

# A COMPREHENSIVE REVIEW ON EXTRACTION, IDENTIFICATION AND PURIFICATION METHODS OF SECONDARY METABOLITES FROM MEDICINAL PLANTS

## Abstract

Antimicrobial resistance's rise and spread has become extremely concerning, demanding search for new and more potent antimicrobials to treat infections brought on by microorganisms that are resistant to them. Medicinal plants have been used globally because of their medicinal qualities in the treatment of numerous ailments since ancient times. In this study, we investigated the extraction techniques and phytochemical screening of medicinal plants leaves. The medicinal efficacy of plant materials is attributed to secondary products present in them, often acting in combination rather than individual compounds. The distinct medicinal properties of plants are specific to particular plant species or groups, which is consistent with the concept of taxonomically distinct combinations of secondary products. This review's findings provide important insights regarding the techniques utilized for antimicrobial activities of herbal medicinal extracts, contributing to the advancement of herbal medicine research.

**Keywords:** In this study, we investigated the extraction techniques and phytochemical screening of medicinal plants leaves.

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## I. INTRODUCTION

Traditional remedies produced from medicinal plants continue to serve 80% of those in need around the world, according to the World Health Organisation (WHO). In comparison to the 28,187 medicinal species used by humans, the expected total number of plants is around 374,000. WHO has also compiled a list of over 20,000 medical plant species and recognised medicinal plants as possible sources of new medications. Many countries have developed rules and regulations. Over 1340 plants with antimicrobial activity have been found, and more than 30,000 antimicrobial compounds have been isolated from plants. Furthermore, higher plant species are therapeutic in 14-28% of cases, and 74% of bioactive plant-derived compounds were found through ethnomedicinal uses. (Vaou, N. et al., 2021).

The medicinal plant industry in India was valued Rs. 4.2 billion during 2019 and is predicted to increase at a CAGR of 38.5% to Rs. 14 billion until 2026, according to an IBEF analysis. (IBEF, 2021). The global market worth of medical plant product surpasses \$100 billion each year. India is abundant in medicinal and herbal plant resources, which fulfill both rural India's healthcare needs and provide raw materials for the pharmaceutical industry (Kala, C.P., 2013).

Traditional or herbal medicines refer to those derived from plants with a long history of existence of safe use in several cultures at the appropriate dosages (Uzodimma, D.E., 2013). These herbal remedies are considered part of alternative and supplementary medicine (CAM) (AlQathama, A., 2016). Traditional medicine is defined by the WHO as "the comprehensive understanding, abilities, and practises rooted in cultural traditions and experiences, whether scientifically explainable or not." These practises are used to preserve health as well as to prevent, diagnose, improve, or treat physical and mental diseases. (Adeeyo, A. O. et al., 2018).

Traditional medicine has a rich heritage in India, and the traditional healthcare system has thrived for centuries. Traditional medicine in India uses twenty five thousand effective plant-based formulas, and more than 1.5 million practitioners use the traditional medicinal system for healthcare (Mukherjee, P. K. et al., 2007). The fact that phytomedicines are prescribed in Western countries for a particular illness or condition draws attention. In contrast, the formulations in the Indian medical system are suggested for a wide range of illnesses (Bhutani, K. K., & Gohil, V. M., 2010). Plants, minerals and animals all are examples of organic products that have been used for centuries to treat a wide range of disorders. Recently, pharmaceutical companies have revised their natural product work strategies in order to identify possible drug development sources and novel compounds (Süntar, I., 2020).

The major processes in generating a high-quality bioactive molecule are the selection of a suitable solvent, extraction methods, phytochemical screening procedures, separation methods, and identification techniques.

## II. EXTRACTION METHODS FOR STUDYING PHYTOCHEMICAL

Extraction procedures include maceration, digesting, decoction, infusion, percolation, Soxhlet extraction, ultrasound-assisted, and microwave-assisted extractions, among others.

For the extraction method, solvents utilized in plant extraction are Polar solvents e.g., alcohols and water, intermediate polar solvents e.g., acetone and dichloromethane; and nonpolar solvents such as n-hexane, chloroform, ether. The different methods are as follows according to Handa S. et. al., 2008; Lu M. et. al., 2017

- 1. Cold Extraction:** A plant substance that has been dried, ground, and finely powdered is mixed with appropriate solvent and keeps in shaking condition. After 5-7 days the extracts will be filtered using Whatman filter paper and then dry using hot plate or oven till it reaches to a powdered form.
- 2. Serial Exhaustive Extraction:** A solvent that changes polarity from a solvent that is non-polar (hexane) to a polar solvent (methanol) is used to assure a broad polarity range of compounds during extraction and to prepare crude extracts.
- 3. Soxhlet Extraction:** Also called as continuous hot extraction. Powdered plant product is keep in a porous bag (thimble) in the extractor. The solvent is then boiled from the round flask, followed by evaporation, and flows down to the final extraction chamber, which then reduces and extracts the therapeutic product by passing through a thimble. The extraction process is again repeated.
- 4. Maceration:** A powdered plant is soaked in the solvent container for a particular time under continuous shaking untill biomass matter is dissolved.
- 5. Decoction:** The powdered plant material is boiled, and then cooled, strained, and sufficient cold water is passed through the medicine to produce the required volume.
- 6. Infusion:** for preparation of fresh extract before use. The powdered plant materials are macerated in cold or boiling water followed by filtration.
- 7. Digestion:** The powdered plant material is macerated while being gently heated at 50°C followed by filtration.
- 8. Percolation:** The powdered plant material along with solvent is placed for around 4 hrs in a covered container. The extract is poured out drip-by-drip from the percolator. Further solvent is incorporated till the percolate is approximately three-quarters the size of the final product. The marc gets pressed and the press liquid is then added to allow it to percolate. Additional solvent is added to create a sufficient volume, and the mixed liquid is filtered by filtration or decanting.
- 9. Sonication:** This process use ultrasound technology to aid in the separation of bioactive compounds at frequencies that range from 20 kHz to 2000 kHz. This technology increases the permeability of the plant cell wall, induces cavitation, and ruptures it.
- 10. Enzymatic:** Enzymes are used during this extraction technique to soften biomass tissues and enable cell breakdown.

**11. Microwave-Assisted Extraction:** Microwave radiation generates microwave energy, which heats the liquids while enhancing extraction kinetics. When heat is given to plant cells, moisture evaporates. The heat from the microwave impact causes the cell wall to break by exerting pressure on it. Exudation occurs, which increases extraction yield.

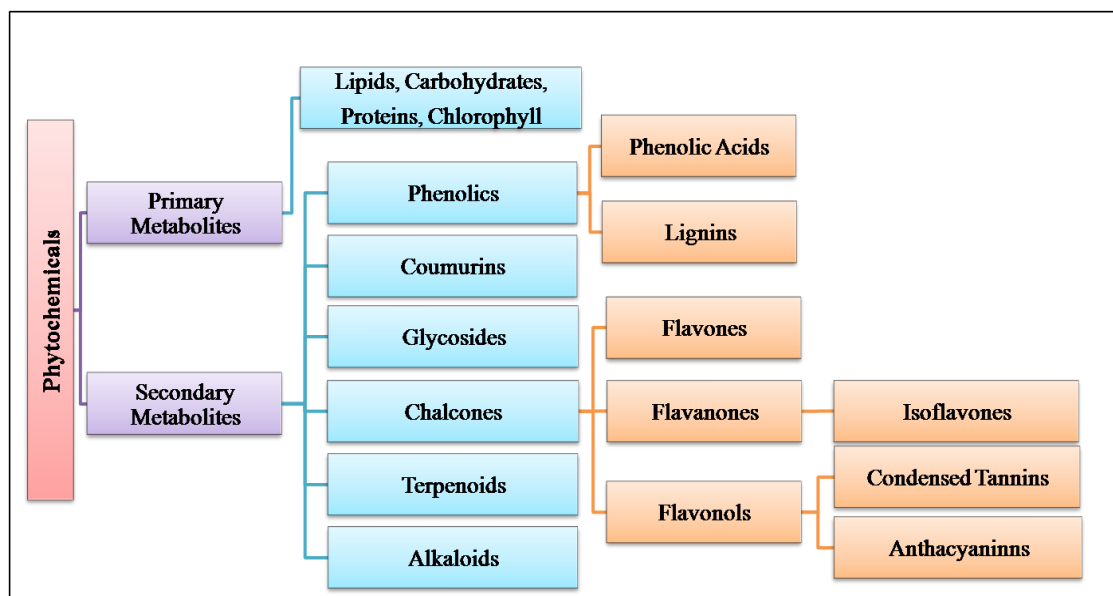
### III. PHYTOCHEMICAL ANALYSIS

Phytochemical analysis is the study of the chemical substances that plants generate and are referred to as phytochemicals. These substances frequently appear in both conventional and complementary medicine and have the potential to be therapeutic. The first stage in studying phytochemicals is extraction, which involves extracting the phytochemicals from the plant material. Phytochemicals can be extracted using a variety of procedures, including solvent extraction, microwave-assisted extraction, and supercritical fluid extraction. The type of phytochemical being extracted and the characteristics of the plant material will determine the extraction technique to be used. A medicinal plant's active compounds can be found through phytochemical analysis, which can also be used to standardize the production of herbal treatments and their quality and safety.

Phytochemicals are non-nutritional bioactive substances found in plants that have therapeutic qualities. They are secondary products from the plant's primary metabolic pathways that have indirect effect on the plant's important functions (photosynthesis, protein synthesis, etc.) and are found only in a single species or a group of related species. They are classified into three classes according to their chemical structure: Terpenes, phenolic compounds, and nitrogen compounds are examples of organic compounds. Despite not being active in the main processes of plant metabolism, phytochemicals play critical roles in plant survival and reproduction. They operate as a defence against infections and herbivores. Some of the key components of medicinal plants include alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds.

- 1. Alkaloids:** are nitrogen-containing compounds. They can prevent degenerative diseases by scavenging free radicals or binding with oxidative reaction catalysts (Roy, 2017).
- 2. Flavonoids:** class of polyphenolic compounds found in many fruits, vegetables, and medicinal plants. Due to their anti-oxidant, antiinflammatory, antimutagenic, flavonoids and anticarcinogenic qualities are crucial components in nutraceuticals, pharmaceuticals, medicine, and cosmetics. Many enzymes, including cyclo-oxygenase (COX), lipoxygenase, xanthine oxidase (XO), and phosphoinositide 3-kinase, can be inhibited by them. They can affect important cellular enzymes (Panche, 2016).
- 3. Tannins:** are water-soluble polyphenolic compounds that have the potential to reduce nutrient bioavailability in the gut as well as have some negative health effects. They do, however, contain a number of health benefits, including anti-inflammatory and antioxidant characteristics. Tannins have a wide range of pharmaceutical applications that are widely distributed in plants. They have astringent properties and are used in the treatment of diarrhea, inflammation, and bleeding (Sharma, 2019).

- 4. Terpenoids:** are Simple hydrocarbons known as terpenes are a wide family of organic molecules. Terpenoids are modified terpenes with various amounts of isoprene units, including sesterpenes, monoterpenes, diterpenes, sesquiterpenes, and triterpenes. Many terpenoids have immunobiological effects and are effective in treating a range of infectious disorders. Certain terpenoids, such Taxol and its derivatives, or malaria, like artemisinin and similar chemicals, also work well against cancer (Negi, 2020).
- 5. Phenolic compounds:** are aromatic compounds found in many plants. They have antioxidant properties and are thought to have a role in preventing chronic diseases. Examples of phenolic compounds found in caffeic acid, which is found in many plants and has antioxidant and anti-inflammatory properties (Kumar et al., 2018). The accompanying figure depicts the relationship between primary and secondary metabolism in plants (Rex JRS et al., 2018).



**Figure 1:** Primary and secondary metabolites of medicinal plant extract.

#### IV. IDENTIFICATION OF PHYTOCHEMICAL COMPOUNDS

- 1. Chromatography:** is a technique for sorting the different chemicals in a plant sample according to their physical and chemical characteristics. Thin-layer chromatography (TLC), gas chromatography (GC), and liquid chromatography (LC) are a few popular chromatographic techniques used for phytochemical analysis. Chromatography is a technique that divides the different components in a plant sample according to their physical and chemical characteristics.
- 2. Spectroscopy:** This technique uses light or other radiant radiation to identify and measure the chemical components that are present in a sample of a plant. Infrared, nuclear magnetic resonance or NMR, and ultraviolet-visible spectroscopy are a few typical forms of spectroscopy utilized for phytochemical study.

- 3. Microscopy:** In this technique, a plant sample's physical features, including the presence of specific phytochemicals, are examined under a microscope. Light, electron, and confocal laser scanning microscopy are a few typical types of microscopy utilized for phytochemical study.
- 4. Other approaches:** Sensory analysis, bioassays, and biochemical assays are other methods that can be utilised for phytochemical analysis. It's vital to remember that various techniques might work better for analysing particular phytochemical kinds. For instance, chromatography is frequently used to analyse molecules with low molecular weight, but spectroscopy is better suited to analyse molecules with higher molecular weights (Sharma, A. 2023).

## V. PURIFICATION OF THE PLANTS EXTRACT

The plant material's raw extract is frequently put through purification processes to get rid of contaminants and isolate the desired chemicals. The extract can be purified using physical method or suitable chemical methods based on the type of functional groups possessed by a compound.

- 1. Column Chromatography:** The most popular technique for removing impurities from compounds on a preparative scale is column chromatography. The process involves preparing the column, placing the collected material on it, eluting it with the mobile phase, and recovering the constituents. There are different forms of column chromatography that can be utilized depending on the stationary phases, including ion-exchange, adsorption, size exclusion chromatography and affinity. Which column type should be utilised is determined by the attributes of the target components to be divided (Jiang, T. et al., 2021).
- 2. High-Performance Liquid Chromatography (HPLC):** Polyphenols can be more precisely identified using HPLC, an improved version of liquid chromatography (LC). Its foundation is the comparison of retention times (RT) between isolated compounds and benchmarks. However, it is more expensive to purchase and operate, has lengthy downtimes, is less precise than gas chromatography (GC), and doesn't have a universally sensitive detector. HPLC can be used with mass spectrophotometry (MS) to increase the sensitivity of the procedure and generate more precise findings based on the unique mass/charge characteristics of atoms. In comparison to LC and HPLC, GC is more accurate, and when paired with MS, it delivers even more accuracy. A significant difficulty is poor compound identification with a large number of unidentified peaks (Dzah, C. S. et al., 2020).
- 3. Other chromatography techniques like Affinity, Adsorption, Ion-exchange, and partition chromatography.**

## VI. CONCLUSION

In conclusion, it is essential to determine the most effective extraction technique along with appropriate extraction solvents in connection to the final medicinal plant extract's specified purpose, such as the identification or separation of bioactive components and tests

for biological activity. It is more desirable to make plant extracts using traditional techniques like maceration extraction because it is a quick and affordable way to get thermolabile medicines. In addition to not requiring longer extraction intervals than the traditional extraction method, this fundamental method also aids in decreasing the amount of solvent needed. The majority of the key bioactive phytochemicals were discovered via phytochemical screening research, which established the therapeutic value of the various plant extracts. Higher total phenolic and flavonoid concentrations have occasionally been seen in methanol extracts compared to aqueous extracts for the assessment of total phytochemical content, and vice versa. In different plant extracts, the qualitative screening test produced varying findings. All plant extracts demonstrated a strong indication of the presence of terpenoids, steroids, and alkaloids. Similar to this, using proper extraction, identification, and purification techniques is crucial to producing a quality medicine product. The identification, isolation, and extraction of bioactive chemicals through various medicinal plants, however, is the fundamental challenge in this procedure.

**Conflicts of Interest:** The authors declare that there is no conflict of interest.

## ACKNOWLEDGMENT

This study was supported by USERC, Dehradun and Combined Institute of medical sciences & Research, Dehradun, India in the form of financial support and space for work.

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