15 Hartmann-Hahn-multiple quantum coherence and nuclear Overhauser multiple quantum coherence spectroscopy. J Magn Reson, 1989; 82(1): 169-173.
50. Rathaur P, SR KJ. Metabolism and pharmacokinetics of phytochemicals in the human body. Curr Drug Metab, 2019; 20(14): 1085-1102.

51. Selby-Pham SN, Miller RB, Howell K, Dunshea F, Bennett LE. Physicochemical properties of dietary phytochemicals can predict their passive absorption in the human small intestine. Sci Report, 2017; 7(1): 1931.
52. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": From kitchen to clinic. Biochem Pharmacol, 2008; 75(4): 787-809.
53. Smith JV, Luo Y. Studies on molecular mechanisms of Ginkgo biloba extract. Appl Microbiol Biotechnol, 2004; 64(4): 465-472.
54. Barnes J, Anderson LA, Gibbons S. Echinacea species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., and *Echinacea purpurea* (L.) Moench): a review of their chemistry, pharmacology and clinical properties. J Pharmacy Pharmacol, 2005; 57(8): 929-954.
55. Izzo A A, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci, 2009; 30(10): 515-527.
56. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea—A review. J Am Coll Nutr, 2006; 25(2): 79-99.
57. Müller WE. Current St John's wort research from mode of action to clinical efficacy. Pharmacol Res, 2003; 47(2): 101-109.
58. Sangwan RS, Chaurasiya ND, Misra LN, Lal P, Uniyal GC, Sharma R. Phytochemical variability in commercial herbal products and preparations of *Withania somnifera* (Ashwagandha). Curr Sci, 2004; 86(3): 461-465.
59. Anyasor GN, Ogunwenmo O, Oyelana OA, Akpofunure BE. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (*Costaceae*). J Med Plants Res, 2009; 3(8): 563-567.
60. Batubara I, Mitsunaga T, Ohashi H. Screening antiacne potency of Indonesian medicinal plants: antibacterial, lipase inhibition, and antioxidant activities. J Wood Sci, 2009; 55: 230-235.
61. Rafi M, Rohaeti E, Miftahudin A, Darusman LK. Differentiation of *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber cassumunar* by thin layer chromatography fingerprint analysis. Indones J Chem, 2011; 11(1): 71-74.
62. Aliakbarzadeh G, Sereshti H, Parastar H. An image analysis of TLC patterns for quality control of saffron based on soil salinity effect: A strategy for data (pre)-processing. Anal Bioanal Chem, 2016; 408(12): 3295-3307.
63. Komsta Ł, Cieśla Ł, Waksmundzka-Hajnos M, *et al*. Investigation of antiradical activity of plant material by thin-layer chromatography with image processing. J Planar Chromatogr, 2008; 21(4): 289-294.
64. Hassan RYA, Khaled R, Farghali A, El-Sherbiny IM. Polyurethane-doped platinum nanoparticles modified carbon paste electrode for the sensitive and selective voltammetric determination of free copper ions in biological samples. Microchem J, 2020; 155: 104765.
65. Ahmad I. Applications of UV/Vis spectroscopy in characterization and catalytic activity of noble metal nanoparticles fabricated in responsive polymer microgels: a review. Comput Theor Chem, 2020; 1179: 112797.

Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Rutvi S*, Salvi S. Advancements in Phytochemical Analysis; Techniques and Applications: A systemic review. J Pharm Adv Res, 2024; 7(11): 2461-2477.

.