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Phytochemical Screening of Medicinal Plants – A Review of Bioactive Compounds and Therapeutic Potentials.

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ABSTRACT

Plants contain bioactive substances called phytochemicals that have a variety of medicinal uses. Tribal people across the world use many plants, which are considered to have beneficial qualities in traditional medicine. Understanding this chemical potential as medicines depends on their discovery and measurement. The major components of phytochemicals are Alkaloids, flavonoids, Terpenoids, Saponins, Phenols, Glycosides, Steroids. In order to test the presence of these phytochemicals, plant extract is extracted from different parts of the plant by performing various extraction techniques such as maceration, percolation, Soxhlet extraction, Reflux extraction significantly. Further the collected plant extract is subjected to phytochemical screening where qualitative and quantitative analysis is acquired. Phytochemicals are used in clinical applications due to their diverse biological activities such as antimicrobial, anti-inflammatory, and anticancer properties. However, conducting phytochemical screening is accompanied by several real-time challenges, such as variability in compound composition, bioavailability, and the need for rigorous standardization and validation in research and clinical use.
Keywords: Plant extract, Secondary Metabolites, Phytochemicals, Anti – microbial, Phytochemical Screening.

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INTRODUCTION

Medicinal plants play a significant role in human health and history. Their incorporation into both conventional and contemporary medicine emphasizes how important it is to preserve and responsibly use these resources. We can guarantee that medicinal plants will continue to help future generations by researching and conserving them. In 1967, the phrase "medical plants" was coined to refer to the study of hallucinogenic plants. Any species of plant whose parts—flowers, leaves, roots, stems, fruits, or seeds—are used either directly or in a preparation as medicine to treat an illness or condition is considered a medicinal plant [1]. Various plant parts, such as bark, flowers, fruits, leaves, resins, rhizomes, roots, seeds, and stems, are utilized in traditional medicine [2]. Bioactive chemicals found in medicinal plants are utilized to treat a variety of human illnesses and are also crucial for recovery [3].

Phytochemicals are the chemical substances that are produced in plants as a result of regular metabolic processes. Phytochemical composition is typically complicated and varies depending on the plant's origin and developmental stage [4]. There are two types of phytochemicals: primary and secondary components. Proteins, sugar, amino acids, and chlorophyll are the main ingredients. Terpenoids and alkaloids are found in secondary components. Antifungal, antibacterial, and anti-inflammatory properties are found in medicinal plants. Numerous phytochemicals have a wide range of functions that can strengthen the immune system and provide resistance to chronic illnesses, shielding the body from dangerous infections. Secondary metabolism typically produces active compounds that give plant species their biological characteristics and are employed for a variety of reasons worldwide, including the treatment of infectious diseases [5].

Major Classes Of Phytochemicals

Biologically active, naturally occurring chemical compounds found in plants are said to be phytochemicals. These offer additional health benefits to humans beyond those associated with macronutrients and micronutrients. They enhance the colour, flavour, and scent of plants while shielding them from harm and disease. Generally speaking, phytochemical are plant chemicals that shield plant cells against environmental threats such pollution, stress, dehydration, UV radiation, and pathogenic invasion. It is now well established that, when their dietary intake is substantial, they play a part in protecting human health. Over a thousand phytochemicals are known to exist, and many more are unknown [6]. Phytochemicals are broadly categorized based on their chemical structures and function as listed in table 1.

Methods Of Plant Extraction

There are several methods for involved in plant extraction, they are:

Maceration: When macerating a fluid extract, whole or coarsely ground plant material is kept in contact with the solvent in a sealed container for a certain amount of time while being constantly stirred until the soluble material dissolves. When dealing with medications that are thermo labile, this approach works well. This uses water or aqueous and non-aqueous solvents, and takes longer time [14].

Decoction: This process involves boiling a crude powder extract in water for 15 minutes, chilling it, straining it, and then passing enough cold water through it to create the necessary volume in order to extract the water-soluble and heat-stable components. This uses water and takes moderate amount of time [15].

Percolation: Percolation is primarily used to extract the active chemical from plant components and is more effective than the maceration technique. This technique often makes use of a thin, cone-shaped jar that is open on both ends. Percolation is a continuous process in which new solvent continuously replaces saturated solvent. This uses water or aqueous and non-aqueous solvents, and takes longer time [16].

Reflux extraction: It is a solid-liquid extraction method that involves repeatable solvent evaporation and condensation at a constant temperature without the loss of solvent. Due to its effectiveness, ease of use, and affordability, the system is extensively utilized in the herbal industries [17].

Microwave-assisted extraction: MAE is one of the modern methods, and employed shortened time of extraction, minimal solvent consumptions, and secures thermo labile compounds. It is typically used to extract bioactive compounds from plant samples. Compared to traditional methods, this method allows for faster recovery of solutes from plant samples with significant extraction efficiency. It is recommended to use solvents with higher efficiency and dielectric factors. When performing MAE, Microwave irradiation uses an electromagnetic field frequency that is between 300 MHz and 300 GHz, which is similar to a photo chemically triggered process [18].

Soxhlet extraction: The Soxhlet extraction process combines the maceration and percolation techniques. In 1879, *Franz von Soxhlet* created the Soxhlet apparatus, a specialized piece of equipment used for the extraction. It has been and continues to be one of the most used extraction techniques [19]. Soxhlet extraction is a type of atmospheric liquid extraction, utilizing solvents at boiling temperature and low pressures, for the selective extraction of targeted compounds. The Soxhlet extraction is a technique which is simple and suitable method for extremely repetitive series of abstraction with a renewed solvent. It is done till complete extraction of the raw material taken from the solute [20, 21].

Modified Methanol extraction: It is an effective technique for removing bioactive substances from biological materials such as tissues, microorganisms, or plants. This method is used for analytical or pharmacological research, in which used to separate polar and semi-polar substances. Benefits include high extraction efficiency and analytical technique compatibility in less duration of time [22].

Methods Of Phytochemical Analysis

Chemical evaluation includes preliminary phytochemical screening. Qualitative and quantitative analysis are the two primary techniques that are employed for any evaluation. The elements can either be identified using the qualitative tests or the quantity of active ingredients present is identified using quantitative assays.

Qualitative Analysis

According to the conventional procedures outlined by Evans (1996) and Brain and Turner (1975), a preliminary phytochemical analysis was performed on the extract by chemical testing. The various chemical tests that are employed for the qualitative analysis are as follows:

Test for proteins

Many of the proteins that have been separated from medicinal plants contain antimicrobial, antioxidant, anti-HIV, anticancer, ribosome-inactivating, and neuro-modulatory properties. To test the presence of proteins the following tests can be made as listed in table 2.

Test for Carbohydrates

The basic blocks of plant and animal cells as well as living processes are powered by the energy-rich molecules known as carbohydrates. The immune system, pathogenesis, blood coagulation, fertilisation, and the folding and positioning of proteins are all significantly impacted by them. Their identification in plants is crucial for quality control and research and development because they are among the bio informative macro-molecules [28]. To test the presence of carbohydrates the following tests can be made as listed in table 3.

Test for Phenols

These are the compounds that act as defence for the plants and these have antioxidant properties. To test the presence of phenols the following tests can be made as listed in table 4.

Test for Flavonoids

These compounds are the major phytochemicals found mostly in plants and known for its property of attracting pollinators and also for protection. To test the presence of flavonoids the following tests can be made as listed in table 5.

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Test for Saponins

Saponins are the plant compounds that have foaming property and have certain medicinal benefits. To test the presence of saponins the following test can be made as listed in table 6.

Test for Alkaloids

These are the nitrogen containing compounds and have good medicinal properties and aid in plant protection. To test the presence of alkaloids the following tests can be made as listed in table 7.

Test for Terpenoids

These compounds are significant for their aromatic properties and aid in signalling properties within the plant. To test the presence of terpenoids the following test can be made as listed in table 8.

Test for Glycosides

This compound aid in molecular bonding in plants and has ample medicinal properties used in traditional medicine. To test the presence of glycosides the following tests can be made as listed in table 9.

Test for Steroids

Plant steroids are the compounds that function in hormonal signalling and help plants in environmental stress management. To test the presence of steroids the following test can be made as listed in table 10.

Table 1: List of phytochemicals and their function.

S.No	Phytochemical	Functions
1	Alkaloids	The alkaloids are the nitrogen-containing compounds known for their analgesic and anticancer properties [7].
2	Flavonoids	They have significant antioxidant properties, which are beneficial for health which reduce inflammation and combat oxidative stress [8].
3	Phenolic Acids	It has anti-aging, anticancer, antibacterial, and anti-inflammatory qualities, they are valuable commercially in the health, cosmetic, and pharmaceutical industries [9].
4	Terpenoids	Terpenoids, also known as isoprenoids, are the most numerous and structurally diverse natural products found in many plants and play a role in anticancer and antimalarial treatments [10].
5	Steroids	Steroids generated from plants, often known as Phyto steroids, have a range of pharmacological effects [11].
6	Saponins	Saponins have biologically active properties such as antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, cholesterol-lowering and anticancer [12].
7	Tannins	These have significant therapeutic potential. Their anti-oxidative, anti-diabetic, antimicrobial, anticancer, and cardio protective properties highlight their importance in health and disease prevention [13].

Table 2: Tests for Protein analysis

S.No	Test	Procedure	Observation	Reference
1	Millon's Test	2ml millon's reagent+ 1ml of plant extract (gentle heating)	A white precipitate is observed.	[23]
2	Ninhydrin Test	2ml ninhydrin reagent+ 1ml plant extract (boiling in water bath)	Purple/violet color is observed.	[24-26]
3	Xanthoproteic test	1ml plant extract+ few drops of concentrated nitric acid.	yellow color is observed	[24, 25, 27]
4	Biuret test	2ml filtrate + 1 drop of 2% copper sulphate solution + 1ml of 95% ethanol + KOH pellets.	A pink colored solution is observed. (in ethanolic layer)	[24]

Table 3: Tests for Carbohydrate analysis

S.No	Test	Procedure	Observation	References
1	Fehling's test	1ml filtrate+ 1ml each of 1ml each of fehling's solution A & B and boiled in water bath	A red precipitate is observed.	[23]
2	Molisch's test	2ml filtrate + 2 drops of alcoholic α–naphthol + 1ml concentrated H2SO4	A violet ring is observed.	[24]
3	Benedict's test	0.5ml filtrate+ 0.5ml benedict's reagent and boiled for 2 min.	Green/yellow/red color is observed.	[24]
4	Iodine test	1ml plant extract+ 2ml iodine solution	A dark blue or purple coloration is observed.	[23]

Table 4: Tests for Phenol analysis

S.No	Test	Procedure	Observation	Reference
1	Ferric chloride test	1ml of plant extract+ few drops of 5% ferric chloride solution.	Dark green/bluish black color is observed.	[24]
2	Lead acetate test	Plant extract is dissolved in 5mL distilled water + 3ml of 10% lead acetate solution.	A white precipitate is observed.	[26]

Table 5: Tests for Flavanoid analysis

S.No	Test	Procedure	Observation	Reference
1	Alkaline reagent test	1ml plant extract + few drops of dilute NaOH solution.	An intense yellow color is observed and becomes colorless on addition of diluted acid.	[29-31]
2	Shinoda test	1ml Plant extract+ 5-10 drops of hydrochloric acid + small pieces of magnesium ribbon added in tube.	Reddish pink or brown color was observed.	[32]

Table 6: Tests for Saponins analysis

S.No	Test	Procedure	Observation	Reference
1	Foam Test	1ml plant extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously.	The formation of stable foam was observed.	[23]

Table 7: Tests for Alkaloid analysis

S.No	Test	Procedure	Observation	Reference
1	Dragendorff's test	1ml sample+ 5ml water+ 2ml of hydrochloric acid until an acid reaction developed. In this add 1ml mixture of dragendorff's reagent (potassium bismuth iodine solution)	Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.	[25]
2	Wagner's test	1 ml filtrate + 1-2 drops of Wagner's reagent (Along the sides of test tube)	A brown/reddish precipitate is observed.	[23]
3	Tannic acid test	1ml plant extract + 10% tannic acid solution	A buff color precipitate is observed.	[33]

Table 8: Tests for Terpenoid analysis

Procedure	Observation	Reference
1ml of Plant extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H2SO4 was added,	A reddish-brown coloration at the interface is observed.	[23]

Table 9: Tests for Glycoside analysis

S.No	Test	Procedure	Observation	Reference
1	Salkowski's test	1ml of extract was mixed with 2ml of chloroform + 2ml of concentrated H2SO4 was added carefully and shaken gently.	A reddish brown color indicated the presence of steroidal ring.	[23]
2	Keller-Killani test	1mL filtrate + 1.5mL glacial acetic acid + 1 drop of 5% ferric chloride + conc. H2SO4 (along the side of test tube)	A blue colored solution is observed.	[30]
3	Liebermann's test	1ml plant extract + 2ml of chloroform + 2ml of acetic acid. The mixture was cooled in ice and carefully concentrated H2SO4 was added.	A color change from violet to blue to green is observed.	[23]
4	Bromine water test	Plant extract + few mL of bromine water	A yellow precipitate is observed.	[33]

Table 10: Tests for Steroid analysis

Procedure	Observation	Reference
Crude extract was mixed with 2ml of chloroform + concentrated H2SO4 was added sidewise.	A red colour produced in the lower chloroform layer is observed.	[23]

Quantitative Analysis

Thin Layer Chromatography (TLC): Thin Layer Chromatography is a technique that uses a liquid as a mobile phase and finely divided adsorbent solids or liquids dispersed across a plate to separate or identify a combination of components into individual components. Identifies compounds based on their retention factor [34].

Spectrophotometry: Spectrophotometry is that all chemical compounds have the ability to absorb, reflect, or transmit light across a particular wavelength spectrum. By measuring the light's intensity as it travels through a sample, this method calculates how much light a chemical component absorbs and determines the concentration of specific phytochemicals based on the obtained value of their wavelength respectively [35].

High-Performance Liquid Chromatography (HPLC): It is a technique that separates substances based on how they interact with the solvent of the mobile phase and solid particles in a densely packed column [36]. The analyte must be eluted via the column at high pressures of up to 400 bars before it can pass through the detector. For substances that dissolve at high temperatures or cannot be evaporated, HPLC is helpful. In a single procedure, HPLC offers both quantitative and qualitative analysis. It is widely used for precise separation and quantification [14].

Gas Chromatography-Mass Spectrometry (GC-MS): Phytochemicals can be analyzed both qualitatively and quantitatively by Gas Chromatography Mass Spectroscopy (GCMS). Gaseous, liquid, and solid materials can all be tested with GCMS [14]. To separate the analytes in gas chromatography, the sample is dissolved in a solvent and then vaporized. Two phases, a stationary phase and a mobile phase are used to disseminate the sample. A chemically inert gas, such as nitrogen, helium, etc., serves as the mobile phase. One of the special types of chromatography that interacts with the analyte without the use of a mobile phase is gas chromatography. Gas-solid chromatography (GSC) uses a solid adsorbent as the stationary phase, while gas-liquid chromatography (GLC) uses a liquid on an inert support. Thermo stability and volatility are needed for the compounds to be examined in GC [37].

Applications Of Phytochemicals In Medicine

Antioxidant properties: Phytochemicals such as flavonoids, phenolic acids, and carotenoids lower oxidative stress and stave off chronic illnesses including cancer and heart disease. Examples include curcumin from turmeric and the catechins found in green tea [38].

Anti-Inflammatory properties: Phytochemicals including terpenoids, alkaloids, and saponins can help treat a variety of ailments, including inflammatory bowel disease and arthritis. Examples include gingerol from ginger and resveratrol from grapes [39].

Anti-microbial properties: By preventing the growth of bacteria, fungi, and viruses, phytochemicals help treat infections and slow the emergence of antibiotic resistance. Examples include garlic's allicin and cloves' eugenol [40].

Anti-cancer properties: Substances like alkaloids, flavonoids, and isothiocyanates reduce tumor growth, prevent angiogenesis, and cause apoptosis. Examples include broccoli's sulforaphane and quercetin [41].

Cardiovascular Health: Polyphenols and other phytochemicals lower blood pressure, prevent atherosclerosis, and enhance lipid profiles. Anthocyanins from berries and epicatechin from cocoa are two examples [42].

Management of Diabetes: By improving insulin sensitivity and modifying glucose metabolism, phytochemicals aid in blood sugar regulation. Examples include fenugreek saponins and berberine from Berberis [43].

Neuro protective properties: By lowering oxidative damage and neuro-inflammation, phytochemicals can prevent neurodegenerative illnesses including Parkinson's and Alzheimer's. Examples include Ginsenosides from ginseng [44].

Skin Health: Phytochemicals in skincare products lessen aging symptoms, encourage healing, and shield the skin from UV rays. Green tea polyphenols and aloe vera gel are two examples [45].

Challenges In Phytochemical Analysis

Although it presents a number of difficulties, phytochemical analysis is essential for researching substances originating from plants. Many secondary metabolites, such as alkaloids, flavonoids, tannins, and terpenoids, are found in tiny concentrations in plants, which make it challenging to isolate and identify them. The extraction and quantification procedures may become more difficult due to matrix interference. Standardization variability in extraction methods (such as temperature, time, and solvent selection) impacts the yield of target chemicals and the reproducibility of results. Comparisons between studies are hampered by the absence of uniform procedures [46].

The identification processes the structural complexity and the existence of isomers, advanced methods like GC-MS, LC-MS, AND NMR demand specialized knowledge and costly equipment [47]. Accurate quantification is hampered by the lack of reference standards for many phytochemicals. Processing, genetic, and environmental factors influence plant composition, leading to variability in analysis. Since several enzymes and intermediates are involved, it is still difficult to comprehend and replicate the biosynthesis processes of phytochemicals [48]. Because of the sensitivity to environmental conditions such as temperature, PH and light, phytochemicals degrade through sample preparation and analysis. Legal and logistical obstacles are increased by the need to source plant materials ethically and to ensure adherence to biodiversity regulation [49].

CONCLUSION

Phytochemical research is crucial to maximizing the therapeutic potential of medicinal plants. Bioactive substances can now be identified and quantified with greater accuracy due to advanced analytical techniques. The wider use of these discoveries in medication development and clinical procedures, however, requires tackling issues like standardization and deterioration [46]. The type of solvent used largely determines whether a phytochemical can be extracted from the plant material. Similarly, a phytochemical's presence or absence in a sample is determined by the test used for phytochemical analysis. To obtain more reliable findings, two or more tests should be conducted [24]. Some plants are necessary for the synthesis of drugs because they provide intermediates, such as the hypothesis for steroids. The medical field makes extensive use of standardized extracts from these plants. Enhancing techniques for both qualitative and quantitative medicinal plant identification is essential for evaluating quality. By tracking harvesting and seasonal variations, phytochemical analysis increases the use of plants as a less expensive raw resource [50]. According to the study, plants contain natural antioxidants in substantial amounts, which may help protect against oxidative stress [32]. Numerous investigations have verified that the existence of these phytochemicals gives the plants under study both physiological and therapeutic qualities that aid in the treatment of various illnesses [23]. Humans have been searching for new drugs to diagnose, prevent, and treat a variety of illnesses for a very long time. It is necessary to find and create a new, potent medication from the various plant parts in order to protect them from deadly illnesses [51].

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