



# The Journal of Multidisciplinary Research (TJMDR)

Content Available at [www.saapjournals.org](http://www.saapjournals.org)

ISSN: 2583-0317



## A REVIEW ON: MODERN METHODS OF EXTRACTION IN THE ESTIMATION OF TERPENOIDS AND RESINS

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### Abstract

Terpenoids are the most abundant compounds among natural products and represent an important group of secondary metabolites in plants with diverse structural forms. They play physiological processes. They are secreted by plants as a protective response to injury or pathogen attack and serve as physical and chemical barriers. The fact that trees producing copious amounts of resin occur predominantly in tropical and subtropical environments may be related to growing evidence that these substances play a role in protecting plants against herbivores and microorganisms. Accurate extraction and quantification of terpenoids and resins are essential for quality control, pharmacological research, and commercial standardization. The complexity of plant matrices and the sensitivity of bioactive compounds necessitate efficient and selective extraction techniques.

**Keywords :** Secondary Metabolites, Microorganisms, Extraction Techniques, Terpenoids, Resins

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DOI: <https://doi.org/10.37022/tjmdr.v5i3.769>

### Produced and Published by

South Asian Academic Publications

### Introduction

Terpenoids and resins are diverse classes of secondary metabolites found across a wide range of plant species, playing vital roles in plant defense, ecological interactions, and human applications. Terpenoids, also known as isoprenoids, are biosynthesized from isoprene units and encompass a vast array of compounds including monoterpenes, sesquiterpenes, diterpenes, and triterpenes. These compounds are responsible for the characteristic aroma, flavor, and therapeutic properties of many herbs, spices, and medicinal plants. They exhibit antimicrobial, anti-inflammatory, antioxidant, and anticancer activities, making them valuable in pharmaceuticals, nutraceuticals, and cosmetics. Resins, on the other hand, are complex mixtures of volatile and non-volatile compounds, including terpenoids, phenolics, and fatty acids. They are secreted by plants as a protective response to injury or pathogen attack and serve as physical and chemical barriers [1]. In industrial contexts,

resins are widely used in the formulation of adhesives, varnishes, paints, incense, and traditional medicines. Their unique chemical composition and polymeric nature make them suitable for diverse applications ranging from art restoration to biomedical devices. Accurate extraction and quantification of terpenoids and resins are essential for quality control, pharmacological research, and commercial standardization. The complexity of plant matrices and the sensitivity of bioactive compounds necessitate efficient and selective extraction techniques. Traditional methods such as steam distillation, Soxhlet extraction, and maceration have been extensively employed. However, these techniques often suffer from significant limitations: High energy consumption due to prolonged heating, Long extraction times that reduce throughput, Degradation of thermolabile compounds, leading to loss of bioactivity, Use of toxic organic solvents that pose environmental and health risks, Low selectivity and poor reproducibility in complex matrices [2]. To address these challenges, modern extraction technologies have emerged, offering cleaner, faster, and more sustainable alternatives. These include: Supercritical fluid extraction (SFE) using CO<sub>2</sub>, which provides high selectivity and preserves compound integrity, Microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), which enhance mass transfer and reduce solvent usage, Pressurized liquid extraction (PLE) and enzyme-assisted extraction (EAE), which improve yield and specificity, Green solvents such

as ionic liquids and deep eutectic solvents, which minimize environmental impact. These advanced methods not only improve extraction efficiency but also align with the principles of green chemistry and sustainable development. Their integration into analytical workflows enables better characterization, standardization, and valorization of plant-derived terpenoids and resins, paving the way for innovations in drug discovery, functional foods, and eco-friendly materials.

Resins Sticky plant secretions that harden when exposed to air are called resins. Resins appeared relatively early in the evolution of trees, as demonstrated by their fossilized form, amber. The fact that trees producing copious amounts of resin occur predominantly in tropical and subtropical environments may be related to growing evidence that these substances play a role in protecting plants against herbivores and microorganisms [3]. Since early times, humans have used resins for diverse purposes and have held amber in high esteem. Classical literature presented many ideas on the possible origins of amber. Pliny, in his *Historia Naturalis* (AD 77), recognized amber as a plant product "produced from a marrow discharged by trees." Despite Pliny's astute observation, amber has been widely considered to be a semiprecious gem. It has even been described in mineralogy textbooks and given mineralogical names. Only in the last few decades has amber been analysed chemically, with the understanding that it is a plant product. New ideas and analytical tools have allowed investigators to place fundamental questions about resinous secretions of plants into the broad perspective of geologic time. Much confusion exists regarding the term resin, because it was defined before rigorous chemical analyses were available. Technically, resins are primarily mixtures of either terpenoids or phenolic compounds, which may be produced in internal ducts or in specialized surface glands of plants. Sometimes they are confused with sap, which is an aqueous solution transported through the xylem or phloem tissues of plants. The term "gum" is also used interchangeably with resin, yet true gums are polysaccharides produced in plants as a result [4].

Terpenoids Terpenoids are the most abundant compounds among natural products and represent an important group of secondary metabolites in plants with diverse structural forms. They play physiological processes. As valuable raw materials, terpenoids are widely utilized in the pharmaceutical, food, and cosmetics industries. These compounds exhibit a wide range of biological activities, including antitumor, anti-inflammatory, antibacterial, antiviral, and antimalarial effects. Additionally, they aid in promoting transdermal absorption, preventing and treating cardiovascular diseases, and exhibiting hypoglycaemic activity [5]. Moreover, studies have revealed several other potential applications of terpenoids, such as insect resistance, immunoregulation, antioxidation, antiaging, and neuroprotection. Due to their complex structures and diverse biological effects,

terpenoids act through multiple mechanisms. This paper reviews the activities and mechanisms of terpenoids and discusses their development and potential applications, providing a valuable reference for new drug discovery and drug design based on terpenoid compounds.

#### Advanced Extraction Techniques

1. **Supercritical Fluid Extraction (SFE)** Supercritical State of CO<sub>2</sub>, CO<sub>2</sub> becomes supercritical above its critical temperature (31.1°C) and pressure (73.8 bar), where it exhibits properties of both a gas and a liquid. Dual Nature In this state, CO<sub>2</sub> has Gas-like diffusivity → penetrates matrices easily. Liquid-like solvating power → dissolves target compounds effectively. Tunable Solvent Power By adjusting temperature and pressure, the solvating ability of CO<sub>2</sub> can be precisely controlled to target specific compounds [6].

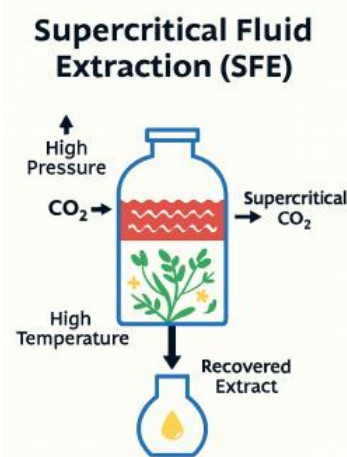


Fig 01: Process Overview

#### Expanded Steps in Supercritical CO<sub>2</sub> Extraction

**Sample Preparation:** Drying Moisture removal is critical to prevent interference with CO<sub>2</sub> solubility and to avoid ice formation under pressure. Grinding/Milling Reduces particle size to increase surface area and improve mass transfer. Sieving Ensures uniform particle size distribution for consistent extraction. Pre-treatment (optional) May include enzymatic treatment or defatting to enhance yield [7].

**Loading into Extraction Vessel** Packing Material is packed to avoid channeling and ensure uniform CO<sub>2</sub> flow. Spacer Materials Inert materials like glass beads may be added to improve flow dynamics. Temperature Control Vessel is pre-heated to maintain supercritical conditions.

**Pressurization and Supercritical CO<sub>2</sub> Generation** Compression CO<sub>2</sub> is compressed using high-pressure pumps. Heating CO<sub>2</sub> is heated above 31.1°C to reach supercritical state. Pressure and temperature sensors ensure precise control.

**Flow Rate Optimization:** CO<sub>2</sub> flow rate is adjusted to balance solubility and through put Residence Time: Duration of exposure affects yield and selectivity.

8Co-solvent Addition (if needed): Ethanol or methanol may be added to enhance polarity and extract hydrophilic compounds.

Recovery and Recycling Extract Collection Compounds are collected in vials or trays.CO<sub>2</sub> Recovery CO<sub>2</sub> is condensed and recycled to reduce operational costs.System Purging Ensures removal of residual compounds before next batch.

### Limitations

Chemical Constraints. Non-polar CO<sub>2</sub> Poor for extracting polar compounds (e.g., sugars, amino acids).Co-solvents Needed Ethanol, methanol, or water improve polarity but add complexity and residue risks.Lipophilic Bias Favors non-polar compounds, missing valuable polar constituents.High CostCapital Investment \$85K-\$300K for systems.Specialized Equipment High-pressure pumps, heaters, separators.InfrastructureRequires reinforced piping and trained personnel.

## 2. Microwave-Assisted Extraction (Mae)

Sample Preparation Drying Moisture content is reduced to prevent dilution of extract and ensure consistent microwave absorption. Grinding/MillingReduces particle size to increase surface area and enhance solvent penetration. Sieving Ensures uniform particle distribution for reproducible extraction. Pre-treatment (optional)May include enzymatic hydrolysis or defatting to improve yield and selectivity [9].

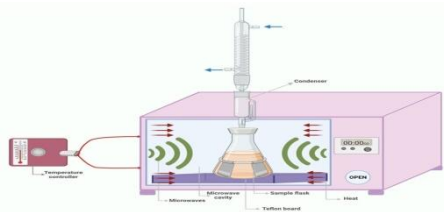


Fig 02 Microwave-Assisted Extraction (Mae)

Solvent Selection Dielectric Properties Solvent must have a high dielectric constant and loss factor to absorb microwave energy efficiently. Common Solvents Polar Water, ethanol, methanol (excellent microwave absorbers).

Microwave Irradiation System Types Closed Vessel System Operates under pressure (up to 20 bar), allowing temperatures above boiling point. Enhances extraction kinetics and yield. Requires pressure monitoring and safety controls.

Cooling and Filtration Cooling Rapid cooling prevents thermal degradation and stabilizes extract. Filtration removes solid residues from the liquid extract [10]. Analysis or Concentration Analytical Techniques Chromatography HPLC, GC-MS for compound identification and quantification.

### Applications of Microwave-Assisted Extraction (MAE)

❖ **Phytochemicals:** It is used to extract bioactive compounds, including terpenes, phenols, and antioxidants, from plant materials.

- ❖ Herbal drug research: MAE is applied to isolate and extract compounds from herbs, which is crucial for developing herbal drugs.
- ❖ Polymer and materials science
- ❖ Additive extraction: MAE is used to extract additives, such as flame retardants, from polymers like polypropylene and polyethylene.
- ❖ Material testing: It is useful for analyzing plastics and other packing materials [12].

**Terpenoid Extraction:** Versatility MAE is particularly effective for extracting both polar and, which are key components inEssential oils (e.g., limonene, non-polar terpenoidsLinalool, pinene)Cannabinoids (e.g., THC, CBD, CBG), Resins and balsams used in perfumes and traditional medicine, Bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant properties [13].

Limitations of Microwave-Assisted Extraction (MAE) Solvent Compatibility Requires polar solvents (e.g., water, ethanol); non-polar solvents like hexane are ineffective unless mixed.Overheating Risk Sensitive compounds may degrade; uneven heating can cause hot spotsn needs precise temperature control. Limited Penetration Microwaves penetrate shallowly in dense samples; irregular shapes reduce heating uniformity. High Equipment Cost Moreexpensive than conventional methods; includes specialized components and regular maintenance [14].

## 3. Ultrasound-Assisted Extraction (UAE)

Ultrasound-Assisted Extraction relies on the phenomenon of acoustic cavitation, which occurs when ultrasonic waves (typically 20–100 kHz) pass through a liquid medium. This creates microscopic bubbles that grow and collapse violently, generating localized high temperatures and pressures. These micro-explosions:

- Disrupt plant cell walls
- Enhance solvent penetration
- Improve mass transfer of target compounds into the solvent

This mechanical effect facilitates the release of bioactive compounds from plant matrices without the need for high temperatures or harsh chemicals.

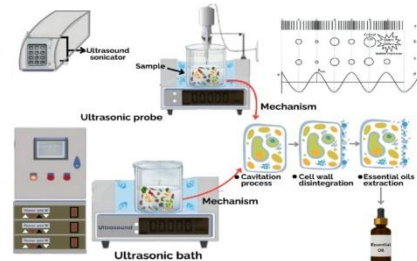


Fig 03: Ultrasound-Assisted Extraction

### Applications

UAE is widely used across various industries due to its efficiency and versatility:

- Pharmaceuticals: Extraction of alkaloids, flavonoids, and polyphenols from medicinal plants
- Food industry: Recovery of antioxidants, essential oils, and pigments from fruits, vegetables, and herbs, Cosmetics, Isolation of natural fragrances, oils, and active ingredients for skincare products.
- Environmental science: Extraction of pollutants or contaminants from soil and water samples.
- Agriculture: Extraction of pesticides or fertilizers from plant tissues for analysis.

### Sample Preparation

**Selection of Plant Material:** Choose appropriate botanical sources based on target compounds (e.g., leaves for flavonoids, roots for alkaloids). **Drying:** Reduces moisture content to prevent microbial growth and improve extraction efficiency. **Grinding/Milling:** Reduces particle size to increase surface area, enhancing solvent contact and cavitation impact. **15,16 Weighing and Solvent Addition:** Accurately weigh the sample and mix with a suitable solvent (e.g., ethanol, methanol, acetone, water) based on polarity and target compound solubility. **Pre-treatment (optional):** Defatting or acid/base treatment may be applied to remove unwanted components or improve selectivity.

**Ultrasound Treatment:** Equipment Setup Use either an ultrasonic bath (low intensity, uniform treatment) or probe-type sonicator (high intensity, localized treatment). **Filtration & Separation Solid-Liquid Separation:** Use filtration (e.g., vacuum filtration, centrifugation) to remove plant debris. **Clarification:** Additional steps like decantation or membrane filtration may be used to remove fine particulates. **Solvent Recovery (optional):** If using volatile solvents, recovery via rotary evaporation or distillation may be performed.

**Concentration & Purification Solvent Evaporation:** Concentrate the extract using rotary evaporator, freeze-drying, or spray drying depending on the application [17, 18].

### Limitations

- Limited Penetration Depth: Effective mainly for surface or near-surface extraction; less efficient for dense or thick samples [19].
- Post-Filtration Required: Solid residues must be removed, adding a processing step
- Equipment Cost: High-power ultrasonic systems can be expensive
- Solvent Compatibility: Not all solvents are suitable for ultrasonic treatment
- Potential Degradation: Prolonged exposure may degrade sensitive compounds
- Energy Consumption: High-intensity ultrasound may require significant energy input [20].
- Pressurized Liquid Extraction (PLE) / Accelerated Solvent Extraction (ASE)

PLE/ASE is widely used across various sectors due to its precision and efficiency: Pharmaceuticals: Extraction of alkaloids, flavonoids, terpenes, and other bioactives from medicinal plants. Food industry: Recovery of antioxidants, essential oils, and nutritional compounds from fruits, seeds, and herbs. Environmental analysis: Extraction of pollutants (e.g., PAHs, PCBs) from soil, sediments, and waste samples. Cosmetics: Isolation of natural oils, fragrances, and active ingredients. Agricultural research: Extraction of pesticides, herbicides, and growth regulators from plant tissue [21].

**Sample Preparation Drying and Grinding:** Reduces moisture and increases surface area for better solvent interaction. **Weighing and Loading:** Sample is accurately weighed and packed into stainless steel extraction cells. **Pre-treatment (optional):** May include defatting or acid/base treatment to improve selectivity

**Solvent Selection** Choose based on target compound polarity: Polar solvents: Water, methanol, ethanol, Non-polar solvents: Hexane, dichloromethane. Solvent mixtures may be used to optimize extraction efficiency.

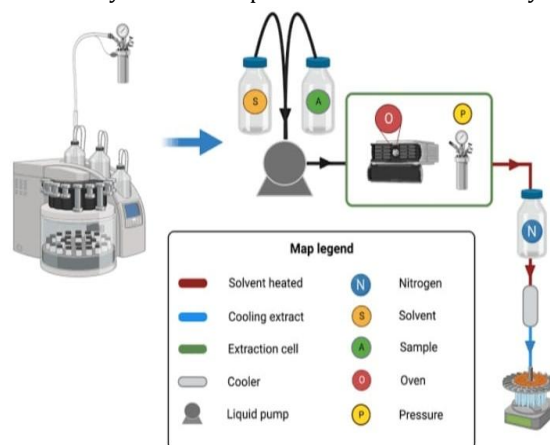


Fig 04: Pressurized Liquid Extraction (PLE) / Accelerated Solvent Extraction (ASE)

**Pressurized Extraction Temperature Control:** Typically 50–200°C depending on compound stability, Pressure Application: Maintains solvent in liquid phase above boiling point, Static Phase: Solvent is held in contact with sample for a set time, Dynamic Phase: Solvent is flushed through the sample to carry extracted compounds

**Collection and Filtration** Extract is collected in vials or tubes, Filtration may be required to remove particulates or residues

**Solvent Recovery and Concentration** Evaporation: Rotary evaporator or nitrogen blow down used to remove solvent, Purification: Chromatography or crystallization may be applied for compound isolation [23].

### Limitations

- Specialized Equipment Required: High-pressure systems are costly and require maintenance.
- Solvent Recovery Needed: Especially important for toxic or expensive solvents.
- Thermal Degradation Risk: Sensitive compounds may degrade at high temperatures.

- Limited Sample Size: Extraction cells have volume constraints.
- Not Ideal for Thermolabile Compounds: Requires careful optimization to avoid degradation.

### Enzyme-Assisted Extraction (Eae)

Enzyme-Assisted Extraction leverages specific hydrolytic enzymes (such as cellulases, pectinases, hemicelluloses, and proteases) to break down structural components of plant cell walls—mainly cellulose, hemicellulose, and pectin. This enzymatic degradation:

- Loosens the cell wall matrix
- Releases intracellular compounds
- Enhances permeability and solvent access

Unlike mechanical or chemical disruption, EAE operates under mild conditions, preserving the integrity of sensitive bioactives.

### Applications

EAE is particularly valuable for extracting compounds that are tightly bound within rigid or lignified plant tissues:

Terpenoids From woody or resinous materials,  
Polyphenols and flavonoids From fruit peels, seeds, and leaves,  
Essential oils From aromatic herbs and spices,  
Proteins and peptides From legumes and cereals.

### Limitations

- Enzyme Cost: High-purity enzymes can be expensive, especially for large-scale use
- Stability Issues: Enzymes may denature under suboptimal conditions (e.g., extreme pH or temperature)
- Longer Processing Time: Compared to rapid physical methods
- Matrix Specificity: Enzyme effectiveness varies with plant type and tissue structure

**ESTIMATION TECHNIQUES FOR TERPENOID AND RESIN** Once terpenoids and resins are extracted from plant matrices, accurate quantification and characterization are essential for quality control, pharmacological evaluation, and product formulation. The following analytical tools are commonly employed: Gas Chromatography-Mass Spectrometry (GC-MS) Separates compounds based on their volatility and polarity. The sample is vaporized and carried by an inert gas (usually helium or nitrogen) through a capillary column coated with a stationary phase. Compounds elute at different times depending on their boiling points and interactions with the column. Mass Spectrometry (MS) Identifies and quantifies compounds by measuring the mass-to-charge ratio ( $m/z$ ) of ionized fragments.

High-Performance Liquid Chromatography (HPLC) is an advanced form of liquid chromatography that operates under high pressure to achieve efficient separation of compounds: A liquid mobile phase (solvent or solvent

mixture) carries the sample through a stationary phase (typically a packed column). Separation occurs based on the differential interactions (adsorption, partitioning, ion exchange, or size exclusion) between the analytes and the stationary phase.

### Application

HPLC is a versatile technique used across many industries and research fields: Non-volatile Terpenoids & Resins Ideal for analyzing complex plant extracts, including diterpenes, triterpenes, and resin acids that are not amenable to GC due to low volatility or thermal instability. Pharmaceuticals Used for drug purity testing, stability studies, and quantification of active pharmaceutical ingredients (APIs). Nutraceuticals Determines bioactive compounds in herbal supplements, vitamins, and functional foods. Cosmetics Analyzes ingredients like antioxidants, preservatives, and fragrances in creams, lotions, and serums. Food Industry Detects additives, contaminants, and nutritional components such as amino acids, sugars, and vitamins. Clinical and Biomedical Research Measures biomarkers, hormones, and metabolites in biological fluids. Environmental Analysis Monitors pollutants, pesticides, and trace organic compounds in water and soil.

Uv-Visible Spectrophotometry is an analytical technique that measures the absorbance of light in the ultraviolet (UV, 200–400 nm) and visible (Vis, 400–700 nm) regions of the electromagnetic spectrum. The core principle is based on the interaction between light and matter—specifically, how molecules with chromophores absorb light at characteristic wavelengths. Chromophores are functional groups within molecules that contain conjugated double bonds or aromatic rings, which can undergo electronic transitions when exposed to UV or visible light.

Nuclear Magnetic Resonance (Nmr) Spectroscopy Nuclear Magnetic Resonance (NMR) Spectroscopy is a powerful analytical technique based on the magnetic properties of certain atomic nuclei. When placed in a strong external magnetic field, nuclei such as  $^1\text{H}$  (proton) or  $^{13}\text{C}$  (carbon-13) absorb radiofrequency radiation at characteristic frequencies. This absorption causes transitions between nuclear spin states, and the resulting signals are detected and translated into a spectrum.

Thin Layer Chromatography (Tlc) Compounds are separated on a silica-coated plate using a solvent system and visualized by staining or UV light.

Applications: Useful for preliminary screening of terpenoids and resins Common in herbal drug standardization and fingerprinting

**Limitations:** Low resolution and sensitivity, Not suitable for precise quantification, Manual interpretation may lead to variability

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) Measures absorption of infrared light by molecular bonds, providing structural information.

Features: Non-destructive and rapid, requires minimal sample preparation, Useful for fingerprint analysis

### Real-World Applications of Terpenoids and Resins

- Medicinal use: Used in drugs like artemisinin (antimalarial), menthol (pain reliever), and camphor (antiseptic, rub for cold).
- Perfume and flavor industry: Terpenoids such as limonene, pinene, and citral are used in perfumes, soaps, and flavoring agents.
- Vitamins: Some terpenoids form parts of vitamin A, E, and K.
- Insect repellents
- Industrial uses
- Varnishes and paints: Natural resins like rosin and copal are used in making varnishes, paints, and adhesives.
- Perfume and incense: Frankincense and myrrh resins are used in perfumes and religious ceremonies.
- Medicinal uses: Some resins have antiseptic and anti-inflammatory properties.
- Electrical and industrial uses: Synthetic resins like epoxy, polyester, and phenolic resins are used as insulators, plastic materials, and adhesives.
- Art and craft: Used in jewelry, sculptures, and preservation [24].

### Conclusion

Modern extraction techniques-such as Ultrasound-Assisted Extraction (UAE), Pressurized Liquid Extraction (PLE), Enzyme-Assisted Extraction (EAE), and Supercritical Fluid Extraction (SFE) have revolutionized the isolation and quantification of terpenoids and resins. Enabled targeted extraction of specific compounds with minimal degradation, improved yield and purity, even from complex or lignified plant matrices Minimized use of toxic solvents, Lowered energy consumption through mild operating conditions, Promoted green chemistry and sustainable processing. Facilitated automation and reproducibility for large-scale production, Supported standardization and quality control in pharmaceuticals, cosmetics, and food industries, Enabled integration with analytical tools like GC-MS and HPLC for precise compound profiling. These advancements not only elevate product quality and safety but also open new frontiers in natural product research, therapeutic development, and eco-friendly innovation.

### Acknowledgement

Not Declared

### Conflicts of Interest

The authors declare no conflicts of interest.

### Author Contribution

All are contribute equally

### Financial Support

None

### Ethical Considerations and Informed Consent

Not Applicable

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