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EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF BIOACTIVE COMPONENTS FROM MEDICINAL PLANTS: A REVIEW

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Abstract:

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds. Bioactive principles are responsible for the therapeutic activities of medicinal plants and provide unlimited opportunities for new drug leads because of their unmatched availability and chemical diversity. For the most part, the beneficial or toxic outcomes of standardized plant extracts depend on the chemical peculiarities of the containing bioactive principles. The focus of this paper is on the analytical methodologies, which include the extraction, isolation and characterization of active ingredients in botanicals and herbal preparations. The common problems and key challenges in the extraction, isolation and characterization of active ingredients in botanicals and herbal preparations are discussed. As extraction is the most important step in the analysis of constituents present in botanicals and herbal preparations, the strengths and weaknesses of different extraction techniques are discussed. The analysis of bioactive compounds present in the plant extracts involving the applications of common phytochemical screening assays, chromatographic techniques such as HPLC and, TLC as well as non-chromatographic techniques such as immunoassay and Fourier Transform Infra Red (FTIR) are discussed.

Key words: *Bioactive principles; Bioassay; Herb extraction; Herb medicinal value; Herb toxicity; Phytomedicine, Chromatography, FTIR.*

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1. INTRODUCTION:

Natural products, such as plants extract, open a new horizon for the discovery of new therapeutic agents [1] . The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed and about 80% of the world's population relies on herbal medicines [2] . Plants contain a wide range of chemical compounds that can be used to treat chronic as well as infectious diseases [3]. Microbial resistance to the chemically synthesized drugs compelled us to move towards the ethnopharmacognosy. They found literally thousands of phytochemicals proved beneficial and have biological activity such as anticancer, antimicrobial, antioxidant, ant diarrheal, analgesic and wound healing activity were reported. This paper mostly highlighted on the analytical methodologies, which includes the extraction methods and the analysis and identification of bioactive compounds present in the plant extracts through the various techniques involving the applications of chromatographic techniques and some detection methods.

1.1. Extraction methods for studying phytochemicals:

Extraction from the plant is an empirical exercise since different solvents are utilized at varying conditions such as time and temperature of extraction. As bioactive components extracted from the plants further their separation from co extractives compounds is essential. Further fractionation of extracted compounds done on the basis of their acidity, polarity or molecular size. The extraction methods mostly used has been discussed below:

1.2 Cold extraction method: The different plants parts dried in an artificial environment at low temperature (50-60 °C) and dried powder then further used for extraction purpose using various solvents. Weigh the dried powder and added into conical flask with respective solvents and allow keeping at room temperature for thirty minute shaking after each twenty four hours for seven days. Finally filter the extract using whatman filter paper under vacuum and dry it at room temperature in watch glass dish. Note down the weight of each dish prior to drying of the extracts and after drying too. Calculate the weight of the extract from the difference [4] .

1.3 Solvent extraction method: Universal Extraction System (Buchi) is recently used for solvent extraction. The dried powder of various plant parts placed in glass thimble for extraction purpose using various solvents. The procedure is carried out for 10 cycles for each extract and adjusts the temperature just below the boiling point of the respective solvents. The resulting solvent extract is filtered, concentrated in vacuum concentrator and used to determine the presence of phytoconstituents [4].

1.4 Supercritical fluid extraction (SFE): Supercritical Fluid Extraction (SFE) involves use of gases, usually CO2, and compressing them into a dense liquid. This liquid is then pumped through a cylinder containing the material to be extracted. From there, the extract-laden liquid is pumped into a separation chamber where the extract is separated from the gas and the gas is recovered for re-use. Solvent properties of CO2 can be manipulated and adjusted by varying the pressure and temperature. The advantages of SFE are, no solvent residues left in it as CO2 evaporates completely [5].

1.5 Microwave-assisted extraction (MAE): It simply termed as microwave extraction, that combines microwave and traditional solvent extraction. Heating the solvents and plant tissue using microwave increases the kinetic of extraction, is called microwave-assisted extraction [6] . The target for heating in dried plant material is the minute microscopic traces of moisture that occurs in plant cells. The heating up of this moisture inside the plant cell due to microwave effect, results in evaporation and generates tremendous pressure on the cell wall. The cell wall is pushed from inside due to the pressure and the cell wall ruptures. Thus the exudation of active constituents from the ruptured cells occurs, hence increasing the yield of phytoconstituents [7, 8].

2. IDENTIFICATION OF PHYTOCHEMICALS:

Plant extracts contains various type of bioactive compounds having different polarities their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds using different separation techniques such as TLC, HPTLC, paper chromatography, column chromatography, Gas chromatography, OPLC and HPLC, should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity [9].

2.1 Chromatography techniques: Chromatography is a technique where the molecules are separated based on their size, shape and charge [10] . During chromatography analyte in solvent and move through solid phase that acts as a sieving material. As molecule proceeds further through molecular sieve it gets separated. Paper and thin layer chromatography

are the chromatographic techniques which readily provides qualitative information and through which it become possible to obtain quantitative data.

2.1.1 Adsorption chromatography: Adsorption chromatography also termed as displacement or liquid/solid chromatography and is based on interactions between the solute and fixed active sites on the stationary phase. The active sites of the stationary phase interact with the functional groups of compounds to be separated by noncovalent bonds, non-polar interactions, van der Waals forces and hydrophobic interactions. The compounds which are loosely bound will be eluted out firstly by the mobile phase at and classes of compounds can be separated.

2.1.2 Partition chromatography: In partition chromatography the molecules to be separated will interact between two immiscible liquid phases according to their relative solubility. This process is also referred as liquid/liquid chromatography.

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2.1.3 Ion-exchange chromatography:
Ion-exchange chromatography allows the separation of ions and polar molecules on the basis of electrical properties of the molecules [11] .

2.1.4 Affinity chromatography: In affinity chromatography, separations are based on the specific interactions between interacting pairs of substances such as macromolecules and it's substrates, cofactor, allosteric effector or inhibitor. During this chromatography, a mixture of substances applied to the columns. Substances that have no affinity with the ligand are washed through with the buffer and desired compound is bind to ligand. Buffer having different pH or an increased ionic strength is used to elute the analyte out.

2.1.5 Size exclusion chromatography: It also termed as gel filtration, gel permeation chromatography and molecular sieve chromatography. In this chromatography, no chemical attraction or interaction occurs between the solutes and stationary phase and the molecules are separated according to their size.

2.1.6 Paper chromatography: In paper chromatography a sheet of paper is used for the inert phase. One of the advantages of paper chromatography is that separations are carried out simply on sheets of filter paper, which acts as both support as well as medium for separation [12] . Another advantage is the considerable reproducibility of Rf (retention factor) values determine on paper. In paper chromatography, filter paper used as solid phase, which is inert phase. A sample is placed near the bottom of the filter paper. Then this filter paper is placed in chromatographic chamber with solvent. The solvent move forwards by capillary action carrying soluble molecules along with it. Low porosity paper will produce a slow rate of movement of the solvent and thick papers have increased sample capacity [13] .

2.1.7 Thin layer chromatography (TLC): The first practical application of thin layer chromatography was given by Stahl [14] . Compared to paper chromatography, the special advantage of TLC is the versatility, speedy and sensitive. TLC is an adsorption chromatography [15] where samples are separated based on the interaction between a thin layers of adsorbent attached on the plate. The technique mostly employed for the separation of low molecular weight compounds.

2.1.8 Column chromatography (CC): Column chromatography involves ion exchange, molecular sieves, and adsorption phenomenon. The flushing in conventional chromatography greatly dilutes the material, and the fractions usually require another step for concentration. A newer method called displacement chromatography elute with some compounds that has great affinity for the adsorbent. Fractions of elute materials can be more concentrated than the original solution applied to column.

2.1.9 Gas chromatography (GC): Gas chromatography is a method for the separation of volatile compounds [16] . In this method, species distribute between gas and a liquid phase. The gas phase is flowing and the liquid phase is stationary. The rate of migration for the chemical species is determined through it's distribution in the gas phase. For example, a species that distributes itself 100% into gas phase will migrate at the same rate as the flowing gas, whereas, a species that distributes itself 100% into stationary phase will not migrate at all. Species that distribute themselves partly in both phases will migrate at an intermediate rate [17] . 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 inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

2.1.10 High performance liquid chromatography (HPLC): 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. [18] . The another name for HPLC is high – pressure liquid chromatography, separates compounds on the basis of their interactions with solid particles of tightly packed column and the solvent of the 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 up to 400 bars is required to elute the analyte through 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 detection of compounds [19] .

2.1.11 High performance thin layer chromatography (HPTLC): High performance thin layer chromatography (HPTLC) is a planar chromatography where separation of sample components 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 plate efficiency as well as nature of separation. HPTLC gives chromatogram i.e. separated samples after chromatography can be inspected by the eyes only in case of HPTLC.

2.1.12 Optimum performance laminar chromatography (OPLC): It is a new concept in parallel chromatography; OPLC combines the advantages of both TLC and HPTLC. OPLC is both an analytical and preparative tool, suitable for research and quality control laboratories. OPLC is a powerful liquid chromatography separation technique that combines the userfriendly interface and resolution of HPLC with the capacity of flash chromatography and multidimensionality of TLC. The basis of OPLC is similar to that of other chromatographic techniques in that a pump is used to force a liquid mobile phase through a stationary phase, such as silica or a bonded phase medium (C8, C18, amino, cyano, diol and ion exchange). The OPLC column housing structure allows flat planar columns to be used in the same way as cylindrical glass or stainless steel ones. The flat column is pressurized up to 50 bars and mobile phase is forced through it at constant linear velocity via a solvent delivery pump. The workstation includes all of the modules required for effective separation of compounds of interest. Phytochemicals are nothing but the large number of secondary metabolic compounds found in plants. The different chemical compounds which are present in plant extracts have been identified through different methods of detection which are discussed as follows and these are important tools in bioactive compound analyses.

3. METHODS OF DETECTION:

3.1 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 for identification and structure determination of the molecule [20, 21] . Samples for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride. The drop forms a thin film between the plates. Solid samples can be milled with potassium bromide (KBr) to and then compressed into a thin pellet which can be analyzed. Otherwise, solid samples can be dissolved in a solvent such as methylene chloride, and the few drop of solution is then placed onto a single High Attenuated Total Reflectance (HATR) plates and spectra was recorded in terms of percentage transmittance. The peaks at specific wave number were assigned by bonding and functional group as per the reference given in Varian FTIR instrument manual [22] .

3.2 Nuclear Magnetic Resonance Spectroscopy (NMR): Nuclear Magnetic Resonance Spectroscopy gives physical, chemical and biological properties of matter. One dimensional technique is routinely used but the complicated structure of the molecules could be achieved through two dimensional NMR techniques. Solid state NMR spectroscopy is used for the determination of molecular structure of solids. Radiolabelled [13] C NMR is used to identify the types of carbon are present in the compound. [1] H-NMR is used to find out types of hydrogen are present in the compound and to find out how the hydrogen atoms are connected.

3.3 Mass spectrometry (MS): Mass spectrometry is a powerful analytical technique for the identification of unknown compounds, quantification of known compounds and to elucidate the structure and chemical properties of molecules. Through MS spectrum the molecular weight of sample can be determined. This method mostly employed for the structural elucidation of organic compounds, for

peptide or oligonucleotide sequencing and for monitoring the existence of previously characterizes compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously.

4. CONCLUSION:

Since, bioactive compounds occurring in plant material consist of multi-component mixtures, their extraction, identification and determination still creates problems. Practically most of them have to be purified by the combination of several chromatographic techniques and various other purification methods to isolate bioactive compound (s).

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