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BASIC CONCEPTS OF PHYTOCHEMISTRY

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➢ ABSTRACT

Phytochemistry is the study of phytochemicals, which are chemicals derived from every part of the plant including roots, stem, leaves, flowers, fruits, seeds etc. 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: Phytochemistry, Extraction, Spectroscopy, 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.[1] The proper understanding of phytochemical is essential for drug discovery and for the development of novel therapeutic agents against major diseases. As per a report by World Health Organization (WHO), over 80% of the people of developing countries are relaying on the traditional medicines that are extracted from the plants for their primary health needs. Use of these traditional medicines for the preparation of modern medical preparations is indispensable and thus 'Phytomedicines' are a link between the traditional and modern medicine.

➢ STEPS IN PREPARATION OF PLANT SAMPLE

selection of plant samples

Proper selection and identification is important for any phytochemical research any defect in this could severely affect the research and may reduce the value of the study.

1. Traditionally used plants by humans for food, medicine or poison based on literature or other sources can be investigated.
2. A random or systematic collection of plants over a large biodiverse area regarding secondary metabolite production can be used
3. Other plant species that are phylogenetically related to the species known to produce a compound of interest
4. Species based on the reports of biological activity in the literature.

Collection of Plant Samples

Plant collection can be done on either from wild forests or from herbariums. But in the case of wild plants there is a risk of plants that are been incorrectly identified. They have an advantage that they do not contain any pesticides or herbicides. After collection they are processed soon to prevent the deterioration of secondary metabolites present in the samples.

Identification of Plant Samples

The collected samples must be identified elsewhere.

1. Reviewing the flora of the region to compile a list of the plants that are in interest and to separate them from the plants that are to be avoided
2. Field identification must be done. They must at least be identified to their level of genus
3. To aid the identification, taxonomic experts should identify the plant species with a permanent scientific record or in case of a voucher specimen, the plant with the reproductive organs must be submitted to the major institutions or herbaria of the source country.

Cleaning of Plants

Proper cleaning of plants is an important step after collection. This process involves the following steps of washing, peeling, stripping leaves from the stems. This is usually done in hands to have better results.

Drying

The plant materials are dried to remove the water content and thus after the removal of water so that they can be stored. This process should be done immediately as soon as the plants are collected so that it is prevented from spoilage. There are two methods in drying the plants.

Natural Process

This process include sun-drying. In this the plants are kept in the shades and are air dried in sheds. This process takes few weeks for complete drying of the moisture. This time depends on the temperature and humidity.

Artificial Drying

Artificial drying is done using the help of artificial driers. This process will reduce the time consumed to few hours or minutes. The common method used for the drying of medicinal plants is warm-air drying. This is done using the hot air oven on which warm air is blown. This method is applicable for drying of succulent parts of plants and fragile flowers. The drying must be done in lower temperature to prevent the thermolabile compounds being disintegrated.

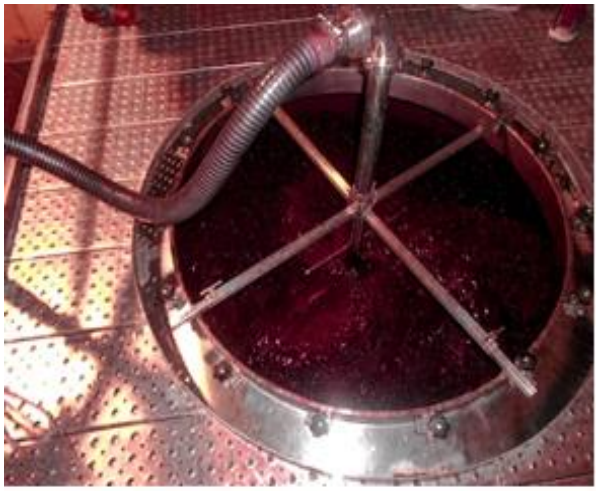
Grinding of Plant Materials

After complete drying of moisture the plant samples are to be powdered for the further analysis. There are different types of powdering, they include the following

1. Grinding can be done by milling in an electric grinder or by a spice mill or can also be in mortar or pestle.
2. Grinding increases the efficiency of the extraction due to increased surface area of the plants. The decrease in the surface area can lead to dense packing of the material.
3. Milling the plants into a fine powder is always ideal but if they are too fine this affects the solvent's flow and also produces more heat which could degrade some thermolabile compound.

- **Techniques for Screening of Phytochemicals from Plants Extraction Methods 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.



Infusion

• In this process the crude drug are macerated with cold or boiling water to obtained fresh infusion.

• These are dilute solutions of the readily soluble constituents of crude drugs.

It is used for extraction of vitamins volatile ingredients and soft ingredients in which the powder drug is extracted with hot/cold water.[3]

Percolation

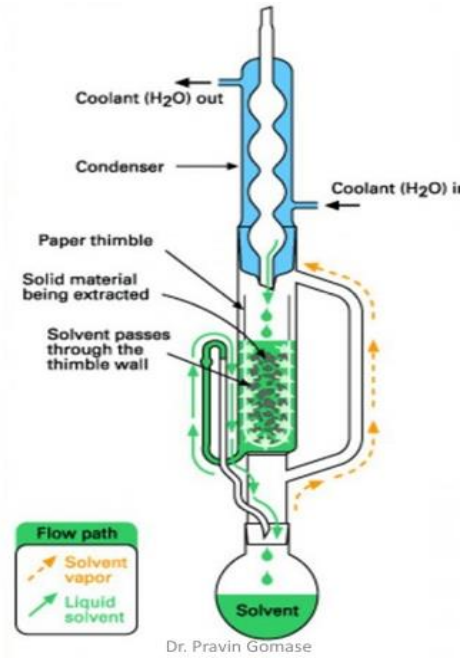
- A percolator having a narrow, V-shaped vessel open at both ends are generally used.
- The solid ingredients are moistened with specified an appropriate amount of the specified menstrum and allowed to stand for approximately 4 h in a well closed container.
- After which the mass is packed and the top of the percolator is closed.
- Additional menstrum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h.
- The outlet of the percolator then is opened and the liquid contained therein is allowed to contained therein is allowed to drip slowly**.**
- Additional menstrum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. Dr. Pravin Gomes
- The marc is then pressed and the expressed liquid is added to the percolate.

- Sufficient menstrum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting.

Decoction

- In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered.
- This procedure is suitable for extracting water soluble, heat stable constituents.

Hot Continuous Extraction (Soxhlet)

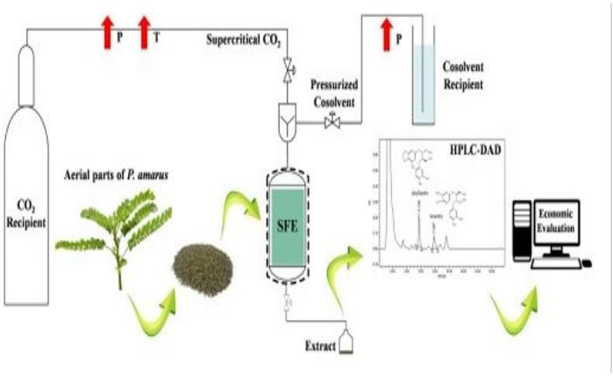


Hot Continuous Extraction (Soxhlet)

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.[4] 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.

Supercritical Fluid Extraction

- The critical point of a substance is the highest temperature and pressure at which substance has vapour- liquid equilibrium.
- Supercritical fluid have liquid like density, gas like viscosity and compressibility and higher diffusivities than liquid which facilitates extraction of wide variety of than liquid which facilitates extraction of wide variety of phytochemical.
- It is a process similar to simple extraction.
- Mostly carbon dioxide uses as extracting fluid because it is safe and abundant and has favourable physical properties.

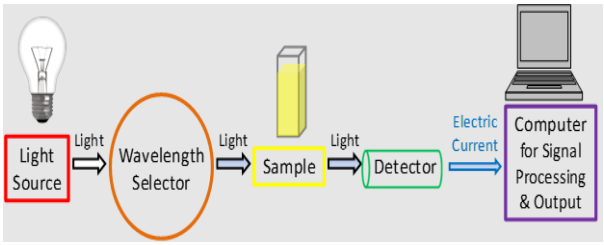


➢ METHODS OF DETECTION

Spectroscopy is used in the detection of phytochemicals.

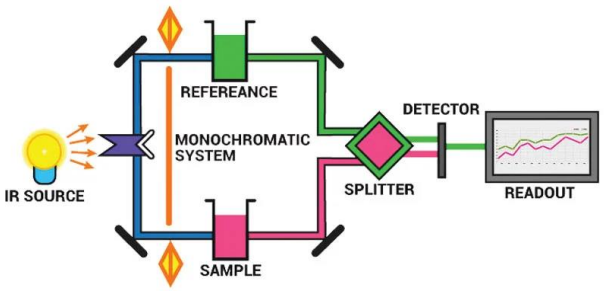
1 Uv Spectroscopy

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.[5] 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.



2. 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.



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. 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.

4. Nuclear Magnetic Spectroscopy: (nmr)

Resonance 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.

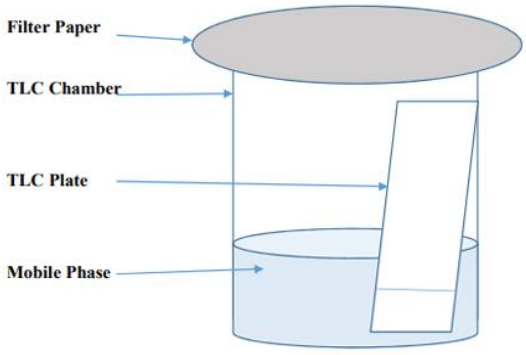
➢ **Quantitative Analysis**

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.[6] High Chromatography Performance is applicable Liquid for compounds soluble in solvents. High performance thin layer chromatography is applicable for the separation, detection, qualitative and quantitative analysis of phytochemicals.

Thin Layer Chromatography (Tlc)

TLC involves separating a mixture into its components using a glass plate coated with a thin layer of an adsorbent, such as silica gel or alumina. The plate, referred to as a chromatographic plate, is used for this purpose.[7] The sample to be separated is applied as a small spot 2 cm above one end of the plate. A liquid eluent is then added to a closed container containing the plate, causing the components of the mixture to rise up the plate and separate at different heights.

Instrumentation

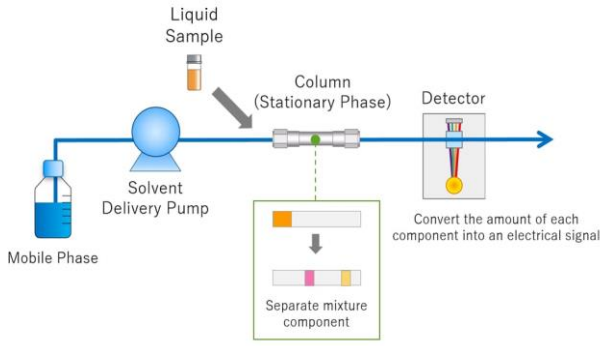


high performance liquid chromatography: (hplc)

High performance liquid chromatography is chromatographers widely and used 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 morphine; papaverine, codeine; emetine, antibiotics, ergot alkaloid, cardiac glycosides, sennosides, and capsaicin are analysed 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.

Figure



Applications of Hplc

1. Analysing dissolving tablets in medicinal dosage forms.
2. Regulating medication stability and estimating shelf life.

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.

Paper Chromatography

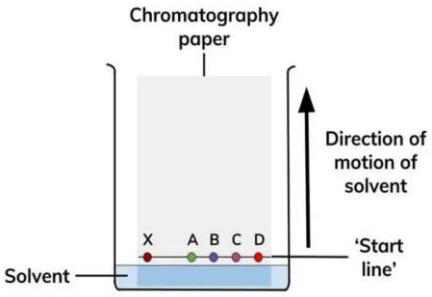
Developed by Richard Laurence Millington Synge and Archer John Porter Martin, paper chromatography was a significant breakthrough. It successfully solved the problem of distinguishing highly similar amino acids. This technique typically employs purified paper sheets, which are known to have absorbed water.[8] The choice of paper varies based on the specific research at hand. Different liquids, such as paraffin oil and silicone, are also used.

Principle of Paper Chromatography

Separation is primarily governed by partitioning rather than adsorption. Substances partition between a mobile phase and a stationary phase. Water occupies the stationary phase within the cellulose layers of the filter paper, while the mobile phase comprises organic solvents and buffers. The developing solution carries the sample up to the stationary phase. The sample components separate based on their solubility in the mobile phase and their affinity for the stationary phase.

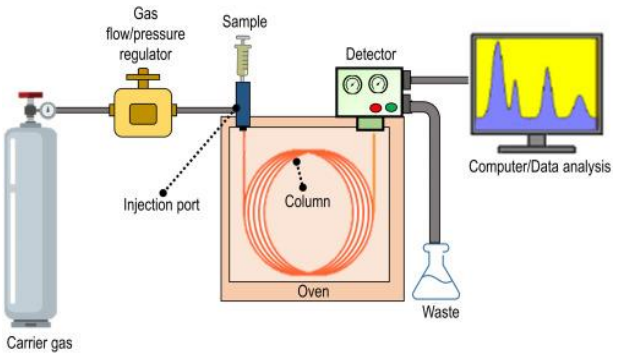
Applications of paper chromatography

1) Counting the components in a sample using the appropriate mobile phase.
2) Effectively separating free amino acids in human serum.



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 up to 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.



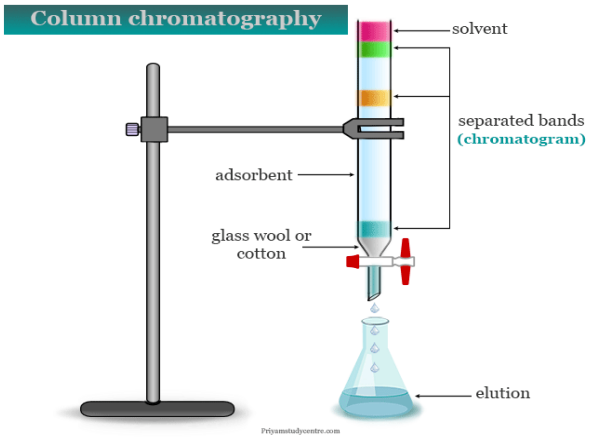
Application of Gas Chromatography

1) Determination of Fatty Acids by Gas Chromatography: Gas chromatography is a widely used

analytical technique in food science, biochemistry, and lipid chemistry, among other domains, for the assessment of fatty acid concentrations.

Column Chromatography

Column chromatography is a method employed for the separation of a mixture's components using a column filled with a suitable adsorbent material, typically packed within a glass tube. The mixture is introduced at the top and washed with an appropriate eluent, which gradually descends down the column. The components are separated based on the degree to which they adhere to the column wall. The most strongly adsorbed component remains at the top, while others are displaced to different heights within the column.

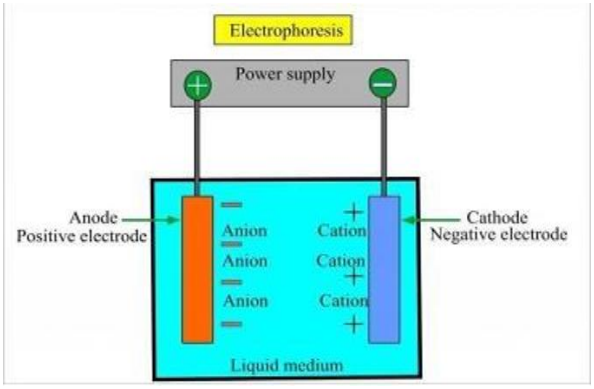


Electrophoresis

Electrophoresis is a laboratory technique used to separate charged molecules, such as DNA, RNA, and proteins, based on their size and charge. This technique relies on the movement of charged particles through a gel or liquid medium under the influence of an electric field.

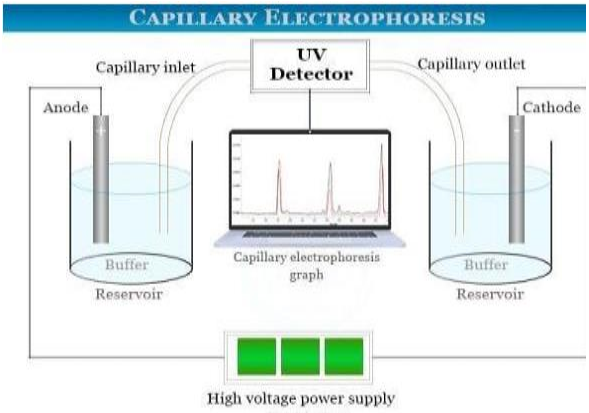
Principle of Electrophoresis

When an electric field is applied, negatively charged molecules (such as DNA) migrate towards the positively charged electrode (anode), while positively charged molecules move towards the negatively charged electrode (cathode). The rate of movement depends on factors like the molecule's charge, size, shape, and the composition of the medium.



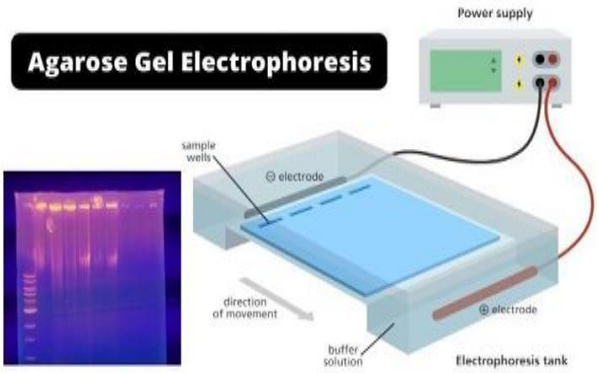
Capillary Electrophoresis

Capillary Electrophoresis (CE) is a high-resolution analytical technique used to separate charged molecules such as DNA, proteins, and small ions based on their size, charge, and mobility in an electric field.[9] Unlike traditional gel electrophoresis, CE utilizes a narrow capillary tube filled with an electrolyte solution, providing faster and more efficient separation.[10]



Agarose Gel Electrophoresis

Agarose Gel Electrophoresis is a widely used technique in molecular biology to separate DNA and RNA fragments based on their size.[10] It utilizes an agarose gel matrix and an electric field to drive negatively charged nucleic acids towards the positive electrode (anode).



CONCLUSION

Plants are an important source of phytochemicals which are an important source of drug and medicine. These phytochemicals have extraordinary properties like antibacterial, antifungal, anti-cancerous, antioxidant,anti inflammatory, anti-diabetic activities etc. The identification of this compound relies on the tools of phytochemical analysis and hence the knowledge about these techniques is important. This article will be helpful in the collection, identification, extraction and analysis of phytochemicals that are extracted from the plants. The methods followed for this analysis should be standard and following non-standard protocols could lead to the false results that are not reproducible. The above article

will help in the qualitative analysis of the phytochemicals.

REFERENCE

1. Egbuna CH, Ifemeje JC, Kryeziu TL, Mukherjee MI, Shah HA, Rao GN, Gido LJ, Tijjani HA. Introduction to phytochemistry. Phytochemistry, 2018; 1: 3-5.
2. Sivanandham, Velavan. (2015). PHYTOCHEMICAL TECHNIQUES - A REVIEW. World Journal of Science and Research, 1: 80-91.
3. Maran JP, Manikandan S, Nivetha CV, Dinesh R. Ultrasound assisted extraction of bioactive compounds from Nephelium lappaceum L. fruit peel using central composite face centered response surface design. Arabian journal of chemistry, 2017; 10: 1145-1157.
4. Majekodunmi SO. Review of extraction of medicinal plants for pharmaceutical research. Merit research journal of medicine and medical sciences, 2015; 3(11): 521 527.
5. Agostini, M., S. Appel, G. Bellini, J. Benziger, D. Bick, G. Bonfini, D. Bravo et al. "Spectroscopy of geoneutrinos from 2056 days of Borexino data." Physical Review D., 2015; 92(3): 031101.
6. Chromatographic Techniques by Dr. ASHWINI WADEGAONKAR.
7. Navjot kaur, Ritika. A review on thin layer chromatography: forensic analytical technique, Pramana Research Journal, ISSN NO: 2249-2976.
8. Mukta Guptaa, Bhupinder Kapoora, Reena Guptaa. Paper Chromatography. Journal of Emerging Technologies and Innovative Research (JETIR), October 2018; 5(10).
9. Stolz A, Jooß K, Höcker O, Römer J, Schlecht J, Neusüß C. Recent advances in capillary electrophoresis-mass spectrometry: Instrumentation, methodology and applications. Electrophoresis, 2019 Jan; 40(1): 79-112.
10. Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. J Vis Exp, 2012 Apr 20; 62.