J Pharm Biopharm Res, 2026, 8(1): 569-578
DOI: 10.25082/JPBR.2026.01.003

REVIEW

A Comprehensive Review on the Isolation of Bioactive Compounds from Plants

Anuansh Tiwari[1] **Anuanshika Tiwari**[2] **Syed Wajahat Abbas Rizvi**[1] **Ritu Tiwar**[3] **Smita Mishra**[4*]

[1] Department of Computer Science and Engineering, Amity School of Engineering and Technology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow, Uttar Pradesh, India
[2] Department of Computer Science (AI & Data Science), Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh, India
[3] Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Government of India, Ghaziabad 201002, Uttar Pradesh, India
[4] Department of Life Sciences, School of Biosciences and Technology, Galgotias University, Greater Noida, Uttar Pradesh, India

Check for updates

Correspondence to: Smita Mishra, Department of Life Sciences, School of Biosciences and Technology, Galgotias University, Greater Noida, Uttar Pradesh, India; Email: smitamishrasoni@gmail.com

Received: July 22, 2026;
Accepted: October 4, 2026;
Published: October 9, 2026.

Citation: Tiwari A, Tiwari A, Rizvi SWA, et al. A Comprehensive Review on the Isolation of Bioactive Compounds from Plants. *J Pharm Biopharm Res*, 2026, **8**(1): 569-578.
https://doi.org/10.25082/JPBR.2026.01.003

Copyright: © 2026 Anuansh Tiwari et al. This is an open access article distributed under the terms of the Creative Commons Attribution-Noncommercial 4.0 International License, which permits all noncommercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract: Plants are an incredible source of diverse chemicals, offering endless possibilities for new drug development. Traditional medicinal plants have the potential to provide cures for various chronic and infectious diseases. Pharmaceutical industries worldwide are actively exploring plant metabolites to develop therapeutic drugs. With up to 80% of people in certain regions relying on herbal medicines, there is immense potential in extracting and isolating bioactive compounds to create innovative new drug candidates. Although challenging, the process can be streamlined through strategic planning and modern techniques, offering promising outcomes in drug discovery research.

Keywords: fraction enrichment, plant metabolites, bioactive compounds, chromatography

1 Introduction

Plants are a vast reservoir of unlimited chemical diversity. Natural products obtained from medicinal plants provide unfailing opportunities for new drug leads, due to which an increasing interest has grown worldwide [1]. Plants used for traditional medicine, such as Ayurvedic, Homeopathic, and other formulations, contain many substances that can cure chronic and infectious diseases [2]. Worldwide, pharmaceutical industries conduct screening programs to procure several chemically diverse plant metabolites to develop therapeutic drugs [3]. According to a WHO survey, up to 80% of the population in many Asian and African countries rely on herbal medicines for primary health care. Herbal formulations from plant raw materials contain several types of bioactive compounds [4, 5]. Only a few are responsible for the therapeutic activity. Hence, isolating the predominant bioactive compound(s) increases their potency and further supports their usage as lead compound(s) for new drug candidature. However, their extraction, isolation, and purification are challenging, as extensive research and method development are required to isolate compounds from plants [6]. Hence, there is a need for standard and defined methodology for the extraction of active compounds that can be used in different commercial sectors, such as the pharmaceutical, food, and chemical industries. The present study covers all conventional and modern techniques adopted by researchers worldwide for isolating bioactive compounds from plant raw materials.

1.1 Plant metabolites

Metabolites are small molecules that are usually intermediate products or end products of metabolic pathways. These are responsible for various cellular functions, such as structure development, signaling, enzyme catalysis or inhibition, cofactor activity, or protective effects [7]. Metabolites can be categorized into two: primary metabolites and secondary metabolites. Primary metabolites are directly involved in the growth, development, and reproduction of an organism. Metabolites that play important key roles in plants' basic life functions such as growth, development, and reproduction are known as primary metabolites. Secondary metabolites are not directly involved in basic functions but have important ecological roles [7, 8]. For example, mono and disaccharides such as fructose and sucrose work as fuel for plants, and amino acids are building blocks; hence, they are categorized as primary metabolites. Actinomycin, a naturally produced antibiotic, is synthesised using tryptophan as a precursor molecule and is known as a

secondary metabolite [9]. These metabolites contribute to the vast and versatile pharmacological effects of medicinal plants. Since ancient times, secondary metabolites have been essential for treating several diseases and have been applied as traditional and complementary medicine [9,10]. In modern medicine, these metabolites act as lead compounds for drug development targeting tuberculosis, diabetes, cancer, and other ailments [11, 12]. Secondary plant metabolites are classified according to their chemical structures into various classes. The classes of secondary metabolites include alkaloids, phenols, saponins, terpenes, and lipids. These classes are further diversified based on their structure. Phenolics can further be classified as simple phenolics, tannins, coumarins, flavonoids, chromones and xanthones, stilbenes, and lignans [8, 12].

1.2 Extraction and isolation of secondary metabolites

As discussed above, secondary metabolites possess pharmacological activities of great interest for drug discovery and development. Hence, researchers strive to extract and purify them while facing challenges in extraction and isolation. The first and foremost step in plant metabolite isolation is extraction with a suitable solvent. It is essential to acquire basic knowledge of the nature and characteristics of the target metabolite before selecting an extraction solvent. Key properties include pH, polarity, thermostability, and miscibility. In chemistry, extraction is a fundamental separation technique that is commonly used to separate the desired substance from its matrix based on the polarity of the extraction solvent [13]. Nonpolar substances such as steroids, terpenoids, quinolines, etc., can easily be extracted using nonpolar solvents such as hexane, petroleum ether, toluene, etc. Whereas polar substances like phenols, flavonoids, glycosides, etc., can readily be isolated by using polar solvents such as water, methanol, or ethanol [13, 14]. Similarly, alkaloids and phenols can be enriched by acidbase extraction techniques. The extraction method must be selected according to the target phytoconstituent(s) to be isolated.

2 Plant extraction (Primary enrichment)

Raw plant material is first extracted in the desired solvent, after which active compounds are isolated from the extract (Table 1). This methodology can also be called primary enrichment of plant materials. Numerous methods are used to prepare plant extracts, which are selected based on the target compound for isolation. The efficiencies of extraction methods mostly depend on critical input parameters, understanding of the plant matrix, chemistry of bioactive compounds, and scientific expertise. Several extraction methods exist; conventional approaches include maceration, percolation, infusion, digestion, and decoction. These methods are timeconsuming and laborintensive [13, 14]. Other extraction techniques utilise specialised extraction apparatus to make the process easier and quicker. These include Soxhlet extraction, Microwaveassisted extraction, Ultrasoundassisted extraction, Supercritical Fluid Extraction, and Pressurized Liquid Extraction [15]. The extraction methods mentioned above are explained in Table 2.

Table 1 List of solvents generally used to isolate target phytoconstituents

Phytoconstituents	Polarity	Solvents for extraction
Alkaloids	Polar	Methanol, Ethanol
Flavonoids	Polar	Methanol, Ethanol, Water
Phenols/Polyphenols	Highly Polar	Methanol, Ethanol, Water
Tannins	Moderate/Nonpolar	Diethyl ether, Hexane, Chloroform, Dichloromethane
Terpenoids	Nonpolar	Hexane, Chloroform, Petroleum ether, Toluene
Glycosides	Polar	Water, Methanol, Ethanol
Anthocyanins	Polar	Water, Methanol, Ethanol
Coumarins	Polar	Water, Methanol, Ethanol
Anthraquinones	Nonpolar	Hexane, Chloroform, Petroleum ether, Toluene
Steroids	Highly Nonpolar	Hexane, Toluene, Petroleum ether

2.1 Soxhlet extraction

Soxhlet extraction, also known as continuous hot extraction, is a process in which finely powdered plant material is placed inside a porous thimble and suspended above extraction solvent held in a roundbottom flask. The solvent is continuously heated using a heating mantle until it evaporates [16]. The evaporated solvent quickly condenses and flows back toward the roundbottom flask connected to a condenser. This repeated cycle speeds up the extraction. The entire process continues until solvent flowing from the extraction chamber leaves no residue behind. The apparatus used is the Soxhlet extractor, consisting of a roundbottom flask, extraction

Table 2 Different methodologies, their advantages, and disadvantages of plant extraction Primary purification

Technique	Rawmaterial nature	Utility maintenance	Solvent nature	Solvent volume	extraction time (min)	Advantages	Disadvantage
Maceration	Coarse		Aqueous, Organic	10	1440	Volatiles extractableHigh yield	Large solvent volumeLong extraction time
Infusion	Fine	0	Aqueous	6	120	Waterextractable compounds	Not applicable for volatiles
Digestion	Coarse	Heat	Aqueous, Organic	6	60	Waterextractable compounds	Not applicable for thermolabile compounds
Decoction	Coarse	Heat	Aqueous	8	20	Waterextractable compounds	Not applicable for thermolabile, midpolar and nonpolar molecules
Percolation	Coarse		Aqueous, Organic	10	1440	Volatiles extractableHigh yield	Large solvent volume Long extraction time
Soxhlet	Fine	Heat and heat exchanger	Organic	4	120	Higher assay yields	Not applicable for thermolabile compounds
Microwaveassisted	Fine	Electromagnets	Organic	2	60	Best for flavonoids; high yield	Not applicable for thermolabile and nonpolar compounds
Ultrasoundassisted	Fine	Ultrasound	Organic	2	60	High yield	Not applicable for thermolabile compounds
Supercritical fluid	Coarse	Supercritical fluid	Organic	2	45	High yield; applicable for thermolabile compounds	Expensive, high maintenance; not applicable for polar substances
Pressurised Liquid	Coarse	High pressure and temperature	Organic	2	30	High yield	Expensive, highmaintenance

chamber, siphon tube, and overhead condenser [17]. This method is suitable for partially soluble plant products mixed with insoluble impurities. It is the most common method used for plant extraction, as it consumes less time and solvent and does not require filtration. However, it is unsuitable for extracting thermolabile bioactive components [14].

2.2 Microwave-assisted extraction

It is one of the advanced techniques relying on dipole rotation and ionic transfer via ion displacement within solvent and plant material. This method is mainly used to extract polar compounds such as flavonoids [17]. Electromagnetic radiation (300 MHz to 300 GHz) is applied to plant material suspended in solvent; energy absorbed by the plantsolvent mixture is converted into heat. This causes dipole rotation and ion migration in the solvent, facilitating solvent penetration and assisting the extraction process. However, this process generates minimal heat with nonpolar solvents owing to low dipole movement and therefore performs poorly under those conditions. This technique minimises solvent consumption and extraction duration [18]. However, it is suitable primarily for phenolic compounds and flavonoids. Compounds such as tannins and anthocyanins may degrade due to their thermolabile nature.

2.3 Ultrasound-assisted extraction

This process applies highfrequency sound energy ($>$ 20 kHz), which disrupts plant cellwall integrity and increases the accessible surface area of plant material for solvent penetration [19]. This leads to the release of secondary metabolites into the solvent. The extraction process suits small sample sizes, reduces extraction time and solvent consumption, and maximises yield. However, the method may lack reproducibility, and highenergy input may degrade phytochemicals through freeradical generation [20].

2.4 Supercritical fluid extraction (SFE)

It is an advanced extraction technique that utilises the unique properties of "supercritical fluids". Supercritical fluids are substances held at temperature and pressure above their critical point, where distinct liquid and gaseous phases no longer exist. Supercritical fluids can diffuse rapidly into porous solid matrices and dissolve target materials efficiently [21]. Supercritical carbon dioxide is mainly used for plant extraction, as it is an ideal solvent featuring low viscosity, high diffusion rate, and high volatility. Thanks to its low critical temperature, it is also used to extract thermolabile compounds. However, it shows low efficiency for polar solutes because of its nonpolar character; therefore, volatile polar modifiers such as methyl acetate, diethyl ether, methanol, or formic acid are added to the system [21].

2.5 Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE) is an advanced extraction technique that performs solventbased extraction at high temperatures and pressures kept below the solvent critical points,

so that solvent remains in the liquid state throughout the whole extraction process [22]. High pressure and temperature alter solvent physicochemical properties, decreasing solvent surface tension and viscosity. This increases analyte solubility in the solvent and delivers significantly higher extraction yields compared with conventional extractions. Most PLE instruments are automated; hence, it is a less laborious but expensive method [22, 23]. SFE and PLE are costly and rarely used for routine plant extraction. Other methods are selected according to the target bioactive compounds.

3 Isolation and purification of active phytoconstituents Secondary enrichment

Before proceeding toward secondary enrichment, it is necessary to determine the phytochemical profile of plant extracts obtained from primary enrichment. Qualitative and quantitative analysis helps identify predominant phytoconstituents and facilitates secondary enrichment [24]. Crude quantification of analytes within extracts can also be performed to assess extractive value. Qualitative and quantitative analysis of phytochemicals present in plant extracts can be completed using a series of biochemical tests summarised in Table 3. When investigating a specific phytochemical ingredient or compound class, solution polarity can be adjusted to precipitate particular chemical classes of interest while leaving undesired molecules dissolved. Compounds bearing primary, secondary, or tertiaryamine groups, carboxylic acids, lactones, and phenols can be selectively extracted by adjusting pH to alter their polarity and solubility. Multiple techniques can be implemented for secondary enrichment of bioactive phytoconstituents according to experimental requirements. Techniques most commonly employed for secondary enrichment are sublimation, crystallisation, acidbase extraction, and chromatography. Within phytochemistry, distillation, differential extraction, and chromatography are more widely used than sublimation and crystallisation. Different compoundisolation techniques are explained below.

3.1 Sublimation

Sublimation describes a phase transition where a solid substance converts directly into the gaseous state without passing through a liquid phase. It is an endothermic process occurring at temperatures and pressures below a substance's triplepoint on its phase diagram. The reverse process of sublimation is deposition (desublimation), in which a substance passes directly from gas to solid phase and crystallises on a cold surface [25]. This technique can be applied to isolate rare compounds such as camphor from *Cinnamomum camphora* and *Ocimum kilimandscharicum* [26].

3.2 Crystallisation

Crystallisation is a separation technique used to obtain purified compounds from supersaturated solutions. In natural crystallisation, plant extract is dissolved in a sparingly soluble solvent under heating. After complete dissolution, the solution is allowed to cool so that crystalline natural compounds precipitate. Most natural compounds are amorphous in the solid state; hence, this method is rarely used for compound separation. Purification can be achieved in four straightforward steps: a) Crystallisation of the product and retention of the filtrate; b) Dissolving the isolated product in a fresh solvent; c) Recrystallisation of the product and retention of the new filtrate; d) Concentrating the filtrate obtained in step 1 to recover additional product, which is then recrystallised using filtrate from step 3. This simple workflow utilises both filtrate streams to maximise product recovery.

3.3 Acid-base extraction

Acidbase extraction is a liquidliquid extraction method typically used to separate organic compounds from one another based on their acidbase properties. The principle is that most organic compounds are more soluble in organic solvents than in water. However, once converted into ionic species, organic compounds gain watersolubility [27]. These compounds can readily be converted into cations or anions by adding acid or base. For example, to separate an organic acid from an organic mixture, the mixture is treated with alkaline solution. Under alkaline conditions, acidic compounds become watersoluble. After phase separation, the aqueous layer contains the organicacid salt. The target acid can be recovered by neutralising the aqueous phase with acid and extracting it into an organic solvent such as chloroform.

As another example, alkaloids are polar but only sparingly soluble in pure water. Their

Table 3 Qualitative and quantitative phytochemical analysis protocol

Tests	Qualitative Test	Quantitative Analysis
ALKALOIDS	Mayer's test: Extracts were dissolved in 5 mL methanol and 5 mL of 2 N HCl and filtered. The filtrate was treated with Mayer's (Potassium Mercuric Chloride) solution. A cream-coloured precipitate indicates a positive test. Wagner's tests: Wagner's reagent (aqueous solution of iodine and potassium iodide) was added to extract dissolved in methanol–HCl solution; a reddish-brown colour indicates a positive test. Dragendorff's test: Spot a drop of extract onto precoated TLC plate. Spray the plate with Dragendorff's reagent. An orange spot indicates a positive test. TLC method: Solvent system: Chloroform : methanol : 25% ammonia (8:2:0.5). Spots are visualised after spraying with Dragendorff's reagent. Orange spots indicate positive results.	The extract was dissolved in acidified alcohol (10% acetic acid in ethanol), incubated for 4 hours, filtered, basified with NH_4OH, and left for 13 hours for alkaloid precipitation. The precipitate is filtered, washed, and weighed. TLC–HPTLC method: Powdered test samples are moistened with half-diluted NH_4OH and macerated with EtOAc for 24 h at room temperature. The organic phase is separated; the aqueous filtrate is acidified then basified with NH_4OH (pH 11–12), and extracted with chloroform (3 ×). The combined chloroform fraction is concentrated by evaporation and used for chromatography. Alkaloid spots are separated using chloroform–methanol (15:1) solvent mixture and detected via HPTLC.
FLAVONOIDS	Shinoda test: Extract mixed with water; magnesium ribbon and concentrated HCl are added. Pink colour indicates a positive test. NaOH test: Treat extract with dilute NaOH, then add dilute HCl. A yellow solution formed in NaOH that turns colourless upon adding dilute HCl indicates flavonoids.	0.5 mL extract is incubated with 0.1 mL 10% $AlCl_3$, 0.1 mL 1 M CH_3COOK and 4.3 mL water for 30 minutes at room temperature. Absorbance is recorded at 415 nm; quercetin is used for the standard calibration curve. Total flavonoids are expressed as mg quercetin equivalent (QE) per gram of extract. TLC–HPTLC method: 1 g powdered sample is extracted with 10 mL methanol in a waterbath (60 °C / 5 min). The filtrate is concentrated by evaporation; water–EtOAc (10:1 mL) mixture is added and mixed thoroughly. The EtOAc phase is retained for chromatography. Flavonoid spots are separated using chloroform–methanol (19:1) solvent mixture and detected by HPTLC.
PHENOLS	Ferric Chloride test: Extract solution treated with $FeCl_3$ solution. Blue-black colouration indicates presence. Folin–Ciocalteu test: Reagent is added to extract suspended in water. Blue-black colouration indicates positive result. Phenol test: Spot extract onto filter paper. Add a drop of phosphomolybdic acid reagent and expose to ammonia vapour. Blue-coloured spot indicates positive result.	0.5 mL sample solution (100 $\mu g/mL$) is mixed with 2 mL 10% Folin–Ciocalteu reagent and 4 mL 7.5% (w/v) Na_2CO_3 solution, then incubated at room temperature for 30 min. The developed blue colour is measured at 765 nm. Gallic acid is used to construct the calibration curve. Total phenol content is expressed as mg gallic acid equivalent (GAE) per gram of dry extract.
TANNINS	Ferric Chloride test: Extract solution treated with $FeCl_3$ solution. Deep bluish-green solution indicates positive test. Braemer's test: 10% alcoholic ferric chloride is added to 2–3 mL methanolic extract (1:1). Dark blue or greenish-grey colouration indicates tannins.	400 μL extract (100 $\mu g/mL$), 3 mL 4% vanillin in methanol, and 1.5 mL concentrated HCl are incubated for 15 min, and absorbance is measured at 500 nm. Catechin is used for calibration. Total tannin is expressed as mg catechin equivalent (CE) per gram of extract.
TERPENOIDS	Salkowski test: Extract mixed with 2 mL chloroform; concentrated H_2SO_4 is added to form a separate layer. Reddish-brown colouration confirms presence. Liebermann–Burchardt test: To 1 mL methanolic extract, add 1 mL chloroform, 2–3 mL acetic anhydride, and 1–2 drops of concentrated sulphuric acid. Pink or red colouration indicates positive test for terpenoids.	100 mg extract is dissolved in 9 mL ethanol and incubated for 24 hours. The mixture is filtered and extracted with 10 mL petroleum ether; the organic fraction is concentrated and weighed.
SAPONINS	Foam test: Extract mixed with distilled water, heated and filtered. Distilled water is added and shaken vigorously. Stable froth and emulsion formed after adding oil signifies saponins. TLC method: 2 g powdered sample is reflux-extracted with 10 mL 70% EtOH for 10 min. Filtrate is concentrated, enriched with saturated n-BuOH and mixed thoroughly. Butanol phase is retained and concentrated for chromatography. Saponins are separated with chloroform : glacial acetic acid : methanol : water (64:34:12:8). Chromatograms are exposed to iodine vapour; spot colour and R_f values are recorded.	5 g extract is mixed with 100 mL 20% aqueous ethanol, heated and stirred at 55 °C for 4 hours and filtered. Residue is re-extracted under identical conditions. Combined filtrates are concentrated, 20 mL diethyl ether is added and shaken vigorously, and the aqueous layer is recovered. 60 mL butanol is added for extraction, followed by two washes with 10 mL 5% NaCl solution (discard NaCl layers). The remaining solution is heated for 30 min, dried, and weighed; results are reported per gram of extract.
STEROIDS	Chloroform and concentrated H_2SO_4 are added to aqueous plant extract. A red lower layer indicates steroids. Liebermann–Burchardt test: To 1 mL methanolic extract add 1 mL chloroform, 2–3 mL acetic anhydride, and 1–2 drops concentrated sulphuric acid. Dark green colouration indicates steroids. Alternative test: To 1 mL extract add 2 mL acetic anhydride and 2 mL concentrated H_2SO_4. A colour change to blue or green indicates steroids. TLC method: 2 g powdered sample is extracted with 10 mL methanol in waterbath (80 °C /15 min). Concentrated filtrate is used for chromatography. Sterols are separated with chloroform : glacial acetic acid : methanol : water (64:34:12:8). After spraying plates with anisaldehyde–sulphuric acid reagent and heating (100 °C /6 min), spot colour (greenish-black to pinkish-black) and hR_f values are recorded under visible light.	A portion of extract (2 g) is dissolved in 90% aqueous ethanol, partitioned against n-hexane, filtered, concentrated, and weighed; results are reported per gram extract sample.

solubility increases in acidic water because of their intrinsic basic character. In neutral conditions, alkaloids are more soluble in chloroform and methanol than in acidic aqueous solutions. Hence, when plant extract is treated with acidic aqueous solution, alkaloids partition into the acidic water layer, while other components can be removed. Alkaloids can then be recovered from the acidic aqueous phase using chloroform extraction. Since chloroform and water are immiscible, two separate layers form; alkaloids preferentially dissolve into the chloroform layer and are collected with this organic fraction [27, 28]. (see in Figure 1)

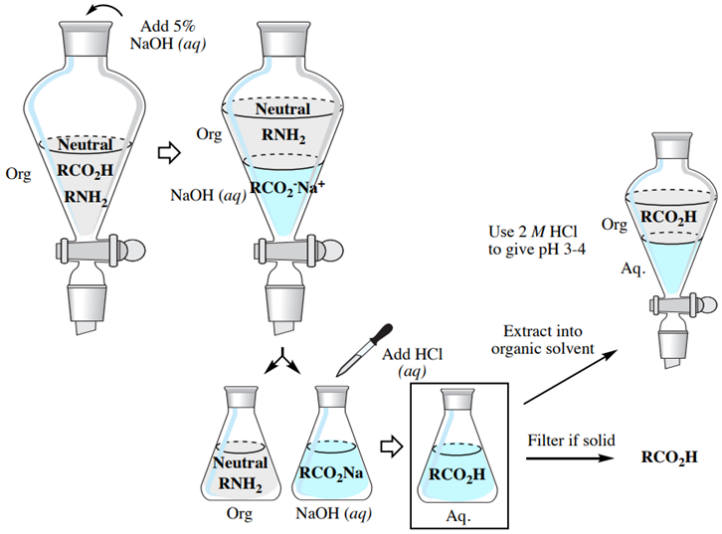

Figure 1 Schematic depiction of acid-base extraction method

3.4 Chromatography

Chromatography is the most common laboratory technique for mixture separation. The mixture is dissolved in a mobile phase that flows through a system (column, plate, or sheet) holding a fixed stationary phase [29]. Compound migration speed depends on compoundstationaryphase affinity, enabling effective separation. Chromatography can broadly be classified as preparative or analytical according to separation objective [29, 30]. Analytical chromatography is mainly used to detect multiple compounds within a mixture. Preparative chromatography is used to separate compounds in bulk for downstream applications [30]. Based on separation principles, chromatography can be divided into adsorption chromatography and partition chromatography.

In adsorption chromatography, mixture components adsorb onto the adsorbent stationary phase (e.g., silica gel or alumina gel) to varying extents. The mobilephase solvent system flows across the stationary phase; compounds travel along with mobile phase according to their miscibility. Thinlayer chromatography and column chromatography fall within this category [30]. Partition chromatography is among the earliest chromatographic approaches developed by chemists and applies partitioncoefficient principles; it relies on liquidliquid interactions and is also known as liquidliquid chromatography.

3.4.1 Liquidliquid partitioning (differential extraction)

This widelyused technique is also known as liquidliquid extraction or partition / liquidliquid chromatography (LLC), used for separation between two immiscible liquids. In general, liquidliquid partitioning performs repeated extractions efficiently [31]. A major advantage of LLC is the huge diversity of available phase systems, enabling finetuned retention and selective fractionation of components. Analytes partition between two immiscible solutions of differing polarity; analyte retention is governed by differential solubility of mixture components in each liquid phase. For instance, hexane and water are immiscible and readily phaseseparated. If a methanolic plant extract is mixed with hexane, compounds with higher solubility in hexane will dissolve into that layer. Since methanol and hexane are immiscible due to opposing polarity, the hexane layer can easily be decanted, and target compounds recovered.

Traditional solidsupport chromatographic media (silica gel, polyamide etc.) can cause solute tailing or irreversible adsorption, leading to low compound recovery. Such losses do not occur in allliquid separation techniques, achieving quantitative compound recovery.

Separation outcomes can be described using the partition coefficient denoted as logP. The

logP value for a compound is $\log_{10}$ of the partition coefficient (P), defined as the ratio of compound concentration in the organic (oil) phase to concentration in the aqueous phase:

$$\text{Partition Coefficient (P)} = [\text{Organic}]/[\text{Aqueous}]$$

Calculated logP values measured against water and a reference organic solvent such as octanol or hexane serve as a guideline for predicting solubility behaviour in other aqueousorganic solvent combinations. A logP value of 1 means a 10:1 concentration ratio between organic and aqueous phases; this compound is hydrophobic and favours dissolution in organic solvents. In contrast, logP = 1 indicates a 1:10 organic-to-aqueous concentration ratio, meaning the compound is hydrophilic and readily soluble in aqueous buffer. A logP = 0 corresponds to equal (1:1) partitioning across organic and aqueous phases; such compounds exhibit appreciable solubility in both phases, and solvent selection depends on application requirements. (see in Figure 2)

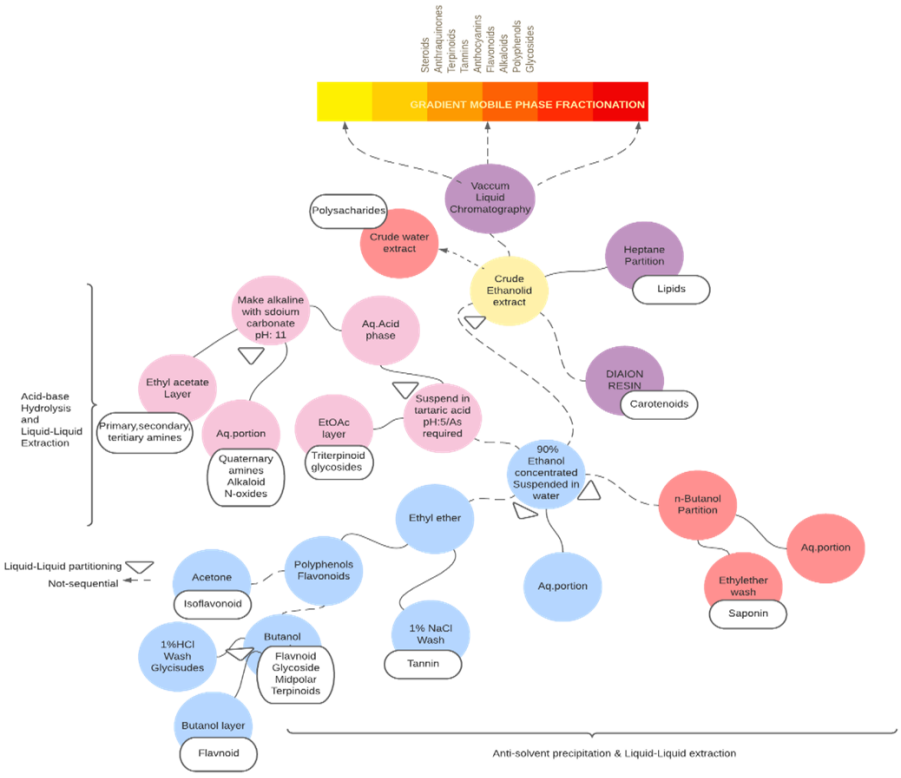

Figure 2 Schematic diagram of purification for secondary enrichment through liquid-liquid partitioning

3.4.2 Thin-layer chromatography

Thinlayer chromatography (TLC) is an analytical method separating biochemicals based on their relative affinity toward stationary and mobile phases. It resembles paper chromatography, except adsorbent material such as silica gel, alumina, or cellulose is coated onto an inert flat substrate as stationary phase instead of paper. TLC offers high versatility: multiple samples can be separated simultaneously on one plate, which is useful for screening applications such as druglevel testing and waterquality analysis. Risk of crosscontamination is low because each separation uses fresh coating material [32].

3.4.3 Column chromatography

In this method, a glass column packed with silica or alumina gel serves as stationary phase. The mixture to be separated is loaded on top of the column and allowed to adsorb onto silica. Mobilephase eluent is then passed through the column. Compounds with higher solubility in the mobile phase elute first, while compounds with stronger stationaryphase affinity remain inside the column for longer. This is a preparative chromatographic technique widely used for bulkscale separation of phytoconstituents from plant extracts [33].

3.4.4 PrepTLC

Preparative TLC (PrepTLC) is a preparative chromatography technique used to purify and isolate compounds at larger laboratory scale. PrepTLC separates moderatequantity samples

for subsequent bioactivity testing and compound characterisation [34]. Compound separation and isolation are rapid, lowcost, and less laborintensive compared with HPLC or conventional column chromatography. Isolated bands or spots are scraped from preparative plates and collected for downstream analysis. PrepTLC plates are prepared by coating silica G (silica mixed with gypsum) onto glass plates. Maximum sampleloading volume depends on silicalayer thickness. Between 1 mg and 1 g of material can be separated in a single PrepTLC run. Conventional analytical TLC must first be performed to optimise the mobilephase system before scalingup isolation with PrepTLC [35].

3.4.5 HighPerformance Liquid Chromatography (HPLC)

Formerly known as highpressure liquid chromatography, HPLC is a powerful advanced separation technique in analytical chemistry used to separate, identify, and quantify individual components within complex mixtures, even for analytes present at low abundance. In HPLC, a pump drives mobile phase through a column holding stationary phase. Each analyte exhibits characteristic retention time inside the column determined by flow rate, analyte miscibility within mobile phase, and analytestationaryphase interactions [36].

4 BAGF: An emerging tool for characterising plant active phytoconstituents

Advances in highthroughput analytical technologies generate large datasets that are often difficult to interpret. Information overload complicates the selection of biologically relevant results. Plant raw materials contain a wide diversity of phytoconstituents; many of these can readily be extracted and identified via mass spectrometry, yet their biological relevance may be limited. Therefore, biological interaction assays are essential to screen and prioritise fractions containing bioactive molecules.

Bioassayguided fractionation (BAGF) of plant extracts is a workflow coupled with chromatographic separation techniques that streamlines isolation of biologicallyactive lead molecules. Instead of targeting predefined compound classes, fractionation strategies for plant extracts are designed according to measured biological activity, and chemical characterisation is performed afterwards on bioactive fractions [37]. Therapeuticallyactive phytoconstituents isolated from plants are primarily obtained through bioassayguided fractionation and purification workflows. Within BAGF, plant extract undergoes successive chromatographic fractionation until a pure biologicallyactive compound is isolated. Retention or rejection of each fraction is determined by bioassay results [38]. Fractions showing high bioactivity are selected for further fractionation; inactive fractions are discarded. Finally, the resulting active fraction is subjected to structural elucidation.

5 Conclusion

Discovering lead compounds for newdrug development is challenging and timeconsuming. However, isolation and identification workflows can be streamlined through strategic planning focused on the physicochemical properties of target compounds. Applying strategies such as bioassayguided fractionation (BAGF) can deliver promising outcomes for drugdiscovery research.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

[1] Chaachouay N, Zidane L. Plant-Derived Natural Products: A Source for Drug Discovery and Development. Drugs and Drug Candidates. 2024, 3(1): 184-207. https://doi.org/10.3390/ddc3010011

[2] Rizvi SAA, Einstein GP, Tulp OL, et al. Introduction to Traditional Medicine and Their Role in Prevention and Treatment of Emerging and Re-Emerging Diseases. Biomolecules. 2022, 12(10): 1442. https://doi.org/10.3390/biom12101442

[3] Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. The Nucleus. 2022, 65(3): 399-411.
https://doi.org/10.1007/s13237-022-00405-3

[4] World Health Organization.Traditional medicine [Questions and answers], 2025.
https://www.who.int/news-room/questions-and-answers/item/traditional-medicine

[5] Mahomoodally MF. Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. Evidence-Based Complementary and Alternative Medicine. 2013, 2013: 1-14.
https://doi.org/10.1155/2013/617459

[6] Bitwell C, Indra SS, Luke C, et al. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Scientific African. 2023, 19: e01585.
https://doi.org/10.1016/j.sciaf.2023.e01585

[7] Sanchez S, Demain AL. Metabolic regulation and overproduction of primary metabolites. Microbial Biotechnology. 2008, 1(4): 283-319.
https://doi.org/10.1111/j.1751-7915.2007.00015.x

[8] Thirumurugan D, Cholarajan A, Raj SSS, et al. An introductory chapter: Secondary metabolites. In V. Ramasamy & S. S. R. Suresh (Eds.), *Secondary metabolites (Ch. 1). IntechOpen. 2018.
https://doi.org/10.5772/intechopen.79766

[9] Selim MSM, Abdelhamid SA, Mohamed SS. Secondary metabolites and biodiversity of actinomycetes. Journal of Genetic Engineering and Biotechnology. 2021, 19(1): 72.
https://doi.org/10.1186/s43141-021-00156-9

[10] Hussein R, El-Anssary AA. Plants secondary metabolites: The key drivers of the pharmacological actions of medicinal plants. In F. B. Philip (Ed.), Herbal medicine (Ch. 2). IntechOpen. 2018.
https://doi.org/10.5772/intechopen.76139

[11] Srivastav VK, Egbuna C, Tiwari M. Plant secondary metabolites as lead compounds for the production of potent drugs. Phytochemicals as Lead Compounds for New Drug Discovery. Published online 2020: 3-14.
https://doi.org/10.1016/b978-0-12-817890-4.00001-9

[12] Seca AML, Pinto DCGA. Biological Potential and Medical Use of Secondary Metabolites. Medicines. 2019, 6(2): 66.
https://doi.org/10.3390/medicines6020066

[13] Barbero GF, Ferreiro-González M, Freitas VCM, et al. Extraction of Natural Products: Principles and Fundamental Aspects. Natural Product Extraction. Published online July 20, 2022: 66-116.
https://doi.org/10.1039/9781839165894-00066

[14] Zhang Q-W, Lin L-G, Ye W-C. Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine. 2018, 13(1).
https://doi.org/10.1186/s13020-018-0177-x

[15] Quitério E, Grosso C, Ferraz R, et al. A Critical Comparison of the Advanced Extraction Techniques Applied to Obtain Health-Promoting Compounds from Seaweeds. Marine Drugs. 2022, 20(11): 677.
https://doi.org/10.3390/md20110677

[16] Redfern J, Kinninmonth M, Burdass D, et al. Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties. Journal of Microbiology & Biology Education. 2014, 15(1): 45-46.
https://doi.org/10.1128/jmbe.v15i1.656

[17] Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. Journal of Pharmacy and Bioallied Sciences. 2020, 12(1): 1-10.
https://doi.org/10.4103/jpbs.jpbs_175_19

[18] López-Salazar H, Camacho-Díaz BH, Ocampo MLA, et al. Microwave-assisted extraction of functional compounds from plants: A Review. BioResources. 2023, 18(3).
https://doi.org/10.15376/biores.18.3.lopez-salazar

[19] Kumar K, Srivastav S, Sharanagat VS. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrasonics Sonochemistry. 2021, 70: 105325.
https://doi.org/10.1016/j.ultsonch.2020.105325

[20] Gil-Martín E, Forbes-Hernández T, Romero A, et al. Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. Food Chemistry. 2022, 378: 131918.
https://doi.org/10.1016/j.foodchem.2021.131918

[21] Ahangari H, King JW, Ehsani A, et al. Supercritical fluid extraction of seed oils – A short review of current trends. Trends in Food Science & Technology. 2021, 111: 249-260.
https://doi.org/10.1016/j.tifs.2021.02.066

[22] Alvarez-Rivera G, Bueno M, Ballesteros-Vivas D, et al. Pressurized Liquid Extraction. Liquid-Phase Extraction. Published online 2020: 375-398.
https://doi.org/10.1016/b978-0-12-816911-7.00013-x

[23] Dobroslavić E, Elez Garofulić I, Šeparović J, et al. Pressurized Liquid Extraction as a Novel Technique for the Isolation of Laurus nobilis L. Leaf Polyphenols. Molecules. 2022, 27(16): 5099.
https://doi.org/10.3390/molecules27165099

[24] Geetha TS, Geetha N. Phytochemical screening, quantitative analysis of primary and secondary metabolites of Cymbopogan citratus (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. International Journal of PharmTech Research. 2014, 6: 521-529.

[25] Sinha D, Mukherjee S, Chowdhury S. Methods of Extraction of Phytochemicals. Isolation, Characterization, and Therapeutic Applications of Natural Bioactive Compounds. Published online June 30, 2022: 250-279.
https://doi.org/10.4018/978-1-6684-7337-5.ch010

[26] Zhang H, Huang T, Liao X, et al. Extraction of Camphor Tree Essential Oil by Steam Distillation and Supercritical CO2 Extraction. Molecules. 2022, 27(17): 5385.
https://doi.org/10.3390/molecules27175385

[27] Marín-San Román S, Rubio-Bretón P, Pérez-Álvarez EP, et al. Advancement in analytical techniques for the extraction of grape and wine volatile compounds. Food Research International. 2020, 137: 109712.
https://doi.org/10.1016/j.foodres.2020.109712

[28] Maldoni B. Alkaloids: Isolation and purification. Journal of Chemical Education. 1991, 68(8): 700.
https://doi.org/10.1021/ed068p700

[29] Strain HH. Chromatography. Analytical Chemistry. 1950, 22(1): 41-48.
https://doi.org/10.1021/ac60037a013

[30] Feng W, Li M, Hao Z, et al. Analytical Methods of Isolation and Identification. Phytochemicals in Human Health. Published online February 12, 2020.
https://doi.org/10.5772/intechopen.88122

[31] Coskun O. Separation Tecniques: CHROMATOGRAPHY. Northern Clinics of Istanbul. Published online 2016.
https://doi.org/10.14744/nci.2016.32757

[32] Silver J. Let Us Teach Proper Thin Layer Chromatography Technique! Journal of Chemical Education. 2020, 97(12): 4217-4219.
https://doi.org/10.1021/acs.jchemed.0c00437

[33] Luska KL. Transformation of a Classic Column and Thin Layer Chromatography Experiment into a Multioutcome, Discovery-Based Activity. Journal of Chemical Education. 2023, 100(5): 1980-1989.
https://doi.org/10.1021/acs.jchemed.2c01196

[34] Primdahl KG, Hansen FA, Solum EJ, et al. Introduction to Preparative Chromatography: Description of a Setup with Continuous Detection. Journal of Chemical Education. 2022, 99(6): 2372-2377.
https://doi.org/10.1021/acs.jchemed.1c00917

[35] Zhang Y, Zhao Z, Li W, et al. Purification of Two Taxanes from Taxus cuspidata by Preparative High-Performance Liquid Chromatography. Separations. 2022, 9(12): 446.
https://doi.org/10.3390/separations9120446

[36] Moldoveanu S, David V. Chromatographic procedures as preliminary separations. Modern Sample Preparation for Chromatography. Published online 2021: 423-437.
https://doi.org/10.1016/b978-0-12-821405-3.00001-0

[37] Mani J, Johnson J, Hosking H, et al. Bioassay Guided Fractionation Protocol for Determining Novel Active Compounds in Selected Australian Flora. Plants. 2022, 11(21): 2886.
https://doi.org/10.3390/plants11212886

[38] Nothias L-F, Nothias-Esposito M, da Silva R, et al. Bioactivity-Based Molecular Networking for the Discovery of Drug Leads in Natural Product Bioassay-Guided Fractionation. Journal of Natural Products. 2018, 81(4): 758-767.
https://doi.org/10.1021/acs.jnatprod.7b00737