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REVIEW ON METHODS USED IN ISOLATIONS OF PHYTOCHEMICALS FROM MEDICINAL PLANTS

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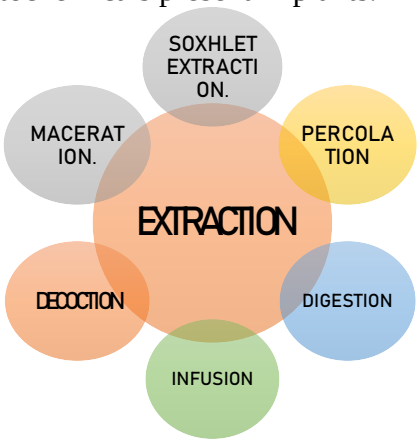
ABSTRACT

Plants contain secondary metabolites such as Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. Today, it is very crucial to develop effective and selective methods for the extraction and isolation of new natural products. Phytochemicals are now determined using a variety of contemporary techniques maceration, infusion, decoction, percolation, digestion, Soxhlet extraction, aqueous-alcoholic extraction by fermentation, supercritical fluid extraction, etc. These types of techniques are very useful in the extraction process. however, qualitative assays are still widely used for basic phytochemical screening of plants. So, for convenience, for newly researcher, the review is focusing on some important methods used to isolate phytochemicals present in plants.



KEYWORDS:- Phytochemicals, Extraction methods, solvents and medicinal plant etc**.**

INTRODUCTIONS

According to World Health Organization (WHO), over 80% of the people of developing countries are relying on the traditional medicines that are extracted from the plants for their primary health needs. Medicinal plants used for different diseases and ailments are the richest bio reservoirs of various phytochemicals. The medicinal properties of plants are determined by

their phytochemical constituents. Many parts of plants, including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains, or seeds, contain chemical components[XXVI]**.** These plants are widely used by all sections of society, whether directly as folk remedies or indirectly as a pharmaceutical preparation for modern medicine [III]**.** Because plants include essential nutrients such as protein, carbs, fats and oils, minerals, vitamins, and water, they are essential for human and animal progress and expansion. [V]**.**

Plant compounds are referred to as phytochemicals. The Greek word "Phyto" means "plant." The role of phytochemicals in plant metabolism determines whether they are classed as primary or secondary elements. Sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls, and other components of primary metabolism are essential for plant growth and development. Secondary metabolism in plants is critical for the plant's survival in its environment [XXV]. Isolation of phytochemicals is a decisive method because it depends on the polarity of the solvent used. The polarity strength of the solvents can be divided into three basic categories which include nonpolar, medium polar, and polar. Soxhlet extraction, maceration, steam distillation, and hydro distillation are some of the most commonly preferred extraction techniques because they are simple and convenient [XVII]. There are various properties of the extraction solvent which influence the extraction efficiency. Such properties may include temperature, solvent ratio, duration of extraction, etc.. Plant metabolites for curable impetus are earning admiration in today's world. The virtual momentous gradation of metabolites is the extraction and isolation of constituent of interest. Nowadays we can designate two units of extraction techniques known as conventional technology and new or green technology. One of which is cheaper and requires a high amount of solvent and acquires a long duration of time whereas the other one is costly with less duration of time respectively. Subsequently post the work of extraction of the secondary metabolites purification and isolation is done via using chromatographic and non-chromatographic techniques [XIV,XII,XIX]

STEPS FOR PLANT COLLECTIONS

1. Collection and identification of plant material.

Firstly, collected plant materials from their natural stage from the forest, wildlife centuries, or study sites. Then identify plant specimens by using available floras such as state flora, district flora, and special flora from libraries. To aid taxonomic experts in confirming or refining the field identification, and as a permanent scientific record, voucher specimens (including reproductive organs, when feasible) should be prepared and deposited in herbaria, including at least one major institution and, if applicable, in a local herbarium in the source country.

2. Drying and Grinding of Plant Materials.

Plant material should be dried at temperatures below 300C to avoid the decomposition of thermolabile compounds. Likewise, it should be protected from sunlight because of the potential for chemical transformations resulting from exposure to ultraviolet radiation.

3. Selections of Solvent.

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Low toxicity, ease of evaporation at low heat, promotion of efficient physiologic absorption of the extract, preservation activity, and inability to cause the extract to complex or dissociate all are quality of a successful solvent in plant extractions. The choice of solvent is influenced by the quality of extract and targeted compound extracted. Some of the solvents used for extraction are surmised below (table -1).

a) Water: Water is a prevalent solvent, used to extract plant products with antimicrobial properties. Plant extracts from organic solvents have been proven to have more consistent antibacterial effects than water extracts, even though traditional healers often use water. [VI].

b) Acetone: Acetone is an excellent extractant since it dissolves several hydrophilic and lipophilic components from the two plants studied, is water-miscible, volatile, and has low toxicity to the bioassay. It's very helpful in antibacterial research where a lot of phenolic chemicals need to be removed. According to a paper, phenolic and flavonoid chemicals can be easily separated in an acetone medium.

c) Alcohol: The presence of higher levels of polyphenols in ethanolic extracts than in aqueous extracts can be attributed to the higher activity of ethanolic extracts compared to aqueous extracts. It means they're more effective in breaking down nonpolar cell walls and seeds, which release polyphenols from cells. The enzyme polyphenol oxidase, which destroys polyphenols in water extracts but is inactive in methanol and ethanol, maybe a more advantageous reason for the decrease in aqueous extract activity. Furthermore, when compared to ethanol, water is a better medium for the existence of microorganisms. [XVII]

d) Chloroform: Terpenoid lactones were obtained through successive extractions of dried barks with hexane, chloroform, and methanol with activity concentrating in chloroform fraction. Occasionally tannins and terpenoids might be found within the aqueous phase, but they are more often obtained with the treatment of less polar solvents [XVI].

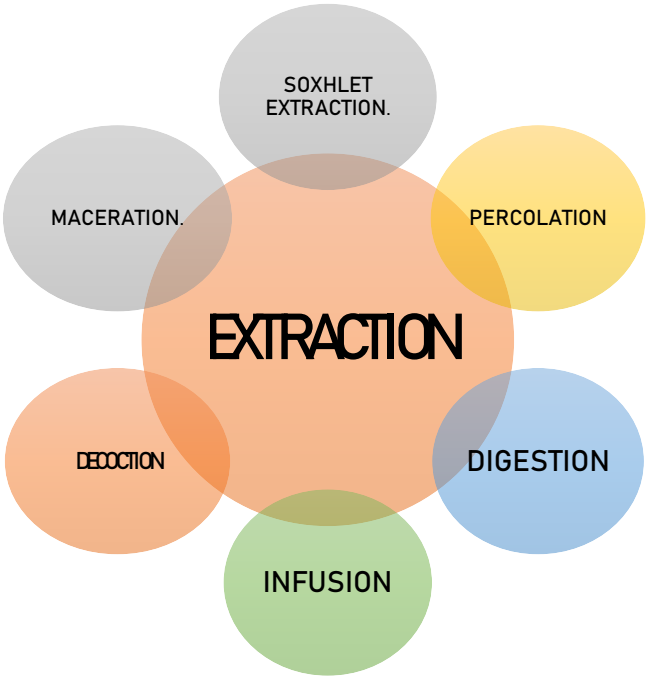
Ether: Ether is normally used selectively for the extraction of coumarins and fatty acids [XVI].

Water	Ethanol	Methanol	Chloroform	Ether	Acetone
Anthocyanins	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonols	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxyllines			
Polypeptides	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			
		Lactones			
		Flavones			
		Phenones			
		Polyphenols			

Table -1 Solvent used for active component extractions.

EXTRACTION PROCESS.

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and photonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques (water distillation, steam distillation, water, and steam distillation), hydrolytic maceration followed by distillation, expression, and enfleurage (cold fat extraction) may be employed. [X].Some of them elaborate below.



1. Soxhlet extraction

Soxhlet extraction is only required where the desired compound has limited solubility in a solvent, and the impurity is insoluble in that solvent [XXI]. It's a continuous liquid/solid extraction. The solid item to be removed is placed in a thimble constructed of a material that can hold solids while only allowing liquids to pass through it (It acts as a filter paper). After that, the thimble is inserted into the extractor. The organic solvent is then heated in a reflux system, causing the vapors to boil. As the vapor rises, it is further condensed by the condenser, filling up the thimble. This procedure is repeated until all of the materials to be removed from the solids have been extracted [XIII].

2. Maceration.

The whole or coarsely powdered plant medicine is kept in contact with the solvent in a stoppered container for a set period (at least 3 days) with regular agitation until the soluble matter is dissolved in maceration (for fluid extract). After standing, the mixture is strained, the Marc (damp solid material) is crushed, and the mixed liquids are purified by filtration or decantation. This approach is best used when dealing with thermolabile medicines. [22,1].

3. Decoction

This method is used for the extraction of the water-soluble and heat-stable constituents from the crude drug by boiling it in water for 15 minutes, cooling, straining, and passing sufficient cold water through the drug to produce the required volume. [XX,XXII]. The sample is boiled in a specified volume of water for a defined time (15 to 60 minutes.) It is then cooled, strained, filtered, and added enough water through the drug to obtain the desired volume. This method is suitable for extracting thermostable (that does not modify with temperature) and water-soluble compounds, and hard plant materials and commonly resulted in more oil-soluble compounds than maceration

4. Infusion

Infusion is a simple chemical process used to extract plant material that is volatile and dissolves readily or releases its active ingredients easily in organic solvents [XX]. It is a dilute solution of the readily soluble components of crude drugs. Fresh infusions are prepared by macerating the solids for a short period with either cold or boiling water [XIX, I]. Fresh infusions are

prepared by macerating the crude drug for a short time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

5. Digestion

This is a type of maceration in which the maceration extraction process is carried out with the use of moderate heat. It's employed when a moderately raised temperature isn't bothersome and the menstrual solvent efficiency is improved as a result [XX]**.** It's employed when a moderately raised temperature isn't bothersome and the menstrual solvent efficiency is improved as a result [XIX]. The most common temperatures are between 35 and 40°C, with a maximum temperature of 50°C. The plant portion to be extracted is placed in a container with the pre-heated liquid at the prescribed temperatures, and the container is shaken periodically for a time ranging from half an hour to 24 hours. This process is used for the herbal material or plant parts that contain poorly soluble substances or polyphenolic compounds.

6. Percolation.

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. The plant material is taken in a percolation tube plugged with cotton or fitted with a filter and a stopcock [XVII]**.** A solvent is added to the plant material and allowed to stand for approximately 4 hours in a well-closed container, after which the mass is packed and the top of the percolator is closed. The whole system is kept for 24 hours at room temperature and the solvent along with the extracted material is collected by opening the stopper below and mixed liquid is clarified by filtration or by standing followed by decanting [XI].

7. Sonication

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like the extraction of rauwolfia root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through the formation of free radicals and consequently undesirable changes in the drug molecules [X].

PHYTOCHEMICAL ANALYSIS (QUALITATIVE AND QUANTITATIVE)

8. Gas Chromatography.

It is an analytical technique for separating compounds based primarily on their volatilities. GC provides both qualitative and quantitative information for individual compounds present in a sample. The gas phase is flowing and the liquid phase is stationary. The rate of migration for the chemical species is determined through its distribution in the gas phase. For example, a species that distributes itself 100% into the gas phase will migrate at the same rate as the flowing gas, whereas, a species that distributes itself 100% into the stationary phase will not migrate at all. Species that distribute themselves partly in both phases will migrate at an intermediate rate [VII]. Gas chromatography involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is then transported through the column by the flow of the inert, gaseous mobile phase. The column itself contains a liquid stationary phase, which is adsorbed onto the surface of an inert solid.

9. High-Performance Liquid Chromatography: (HPLC)

It is a versatile, robust, and widely used technique for the isolation of natural products. HPLC is an analytical technique for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial, etc. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of medicinal plants. To identify any

compound by HPLC, a detector must first be selected, the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase. Modern HPLC uses a non-polar solid phase, like C18, and a polar liquid phase, generally a mixture of water and another solvent. High pressure of up to 400 bars is required to elute the analyte through a column before they pass through a diode array detector (DAD). A DAD measures the absorption spectra of the analytes to aid in their identification. HPLC is useful for compounds that cannot be vaporized or that decompose under high temperature and it provides a good complement to gas chromatography for the detection of compounds [XXIII].

10. High-Performance Thin Layer Chromatography: (HPTLC)

It is a planar chromatography where separation of natural compounds is achieved on high-performance layers with detection and data acquisition. These high-performance layers are pre-coated plates coated with a sorbent of particle size 5-7 microns and a layer thickness of 150-200 microns. The reduction in thickness of layer and particle size results in increasing the plating efficiency as well as the nature of separation. HPTLC plates are substantially more expensive (4- to 6-times more) than normal plates but are an efficient alternative when high sensitivity, accuracy, and precision are required in situations demanding high performance [XV].

11. Optimum Performance Laminar Chromatography: (OPLC)

OPLC is a revolutionary parallel chromatography concept that combines the benefits of both TLC and HPTLC. OPLC is a versatile analytical and preparative instrument that can be used in research and quality control labs. It's a versatile liquid chromatography separation technique that combines HPLC's user-friendly interface and resolution with flash chromatography's capacity and TLC's multidimensionality. OPLC works on the same principle as other chromatographic procedures in that it uses a pump to drive a liquid mobile phase through a stationary phase, such as silica. Flat planar columns can be used in the same way as cylindrical glass or stainless-steel columns thanks to the OPLC column housing construction. The mobile and the flat column are both pressured to 50 bars. [II].

12. Ultraviolet/Visible (UV/VIS) Spectroscopy

UV-Visible Spectroscopy UV-visible spectroscopy can be performed for qualitative analysis and identification of certain classes of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range. Natural compounds can be determined by using UV-visible spectroscopy. Moreover, spectroscopic UV-Vis techniques were found to be less selective and give information on the composition of the total polyphenol content. This technique is not time-consuming and presents a reduced cost compared to other techniques [VII].

13. Thin-Layer Chromatography

TLC is the most commonly used planar chromatographic method in natural product research. This is the easiest and cheapest technique and can be applied in the analysis, isolation, and setting of the parameters for column chromatography [IV].

The sample is applied to the surface first, and then the solvent is absorbed onto the plate's surface. This method can be used to keep track of the process, identify components, and check the purity of the compound. Polar compounds that are more polar have significant interlinking with the motion phase, whereas less polar elements spread to the surface's highest point. If the motion phase is replaced with a more polar chemical or solvent, it will be more effective at dispelling analytes from silica gel and will shift to a higher resolution. [XXIV].

14. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier- transform infrared spectroscopy is a valuable tool for the identification of functional groups present in the plant extract. It helps in the identification and structure determination of

the molecule. It is a high-resolution analytical tool to identify the chemical constituents and elucidate the structural compounds. FTIR offers a rapid and non-destructive investigation of fingerprint herbal extracts or powders [VII&VIII].

15. Nuclear Magnetic Resonance Spectroscopy (NMR)

Magnetic Resonance of Nuclei Physical, chemical, and biological aspects of the matter are determined through spectroscopy. The one-dimensional approach is commonly employed, however two-dimensional NMR techniques could be applied to achieve the intricate structure of the molecules. The molecular structure of solids has been determined using solid-state NMR spectroscopy. The types of carbon contained in the molecule are identified using radiolabeled 13C NMR. 1 H-NMR is utilized to determine the types of hydrogen contained in a chemical as well as the connections between the hydrogen atoms. [VII&VIII]

16. Mass Spectroscopy

Mass spectrometry is a strong analytical technique for determining the structure and chemical characteristics of molecules, as well as identifying novel compounds and quantifying known chemicals. The molecular weight of the sample can be determined using the MS spectrum. This method is commonly used to deduce the structure of organic compounds, sequence peptides or oligonucleotides, and monitor the presence of previously identified compounds in complex mixtures with high specificity by simultaneously determining the molecular weight and a diagnostic fragment of the molecule. [VII&VIII].

CONCLUSION

Plants are a rich source of phytochemicals, which are used to make drugs and medicines. These phytochemicals have antibacterial, antifungal, anti-cancer, antioxidant, anti-inflammatory, and anti-diabetic properties, among others. The instruments of phytochemical analysis are used to identify this molecule, thus familiarity with these procedures is essential. This article will assist you in collecting, identifying, extracting, and analyzing phytochemicals taken from plants. The methodologies used in this research should be conventional, as using non-standard protocols could result in inaccurate and unreplicable results. The preceding information will assist with phytochemical qualitative analysis.

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