A REVIEW ON: TECHNICAL APPROACH FOR SEPARATION AND PURIFICATION OF PLANT CONSTITUENT

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❖ **Abstract:**

- For several disciplines, such as phytochemistry, pharmacognosy, and nutrition, the study of plant components is essential. Clarifying these chemicals' structures, comprehending their roles, and assessing their possible health advantages all depend on analytical methods for their separation and purification. Key analytical methods are reviewed in this study, including mass spectrometry, spectroscopic techniques (such as NMR and UV-Vis spectroscopy), and chromatography (such as gas chromatography, liquid chromatography, and thin-layer chromatography). The fundamentals and process of each approach are examined in light of how well they isolate and describe phytochemicals. Combining these methods guarantees excellent purity and specificity and permits thorough analysis, both of which are essential for additional biological testing and the creation of therapeutic medicines. Complex plant matrices can now be analysed much more easily because to recent developments in analytical techniques as high-performance liquid chromatography coupled (HPLC). A fundamental basis for further investigation into the many and advantageous chemicals present in plants is provided by this synthesis of analytical methods.

❖ **Keywords:**

- Plant Constituents, Separation Techniques, Purification Techniques, Chromatography, Mass Spectrometry (MS)

❖ Introduction:

- Naturally occurring substances from prebiotic, microbial, plant, and animal sources have always piqued human curiosity. Plant extracts of various sections have been used extensively in traditional remedies, fragrances, food flavourings, and preservatives. They are also more frequently used in both common and chronic illnesses. Alkaloids, steroids, tannins, glycosides, volatile and fixed oils, resins, phenols, and flavonoids are among the active substances found in plants and are deposited in various areas of the plant. The combination of these active chemicals gives the plant its therapeutic and medical properties [1]. Numerous plant extracts have been utilized extensively as food flavourings, preservatives, traditional remedies, and fragrances. Bioactive natural ingredients are more frequently used in anti-inflammatory, analgesic, and antipyretic treatments, as well as substitutes for hormone replacement therapy [3,4,5] and in the treatment of infectious and chronic diseases [2] such as cancer, diabetes, and asthma. Additionally, it is used as a treatment for bronchitis, arthritis, oedema, fever and cough of pneumonia, hepatitis, nephritis, gastropathy, and bronchitis [6]. In addition to meeting the majority of people's basic medical needs in underdeveloped nations, natural medicines are also becoming more and more popular in wealthy nations because of its low or nonexistent side effects and high cost of care. About 49% of people in the USA have experimented with natural remedies for illness prevention and treatment. Over 80% of the world's population, according to the World Health Organization (WHO), gets their primary medical care from traditional medicine, which primarily uses plant extracts [7]. The most abundant bioresource of pharmaceutical intermediates, nutraceuticals, nutritional supplements, and chemical entities for both synthetic and conventional medications is medicinal plants [8]. The bioactive natural compounds are insufficient, despite their importance and widespread use. These days, there is a pressing need to promote research on natural products and create efficient and selective ways. Since ancient times, medicinal plants have been the cornerstone of traditional herbal therapy used by rural populations all over the world. In the third millennium BC, the Sumerian and Akkadian civilizations undoubtedly began using plants for therapeutic purposes. Approximately 400 different plant species were identified for medical applications by Hippocrates (c. 460–377 BC), one of the ancient authors who reported medicinal natural products of plant and animal origins. Ancient traditional medical systems, such as Chinese, Ayurvedic, and Egyptian, have relied heavily on natural products [9]. Over time, they have taken center stage in contemporary society as a natural source of chemotherapy and among researchers looking for different medication sources. In the poor world, some 3.4 billion people rely on traditional plant-based remedies. This amounts to around 88% of the global population, who mostly receive their primary medical treatment from conventional medicine [10, 11].

- The many analytical techniques used in the separation and purification of plant constituents are thoroughly examined in this review study.

❖ **Analytical Techniques:**

- **Separation Techniques:**

1. Capillary Electrophoresis (CE)
2. Micellar Electrokinetic Chromatography (MEKC)
3. Isoelectric Focusing (IEF)
4. Nanofiltration (NF)
5. Reverse Osmosis (RO)
6. Microdialysis

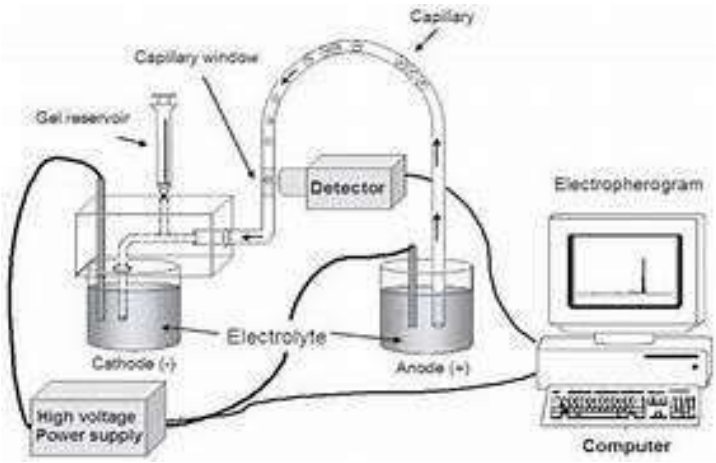
- **Purification Techniques:**

1. Ultrasonic Extraction
2. Sonication
3. High-Intensity Focused Ultrasound (HIFU)
4. Electrodialysis (Ed)
5. Electrofiltration
6. Enzyme-Catalyzed Reactions
7. Enzyme-Linked Immunosorbent Assay (Elisa)
8. Magnetophoresis

❖ SEPERATION TECHNIQUE:

1. CAPILLARY ELECTROPHORESIS (CE):

- Based on the differential migration of charged species through a capillary under the influence of an electric field, capillary electrophoresis (CE) is a very effective separation method. CE has become widely employed in a variety of sectors, including pharmaceutical analysis, biochemistry, environmental science, and clinical diagnostics. Its primary function is to separate ionic species according to their size and charge.



➢ **Principles:**

- In CE, an electrolyte solution is poured into a capillary tube, usually composed of fused silica. Analytes migrate toward the oppositely charged electrode at varying speeds depending on their electrophoretic mobility when a high voltage is placed across the capillary. The charge-to-mass ratio, molecular size, and shape are some of the variables that affect mobility. Conversely, the electro osmotic flow (EOF), or the bulk flow of the buffer solution towards the cathode, separates neutral molecules.

➢ **Procedure:**

i. Capillary Preparation:

- **Capillary Selection:** Select a fused silica capillary, which usually has a length of 30 to 100 cm and an inner diameter between 25 and 100 µm.
- **Conditioning:** A sequence of solvent rinses is used to condition the capillary. To maintain cleanliness and avoid unintended interactions with analytes, a standard protocol involves flushing the capillary with 1M sodium hydroxide (NaOH) for five minutes, followed by deionized water and a running buffer.

ii. Sample Preparation:

- Samples should be prepared by diluting them in the proper buffer solution. Because particles can obstruct the narrow capillary and reduce separation performance, make sure the samples

are filtered (typically using 0.45 µm filters) to get rid of any particulate matter.

- The buffer used should be tailored to accomplish the desired separation and have a pH that facilitates analyte ionization.

iii. Filling the Capillary with Buffer:

- A running buffer, frequently an electrolyte solution, is poured into the capillary to supply the conductivity required for separation. The choice of buffer is essential for figuring out analytes' electrophoretic mobility.
- Phosphate, borate, or acetate systems are examples of common buffers; the analyte determines how the pH should be adjusted.

iv. Sample Injection:

- Hydrodynamic injection, which applies pressure for a predetermined amount of time, or electrokinetic injection, which applies voltage for a brief length of time, are the two methods used to introduce samples into the capillary.
- In order to prevent capillary overload, which may result in poor resolution, injection parameters are carefully regulated.

v. Application of Electric Field:

- When a strong electric field, usually between 10 and 30 kV, is applied across the capillary, the charged analytes move in the direction of the opposite electrode. The analyte's size and charge determine the migration rate; larger and less charged molecules migrate more slowly.
- The variation in electrophoretic mobility between species is what causes the separation.

vi. Detection:

- At the end of the capillary, analytes are detected, usually using UV-Vis spectrophotometry, fluorescence, or mass spectrometry (CE-MS).
- The system creates an electropherogram, with peaks representing distinct analytes, as each analyte arrives to the detector at a separate time (retention time).

vii. Data Analysis:

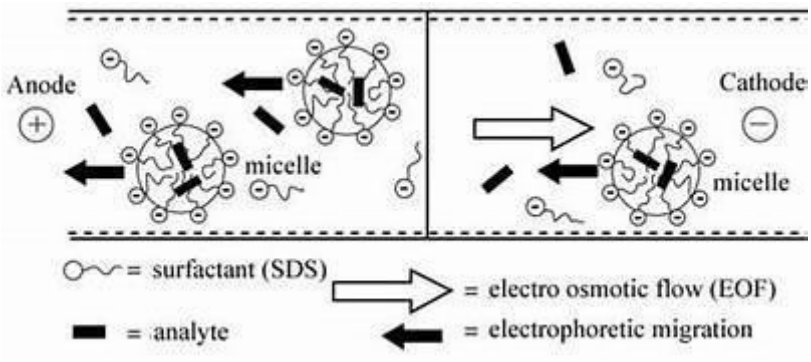
- The analytes included in the sample are identified and quantified by analyzing the migration time and peak regions or heights.
- Electrophoretic mobility, which aids in the qualitative and quantitative identification of the separated compounds, is computed using the data. [12]

2. MICELLAR ELECTROKINETIC CHROMATOGRAPHY (MEKC):

➢ **Principle:**

- Neutral and charged molecules are separated using a capillary electrophoresis (CE) technique called Micellar Electrokinetic Chromatography (MEKC). To separate compounds according to their varying interactions with micelles and the aqueous media, it combines micelle production with electrophoretic mobility. Sodium dodecyl sulfate, or SDS, is one

example of a surfactant that is added to the buffer above its critical micelle concentration (CMC) in MEKC. Micelles, which resemble pseudostationary phases, are formed by these surfactants. While charged molecules are separated based on both their electrophoretic mobility and interaction with the micelles, neutral molecules—which are typically not separated by electrophoresis—are retained by the micelles due to their hydrophobic interactions.



➢ **Procedure:**

i. **Preparation of the Electrolyte Buffer**: A appropriate buffer solution, usually with a pH of 7 to 9, is made. The buffer is supplemented with a surfactant, such as sodium dodecyl sulfate (SDS), at a concentration greater than the critical micelle concentration, which is normally between 10 and 50 mM.

ii. **Sample Introduction**: A appropriate buffer solution, usually with a pH of 7 to 9, is made. The buffer is supplemented with a surfactant, such as sodium dodecyl sulfate (SDS), at a concentration greater than the critical micelle concentration, which is normally between 10 and 50 mM.

iii. **Application of Voltage**: A strong voltage is delivered across the capillary, usually between 10 and 30 kV. The components of the sample are carried toward the detector by the EOF as it travels from the anode to the cathode.

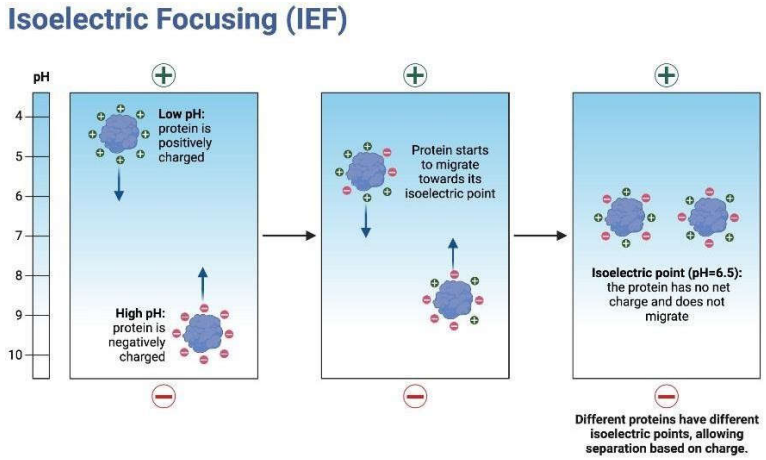
iv. **Separation**: Analytes that are charged move in accordance with their electrophoretic mobility as they pass through the capillary. On the other hand, neutral analytes interact with the micelles. Their hydrophobicity determines the degree of contact, which results in varying migration times.

v. **Detection**: Depending on the kind of analyte, UV-VIS detectors, fluorescence, or mass spectrometry are used to find the separated analytes at the capillary output.

vi. **Data Analysis:** Plotting the results as electropherograms reveals how long it took for each chemical to get to the detector. The interaction between the analyte and the micelles affects the retention time. [13]

3. ISOELECTRIC FOCUSING (IEF):

- Proteins and other amphoteric molecules can be separated using the potent and incredibly accurate electrophoretic method known as isoelectric focusing (IEF), which is based on the molecules' isoelectric points (pI). Proteins can carry both positive and negative charges, depending on the pH of their surroundings, which is the foundation of the IEF principle. When a protein is exposed to an electric field and a pH gradient, it migrates until it reaches the isoelectric point, or the point at which its net charge is zero, at which point it stops moving. This unique property of each protein forms the basis of separation in IEF.



➢ Principle:

- **Amphoteric Nature of Proteins:** Because proteins contain both basic and acidic groups, they can donate or receive protons, resulting in varying charges at different pH values.
- **Isoelectric Point (pI):** The pH at which a protein has no net electrical charge is known as the isoelectric point (pI). Proteins will go to the area where the pH matches their pI when they are exposed to an electric field and a pH gradient.
- **Separation:** Because proteins become electrically neutral at their pI, they cease to move. This makes it possible to separate proteins precisely, even ones with very similar charges and sizes.

➢ Procedure:

i. **Preparation of the pH Gradient**: A combination of ampholytes, which are tiny, soluble amphoteric molecules, is used to create a pH gradient in a gel (often polyacrylamide). The gradient, which normally spans from pH 3 to pH 10, can be either basic or acidic.

ii. **Sample Application**: The gel is coated with the protein sample. Depending on their initial charge, samples are introduced close to the anode for acidic samples or the cathode for basic ones.

iii. **Application of Electric Field**: The gel is subjected to an electric field. Depending on their charge, proteins start to migrate: negatively charged proteins migrate toward the anode, and positively charged proteins migrate toward the cathode. The proteins come into contact with

various pH zones as they travel. They have no net charge when they reach their isoelectric point, which causes them to stop moving.

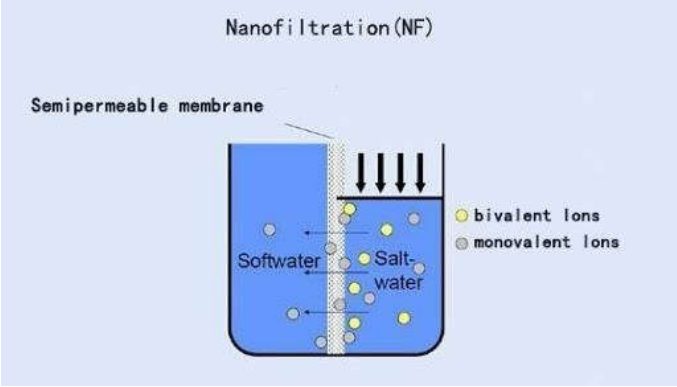
iv. **Focusing Process**: Proteins maintain a sharp focus at their pI points because they would regain a charge and migrate back to their pI if they deviated from this point. Each protein's pI is represented by a highly concentrated band as a result.

v. **Detection and Analysis**: Proteins can be seen using staining methods (such as Coomassie Brilliant Blue or silver staining) once focussing is finished. SDS-PAGE can be used after IEF in two-dimensional gel electrophoresis to further separate proteins according to size. [14]

4. NANOFILTRATION (NF):

➢ **Principle:**

- The main purpose of nanofiltration (NF), a membrane filtration technique, is to separate molecules according to their charge and size. In terms of pore size and filtering capabilities, the method falls somewhere between ultrafiltration and reverse osmosis, usually enabling the removal of particles of a size between 0.01 and 0.01 microns. Numerous industrial and environmental applications can benefit from the ability of nanofiltration membranes to separate dissolved salts, organic compounds, and multivalent ions from solutions.



➢ **Procedure:**

i. **Feed Preparation:** To eliminate suspended particles and lessen membrane fouling, the feed solution which contains the mixture to be separated is pre-treated. To get rid of big particles, pre-filtration procedures can use ultrafiltration or microfiltration.

ii. **Membrane Selection:** The right nanofiltration membrane is selected based on the application. Polymers with selective permeability based on pore size and charge properties, such as polyamide or thin-film composites, are examples of membrane materials.

iii. **System Setting:** The nanofiltration membrane is contained in a membrane module that receives the pumped feed. The liquid is forced through the membrane by the pressure that is given to the feed stream, which is usually between 5 and 30 bar.

iv. **Filtration Process:**

- **Permeate:** The ions and smaller molecules enter the permeate stream after passing through the membrane. Water and monovalent ions (such as sodium and chloride) are commonly included in this.
- **Retentate:** Depending on the application, the membrane retains larger molecules, multivalent ions, and organic compounds, creating the retentate stream, which is either disposed of or undergoes additional processing.

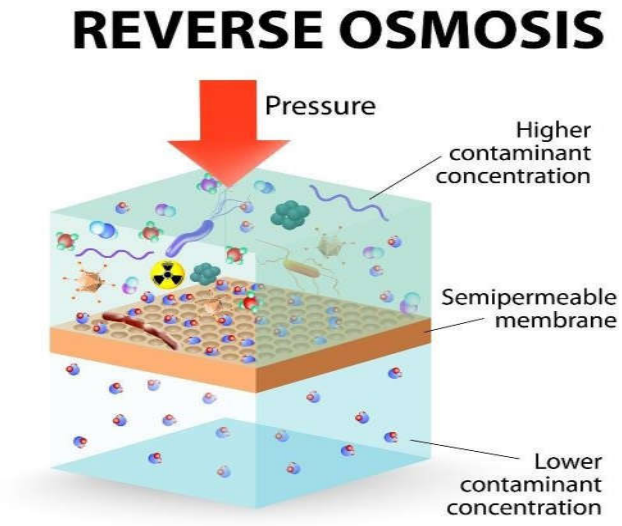
v. **Continuous or Batch Operation:** Depending on the size of the operation, nanofiltration can be carried out in batch or ntinuous modes. In continuous mode, permeate and retentate leave the system at the same time as fresh feed enters continually. When using batch mode, all of the feed is processed at once.

vi. **Post-Treatment:** Depending on the particular separation needs, further processing stages are frequently applied to the permeate and retentate. While retentate can be treated to extract valuable components or disposed of, permeate can be used for additional purification or desalination. [15]

5. REVERSE OSMOSIS (RO):

➢ **Principle:**

- Water or a solvent is pushed through a semi-permeable membrane in the pressure-driven membrane separation process known as reverse osmosis (RO), which leaves behind dissolved solids and other impurities. The procedure applies pressure to one side of the membrane that is higher than the solution's osmotic pressure. In order to successfully separate the solute from the solvent, this pressure forces the solvent, usually water, from an area of higher solute concentration (more contaminants) to an area of lower solute concentration (purified solvent). Until equilibrium is reached, the solvent in natural osmosis travels across a membrane from a region of low solute concentration to one of high solute concentration. By applying an external pressure, RO reverses this process, allowing the solvent to flow through the membrane while the majority of solutes, including organic molecules, microorganisms, and salts, remain on the pressured side.



➢ **Procedure:**

i. **Feed Preparation:** Large particle matter, chlorine, and other substances that might

contaminate or harm the RO membrane are first eliminated from the feed, or solution to be filtered. Filtration, carbon filtering, and occasionally chemical conditioning are common pre-treatment procedures used to stop the production of scale.

ii. **Application of Pressure:** The feed solution is subjected to pressure, which normally ranges from 3–10 bar for fresh water to as much as 80 bar for seawater desalination. The solvent is forced through the semi-permeable barrier by this pressure.

iii. **The process of separation:** The solvent, usually water, passes through the membrane and moves from the feed side, which has a high concentration, to the permeate side, which has a low concentration. Salts, organic substances, and microorganisms are among the solutes that are retained on the membrane as a concentrate (sometimes called the refuse or brine).

iv. **Permeate and Concentrate Collection:** The concentrate is either disposed of or undergoes additional processing, whereas the pure solvent (permeate) is gathered on the opposite side of the membrane.

v. **Membrane Maintenance:** To ensure the system's long-term effectiveness, RO membranes need to be cleaned and maintained on a regular basis to avoid fouling, scaling, and biofilm formation.[16]

6. MICRODIALYSIS:

➢ **Principle:**

- By simulating the operation of a capillary system, microdialysis is an in vivo sampling technique used to track biochemical changes in tissues or fluids. According to their concentration gradient, solutes diffuse over a semi-permeable membrane. This procedure makes it possible to continuously gather tiny molecules from the interstitial space of tissues or bodily fluids, such as hormones, medications, and neurotransmitters. The method operates on the dialysis principle, which states that larger molecules are blocked from passing through the membrane while smaller solutes do. Once inside the target tissue, the microdialysis probe is pumped with a perfusion fluid, which is typically comparable to extracellular fluid. The exchange of solutes between the extracellular space and the perfusion fluid is made possible by the membrane at the probe's tip. The perfusion fluid is collected for additional investigation once molecules of interest diffuse into it.

➢ **Procedure:**

i. **Insertion of the Probe:** A microdialysis probe is carefully inserted into the target biological fluid or tissue (such as the brain, blood, or muscle). The position of the semi-permeable membrane at the tip of the probe is essential for efficiently sampling molecules.

ii. **Perfusion:** At a regulated flow rate (generally between 0.1 and 5 µL/min), a perfusion solution typically an isotonic buffer, like Ringer's solution is delivered into the probe.

This solution's composition is intended to be similar to that of the interstitial fluid in order to preserve osmotic balance and promote passive diffusion.

iii. **Diffusion Process:** Drugs, neurotransmitters, and metabolites from the extracellular fluid permeate the semi-permeable membrane and enter the perfusion fluid. The concentration differential across the membrane acts as the driving force, permitting only tiny molecules to flow through.

iv. **Collection:** Fractions of the dialysate, or perfused fluid with the disseminated molecules, are gathered for further examination. Although a correction factor (relative recovery) is frequently required to estimate the true extracellular concentration, the analyte concentration in the dialysate represents the molecule's interstitial concentration.

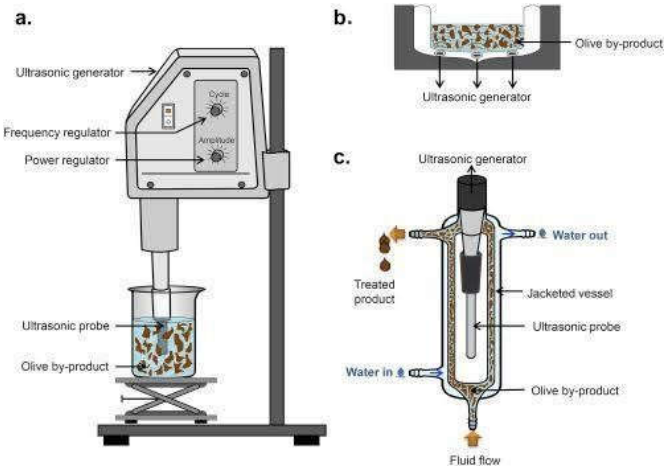
v. **Analysis**: To determine the analyte concentration, the collected dialysate is subjected to separation techniques including mass spectrometry (MS), gas chromatography (GC), or high-performance liquid chromatography (HPLC). High sensitivity and specificity for a range of biological substances are made possible by these methods.[17]

❖ PURIFICATION TECHNIQUES:

1. ULTRASONIC EXTRACTION:

➢ **Principle:**

- Ultrasonic extraction, sometimes referred to as ultrasound-assisted extraction (UAE), is a method that improves the extraction of chemicals from plant materials or other sources by using ultrasonic vibrations. Cavitation—the creation, expansion, and collapse of bubbles in a liquid media that results in localized high temperature and pressure—is the basis for the idea. The release of bioactive chemicals is facilitated by these effects, which break cell walls and promote mass transfer. Plant cells are broken down and solvent penetration into the cells is improved when ultrasonic waves travel through the liquid and create mechanical vibration. This procedure increases the extraction's yield and efficiency. The cavitational forces improve the solvent-target compound interaction, which enhances the desired constituents' solubilization and desorption.



➢ **Procedure:**

i. **Sample preparation:** To enhance the surface area for extraction, the plant material (such as Picrorhiza kurroa or other botanicals) is dried and ground into a fine powder.

ii. **Solvent Selection:** Depending on the solubility of the target compound, an appropriate solvent (such as ethanol, methanol, water, or a solvent mixture) is selected. The best extraction efficiency can be achieved by optimizing the solvent ratio.

iii. **Sonication:** In a beaker or ultrasonic extraction vessel, the solvent and powdered plant material are combined. An ultrasonic bath or probe is used to set up ultrasonic equipment. The ultrasonic waves' strength and frequency, which are typically between 20 and 100 kHz, are changed. Since greater temperatures can damage thermolabile

chemicals while also improving extraction, the sonication time and temperature are also tuned.

iv. **Cavitation and Cell Disruption:** The solvent's cavitation bubbles are created by the ultrasonic waves, and when they burst, they produce confined areas of high temperature and pressure. Plant cell walls are broken down as a result, which makes it easier for intracellular substances to escape into the solvent.

v. **Filtration and Collection:** After sonication, the liquid is filtered to get rid of any solids, leaving the extract in the solvent that contains the bioactive chemicals.

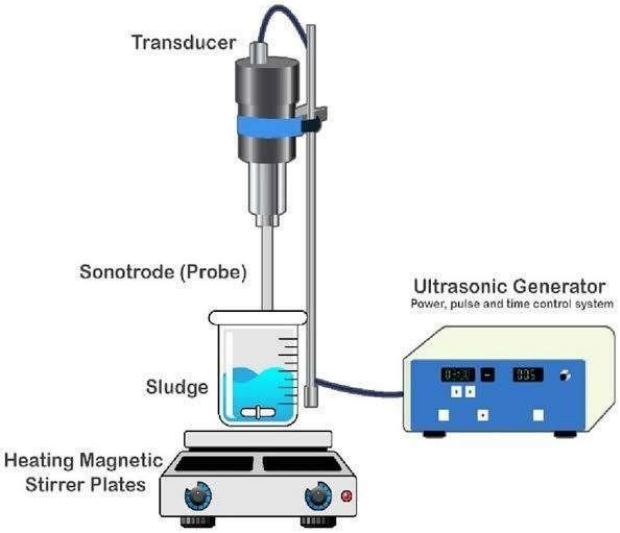
vi. **Purification of Extracted Compounds:** Depending on the target component and the necessary purity level, purification can be accomplished by methods like thin-layer chromatography (TLC), solid-phase extraction (SPE), or column chromatography.

vii. **Concentration and Drying**: To concentrate or separate the extracted chemicals, the solvent can be evaporated using a rotary evaporator or alternative drying methods such lyophilization (freeze-drying).[18]

2. SONICATION:

➢ **Principle:**

- High-frequency sound waves, typically between 20 and 100 kHz, are used in the purifying process known as "sonication" to stir up particles in a sample. The creation, expansion, and implosive collapse of bubbles in a liquid media are all part of this process, which is frequently called acoustic cavitation. When these bubbles burst, locally high temperatures, pressures, and shear forces are created. These conditions have the potential to damage cells, break chemical connections, and disintegrate or dissolve undesirable substances.



➢ **Procedure:**

i. **Sample Preparation:** The sample that needs to be purified such as cell lysate, a particle suspension, or an impurity solution is put in an appropriate container. Make sure the container is made of a material that can withstand sonication, such as plastic or glass that doesn't absorb sound.

ii. **Addition of Solvent (if required):** To facilitate the sonication process, a solvent may be added to the sample, contingent on the type of contaminants. For instance, organic solvents may be utilized for chemical samples, but water or buffer solutions are frequently utilized in biological applications.

iii. **Sonication Setup**: The container is either probed with an ultrasonic horn or immersed in a sonicator bath. A probe sonicator is frequently chosen for precision applications because it more precisely delivers the energy into the sample.

iv. **Setting Sonication Parameters:**

- **Frequency:** Select an appropriate frequency, usually between 20 and 50 kHz for the majority of purifying applications.
- **Amplitude:** Modify sound wave amplitude according to the intended result and sample sensitivity (greater for robust materials, lower for sensitive samples).
- **Temperature Control:** The sample may become heated by high-energy sonication, so it's important to either work in brief bursts or chill the sample—typically with an ice bath—to prevent overheating.

v. **Sonication Process**: Short bursts of sonication (e.g., 30 seconds on, 30 seconds off) will allow the sample to cool and avoid heat damage. During sonication, keep an eye on the sample to make sure cavitation happens without causing it to deteriorate from too much heat or overprocessing.

vi. **Centrifugation or Filtration**: Impurities like cellular debris or aggregated chemicals can be separated by centrifugation or filtering following sonication. For usage or additional analysis, the purified solution or supernatant is gathered.

vii. **Post-purification Analysis**: Examine the purified sample to make sure the sonication procedure worked. For biological materials, common analytical methods include protein assays, spectrophotometry, and High-Performance Liquid Chromatography (HPLC).[19]

3. HIGH-INTENSITY FOCUSED ULTRASOUND (HIFU):

➢ **Principle:**

- High-Intensity Focused Ultrasound (HIFU) is a method that sonicates molecules to purify them using concentrated ultrasonic vibrations. HIFU uses the mechanical effects of acoustic cavitation, in which high-frequency sound waves cause microbubbles to develop, grow, and collapse in a liquid media, to function at frequencies between 0.8 and 3.5 MHz. Localized high temperatures and pressures produced by these cavitation effects cause cellular

structures to break down, intracellular chemicals to be released, and molecular component separation to occur. By increasing mass transfer and improving solubility, the approach is frequently used to purify biomolecules, nanoparticles, or to improve chemical extraction, enabling a more selective separation of desired compounds from contaminants. Because HIFU is focused, the ultrasonic energy is concentrated in a single location, causing less damage to the surrounding material and lowering the possibility of unintended degradation or modification of sensitive substances.

- **Procedure:**

i. **Sample Preparation:** The target material (such as biomolecules, herbal extracts, or nanoparticles) is dissolved in a suitable solvent or buffer solution to create the sample. Before sonication, the solution might need to be filtered to get rid of unwanted particles.

ii. **Sonication Setup:** To prevent energy loss, the prepared sample is put in a container, usually a glass or quartz vial. Using a water bath or coupling medium, the vial is placed in the path of the concentrated ultrasonic beam to guarantee effective ultrasonic wave transmission. Based on the type of sample and the purification target, the HIFU device is calibrated to the desired frequency, usually between 1-3 MHz.

iii. **Ultrasound Application:** The sample is exposed to ultrasound energy at predetermined intervals. The volume of the sample and the intended result determine the length and level of intensity. The cavitation mechanism encourages the release and separation of chemicals by upsetting molecular clumps. By ensuring localized and precise action, the focused ultrasound enhances purification process selectivity.

iv. **Cavitation-Induced Purification:** As cavitation bubbles burst, contaminants are broken down or undesirable molecules are easier to remove thanks to the creation of extremely high localized temperatures (up to several thousand Kelvin) and pressures (hundreds of atmospheres). Additionally, HIFU improves diffusion and speeds up the purification process by strengthening the bond between the solvent and solutes.

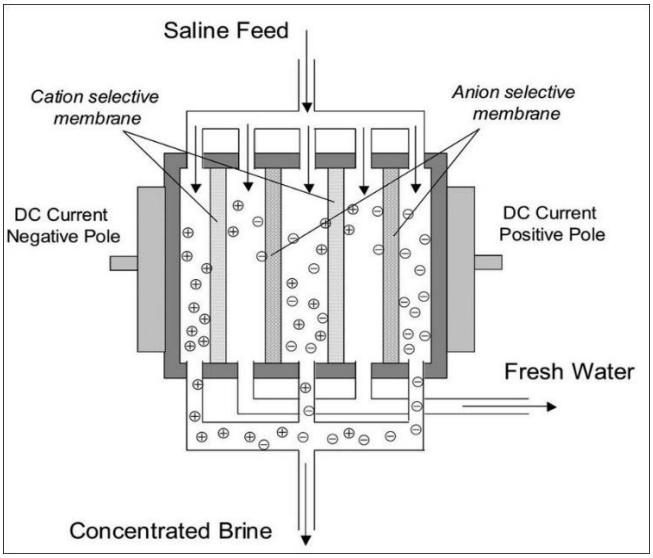
v. Separation and Collection: To separate the purified component from the solvent or contaminants, the solution is put through an appropriate separation process (such as filtering, centrifugation, or chromatographic procedures) after sonication. The purity of the finished product may occasionally be improved by employing extra purification procedures like phase separation or recrystallization.

vi. Post-processing: If necessary, the refined chemical is dried or condensed. To confirm the finished product's purity and chemical makeup, analytical methods like nuclear magnetic resonance (NMR), mass spectrometry, and high-performance liquid chromatography (HPLC) can be used.[20]

4. ELECTRODIALYSIS (ED):

➢ Principle:

- Ion migration across ion-selective membranes is accelerated by an electric field in the membrane-based separation technique known as electrodialysis (ED). The removal or separation of charged species, like salts, from a solution is its main use. Both cation-exchange membranes, which permit the flow of positively charged ions, or cations, and anion-exchange membranes, which permit the passage of negatively charged ions, or anions, are essential to the process. Ions go through the corresponding membranes toward electrodes of opposite charge when an electric potential is applied across the system, leaving behind a pure solution.



➢ Procedure:

i. **Setup Preparation**: Multiple cells made of alternating cation- and anion-exchange membranes are positioned between two electrodes (the anode and the cathode) to construct the electrodialysis system. The cell is filled with the feed solution that contains the ions that need to be eliminated.

ii. **Ion Migration Under Electric Field:** Ions in the feed solution move through cation-exchange membranes toward the negatively charged cathode and anion-exchange membranes toward the positively charged anode when a direct current (DC) electric field is applied. The ions gather in the concentration compartment after being selectively extracted from the feed solution as they travel.

iii. **Ion Removal and Collection:** The desalinated product is the purified stream that has had its ions removed. The concentrate compartment collects a different stream of concentrated ions.

iv. **Recirculation:** To improve purifying effectiveness, the feed stream is frequently

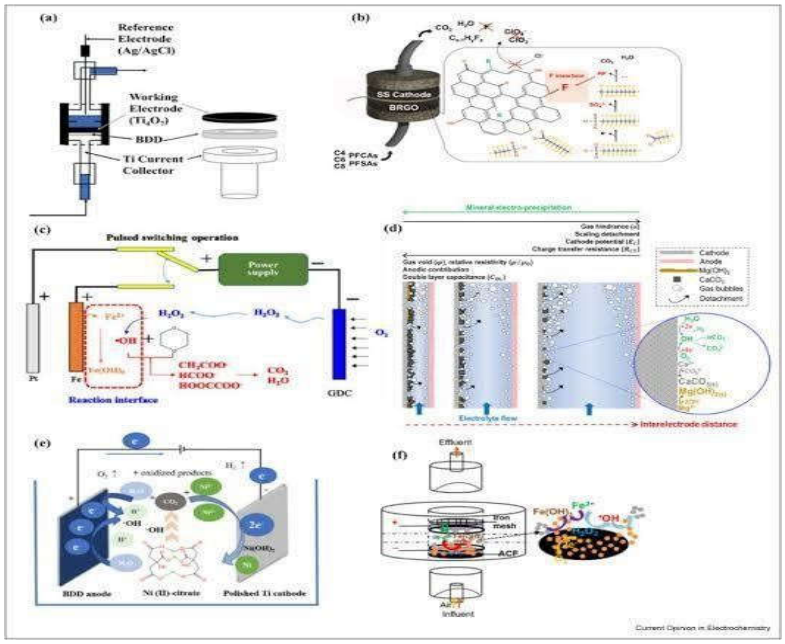
recirculated through the system.

v. **Membrane Maintenance and Cleaning:** To avoid fouling, which can impair performance, the membranes must be cleaned on a regular basis. To maximize ion removal and efficiency, the system might also need to have its voltage or flow rates adjusted. [21]

5. ELECTROFILTRATION:

➢ **Principle:**

- A purification method called electrofiltration improves the removal of charged particles from a solution by combining filtration and electrokinetic processes. The idea is based on applying an electric field across a filtration membrane, which facilitates the flow of charged particles—like ions or macromolecules—to the electrode that is oppositely charged. In addition, the membrane separates larger particles according to size by acting as a physical barrier. Compared to conventional filtration, this method permits better separation, lowers membrane fouling, and improves filtering efficiency.



➢ **Procedure:**

i. **Creating the Feed Solution:** Create the solution that contains the target particles or compounds that require separation, such as proteins, colloids, or ionic species. Depending on the particle charge, optimize the separation by adjusting the solution's pH and ionic strength as needed.

ii. **Electrofiltration System Setup:** Make use of an electrofiltration cell with a porous membrane serving as the medium for separation. To generate an electric field across the membrane, position electrodes on either side of it. The size of the particles to be separated should be taken into consideration while choosing the membrane's pore size.

iii. **Electric field application:** Move the charged particles in the solution toward the electrode with the opposite charge by applying a regulated voltage between the electrodes. The electric field minimizes membrane fouling while improving particle mobility and filtration rate.

iv. **Filtration Process:** The electric field pulls charged particles as the solution moves across the membrane, separating neutral particles according to size. On one side of the membrane, gather the filtrate, or permeate, that contains the purified solution.

v. **Gathering Retentate and Permeate:** Following the filtration procedure, the solution is separated into two parts: permeate, which contains the purified chemicals, and retentate, which contains bigger particles or those that are not adequately charged. Depending on the system's size and configuration, the procedure may be batch or continuous.

vi. **Post-filtration Analysis:** Examine the concentration, purity, and separation effectiveness of the target chemicals in the retentate and permeate. Chromatography, electrophoresis, and spectrophotometry are examples of common analytical methods. [22]

6. ENZYME-CATALYZED REACTIONS:

➢ **Principle:**

- Because of their efficiency, gentle reaction conditions, and specificity, enzyme-catalyzed reactions are frequently employed in purification. The ability of the enzyme to specifically identify and catalyze the conversion of particular substrates into products, enabling the isolation of these products from the reaction mixture, is the foundation of the enzyme-catalyzed purification principle. The elimination of undesirable contaminants or the isolation of target chemicals in a more refined form are made easier by this selective biocatalysis.

➢ **Procedure:**

i. **Selection of Enzyme**: Based on the target molecule's characteristics and the enzyme's substrate specificity, select the right one. For instance, lipases are employed for fatty acids or esters, whereas proteases can be utilized to split proteins specifically.

ii. **Preparing the Reaction Mixture:** Put the substrate a raw material that contains the target compound into an appropriate buffer solution. The buffer's pH and temperature should be adjusted to maximize the activity of the enzyme. At the ideal concentration, add the enzyme to the reaction mixture. Although they can change based on the reaction, enzyme concentrations usually fall between 0.1% and 1% of the substrate's weight.

iii. **The Enzymatic Process:** The reaction mixture should be incubated at the ideal temperature, pH, and other parameters for enzyme activity. Depending on the enzyme and substrate, this could go on for a few minutes or several hours. Using methods like thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), or spectrophotometry, sample the reaction and examine how the substrate is transformed into the intended product.

iv. **Product Isolation:** The target product can be extracted from the reaction mixture when the enzymatic reaction is finished. This stage frequently entails:

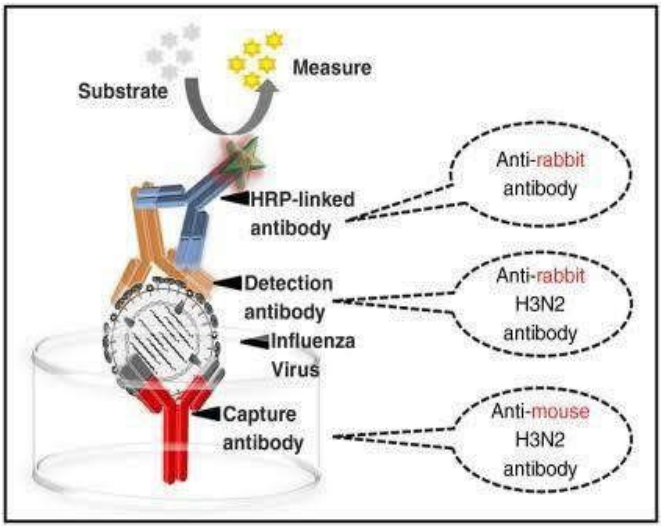
- **Precipitation:** This can be a quick and efficient method of product isolation if the enzyme or contaminants are precipitable by altering the pH or adding salts (such as ammonium sulfate).
- **Filtration:** To get rid of enzyme clumps or undissolved contaminants, solid-liquid separation methods such membrane filtration can be applied.
- **Chromatographic Separation:** The reaction product can be further purified from any residual contaminants using methods such affinity chromatography, ion-exchange chromatography, or size-exclusion chromatography.

v. **Enzyme Removal (if required):** Following the reaction, the enzyme might need to be eliminated if it is not a component of the intended result. This can be accomplished by:

- **Heat denaturation:** By increasing the temperature, the enzyme can be rendered inactive if the result is heat-stable.
- **Affinity or size-exclusion chromatography:** Size-based separation or trapping enzymes on affinity resins are two more methods for removing them.

vi. **Product Concentration and Drying:** Following purification, techniques such as solvent removal, rotary evaporation, or lyophilization (freeze-drying) can be used to concentrate the finished product. Depending on the intended use, the product may undergo additional drying and preparation to reach its ultimate state. [23]

7. ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA):



➢ **Principle:**

- A biochemical method called the Enzyme-Linked Immunosorbent Assay (ELISA) uses particular antigen-antibody interactions to find out whether an antigen—a protein, peptide, or antibody—is present in a sample. This method depends on an enzyme's capacity to catalyse a reaction that results in a detectable signal—typically a

change in color signaling the presence of the target analyte. A particular antibody is utilized to bind to an immobilized antigen on a solid surface in a standard ELISA. When an enzyme is exposed to a substrate, the antibody is coupled to it and catalyses a reaction that results in a measurable signal, such as chemiluminescent, colorimetric, or fluorescent output. ELISA is frequently used for antigen detection and quantification in clinical diagnostics, research, and pharmaceutical development.

- **Procedure:**

i. **Coating:** The purified antigen (or capture antibody in sandwich ELISA) is applied to a 96-well plate, which is then incubated for one to two hours at 37°C or overnight at 4°C. By using a buffer (like PBS) to wash the wells, excess unbound antigen is eliminated.

ii. **Blocking:** To cover unused well surface sites and stop nonspecific binding, a blocking solution (such as 1% BSA or non-fat dry milk) is applied to the wells.

iii. **Primary Antibody Binding:** The antigen-containing sample is added to the wells, and in either direct or indirect ELISA, the antigen binds to the antibody; in sandwich ELISA, the immobilized antibody captures the antigen. This process is known as primary antibody binding. After one to two hours of incubation at room temperature, the unbound material is washed off.

iv. **Indirect ELISA Secondary Antibody:** The wells are filled with a secondary antibody that binds to the primary antibody after being conjugated to an enzyme (such as alkaline phosphatase or horseradish peroxidase, or HPA). Additional washing is performed after incubation to get rid of any unbound secondary antibodies.

v. **Substrate Addition:** The wells are filled with an enzyme-specific substrate (such as TMB for HRP), which causes a color shift that corresponds to the quantity of antigen or antibody in the sample. A predetermined amount of time, usually 10 to 30 minutes, is given for the reaction to develop.

vi. **Detection:** To fix the color intensity, a stop solution, such as sulfuric acid, is used to stop the enzymatic reaction. Using a microplate reader set to the proper wavelength (450 nm for colorimetric ELISA), the intensity of the color shift is determined. [24]

8. MAGNETOPHERESIS:

- **Principle:**

- In order to remove and purify magnetically responsive particles from a mixture, magnetopheresis is a purification method that uses magnetic fields. Its main foundation is the magnetic characteristics of particles, such as those functionalized or coated with magnetic nanoparticles (iron oxide, for example). These particles can be distinguished from non-magnetic components in the solution by migrating toward the magnetic field when

exposed to an external magnetic field. When the magnetic particles are functionalized with certain ligands or antibodies that bind to target molecules preferentially, this technology is especially helpful for biological or chemical purification procedures. Following binding, the bound target can be easily separated by the external magnetic field and subsequently cleaned and processed further.

- **Procedure:**

i. **Magnetic Nanoparticle Preparation:** Create or buy magnetic nanoparticles, such as iron oxide nanoparticles. To ensure appropriate binding affinity, functionalize the nanoparticles using a ligand or antibody unique to the target molecule.

ii. **Reaction with Sample:** Include the target molecule-containing solution with the functionalized magnetic nanoparticles. To enable binding between the nanoparticles and the target molecules, incubate the mixture for a predetermined amount of time, typically 30 minutes to 2 hours.

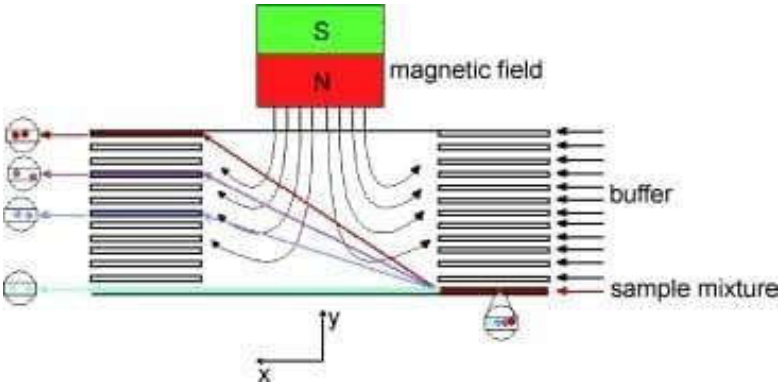
iii. **Magnetic Separation:** Apply an external magnetic field following incubation, typically with a magnet or magnetic separator. While the rest of the solution is unaffected, let the particles be drawn to the magnetic field so they can collect along the magnet.

iv. **Supernatant Removal:** Gently drain the supernatant, which comprises the unbound, non-magnetic materials, leaving the magnetic particles at the container's bottom.

v. **Washing:** To keep the magnetic particles apart, add a buffer solution, shake the container gently, and use the magnetic field to wash the particles. To guarantee complete cleaning, repeat this procedure two or three times.

vi. **Target Elution:** To free the bound target molecules from the magnetic particles, add the proper elution buffer after washing. After removing the magnet, gather the target molecules that have been eluted for use in subsequent processes.

vii. **Final Purification**: Use further methods like filtration or chromatography to further purify the eluted sample if necessary. [25]



❖ CONCLUSION:

- To improve our knowledge of the chemical characteristics and biological activities of plants, it is essential to separate and purify their constituents. High-precision and accurate phytochemical isolation and characterization are made possible by analytical techniques like mass spectrometry, chromatography, and several spectroscopy methods. Because every methodology has different benefits and drawbacks, it is essential to choose the best one for the particular needs of the investigation. The advancement and improvement of these analytical techniques will be essential to guaranteeing the efficacy and purity of compounds derived from plants as the market for natural goods in medicines, nutraceuticals, and functional foods continues to grow. In addition to making it easier to explore plant biodiversity, ongoing research and advancements in analytical techniques will help find new medicinal compounds. In the end, these initiatives will strengthen our ability to utilize plant components for a variety of purposes, advancing sustainability and health in a world growing more complicated by the day.

❖ **Reference:**

1. P. Tonthubthimthong, S. Chuaprasert, P. Douglas and W. Luewisutthichat.(2011, March). Supercritical CO2 extraction of nimbin from neem seeds an experimental study, Journal of Food Engineering. 47 (4), pp. 289-293. https://doi.org/10.1016/S0260-8774(00)00131-X.
2. V. Duraipandiyan, M. Ayyanarand S. Ignacimuthu. (2006, October). Antimicrobial activity of some ethnomedicinal plants used by Paliyartribe from Tamil Nadu, India. BMC Complementary Altern. Med. 6 (35), pp. 35-41. http:// doi.org/10.1186/1472-6882-6-35]
3. J.J. Roy Burman, "Tribal Medicine" 1st ed. A Mittal Publication, 2003, New Delhi, pp. 12.
4. JX Li and ZY. Yu (2006). Cimicifugaerhizoma: from origins, bioactive constituents to clinical outcomes, Curr Med Chem. 13 (27), pp 2927-51.
5. JF. Liao, Y.M Jan, SY. Huang, HH. Wang, LL. Yu and CF. Chen. (1995, July). Evaluation with receptor binding assay on the water extracts of ten CNS-active Chinese herbal drugs, Proceedings of the National science Council, Republic of China. 19 (3), pp. 151-158.
6. A. Ahmad, A.F.M. Alkarkhi, S. Henaand L.H. Lima. (2009, February). Extraction, Separation and Identification of Chemical Ingredients of ElephantopusScaber L Using Factorial Design of Experiment, International Journal of Chemistry. 1(1), pp. 36-49.
7. B. Sandhya, S. Thomas, W. Isabel and R. Shenbagarathai (2006). Ethnomedicinal plants used bythe valaiyan community of piranmalai hills (reserved forest), tamilnadu, india. - a pilot study. African Journal of traditional, Complementary and alternative medicines.3 (1), pp. 101-114.
8. A. Pandey and S. Tripathi. (2001, January). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug, Journal of Pharmacognosy and Phytochemistry. 2(5), pp. 115-119.
9. Daffre, S, Bulet, P, Spisni, A, Ehret-sabatier, L, Rodri-gues, EG. & Travassos, L.R. Studies in Natural Prod-ucts Chemistry. Vol. 35. 2008, pp 597- 691.
10. Doughari, J.H, Human, I.S, Bennade, S. & Ndakidemi, P.A. Phyto chemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. Journal of Medicinal Plants Research. 3 (11), 2009 pp 839-848.
11. Yadav, N.P. &Dixit, V.K. Recent approaches in herbal drug standardization. 2 (3), 2003 pp 195-203.

12. Righetti, P. G., Capillary Electrophoresis in Analytical Chemistry, Marcel Dekker Inc., 2001.
13. Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A., & Ando, T. (1984). Electrokinetic separations with micellar solutions and open-tubular capillaries. Analytical Chemistry, 56(1), 111-113.
14. Garfin, D. E. (1990). Isoelectric focusing. Methods in Enzymology, 182, 459-477. https://doi.org/10.1016/0076-6879(90)82046-T
15. S. K. Sikder, A. M. Mohanty, P. Ghosh, and M. N. Islam, "Nanofiltration Process: Applications in Industrial Wastewater Treatment," Separation and Purification Technology, vol. 107, pp. 1-15, 2013.
16. Mulder, M. (1996). Basic Principles of Membrane Technology. Kluwer Academic Publishers. This book explains the theory and applications of various membrane technologies, including reverse osmosis.
17. Borsdorf, H., & Eiceman, G. A. (2006). Ion Mobility Spectrometry: Principles and Applications. Applied Spectroscopy Reviews, 41(4), 323-375.
18. Chemat, F., Abert Vian, M., & Cravotto, G. (2012). Green extraction of natural products: concept and principles. International Journal of Molecular Sciences, 13(7), 8615-8627. doi:10.3390/ijms13078615.
19. Mason, T. J., & Lorimer, J. P. (2002). Applied Sonochemistry: The Uses of Power Ultrasound in Chemistry and Processing. Wiley-VCH.
20. Chen, H., & Wang, S. (2017). "High-Intensity Focused Ultrasound: Principles, Mechanisms, and Applications in Extraction and Purification." Ultrasonics Sonochemistry, 40, 546-553.
21. Tanaka, Y. (2014). Ion Exchange Membranes: Fundamentals and Applications. Elsevier. (Principles and detailed discussion on ED membranes).
22. Bowen, W. R., & Welfoot, J. S. (2002). Modelling the performance of membrane nanofiltration—critical assessment and model development. Chemical Engineering Science, 57(7), 1121–1137.
23. Otte, J., et al. (2000). Enzyme-assisted protein recovery and purification from waste by- products. Trends in Food Science & Technology, 11(10), 447-458.
24. Engvall, E., & Perlmann, P. (1971). Enzyme-linked immunosorbent assay (ELISA): Quantitative assay of immunoglobulin G. Immunochemistry, 8(9), 871–874.

25. Tran, N., & Webster, T. J. (2010). Magnetic nanoparticles: biomedical applications and challenges. Journal of Materials Chemistry, 20(40), 8826-8833. doi:10.1039/c0jm00994f.