# Phytochemical Analysis of Medicinal Plants

**Manonmani R, Shilpa L S, Haripriya B, Praveena Anitta A**

This chapter presents an in-depth phytochemical investigation of medicinal plants, emphasizing major bioactive groups such as alkaloids, flavonoids, tannins, glycosides, terpenoids, saponins, steroids, and phenolic compounds. It documents local names, plant parts and extraction methods, using both classical chemical tests (e.g., Dragendorff's, Mayer's, Fehling's) and advanced techniques such as TLC, HPLC, and NMR. Standardized procedures with characteristic observations are provided to aid herbal drug quality control. The study highlights how plant part selection, geography and solvent choice affect phytochemical yields. Integration of traditional knowledge with modern analytical tools supports drug development, ethnobotanical conservation and validation of medicinal plants for pharmacological use.

***Keywords:*** *Phytochemical screening, Medicinal plants, Secondary metabolites, Chromatographic analysis, Bioactive compounds, Herbal drug standardization*

Manonmani R[1*], Shilpa L S[2], Haripriya B[2], Praveena Anitta A[2]
[1]Assistant professor, PG & Research Department of Botany, Holy Cross College (Autonomous), Affiliated to Bharathidasan University, Trichy, Tamil Nadu, India.
[2]Research Scholar, PG & Research Department of Botany, Holy Cross College (Autonomous), Affiliated to Bharathidasan University, Trichy, Tamil Nadu, India.

[*]Email: manonmani.s22@gmail.com


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## Introduction

Since ancient times, plants have served as medicinal resources because of the bioactive phytochemicals they contain (Sinha et al., 2022). These naturally occurring compounds, found in roots, bark, leaves, seeds and other plant parts, are primarily responsible for plants' therapeutic properties. Extracting, quantifying and analyzing phytochemicals is essential for discovering new drug leads and ensuring the quality of medicinal plant products (Ingle et al., 2017).

Phytochemicals play a vital role in shielding plants from pathogens and environmental stressors, while also influencing their characteristic colour, aroma, and flavour (Haseen et al., 2024). Advances in phytochemical analysis using extraction, chromatography and spectroscopy enable researchers to evaluate medicinal potential, identify novel compounds and improve quality control. Understanding plant chemistry in this way is crucial for disease prevention, immunity enhancement and developing new therapeutics.

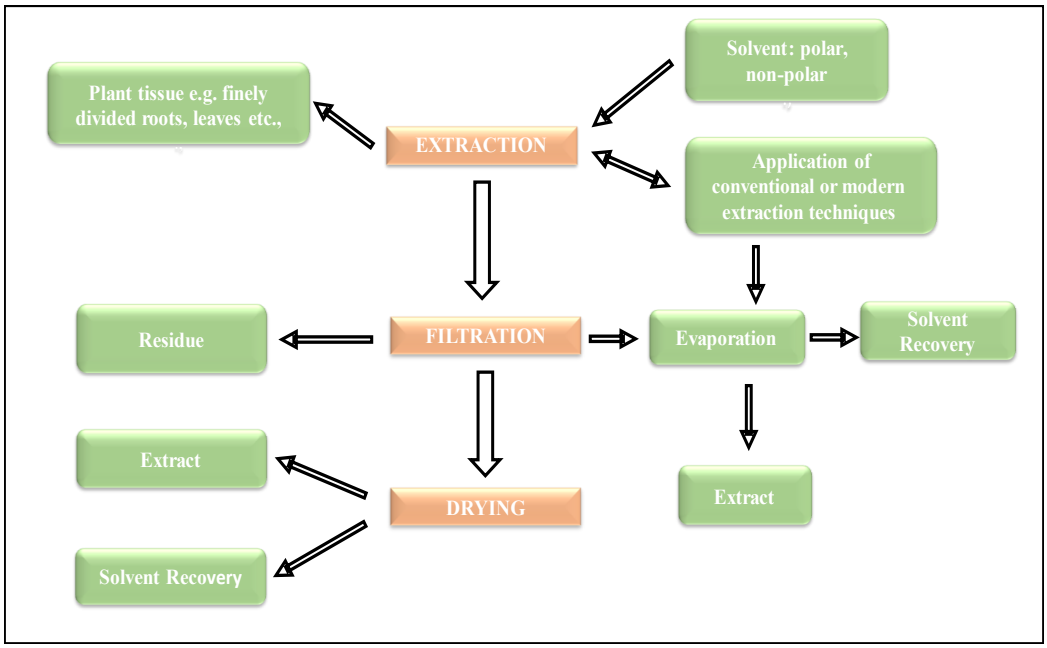


**Figure 1. Flowchart illustrating the steps involved in extracting phytochemicals from plant materials (Bitwell et al., 2023).**

**Table 1. List of selected medicinal plants and the specific parts utilized for phytochemical analysis (Akhtar et al., 2018).**

| S. No. | Name of the Plant | Common Name | Plant Parts Utilized |
|---|---|---|---|
| 1. | *Albizia labeek* L. | Frywood | Brak |
| 2. | *Anethum graveolens* L. | Dill | Aerial parts |
| 3. | *Azadirachta indica* (A. Juss.) | Neem Tree | Plant Leaves |
| 4. | *Berberis lycium* Royle | Zarch | Bark |
| 5. | *Carthamus oxyacantha* M.Bieb. | Kandiari | Flower |
| 6. | *Celosia cristata* (L.) | Kalgha, Cockscomb | Aerial Parts |
| 7. | *Colebrookea oppositifolia* Sm. | Dhusure | Leaves, Flower |
| 8. | *Dodonaea viscosa* (L.) Jacq. | Hopbush | Plant Leaves |
| 9. | *Fagonia cretica* (L.) | Dhman Booti | Stem and Spines |
| 10. | *Jasminum officinale* (L.) | Chambeli | Leaves and Stem |
| 11. | *Justicia adhatoda* L. | Arusa | Leaves |
| 12. | *Moringa oleifera* (Lam.) | Drumstick Tree | Leaves and Bark |
| 13. | *Murraya koenigii* (L.) Spreng. | Curry Tree | Plant Leaves |
| 14. | *Nasturtium officinale* (W.T. Aiton) | Watercress | Leaves and Stem |
| 15. | *Ocimum basilicum* (L.) | Tulsi | Aerial Parts |
| 16. | *Phyllanthus emblica* L. | Amla | Leaves |
| 17. | *Pinus roxburghii* (Sarg.) | Chir Pine | Pine Needles |
| 18. | *Ricinus communis* (L.) | Castor Plant | Plant Leaves |
| 19. | *Rosa indica* (L.) | Gulab | Leaves and Stem |
| 20. | *Rumex hastatus* (L.) | Khatti buti | Bark |

**Types of Phytochemicals**

Phytochemicals are naturally occurring plant compounds, grouped into primary metabolites such as carbohydrates, proteins, lipids and amino acids, and secondary metabolites that include alkaloids, terpenoids, flavonoids, lignins, steroids, curcumins, saponins, phenolic compounds and glycosides. Secondary phytochemicals often act as antioxidants and antibacterial agents (Siddiqui and Noid; 2022).

Phenolic compounds such as flavonoids, phenolic acids and polyphenols contain hydroxyl groups on aromatic rings and are valued for their health-protective and food-stabilizing roles. Flavonoids provide plant coloration and exhibit antimicrobial, anti-inflammatory and anticancer effects. Tannins, found in leaves, fruits and stem coverings, protect plants, aid in beverage clarification and act as strong antioxidants.

Alkaloids are nitrogen-containing plant metabolites like morphine and nicotine, with strong physiological activity and protective roles against herbivores. Terpenoids (isoprenoids), made from isoprene units, add aroma/flavour and have medicinal uses (e.g., artemisinin, taxol). Saponins, found in many plants, create foam in water, have haemolytic effects and are classified as steroidal or triterpenoid.

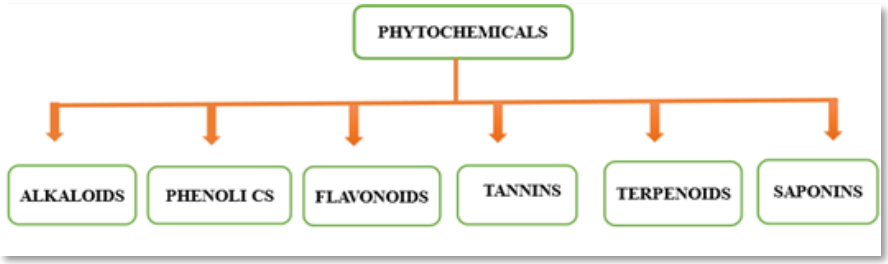


**Figure 2. Types of Phytochemicals**

**Solvents in Phytochemical Extraction**

Phytochemicals are bioactive plant-derived compounds with medicinal benefits. Efficient extraction of these compounds is essential for their use in pharmaceuticals and nutraceuticals, with solvent choice significantly impacting extraction yield and efficiency (Dhawan and Gupta, 2017).

**Types of Solvents** (Rao et al., 2023 and Oluto et al., 2025).

**a) Polar Solvents**

- **Water:** Suitable for isolating polar constituents, including phenolic acids, flavonoids and glycosides.
- **Methanol:** A flexible solvent capable of extracting many phytochemicals, such as alkaloids and saponins.
- **Ethanol:** Similar to methanol, used for extracting various phytochemicals and is less toxic.
- **Acetone:** Often used for extracting alkaloids and other polar compounds.
- **Acetonitrile:** Used for certain types of phytochemical extractions.
- **Dimethylformamide (DMF):** Effective for extracting specific polar compounds.
- **Dimethylsulfoxide (DMSO):** Used for its high solvency power with polar compounds.
- **Isopropanol:** Commonly used in phytochemical extractions.

**b) Non-polar Solvents**

- **Hexane:** Used for extracting nonpolar lipids and essential oils.
- **Chloroform:** Effective for extracting nonpolar compounds such as steroids and terpenoids.
- **Petroleum Ether:** Commonly used for extracting nonpolar lipids.
- **Diethyl Ether:** Used for extracting nonpolar compounds.
- **Ethyl Acetate:** Effective for moderate polarity compounds.
- **Benzene:** Used for extracting nonpolar compounds, though less common due to toxicity.
- **Toluene:** Another nonpolar solvent used in phytochemical extractions.

**Modern Extraction Techniques** (Bitwell et al., 2023).

**1. Maceration**

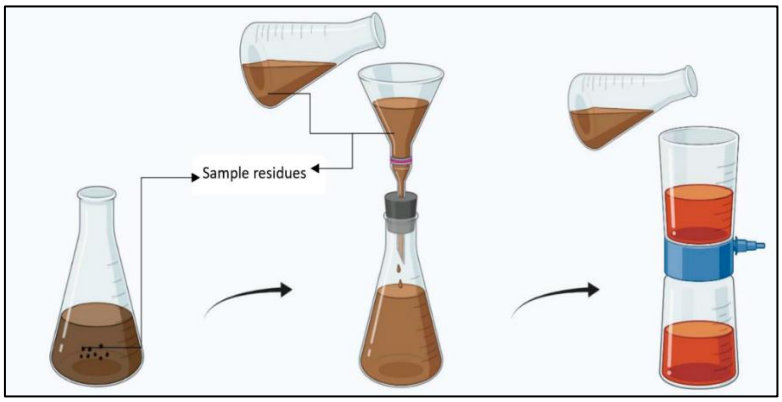


**Figure 3. A diagrammatic representation of Maceration**
Source: https://share.google/images/jOySAfk68zl4g5ObN

Maceration is a straightforward and economical extraction technique in which powdered plant material is immersed in a solvent for an extended period, enabling bioactive compounds to gradually diffuse into the liquid. The process involves soaking, occasional stirring, filtration, and concentration of the extract. Advantages include preservation of heat-sensitive compounds, minimal equipment and suitability for various plants and solvents.

**2. Percolation Method**

Percolation is an effective extraction technique in which coarsely powdered plant material is first moistened and then continuously washed by solvent flowing through it by gravity, allowing active compounds to dissolve efficiently. The process involves imbibition (wetting), maceration, and percolation, using minimal solvent and preserving heat-sensitive compounds. It yields concentrated extracts and is widely used in pharmaceutical and herbal industries.

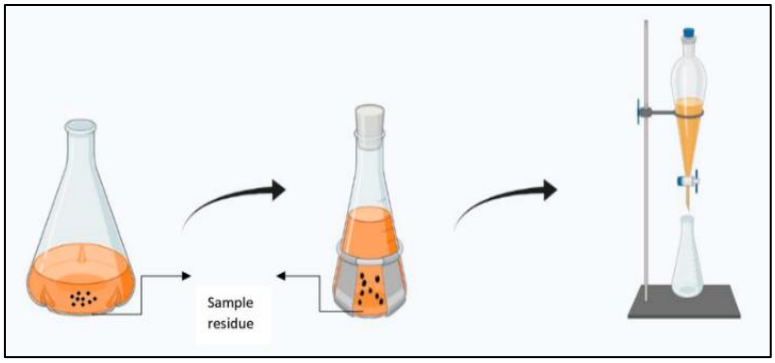


**Figure 4. A diagrammatic representation of Percolation**
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**3. Soxhlet extraction**

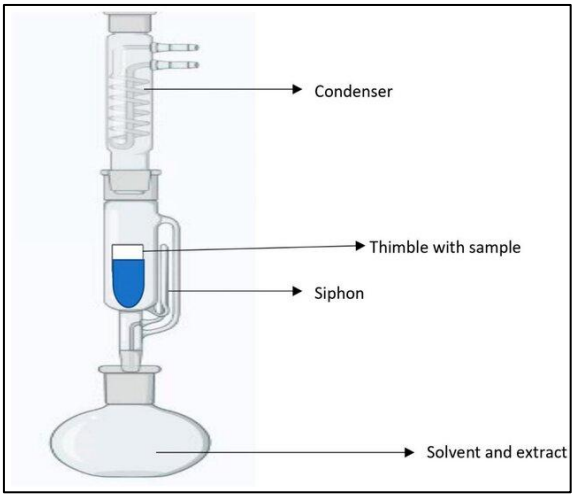


**Figure 5. A diagrammatic representation of Soxhlet extraction**
Source: https://share.google/images/STuUXCWVZStkZWNTt

Soxhlet extraction is an automated, efficient method where a finely powdered sample in a porous thimble is repeatedly washed by condensed solvent vapor cycling from a heated flask. The solvent dissolves target compounds and siphons back for continuous extraction. Advantages include high efficiency, minimal supervision, solvent recycling, broad applicability and suitability for various scales.

**4. Supercritical fluid extraction**

Supercritical fluid extraction (SFE) employs supercritical gases, usually carbon dioxide, under precise temperature and pressure conditions to isolate bioactive compounds from dried, powdered plant materials. The fluid dissolves target compounds, which precipitate when pressure and temperature decrease. Optimization methods like response surface methodology enhance yield. Advantages include high solubility, environmental friendliness, selective extraction, efficient separation and minimal thermal degradation.

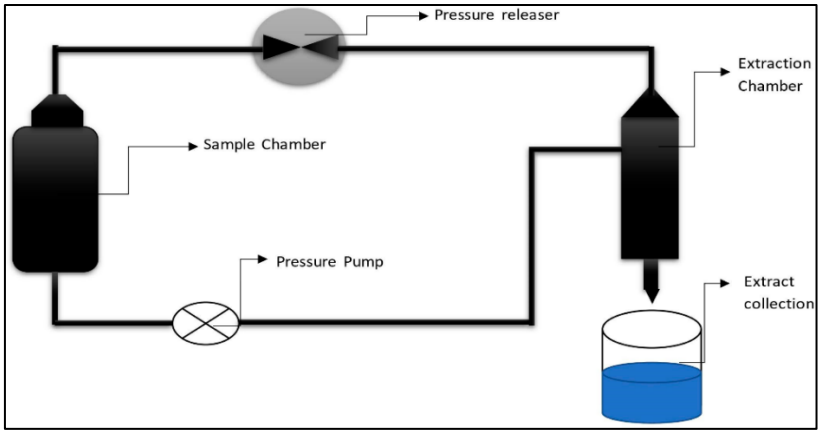


**Figure 6. A diagrammatic representation of Supercritical fluid extraction**
Source: https://www.mdpi.com/molecules/molecules2800887/article_deploy/html/images/molecules-28-00887-g011.png

**5. Microwave assisted extraction**

Microwave-assisted extraction (MAE) applies microwave energy to quickly heat the solvent and dried plant material, improving solvent penetration and disrupting bonds to enhance extraction efficiency. The process leads to faster extraction, higher yields, reduced solvent use and selective extraction through temperature and time control, followed by filtration and concentration.

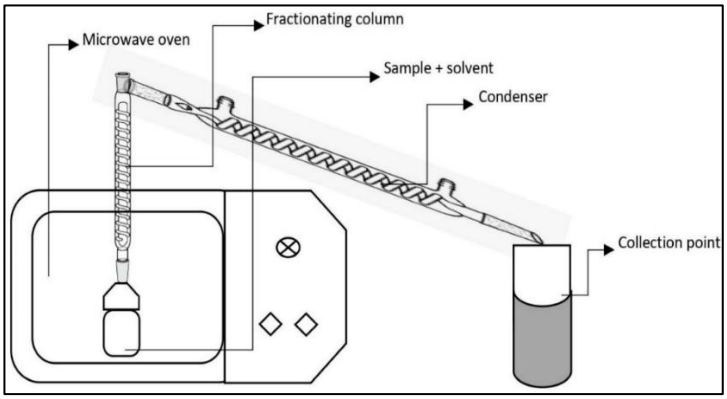


**Figure 7. Illustration showing the process of microwave-assisted extraction**.
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**6. Ultrasound assisted extraction**

Ultrasound-assisted extraction (UAE) employs ultrasonic waves to form cavitation bubbles that break plant cell walls, improving solvent penetration and speeding up the release of bioactive compounds. The process involves dried, ground plant material mixed with solvent, ultrasound application at controlled settings, followed by filtration and concentration. Advantages include faster extraction, higher yields, lower temperatures protecting heat-sensitive compounds and reduced solvent use.

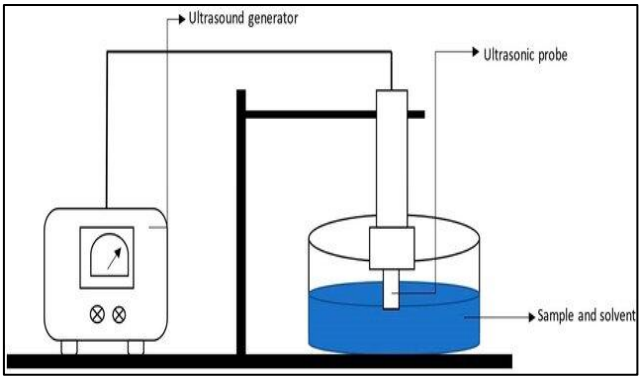


**Figure 8. A diagrammatic representation of Ultrasound assisted extraction.**
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**Filtration**

Filtration is essential in phytochemical analysis for separating bioactive compounds from impurities, aiding purification for pharmaceutical and nutraceutical use.

**Types of Filtration Methods:(**Mendoza et al., 2024)

1. **Simple Filtration:** Uses filter paper (e.g., Whatman No. 1) to separate solids from liquids by gravity or gentle suction during initial extraction.

2. **Vacuum Filtration:** Applies vacuum (e.g., Buchner funnel with Whatman No. 1) to speed filtration and remove fine particles from concentrated extracts.

3. **Centrifugation:** Separates mixture components by density via high-speed spinning, such as isolating chloroplasts from plant samples.

4. **Ultrafiltration:** Uses membranes with tiny pores to separate molecules by size, concentrating and purifying compounds like flavonoids.

5. **Chromatographic Filtration:** Combines filtration and chromatography (e.g., column chromatography) to separate compounds according to their chemical properties, making it effective for isolating alkaloids.

**Drying in phytochemicals:** (Gulati et al., 2020)

**Methods of Drying Herb**

Drying is a critical step in herb processing to reduce moisture content, preserve bioactive compounds and extend shelf life. Common drying methods include:

**Sun Drying**

In this method, fresh herbs are placed on an agro-shade net and sun-dried with frequent turning to ensure uniform drying. At night, the material is covered to avoid moisture uptake. The drying progress is checked by weighing the samples periodically until a constant weight is reached.

**Figure 9. Sun Drying**
Source: https://share.google/images/DmcOdhLef35eQv6KL

**Shade Drying**

Shade drying begins with briefly sun-drying the washed herbs to remove surface moisture, after which they are dried at room temperature in the shade. The material is spread on an elevated agro-shade net and turned several times daily. Drying continues until the samples show a constant weight on repeated measurements.

**Figure 10. Shade drying**
Source: https://share.google/images/53St5TMKz0tD6YQbh

**Hot Air Drying**

In this method, herbs are dried using a hot air blower. Trays holding the material are positioned in front of the blower inside an enclosed box to reduce heat loss. The drying chamber is kept at about 50°C with a relative humidity of around 78%.

**Figure 11. Illustration of hot-air drying.**
Source: https://share.google/images/1UOesFHji5nled3Pb

**Oven Drying**

Oven drying involves spreading the plant material evenly in a hot-air oven maintained at 45 ± 2°C. The samples are weighed periodically, and drying stops once their moisture content falls to about 8–10%.

**Figure 12. Oven drying**
Source: https://share.google/images/iJI54LRfg8smA6LCo

**Microwave Drying**

This method applies electromagnetic radiation at 915 or 2,450 MHz to dry the herb. Small batches of the sample are placed in the microwave and processed until the required moisture level is reached.

**Figure 13. Microwave drying**
Source: https://share.google/images/pa3Njzqdwzf2ryU4f

In all these drying methods, the target is to reduce the moisture content to less than 10 percent. Drying time and changes in herb color are noted during the process. After drying is complete, the samples are prepared for phytochemical analysis.

**Qualitative Techniques for Determination of Phytochemicals:** (Shaikh and Patil. 2020)

**Table 2. Preparation of reagents used for phytochemical analysis.**

| **Reagents and Solutions** | **Constituents** |
|---|---|
| Dragendorff reagent | Stock solution: Dissolve 5.2 g of bismuth carbonate and 4 g of sodium iodide in 50 mL of glacial acetic acid and heat briefly. After standing for 12 hours, filter off the sodium acetate crystals using a sintered glass funnel. To the filtrate, add 40 mL of this solution to 160 mL of ethyl acetate and 1 mL of distilled water. Store in an amber bottle.<br>Working solution: Mix 10 mL of the stock solution with 20 mL of acetic acid and add distilled water to a final volume of 100 mL. |
| Mayer reagent | Solution A: Dissolve 1.358 g of mercuric chloride in 60 mL of distilled water.<br>Solution B: Dissolve 5 g of potassium iodide in 10 mL of distilled water.<br>Working solution: Combine Solutions A and B, then add distilled water to a final volume of 100 mL. |
| Wagner reagent | Dissolve 1.27 g of iodine and 2 g of potassium iodide in distilled water, then add more distilled water to make a final volume of 100 mL. |
| Benedict reagent | Solution A: Dissolve 173 g of sodium citrate and 100 g of sodium carbonate in 800 mL of water and boil until the solution becomes clear.<br>Solution B: Dissolve 17.3 g of copper sulfate in 100 mL of distilled water.<br>Working solution: Combine Solutions A and B. |
| Fehling solutions | Solution A: Dissolve 34.66 g of copper sulfate in distilled water and make up to 100 mL.<br>Solution B: Dissolve 173 g of potassium sodium tartrate and 50 g of NaOH in distilled water and adjust to 100 mL.<br>Working solution: Combine equal volumes of Solutions A and B. |

**Table 3: Qualitative assays for Phytochemical detection**

**Alkaloid detection: (Silva et al., 2017 and Oluto et al., 2025)**

| **S.No** | **Tests** | **Procedure** | **Observation** |
|---|---|---|---|
| 1. | Dragendorff (Kraut) test | Add a few millilitres of the filtrate to 1–2 mL of Dragendorff reagent. | Formation of a reddish-brown precipitate. |
| 2. | Mayer (Bertrand/Valser) test | Add a few millilitres of the filtrate and 1–2 drops of Mayer reagent along the inner wall of the test tube. | A creamy white or yellow precipitate appears. |
| 3. | Wagner test | Add a few millilitres of the filtrate and 1–2 drops of Wagner reagent along the side of the test tube. | A brown or reddish precipitate forms. |

**Carbohydrate detection:** (Pandey and Tripathi, 2014)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Barfoed test | Mix 1 mL of filtrate with 1 mL of Barfoed reagent and heat for 2 minutes. | A red precipitate indicates the presence of monosaccharides. |
| 2. | Molish test | Combine 2 mL of filtrate with 2 drops of alcoholic α-naphthol, then carefully add 1 mL of concentrated $H_2SO_4$ along the test tube wall. | A violet ring form. |
| 3. | Seliwanoff test | Mix 1 mL of the extract with 3 mL of Seliwanoff reagent and heat in a water bath for 1 minute. | A rose-red color indicates the presence of ketoses. |

**Detection of reducing sugar:** (Singh and Kumar, 2017; Oluto et al., 2025)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Benedict test | Mix equal volumes (0.5 mL) of the filtrate and Benedict reagent, then heat the mixture for 2 minutes. | A green, yellow, or red color develops. |
| 2. | Fehling test | Mix 1 mL each of Fehling solutions A and B with 1 mL of filtrate, then heat the mixture in a water bath. | A red precipitate is formed. |

**Glycoside detection:** (Kumar et al., 2013; Pant et al., 2017)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Borntrager test | Mix 2 mL of the hydrolyzed filtrate with 3 mL of chloroform and shake thoroughly. Separate the chloroform layer and add 10% ammonia solution. | A pink-colored solution is produced. |
| 2. | Sodium hydroxide (10%) test | Mix 1 mL of diluted $H_2SO_4$ with 0.2 mL of the extract and boil for 15 minutes. After cooling, neutralize with 10% NaOH and add 0.2 mL of Fehling solutions A and B. | A brick-red precipitate appears. |
| 3. | Concentrated $H_2SO_4$ test | Combine 5 mL of plant extract with 2 mL of glacial acetic acid, add one drop of 5% $FeCl_3$, and then carefully introduce concentrated $H_2SO_4$. | A brown ring is formed. |

**Cardiac glycoside detection:** (Pandey and Tripathi, 2014)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Keller–Killiani test | Mix 1 mL of filtrate with 1.5 mL of glacial acetic acid and one drop of 5% ferric chloride, then carefully add concentrated $H_2SO_4$ along the side of the test tube. | A blue-colored solution appears in the acetic acid layer. |
| 2. | Kedde test | Evaporate 4 mL of the extract to dryness, add 1–2 mL of methanol and 1–2 mL of alcoholic KOH, followed by 3–4 drops of 1% alcoholic 3,5-dinitrobenzene, then heat the mixture. | A violet color that gradually fades, indicating cardenolides. |
| 3. | Bromine water assay | Add a few millilitres of bromine water to the plant extract. | A yellow precipitate is formed. |

**Detection of proteins and amino acids:** (Gulati, 2020)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Biuret assay | Mix 2 mL of filtrate with one drop of 2% copper sulfate solution, add 1 mL of 95% ethanol, then introduce a few KOH pellets. | A pink-colored solution appears in the ethanolic layer. |
| 2. | Millon test | Add a few drops of Millon reagent to 2 mL of the filtrate. | A white precipitate forms. |
| 3. | Ninhydrin test | Add 2 mL of filtrate to 2 drops of ninhydrin solution (10 mg ninhydrin in 200 mL acetone). | A purple-colored solution indicates the presence of amino acids. |

**Detection of Flavonoids:** (Pant et al., 2017; Tyagi, 2017)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Alkaline reagent assay | Mix 1 mL of extract with 2 mL of 2% NaOH solution, then add a few drops of diluted HCl. | A strong yellow color appears, which turns colorless after adding diluted acid. |
| 2. | Ferric chloride assay | Add a few drops of 10% ferric chloride solution to the aqueous extract. | A green precipitate forms. |
| 3. | Ammonia assay | Add 5 mL of diluted ammonia solution to the filtrate, then carefully introduce concentrated $H_2SO_4$. | A yellow color develops. |
| 4. | Concentrated $H_2SO_4$ assay | Add concentrated $H_2SO_4$ to the plant extract. | An orange color appears. |

**Phenolic compound detection:** (Olotu et al., 2025)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Iodine assay | Mix 1 mL of the extract with a few drops of diluted iodine solution. | A brief red coloration is observed. |
| 2. | Ferric chloride assay | Add a few drops of 5% ferric chloride solution to the aqueous extract. | A dark green or bluish-black color develops. |
| 3. | Lead acetate assay | Dissolve the plant extract in 5 mL of distilled water, then add 3 mL of 10% lead acetate solution. | A white precipitate forms. |
| 4. | Potassium dichromate assay | Add a few drops of potassium dichromate solution to the plant extract. | A dark coloration appears. |

**Detection of Tannins:** (Njoku and Obi, 2009; Rahman and Ahmed, 2013)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Braymer's assay | Take 1 mL of the filtrate, dilute it with 3 mL of distilled water, and then add three drops of 10% ferric chloride solution. | Blue-green coloration |
| 2. | 10% NaOH test | Mix 0.4 mL of the plant extract with 4 mL of 10% sodium hydroxide solution and shake the mixture thoroughly. | Formation of an emulsion indicates the presence of hydrolysable tannins. |
| 3. | Lead Sub-acetate Test | Add 1 mL of the filtrate to three drops of lead sub-acetate solution and mix gently. | The appearance of a creamy, gelatinous precipitate confirms tannins. |

**Detection of Saponins:** (Olotu et al., 2025)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Foaming Ability Assay | Take 0.5 g of the plant extract and add 2 mL of water, then shake the mixture vigorously | Stable foam that remains for about 10 minutes indicates saponins |
| 2. | Sodium Bicarbonate ($NaHCO_3$) Test | Add the plant extract to a few millilitres of sodium bicarbonate solution, dilute with a little distilled water, and shake the mixture vigorously. | Appearance of a stable, honeycomb-like froth indicates Saponins. |
| 3. | Olive oil foaming assay | Combine the aqueous extract with 5 mL of distilled water and shake the mixture vigorously. Then add a few drops of olive oil and shake again. | The formation of foam on the surface indicates Saponins. |

**Detection of Phytosterols:** (Tyagi, 2017; Uma et al., 2017)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Salkowski reaction | Add a few drops of concentrated sulfuric acid ($H_2SO_4$) to the filtrate, shake gently, and allow the mixture to settle. | Red coloration in the lower layer |
| 2. | Libermann–Burchard reaction | Prepare a mixture of 50 g extract with 2 mL acetic anhydride, and gently introduce 1–2 drops of conc. $H_2SO_4$ down the side of the tube. | Multiple sequential colour transitions |
| 3. | Test with Acetic Anhydride | To 0.5 mL of the extract, add 2 mL acetic anhydride, followed by 2 mL conc. $H_2SO_4$. | A violet coloration shifting to blue/green |

**Detection of cholesterol:** (Gul et al., 2017)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | | Combine 2 mL extract and 2 mL chloroform, then introduce acetic anhydride (10 drops) and finally 2–3 drops of conc. $H_2SO_4$. | A rose-red coloration |

**Detection of Terpenoids:** (Gul et al., 2017)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | | Combine 2 mL chloroform with 5 mL extract and evaporate the mixture using a water bath.<br>Then introduce 3 mL conc. $H_2SO_4$ and heat the mixture. | A grey colour confirms Terpenoids |

**Detection of Triterpenoids:** (Singh and Kumar, 2017)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Salkowski's test | Filtrate + few drops of conc. $H_2SO_4$ (Shaken well and allowed to stand) | Golden yellow layer (at the bottom) |

**Detection of Diterpenes:** (Singh and Kumar, 2017)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Copper Acetate reaction | After dissolving the extract in distilled water, introduce 3 to 4 drops of copper acetate solution. | A distinct emerald-green colour |

**Detection of lignin's:** (Shaikh and Patil, 2020)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Labat reaction | Prepared extract with gallic acid and allow the mixture to stand. | An olive-green coloration |
| 2. | Furfuraldehyde reaction | Mix the plant extract solution with 2% furfuraldehyde reagent. | Development of a red colour |

**Detection of Quinones:** (Pooja and Vidyasagar, 2016)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Alcoholic Potassium Hydroxide test | To 1 mL of extract, add alcoholic KOH and observe the mixture. | A transition from red to blue |
| 2. | Concentrated Hydrochloric Acid test | The extract with conc. HCl and observe. | Formation of a green colour |
| 3. | Conc. Sulphuric acid test | Prepare a solution by mixing 10 mg extract with isopropyl alcohol, followed by the addition of a drop of conc. $H_2SO_4$. | Development of a red colour |

**Detection of Anthraquinones:** (Hazra et al., 2007)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Borntrager reaction | Mix 10 mL of 10% ammonium solution with a few millilitres of the filtrate and shake the mixture vigorously for about 30 seconds. | A pink, violet, or red coloration |
| 2. | Ammonium hydroxide reaction | Prepare a solution of 10 mg extract in isopropyl alcohol, followed by the addition of conc. $NH_4OH$. | After 2 minutes, the mixture develops a red coloration |

**Detection of Coumarins:** (Njoku and Obi, 2009; Pooja and Vidyasagar, 2016)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Sodium Hydroxide paper assay | 0.5 g of moist extract into a test tube, seal the top with 1N NaOH-impregnated filter paper, and heat in a water bath | When examined under UV illumination, the paper exhibits a yellow fluorescent signal |
| 2. | Sodium Hydroxide test | Plant extract with 10% NaOH, followed by chloroform, and observe the colour | A yellow coloration |

**Detection of Resins:** (Olotu et al., 2025)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Acetic anhydride reaction | Mix 1 mL of the plant extract with acetic anhydride solution, then add 1 mL of concentrated sulfuric acid. | An orange to yellow colour change |
| 2. | Turbidity formation | Combine 10 mL extract with 20 mL of 4% HCl | Cloudiness or Turbidity |

**Detection of Fixed oils and Fats:** (Olotu et al., 2025)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | Stain Formation test | Press a small portion of the extract between filter papers | An oily spot on the paper |
| 2. | Saponification assay | Combine the plant extract with 0.5N alcoholic KOH, introduce phenolphthalein indicator, and heat for approximately 2 hours. | soap formation or reduction in alkalinity |

**Detection of Volatile oils:** (Uma et al., 2017)

| S.No | Tests | Procedure | Observation |
|---|---|---|---|
| 1. | UV Fluorescence assay | Saturated 10 mL extract is filtered and then examined under ultraviolet light. | A distinct pink fluorescence |

**Quantitative test for Phytochemical Screening**
**Chromatographic Techniques:** (Banu and Cathrine, 2015).

**1. Gas Chromatography (GC)**

It analyses volatile compounds by separating them between a liquid stationary phase and a gas mobile phase. Components migrate at different rates based on their affinity to each phase, with more volatile molecules moving faster. GC is primarily used for quantitative analysis of volatile substances like essential oils, terpenes and alkaloids in plant samples.

**2. High Performance Liquid Chromatography (HPLC)**

This analyses non-volatile compounds by dissolving samples in a solvent and passing them under high pressure through a column. Separation depends on interactions with the stationary and mobile phases. Coupled with detectors like UV-Vis, fluorescence, or mass spectrometry, HPLC effectively analyses and quantifies polar phytochemicals such as flavonoids, phenolics and alkaloids.

**3. High Performance Thin Layer Chromatography (HPTLC)**

It's a low-cost technique where samples are spotted on a stationary phase (silica gel or alumina) and developed with solvents to separate compounds by affinity. Visualization via UV light or colour reagents enables qualitative and semi-quantitative analysis. It is effective for screening plant extracts and with densitometry, allows identification and quantification of phytochemicals.

**4. Thin layer Chromatography**

It's a simple, cost-effective method for separating non-volatile compounds on a silica gel or cellulosic plate using a solvent mobile phase. Compounds separate by polarity, with more polar ones migrating slower. Visualization is done by UV light or chemical stains. TLC is ideal for rapid qualitative analysis and phytochemical screening.

**5. Supercritical fluid separation**

It's a green technology using supercritical $CO_2$ (above 31.1°C and 73.8 bar) to selectively extract bioactive phytochemicals with minimal thermal degradation and solvent residues. Supercritical $CO_2$ combines gas-like diffusivity for deep penetration and liquid-like density to dissolve non-polar/moderately polar compounds. Extraction efficiency depends on pressure, temperature, co-solvent (e.g., ethanol) and plant matrix, enabling fine-tuning to target polar phytochemicals.

**Spectroscopic analysis**

Spectroscopy plays a crucial role in detecting and analysing phytochemicals by identifying their structural and functional properties. The commonly used spectroscopic techniques include: (Ingle et al., 2017)

- **Mass Spectroscopy**

  It's a powerful tool for identifying, quantifying and characterizing phytochemicals by determining molecular weight and generating diagnostic fragments. It enables accurate analysis of compounds in complex mixtures and is essential in phytochemical research, including peptide and organic compound structural analysis.

- **Nuclear Magnetic Resonance Spectroscopy (NMR)**

  Nuclear Magnetic Resonance (NMR) spectroscopy reveals molecular, genetic and mechanical properties. One-dimensional NMR is common, while two-dimensional NMR provides detailed molecular structures. Solid-state NMR analyses solid structures. Radiolabelled C-NMR identifies carbon types and H-NMR determines hydrogen types and their connectivity within compounds.

- **Fourier-transform infrared spectroscopy (FTIR)**

  This identifies functional groups and molecular structures in plant extracts. Samples are prepared by placing a liquid drop between sodium chloride plates, grinding solids with potassium bromide into pellets, or dissolving solids in solvents and applying onto a High Attenuated Total Reflectance (HATR) plate.

Spectra show transmittance percentages, with characteristic peaks corresponding to specific bonds and functional groups.

- **UV Spectroscopy**

It measures light attenuation after passing through or reflecting off a sample, utilizing UV light to excite outer electrons in biomolecules or inorganic solutions. Absorbance at specific wavelengths, governed by Beer-Lambert's law, enables concentration determination. This technique characterizes optical and electronic properties of materials, including pigments and plant compounds, by recording parts of the UV-Vis spectrum.

## Conclusion

The study of phytochemical analysis highlights the importance of bioactive compounds in medicinal plants for modern medicine. Extraction efficiency depends greatly on solvent polarity. By comparing different solvents and using varied extraction, drying, and filtration methods, researchers can isolate key phytochemicals like alkaloids, flavonoids, tannins and saponins, which underlie therapeutic effects. Advances in qualitative and quantitative techniques have deepened understanding of plant-based medicines, supporting the development of pharmaceuticals and natural remedies. Ongoing phytochemical analysis is vital to fully exploit medicinal plants for disease prevention and treatment.

## Declaration of conflict of interest

The authors declare that they have no conflicts of interest related to this work.

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