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Research

A REVIEW ON INNOVATIVE TECHNIQUES IN PHYTOCHEMICAL ANALYSIS: TRENDS AND APPLICATIONS

K. Grishma sai[*1] P. Prabhavathi[2], Y. Prapurnachandra[3],

1Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524346 A. P., India

2Department of Pharmaceutical Chemistry, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524346 A.P., India.

3Department of Pharmacology, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore Dt.524346 A.P., India.

Corresponding Author:
K. Grishma sai

Email: *NA*

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Abstract:
Phytochemicals, bioactive compounds derived from plants, have garnered significant attention due to their diverse therapeutic and nutraceutical properties. Accurate and efficient analysis of phytochemicals is crucial for quality control, drug development, and functional food production. Traditional analytical methods, such as chromatography and spectrophotometry, though widely used, often face limitations regarding sensitivity, specificity, and throughput. Consequently, innovative techniques in phytochemical analysis have emerged, offering improved accuracy, precision, and efficiency.
This review highlights recent advancements in phytochemical analysis, emphasizing trends and applications. Cutting-edge chromatographic techniques like ultra-high-performance liquid chromatography (UHPLC) and hyphenated methods, such as liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS), provide superior resolution and detection capabilities. Spectroscopic innovations, including nuclear magnetic resonance (NMR) and Fourier-transform infrared spectroscopy (FTIR), offer non-destructive and rapid analysis. Emerging methods, such as metabolomics, biosensors, and machine learning-driven approaches, enable comprehensive profiling and quantification of phytochemicals in complex matrices.
Additionally, microextraction techniques, such as solid-phase microextraction (SPME) and dispersive liquid-liquid microextraction (DLLME), enhance sample preparation efficiency and environmental sustainability. The integration of green analytical chemistry principles further optimizes resource use and minimizes waste. These advancements have been pivotal in various fields, including food safety, pharmaceuticals, cosmetics, and environmental sciences.

Key words: Phytochemical Analysis, Chromatographic Techniques, Spectroscopic Innovations, Metabolomics, Green Analytical Chemistry.

Introduction:

Phytochemicals (bioactive compounds originated from plants) as potential drugs have created the new pharmacological research field as first such globally attractive area of scientific interests. This broadened review covers new research in various analytical methods, pharmacological activities, chemistry, pharmacodynamics, pharmaceutical applications, interdisciplinary studies, industrial considerations, and ethics of safer and effective medicine using phytochemicals . Through the course of looking into these issues, our group would like to have a comprehensive view of the current state of phytochemical research and its possible outcomes for the public health . With the aid of the multi-disciplinary approach that combines chemistry, pharmacology, and the other related science domains, scientists have succeeded in learning about the chemical constitution, efficacy, and mechanisms of action of natural components . The analytical methods like spectroscopy, chromatography, and mass spectrometry play vital roles in characterizing the exact profiles and accurate quantities of phytochemicals by pharmaceutical laboratories.1

Development of separation techniques such as HPLC, HPTLC, Gas chromatography, Paper chromatography, electrophoresis and new generation hyphenated techniques gave momentum to phytochemical research. Spectroscopy plays a critical role in Phytochemistry. Spectroscopic techniques such as IR, UV-Vis, 2D-NMR, FABMS etc. plays crucial role in characterization of plant molecules. Phytochemistry is mainly used in the field of herbal medicine. Phytochemical techniques mainly apply to the quality control of herbal medicine of various chemical components such as saponins, alkaloids, volatile oils, flavonoids and anthraquinones.2

Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as “manfriendly medicines”. This paper mainly deals with collection, extraction, qualitative and quantitative analysis of phytochemicals.3

Traditional tecniques:

Maceration:

Maceration is a simple extraction method that involves soaking the plant prepared raw material in a coarse or powder form in a solvent of interest at room conditions for at least three days with intermittent agitation. After the extraction is completed, the mixture is strained either through sieves or a net with tiny holes. Subsequently, the marc is pressed, and the liquid extract is cleaned using either filtration or decantation after standing. Maceration is preferably carried out in a stoppered container to minimize solvent loss through evaporation.4

Percolation:

Percolationimplies a slow passage of the menstruum under the influence of gravity through a column of drug powder and during this movement it goes on extracting the drug molecules layer wise. This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used.5

Soxhelt extraction :

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds.6

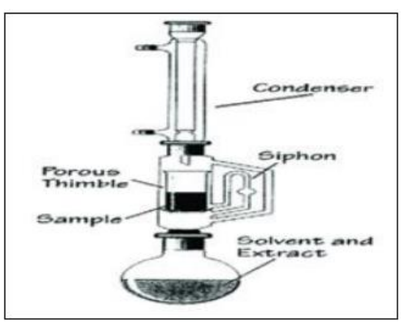

Fig :1 Soxhelt Apparatus

Digestion:
Digestion is an extractive method similar to maceration and uses slight warming in the extraction process. Care is, however, exercised to avoid the temperature altering the bioactive phytochemicals of given plant material. Therefore, there is increased efficiency in using the extraction solvent due to warming.7

Infusion:
It is a very simple method of extraction used for vitamins, volatile ingredients and softingredients in which powdered drug is extracted with hot or cold water. In this method, the powdered drug is soaked in hot water for the specified period with or without stirring and then filtered. Once the powdered drug is added to hot water no further heating is done and kept aside.8

Decoction:
This method is used for the extraction of the water soluble and heat stable constituents from 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.9

Moderen techinques:

High-Performance liquid chromatography:
HPLC remains a cornerstone in phytochemical analysis due to its precision, sensitivity, and versatility. Recent advancements in HPLC include ultra-highperformance liquid chromatography (UHPLC), which offers faster analysis and higher resolution. These improvements are crucial for the rapid screening of compound plant matrices.10

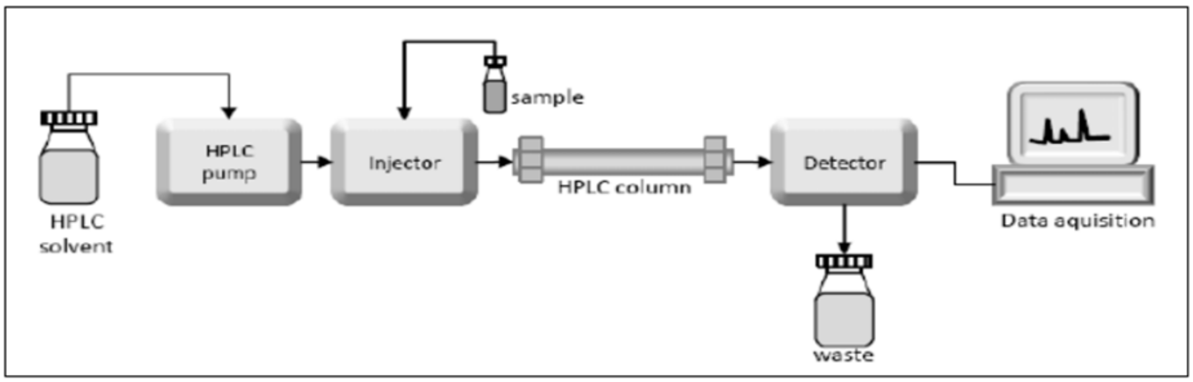

Fig:2 High Performance Liquid Chromatography

Gas chromatography-Mass spectroscopy:
Gas chromatography is applicable for volatile compounds. In this method, species distribute between a gas and a liquid phase. The gas phase is flowing and the liquid phase is stationary. When the sample molecules are in liquid phase they are stationary. The rate of migration depends on how much of chemical species is distributed into liquid phase. Higher the percentage of material in the gaseous state faster will be the migration. The species which distributes itself 100% in the stationary state will not migrate. If a sample distributes itself in both phases, it will migrate at an intermediate rate. This gas chromatography gives the total amount of vapour.[3]

Microwave- Assisted extraction:
Microwaveassisted extraction (MAE) is a process of using microwave energy to heat solvents in contact with a sample in order to partition analytes from the sample matrix into the solvent. The ability to rapidly heat the sample solvent mixture is inherent to MAE and the main advantage of this technique.

Microwave power and extraction time are the other parameters that impact extraction efficiency in MAE in addition to temperature. During the extraction of carotenoids from carrot waste, it was observed that both microwave power and extraction time were

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significantly useful in obtaining carotenoids from carrot waste.[11]

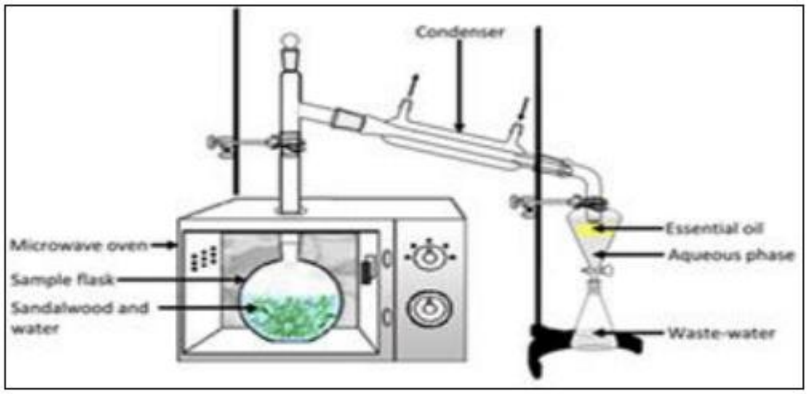

Fig:3 Microwave Assistd Extractor

Ultrasound- Assisted extraction:

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 extraction of rauwolfia root, its large-scale application is limited due to the higher costs.[12]

Supercritical fluid extraction:

The supercritical CO_2 extraction method is applied to commercial extractions from natural resources. However, adjusting the temperature and pressure parameters is critical to enhancing the yield and uncompromised biological activities . The higher temperature promotes the solubility of solutes in supercritical CO_2, but it is suggested that due consideration should be given for compromised temperature in the case of thermolabile molecules. In the case of thermolabile phytochemicals, keeping the temperature at unaltered low values while increasing the pressure helps the phytochemicals not degrade.[13]

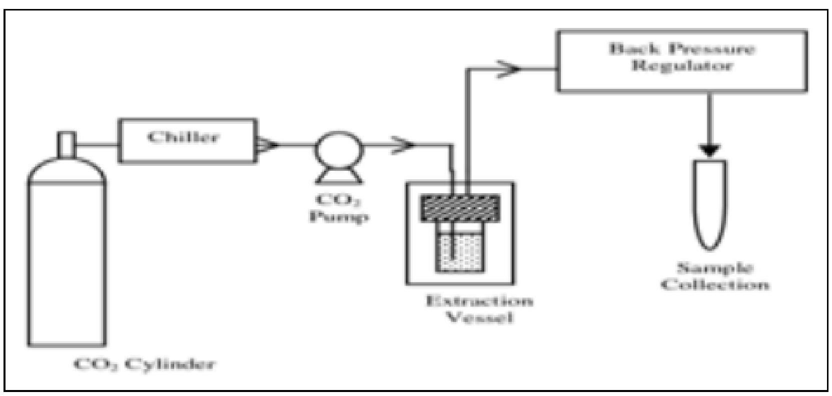

Fig:4 Supercritical Fluid Extractor

Thin layer chromatography:

The classical TLC studies were done by Izmailove, Shraiber and Stahl. In 1938 Izmailove and Shraiber separated certain medicinal compounds on unbound alumina spread on glass plate. Mainhard and Hall in 1949 used a binder to adhere alumina on slides. In early 1950`s Kirchner and colleagues developed TLC as we know it today. The wide applicability of TLC was demonstrated by Stahl in 1958. Stahl introduced the term "Thin Layer Chromatography" in the late 1950`s. His major contribution was the standardization of the materials, procedure and nomenclature and description of selective solvent systems for the resolution of important class of compounds.[14]

High performance thin layer chromatography:

HPTLC is a chromatographic technology that can be utilised for many purposes such as constituent identification, impurity identification and determination, and active substance quantitative determination. Compared to conventional TLC, HPTLC offers improved accuracy, reproducibility, and record-keeping capabilities, making it one of the best TLC methods for analytical applications. A type of thin-layer chromatography (TLC) known as high-performance thin-layer chromatography (HPTLC) uses an optimised coating material, automated processes for feeding the mobile phase, layer preconditioning, precise sample application, scanning of the chromatogram development, and photo documentation to provide superior separation power.[15]

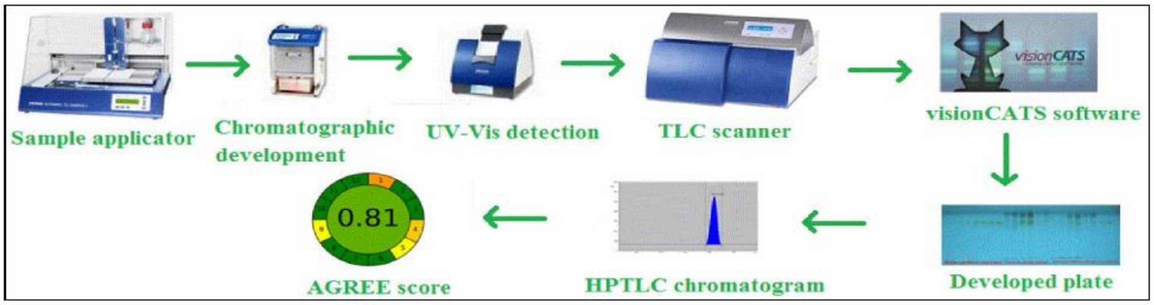

Fig:5 High Performance Thin Layer Chromatography

Paper chromatography:

It consists of a layer of cellulose highly saturated with water. In this method a thick filter paper comprised the support, and water drops settled in its pores made up the stationary "liquid phase." Mobile phase consists of an appropriate fluid placed in a developing tank. Paper chromatography is a "liquid-liquid" chromatography.[16]

Accelerated solvent extraction:

Accelerated solvent extraction is a technique for extracting organic compounds from solid and semisolid samples with liquid solvents. The extraction cell is filled with the solid sample to be examined and placed in a temperature-controllable oven. After adding the solvent, the cell is heated at constant pressure (adjustable between 0.3 and 20 MPa) up to a maximum temperature of 200°C and kept at constant conditions for a while so that equilibrium can be established. The extract is then transferred to a sample tube.[9]

Optimum performance laminar chromatography:

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.

Summary:

The field of phytochemical analysis has witnessed remarkable advancements in recent years, driven by the need for more accurate, efficient, and environmentally sustainable techniques. Innovative methods, including high-resolution spectroscopy, chromatography, metabolomics, and integrated hyphenated techniques, have revolutionized the identification, quantification, and characterization of phytochemicals. Emerging technologies like microfluidics, biosensors, and artificial intelligence-based data analysis are further enhancing the precision and throughput of phytochemical studies.

These advancements not only improve the understanding of bioactive compounds but also foster their application in pharmaceuticals, nutraceuticals, cosmetics, and food industries. Furthermore, trends such as green analytical chemistry emphasize the adoption of eco-friendly solvents and techniques, aligning with sustainable research practices.

Overall, innovative techniques in phytochemical analysis continue to evolve, addressing challenges related to sensitivity, complexity, and scalability while opening new avenues for natural product discovery and utilization. The integration of cutting-edge technologies promises to expand the horizons of phytochemical research, ensuring its relevance and applicability in diverse scientific and industrial domains.

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