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PHYTOCHEMISTRY: RECENT TRENDS AND APPLICATION

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ABSTRACT

Phytochemistry is the study of phytochemicals, which are chemicals derived from plants. Phytochemicals present in medicinal herbs and spices have used as natural remedies against illness. The proper understanding of phytochemical is essential for drug discovery and the development of novel therapeutic agents against diseases. The extraction procedures are vitally important in the analysis of phytochemicals. There are some conventional extraction method and advanced extraction method. The modern development in the instrumental techniques of analysis and chromatographically methodologies has added numerous complex and rare natural products to the armoury of phytomedicine. This paper mainly deals with the new methods used in phytochemistry for the extraction of phytochemicals and their application.

Keywords: Phytochemicals, Extraction, Chromatography, Quantitative analysis

INTRODUCTION

Phytochemistry is the study of the chemicals produced by plants, particularly the secondary metabolites. Phytochemicals synthesised for self-defence against insects, pests, pathogens, herbivores, UV exposure and environmental hazards. Phytochemistry is concerned with two aspects the study of the chemical composition of plants and the explanation of various plant processes in which chemical phenomena are concerned. The first part includes the qualitative detection of plant component, the actual isolation of plant component and the qualitative estimation of plant component

without isolation. The study of prior trends and impact on research on phytochemistry of medicinal plants is desirable. The extraction procedures are vitally important in the analysis of phytochemicals. Maceration, percolation and soxhlet extraction methods prominently used in phytochemical screening studies. But there are some advanced methods such as supercritical fluid extraction (SFE), microwave-assisted (MAE), ultrasound-assisted extraction (UAE) and accelerated solvent extraction **[1]**.

Techniques For Screening of Phytochemicals From Plants

Extraction methods

1. Maceration

A whole or coarsely powdered crude drug is allowed to contact the solvent. The powder kept in a stoppered container for a particular period with frequent agitation **[2]**. In the end, the solvent drained, and the remaining miscella removed from the plant material through pressing or centrifuging. Maceration is not an advanced technique since active ingredients cannot be extracted **[3]**.

2. Percolation

Percolation is used to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator which has a narrow cone shaped vessel open at both ends is used for this technique **[4]**. The solid crude plant material are moistened with a suitable quantity of the specific solvent and permitted to stand for around 4 h in a well stoppered container, after that the mass is packed and the top of the percolator is closed. Supplementary solvent is added to form a shallow layer above the mass, and the mixture is allowable to macerate in the closed percolator for 24 h. The vent of the percolator then is opened and the liquid contained therein is allowed to drip slowly. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting **[5]**.

3. Soxhlet extractions

Soxhlet extraction is only essential where the preferred compound has partial solubility in a solvent, and the impurities are insoluble in that solvent. If the suitable content has a high solubility in a solvent then a simple filtration can be used, separate the compound from the insoluble substance. In this process, the finely powdered crude drug placed in a thimble. Then it placed in the chamber of the Soxhlet apparatus. The extracting solvent is heated, and its vapours condensed in a condenser. Condensed extract drips into a thimble containing the crude drug, and extracts it by contact. When the level of liquid in the chamber rises to the top of the siphon tube, the liquid contents of the

chamber siphon into the flask. Procedure is continuous and carried out until a drop of solvent from the siphon tube does not leave a residue when evaporated **[6]**.

4. Decoction

In this the crude drug is boiled in specific amount of water for 15 minutes. Then it is cooled and strained and passed sufficient volume of cold water through the drug to produce the required volume. This method is appropriate for extracting water-soluble, heat-stable constituents. For preparation of Ayurvedic formulation 'quath' this method is used **[7]**.

5. Digestion

This is a type of maceration in which mild heat is applied during the maceration extraction process. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstruum is increased thereby **[8]**.

6. Infusion

It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period of time with either cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs **[8]**.

7. Plant tissue homogenization

The fresh plant, dried or wet parts are grind in a blender to fine particles, placed in a certain quantity of solvent and shaken vigorously for 5 - 10 min or left for 24 h after which the extract filtered. The filtrate may be dried under reduced pressure and redissolved in the solvent to determine the concentration **[9]**.

Quantitative Analysis

Qualitative and quantitative analysis of phytochemicals can be done using gas chromatography, mass spectroscopy (GC-MS). GC-MS can be applied to solid, liquid and gaseous samples. First the samples are converted into gaseous state then analysis is carried out on the basis of mass to charge ratio. High Performance Liquid Chromatography is applicable for compounds soluble in solvents. High performance thin layer chromatography is applicable for the separation, detection, qualitative and quantitative analysis of phytochemicals.

1. Gas liquid chromatography

Gas liquid chromatography separates volatile substances by percolating a gas stream over stationary phase. The basis of separation in GLC is the partitioning of the sample in and out of the film of liquid spread over an inert solid. GLS is the most selective and versatile form of gas chromatography since there exists a wide range of liquid phases usable upto 450°C. the important applications of GLC include examination of many volatile oil, plant acids, alkaloids of opium, tobacco, conium and belladonna; the resins of cannabis, steroidal compounds, cardioactive

glycosides and aglycones, sugar and amino acids.

A drug containing carboxylic acid or primary amine functional groups gives tailing peaks due to interaction of the functional groups with stationary phase. This is overcome by the problem faced by these compounds, special technique such as pyrolysis GC, photolysis GC **[10]**.

2. High Performance Liquid Chromatography: (HPLC)

High performance liquid chromatography (HPLC) is widely used by chromatographers and by the pharmaceutical industry for the accurate and precise analysis of chemicals and drugs of diverse nature. The systematic scale-up from analytical to preparative and process scale and further scale-up to industrial scale can be used in the medicinal and aromatic plant industry for the isolation and purification of phytomolecules of therapeutic and commercial interest. Due to the gradual increase in the demand for phytomolecules, the importance of process-scale HPLC as a purification tool has been increasing. Drugs like morphin, papaverine, codeine; emetine, antibiotics, ergot alkaloid, cardiac glycosides, sennosides, and capsaicin are analyzed by HPLC.

Separation of chemical compounds is carried out by passing the mobile phase, containing the mixture of the components, through the stationary phase, which consists of a column packed with solid particles. The cause for retention is physical and chemical forces acting between the solute and the two phases, on the chromatographic column. The reason for retention is the difference in the magnitude of forces; this results in the resolution and hence separation of the individual solutes. The separation of compounds occurs by distribution of solutes between the two phases **[11]**.

3. High Performance Thin Layer Chromatography: (HPTLC)

High Performance Thin layer Chromatography is a modified version of thin layer chromatography. High Performance Thin layer Chromatography is planer chromatography where separation of sample components is done on high performance layers with detection and acquisition using an advanced work-station. These high performance layers are pre-coated with a sorbent of particle size 5-7 microns and a layer thickness of 150-200 microns. The reduction in the thickness of the layer and the particle size results in increasing the plate efficiency along with nature of separation. HPTLC is suitable for qualitative, quantitative and micro-preparative chromatography.

HPTLC is one of the most widely applied methods in phytochemical analysis. It is due to its numerous advantages, e.g., it is the only chromatographic method offering

the option of presenting the results as an image. Other advantages include simplicity, low costs, parallel analysis of samples, high sample capacity, rapidly obtained results, and possibility of multiple detection. HPTLC provides identification as well as quantitative results. It also enables the identification of adulterants. In case of complex samples, the resolving power of traditional one-dimensional chromatography is usually inadequate; hence special modes of development are required **[12]**.

4. Optimum Performance Laminar Chromatography: (OPLC)

OPLC combines the advantages of TLC and HPLC. The system separates about 10-15 mg samples, with simultaneous processing of up to 4 or 8 samples at a time depending on the model. In OPLC a pump is used to force a liquid mobile phase through a stationary phase, such as silica or a bonded-phase medium. 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 work station includes all of the modules required for effective separation of the compound sample of interest, including two 96- well plate sample holders and automated sampling system that withdraws a sample from each well and places it on the OPLC planar sorbent bed, a solvent delivery system including a mobile phase degasser and pump, OPLC purification unit, a four channel diode array detector to monitor the eluent and fraction collector to six 96- well plates to hold the separated compounds **[13]**.

METHODS OF DETECTION

Spectroscopy is used in the detection of phytochemicals.

1. UV Spectroscopy

Ultraviolet and visible spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Ultraviolet radiation is energetic enough to promote outer electrons to higher energy level and UV spectroscopy is usually applied to molecules or inorganic complexes in solution. This results from transition between the electronic energy levels. Measuring the absorbance at some wavelength by applying Beer- Lambert's law can determine the concentration of the analyte solution. It is useful to characterize the absorption, transmission and reflectivity of a variety of important materials such as pigments and other compounds from plants. This qualitative application requires recording at least a portion of the UV-Visible spectrum for characterization of the

optical or electronic properties of materials **[14]**.

2. IR Spectroscopy

IR spectroscopy is used to determine the functional group present in the sample. Infrared absorption spectroscopy is the measurement of the wavelength and intensity of the absorption of mid-infrared light by a sample. Mid-infrared light is energetic enough to excite molecular vibrations to higher energy levels. The wavelength of many IR absorption bands are characteristics of specific types of chemical bonds, and IR spectroscopy finds its greatest utility for qualitative analysis of organic and organometallic molecules. IR spectroscopy is used to confirm the identity of a particular compound and as a tool to determine the newly synthesized molecule **[14]**.

3. Mass Spectroscopy

Mass spectroscopy is a powerful tool for the identification of materials. Mass spectrometry has become one of the most important tools in the biochemical sciences with capability ranging from small molecule analysis to protein characterization. Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds, to quantify known compounds and to elucidate the structure and chemical properties of molecules. The molecular weight of sample can be determined from MS Spectrum. Structural information can also be generated from certain types of mass spectrometers. This procedure is useful for the structural elucidation of organic compounds, for peptide or oligonucleotide sequencing and for monitoring the existence of previously characterize compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously **[14]**. The complexity of the extraction mixture determines the proper quantitative device. Regular chromatographic and CE detectors can normally be used for all but the most complex samples, for which mass spectrometry (MS) should be applied **[15]**.

4. Nuclear Magnetic Resonance Spectroscopy: (NMR)

Nuclear Magnetic Resonance Spectroscopy gives physical, chemical and biological properties of matter. Chemists to study chemical structure using simple one-dimensional techniques routinely use NMR spectroscopy. Two dimensional techniques are used to determine the structure of more complicated molecules. These techniques are replacing X-ray crystallography for the determination of protein structure. Time domain NMR spectroscopic techniques are used to probe molecular dynamics in solutions. Solid state NMR spectroscopy is used to determine the molecular structure of solids.13C- NMR is used to identify the

types of carbon are present in the compound. 1H- NMR is used to find out types of hydrogen are present in the compound and to find out how the hydrogen atoms are connected.

Although this technique is usually associated with structure-determinations of organic compounds the use of 1 H-NMR spectroscopy has been described for the assay of atropine and hyoscine in extracts of belladonna, hyoscyamus and stramonium. It has also been used for the quantitative determination of strychnine and brucine in Strychnos nux-vomica, affording a number of advantages over other methods **[16]**.

5. X-Ray Crystallography

x-ray crystallography is an experimental technique that exploits the fact that X- ray are diffracted by crystals. X- rays have the proper wavelength(in the Angstrom range 10-8) to be scattered by the electron cloud of an atom of comparable size. Based on the diffraction pattern obtained from X-Ray scattering off the periodic assembly of molecules or atoms in the crystal, the electron density can be reconstructed. Additional phase information can be extracted either from the diffraction data or from supplementing diffraction experiment to complete the reconstruction. A model is then progressively built into the experimental electron density, refined against the data and the result is a quite accurate molecular structure **[3]**.

Advanced Methods

1. Supercritical fluid extraction

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. SFE can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). These essential oils can include limonene and other straight solvents. Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. Extraction conditions for supercritical carbon dioxide are above the critical temperature of 31 °C and critical pressure of 74 bar. Addition of modifiers may slightly alter this. The discussion below will mainly refer to extraction with CO_2, except where specified.

The use of supercritical fluids for the extraction of a range of materials including plant products of medicinal, flavouring and cosmetic interest has, during the last decade, become of increasing economic and research interest. A certain temperature, and pressure, single

substances do not condense or evaporate but exist as a fluid. Under these conditions the gas and liquid phases both possess the same density and no division exists between the two phases. Extraction of oil from evening primrose, Extraction of taxol from Taxus brevifolia, Extraction of annatto seeds and Decaffeination of green coffee is done Supercritical fluid extraction **[17]**.

2. Microwave Assisted Extraction (MAE)

Microwave assisted extraction (MAE) is based on heating the solvent through absorption of microwave energy by polar molecules, thus increasing the solvent penetration into the sample matrix. MAE has been applied to the extraction of organic compounds from very different types of matrix because it saves solvent and it is rapid and efficient in terms of energy use. This method allows the acceleration of energy transfer, facilitating the solvation of analytes, and also promoting the disruption of weak hydrogen bonds **[18]**. , the use of microwave for extraction of constituents from plant material has shown tremendous research interest and potential. Conventional techniques for the extraction of active constituents are time and solvent consuming, thermally unsafe and the analysis of numerous constituents in plant material is limited by the extraction step **[19]**.

3. Accelerated solvent extraction

Accelerated solvent extraction (ASE) is a method for extracting various chemicals from a complex solid or semisolid sample matrix. The process uses high temperature and pressure, which results in the extraction taking less time and requiring less solvent, and possibly also giving better analyte recovery, than traditional methods that use less extreme conditions. The elevated temperature is employed to increase extraction efficiency of the analyte of interest and the elevated pressure is used to keep the solvent in a liquid state as the temperature is increased above its boiling point. Accelerated solvent extraction (ASE) has been used in many applications such as extraction of pesticides and bioactive/nutritional compounds. This method utilizes higher temperatures and pressures during the extraction process. Elevated pressures (>1000 psi) allow for solvents to be heated at temperatures higher than their boiling point which increases diffusion rates, disrupts the strong solute matrix interactions and decreases liquid solvent viscosity, allowing better penetration into the matrix and then improving extraction **[20, 21]**.

4. Ultrasound assisted extraction

Ultrasound-assisted extraction (USAE) is an interesting process to obtain high valuable compounds and could contribute to the increase in the value of some food by-products when used as sources of

natural compounds. This is an advanced technique which has the capability of extracting large amount of bioactive compounds within shorter extraction time. For a successful application of the USAE, it is necessary to consider the influence of several process variables, the main ones being the applied ultrasonic power, the frequency, the extraction temperature, the reactor characteristics, and the solvent–sample interaction. The highest extraction rate is usually achieved in the first few minutes, which is the most profitable period. To optimize the process, rate equations and unambiguous process characterization is needed aspects that have often been lacking **[23]**.

New Trends

1. Phytochemical genomics

Phytochemical genomics is a recently emerging field, which investigates the genomic basis of the synthesis and function of phytochemicals (plant metabolites), particularly based on advanced metabolomics. In this, the biosynthetic mechanism and regulation, function and evolution of plant metabolites (phytochemicals) are investigated by the systematic integration of genomics and related '-omics' such as transcriptomics, proteomics and metabolomics. Testable hypotheses can be generated by this integrated systems analysis. Subsequently the hypotheses must be validated by reverse genetics/biochemistry/chemistry for further application by biotechnology. The secrets of the origin of huge chemical diversity of plants, that is, 200 000 to one million metabolites estimated, can be unveiled by these studies. Furthermore, knowledge obtained through the studies would be the basis for further application of plants' function to agriculture, medicine and chemical industries. Obviously this study was initiated with a few model plants like Arabidopsis, of which completed genome sequences are available; however, the studies have been extended to crops and medicinal plants, in which no genome sequences are readily available **[24]**.

2. Up-to-date technology advance in metabolomics

Metabolomics is a key component tin phytochemical genomics .One of the major bottle necks of current metabolomics is the annotation of metabolite peaks detected by mass spectrometry (MS) or nuclear magnetic resonance (NMR). In the last few years ,progress has been made in this annotations strategy, in particular, by computational application as exemplified in. Several data bases for plant metabolites and their mass spectra have recently become available **[25]**.

3. Plant Tissue Culture

Plant tissue culture represents an alternative to whole plants as a source of phytochemicals. Tissue culture is in vivo

cultivation of plants cell or tissue under aseptic and controlled environmental conditions in liquid or an semisolid well defined nutrient medium for the production of primary and secondary metabolite or to regenerate plant. This technique affords alternative solution to problem arising due to current rate of extinction and decimation of flora and ecosystem to whole process requires well equipped culture laboratory and nutrient medium. This process involves various steps viz. preparation of nutrient medium containing inorganic and organic salts, supplemented with vitamins. Plant growth hormones and amino acids as well as sterilization of explants (source of plant issue), glassware and other accessories inoculation and incubation **[26]**.

4. Biochromatography

Biochromatography has increasingly gained in importance, particularly in the pharmaceutical industry for the development, analysis, and production of active pharmaceutical ingredients, the so called APIs, with a significantly increased number of biomolecules. Traditionally, biochromatography was mostly carried out on polymer based materials with larger particles in the low or medium pressure range. They mainly consist of carbon and hydrogen, which form chemical compounds with oxygen, nitrogen, phosphorus, or sulfur. It discusses the different parameters for the development and adaptation of reversed phase chromatography of peptides and proteins. Size exclusion chromatography (SEC) is a special form of chromatography, which differs in the fact that there are (ideally) no interactions with the stationary phase. Also the high performance of the UHPLC systems and the increased peak capacity in combination with fast and high resolution mass spectrometers will drive this progress **[27]**.

CONCLUSION

Nature is a unique source of structures of high phytochemical diversity, many of them possessing interesting biological activities and medicinal properties. Phytochemistry is the study of phytochemicals which includes various techniques for screening, isolation and detection of phytochemicals from plants. These techniques include various conventional and advanced extraction methods. Qualitative and quantitative analysis of extract and active constituents is done by various Spectroscopic techniques. These are also new fields which are included in phytochemistry where we use phytochemical studies in field of treatment and cure of disease.

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