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

Sapna Joshi1, Parmeshver Bhagat2, Rajeev Singh Kushwaha3, Ranjeet Kumar1, Deepika Biswas\*1

1Combined Institute of Medical Science & Research, Kuanwala, Dehradun, Uttarakhand- 248001

2 Department of Biotechnology, Sant Gahira Guru University, Ambikapur, Surguja (C.G.).

3Government Doon Medical College, Dehradun, Uttarakhand- 248001

**\*Corresponding Author**

Dr. Deepika Biswas

Assistant Professor

Department of Biochemistry

Combined Institute of Medical Sciences & Research

Dehradun, India 248001

E-mail: biswas.deepika89@gmail.com

Telephone no: +919968413275

**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 for their therapeutic properties in treating various diseases 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 actions of plants are specific to particular plant species or groups, which is consistent with the concept of taxonomically distinct combinations of secondary products. The findings from this review provide valuable insights into the techniques utilized for antimicrobial activities of herbal medicinal extracts, contributing to the advancement of herbal medicine research.

1. **Introduction**

According to the World Health Organization (WHO), traditional medicines derived from medicinal plants still benefit 80% of the developing world. In comparison to the 28,187 medicinal species used by humans, the total estimated number of plants is around 374,000. WHO has also recorded the names of over 20,000 medicinal plant species and identified medicinal plants as potential sources of new drugs for medicinal plants. Many countries have developed rules and regulations. Over 1340 plants have been identified as having antimicrobial action, and over 30,000 antimicrobial molecules have been extracted from plants. Furthermore, 14-28% of higher plant species are medicinal, and 74% of bioactive plant-derived chemicals were discovered based on ethnomedicinal applications **(Vaou, N. et al., 2021**).

 According to an IBEF report, the medicinal plant market in India was worth Rs. 4.2 billion (US$ 56.6 million) in 2019 and is expected to grow at a CAGR of 38.5% to Rs. 14 billion (US$ 188.6 million) by 2026 (**IBEF, 2021)**. The global market value of medical plant products 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 that have a long history of safe use in various cultures at the appropriate dosages **(Uzodimma, D.E., 2013**). These herbal remedies are considered part of complementary and alternative medicine (CAM) **(AlQathama, A., 2016**).The World Health Organization defines traditional medicine as the comprehensive knowledge, skills, and practices rooted in cultural beliefs and experiences, whether scientifically explainable or not. These practices are used for maintaining health and preventing, diagnosing, improving, or treating physical and mental illnesses (**Adeeyo, A. O. et al., 2018**).

Traditional medicine has a rich heritage in India, and the traditional healthcare system has thrived for centuries. Traditional and folk medicine in India uses 25,000 effective plant-based formulations, and more than 1.5 million practitioners use the traditional medicinal system for healthcare (**Mukherjee, P. K. et al.**, 2007). The fact that phytomedicines used in Western countries are indicated for a specific disease or condition attracts attention. This is in contrast to the Indian medical system, where the formulations are indicated for a wide range of conditions (**Bhutani, K. K., & Gohil, V. M., 2010**). Plants, animals, and minerals are examples of natural products that have been used for centuries to treat a variety of diseases. Recently, pharmaceutical companies have revised their natural product research strategies in order to identify potential sources and new molecules for drug development (**Süntar, I., 2020).**

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

1. **Extraction methods for studying phytochemical**

Maceration, digestion, decoction, infusion, percolation, Soxhlet extraction, , ultrasound-assisted, and microwave-assisted extractions etc are all examples of extraction processes. For the extraction method, solvents often utilised in medicinal plant extraction are Polar solvents (e.g., water, alcohols), intermediate polar solvents (e.g., acetone, dichloromethane), and nonpolar solvents (e.g., n-hexane, ether, chloroform). The different methods are as follows according to Handa S. et. al., 2008; Lu M. et. al., 2017

* 1. *Cold Extraction*: A dried, grinded, and finely powdered plant material is mixed with appropriate solvent and keeps in shaking condition. After 5-7 days filter the extract using Whatman filter paper and dry using hot plate or oven till it reaches to a powdered form.
	2. *Serial Exhaustive Extraction*: To ensure a broad polarity range of chemicals being extracted and to prepare crude extracts, a solvent of increasing polarity from a non-polar solvent (hexane) to a polar solvent (methanol) is utilised.
	3. *Soxhlet Extraction*: Also known as continuous hot extraction. Powdered plant material is placed in a porous bag (thimble) in the extractor. The solvent is then heated from the bottom flask, evaporates, and flows down to the extraction chamber, which then condenses and extracts the medicinal product by coming into thimble. The extraction is repeated
	4. *Maceration:* A whole or coarsely powdered plant is soaked in the solvent in a container for a period under continuous mixing until agitation until the biomass matter is dissolved.
	5. *Decoction:* The powdered plant material is brought to a boil in water, then cooled, strained, and enough cold water is passed through the medicine to produce the desired volume.
	6. *Infusion*: for preparation of fresh extract before use. The powdered plant material are macerated with either 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 approximately 4 h in a closed container. The extract is poured out drip-by-drip from the percolator. More solvent is added until the percolate is about three quarters the volume of the finished product. The marc is pressed, and the liquid from the press is added to the percolate. To obtain a sufficient volume, additional solvent is added, and the mixed liquid is purified by filtration or decanting.
	9. *Sonication*: Ultrasound technology is used in this procedure to aid in the extraction of bioactive substances at frequencies ranging from 20 kHz to 2000 kHz. Ultrasound increases cell wall permeability, causes cavitation, and ruptures the plant cell wall.
	10. *Enzymatic Extraction*: Enzymes are utilised in this extraction procedure to soften the tissues of biomass and enable cell breakdown.
	11. *Microwave-Assisted Extraction*: Microwave energy is created by microwave radiation, which heats the solvents while improving the extraction kinetics. When heat is given to plant cells, moisture evaporates. The microwave impact exerts pressure on the cell wall, causing it to burst. Exudation occurs, which increases extraction yield.
1. **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 initial step in the examination of phytochemicals is extraction, which entails isolating the phytochemicals from the plant material. Solvent extraction, microwave-assisted extraction, and supercritical fluid extraction are a few of the techniques that can be used to extract phytochemicals. 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 metabolites from the plant's primary metabolic pathways that have no direct 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 based on 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:* are a class of polyphenolic compounds found in many fruits, vegetables, and medicinal plants. Due to their anti-oxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic qualities, flavonoids are crucial components in nutraceuticals, pharmaceuticals, medicine, and cosmetics. Several enzymes, including xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase, 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, have a variety of health benefits, including antioxidant and anti-inflammatory properties. 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 monoterpenes (2), sesquiterpenes (3), diterpenes (4), sesterpenes (5), and triterpenes (6). 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 anti-inflammatory and antioxidant effects **(Kumar et al., 2018**).Here is the Relationship between primary and secondary metabolism in plants in given figure **(Rex JRS et al., 2018**).



Figure: Primary and secondary metabolites of medicinal plant extract.

1. **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 (IR), nuclear magnetic resonance (NMR), and ultraviolet-visible (UV-vis) 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).
2. **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 steps in the technique are to prepare the column, place the sample on it, elute the column with the mobile phase, and then recover the constituents. Based on the various stationary phases, there are numerous types of column chromatography that can be used, including adsorption, ion-exchange, affinity, and size exclusion chromatography. The properties of the target components to be split determine which column type should be used. Plant products is frequently separated using a technique known as adsorption chromatography, which bases the process on the distinct ways that the compounds bind to the stationary phase **(Jiang, T. et al., 2021).**
	2. *High-performance liquid chromatography (HPLC):* Polyphenols can be more precisely identified using high-performance liquid chromatography (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 Adsorption chromatography, Ion-exchange chromatography, Affinity chromatography, partition chromatography
1. **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 reducing 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 from various medicinal plants, however, is the fundamental challenge in this procedure.

**Conflicts of interest**

The authors declare that there is no conflict of interest.

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