A Review on Advanced Herbal Technology

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***Abstract*—Due to their natural composition and medicinal potential, the usage of herbal medicines has been growing worldwide. The significance of medicinal plants, as well as their identification, authentication, extraction, and analysis, are the main topics of this review. A number of methods for extracting bioactive chemicals from plant sources have been thoroughly explained. These methods include Soxhlet extraction, maceration, and percolation, as well as more recent methods like microwave and ultrasonic-assisted extraction. To guarantee the effectiveness, safety, and purity of herbal remedies, verification is crucial. This can be accomplished by using a variety of methods, such as DNA markers. It has been explained how chromatographic methods are used to separate and identify bioactive substances. The study comes to the conclusion that current authentication methods and standards are crucial.**

I. INTRODUCTION

The majority of people know the names of a few fresh herbs, which are the most common. Basil, thyme, and rosemary have a very distinct look, so it is easy to remember what they look like. Herbal medicinal materials have been used worldwide for the maintenance of health and treatment of diseases. Credentials of herbal medicinal ingredients by DNA technology has been widely used, and the practice began in the mid-1990s. The basic resources of medicines come from nature, and they have been used as medicaments since ancient times. Now days, people are getting attracted to herbal medicine due to its advantages. Herbal formulations have gained extensive acceptability as medicinal agents for various diseases. Although these uses are unnatural, it is a known fact that more than 80% of the world's population relies on herbal medicines and development for healthy living. This increased use of herbal products has led to the abuse and adulteration of these herbal products, which has caused disappointment for both the consumer and the manufacturer. The development of reliable analytical tools to profile the phytochemical composition, quantification of the herbal active and other major constituents, is a major challenge for scientists. Standardization is an important step for establishing a standard biological activity, a standard chemical profile, or simply a standard quality assurance program for production and manufacturing of herbal drugs. Various conventional methods and recent advances are described in the present review.

II. CRUDE PLANT MATERIAL

The elements of the botanic report, with genus, types, sovereignty, description, part of the plant, vigorous, and appearances should be stubborn, and, if thinkable, scope limits may be specified. Unversed, adulterant, and microbial elements should be clarified or limited. Voucher examples, representing each lot of plant material handled, would be determined by a capable physiologist and must be kept for at least a 10-year period. A portion number ought to be assigned, and this should occur on the product title. The mode of manufacturing should be described in detail. If other implications are added during the manufacturing process in order to alter the plant preparation to a particular level of active or factors members or for any other goal, the added ingredients should be stated in the mode of trade. A mode for documentation, and where possible, assays of the plant research should be included. If it is not thinkable to designate a dynamic principle, it should serve to designate a precise substance or variability of consequences to ensure quality of the pre substance

Different methods of identification of plant:
1)Expert determination
2)Recognition
3)Comparison.
4)The use of key and similar instruments

Designation is the basic action and one of the chief functions of systematics. Though identification is a distinct action or technique, from the point of view of method, it is related to classification and nomenclature. Documentation is the process of defining the resemblances or dissimilarities between two items, i.e., two items are similar or dissimilar. When the mystery plant is compared with the identified plant and it is identified that both the items are the same, then the process of classification is used, i.e., if one is able to agree on the classification of the unknown plant as belonging to the same group as the known plant, then the information accumulated from the classification process is at one's disposal. Both of these processes of identification and classification include comparison and conclusion, and the determination of the standards of similarities is required. Therefore, designation is considered an elementary process of classification, and terminology plays an important part in the recovery of information and as a medium of transmission. According to Blackwelder (1967): "proof of identity simplifies us to recover the appropriate facts from the process (classification) to be connected with some trial at hand" and is "reasonable described as the healing side of taxonomy." In exercise, one may easily identify a plant with the benefit of comparison or the aid of keys and may reach the name of the plant. The empirical aspects of plant identification and the techniques of plant identification are discussed in this chapter.

How To Identify Plants

Recognition:

The best method of identification is expert determination in words of trustworthiness or accuracy in public the professional has formulated medications (monograph revision synopses) of the group in question, it's possible that the better current flours or manual include the expert idea of taxable.

Authentication of plant

Formal medical systems are shifting base to the level of stylish medicines in treatment and prevention factors. The increased trade in medicinal plants is providing revenue citation for herbalists, and the substitution of occasional ingredients with cheaper and more readily functional ones is deluding the end users. The unsettled source of the difficulties allied with the calibration of remedial plants is the complicated arrangement of herbal drugs used in the form of whole plants, parts of the plants, or their extracts. Intentional adulteration of deliberate components is posing problems in identifying the real aids. The authentication of medicinal plants by recent molecular approaches is inevitable for herbal drug projects, research, and academia. In recent times, herbal genomics, molecular studies of medicinal plants, and potent next generation sequencing approaches have been employed to revolutionize the recent knowledge. A display of variusing our molecular indications used, their efficiency in barcoding for the resolution of correct confirmation of drugs has been struggled in this study. Data lived collected from last publications and online storages like NCBI, Pubmed etc. There are several different molecular approaches that can be exploited for authentication of medicinal plants like "Regulation Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Element Length Polymorphism (AFLP), Sequence Characterized Amplified Regional (SCAR), Selective Amplification of Microsatellite polymorphic loci (SAMPL), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeat (ISSR), DNA barcoding, Next Generation Sequencing Techniques etc." Few of medicinal plants were documented to have molecular data that can be exploited for the designation of medicinal plants. The genomics statistics of poly herbal preparations helps in the scientific validation and worldwide distinction of the medicinal products. Even though the difficulties in reprehensibility, designing, and derivation of molecular markers and their amplification, and problems related to DNA separateness and purification act as the main barrier in front of the experimenters. It is high time to focus these new techniques for proper identification to provide the fidelity of conventional herbal products and thereby paving a way towards worldwide approval of our indigenous medicinal systems.

Authentication of medicinal plant using molecular biology techniques:

Medicinal plants have gained tremendous popularity in the USA, especially as botanical supplements, herbal medicine, and as a source for drug development. It has been evaluated that in 1997, Americans used or consumed 5.1 billion US dollars' worth of herbal medicine. In order to protect

consumers, the authentication of medicinal plants assumes immense importance. In an ideal situation, it should begin from the time the plant material is harvested to the final product. Though there does not exist any single or exceptional technique to achieve 100 percent authentication during the entire process, it can be accomplished by adopting multiple methodologies. The entire process starts with the use of good voucher specimens, which are used to prove the chain of detention. Macroscopic and microscopic techniques can be employed as designation techniques. Chemical examination can be employed to lay out the best method to detect contaminants. It can also be an excellent method for plant identification. All these methodologies have limitations, and additional analytical techniques are needed to aid in the process. The field of molecular biology has a number of techniques that are quite helpful for the authentication of medicinal plants. Various aspects of the method of authentication are included in this study with priority to the method of molecular biology.

1. The application of the polymerase chain reaction (PCR) to produce DNA markers (DNA) for authenticating botanicals has proven to be very popular and may be the best method to determine the identity and purity of botanicals. DNA markers have proven to be a valuable addition to the utilization of morphological/anatomical and chemical markers. There are several reasons for this. First, DNA is the most unambiguous indicator of genetic identity.

2. One is that here can be incapable magnification of DNAs brought about by competition for primer sites and by low annealing temperatures (Wising et al., 2005). Another serious problem is the lack of reproducibility from lab to lab in part due to changes in PCR parameters (Itchen et al., 2004). Additionally, although it is not necessary to know anything about the genome, the plant material, and although the primers are arbitrarily designed, RAPDs and related PCR techniques require knowledge of the identity of the plants being analyzed.

3. In the therapeutic or botanical complemental purposes of plant, particularly medicinal plants, the authentication of plant resources is a precarious issue. Authentication should be carried out through all the various methods for the safety confirmation of plant products since a single method would not be enough to provide exact plant authentication. These are outer morphological and microscopic methods, physicochemical methods such as TLC, HPLC, NMR, and X-ray, and molecular methods

4. Botanical authentication sometimes involves access to voucher specimens stored within herbaria. Reference standards for botanical and microscopic analysis are not always smoothly functional and should be stored under optimal conditions to avoid degradation. Both macroscopic and microscopic methods are limited when analyzing powdered multi-herb combinations, when analyzing material showing little or no cellular differentiation between closely associated species and genera, and when analyzing material that has been processed to a degree beyond which morphological differentiation is possible.

Authentication of the medicinal plant using the DNA markers

Medicinal plants have been in use for centuries in the global community for the conservation of health and the management of diseases, especially the chronic ones. However, the impurities and the use of spurious materials as substitutes have become a problem of concern for the users of the materials, as well as the industry, based on the need for safety and efficacy. Therefore, the authentication of the medicinal plants is of utmost importance. The problem is resolved using the morphological, anatomical, and chemical markers, as well as the DNA markers. The last method is the best choice based on the preference of the method over the other two, as the method is not age dependent and is tissue-specific with higher discriminating power. Therefore, the characterization of the plants using the markers is the best method of identifying the curative plant types as well as the changes of the identical species. The availability of certified taxonomic specimens in the herbaria is certainly required for the confirmation of the identity of the plant using the method over the closing painterly assessment and analysis. Medicinal plant species are known to be in use since time immemorial for the treatment and cure of hurman and animal ailments. Though there has been a decline in the usage and practice of natural medicine

with the advent of antibiotics and sulfa drugs, the toxicity and harmful effects of synthetic medicine and antibiotics have given a new lease of life to herbal systems of medicine. again

III. EXTRACTION OF HERBS

Natural medicines were the only choice for the prevention and treatment of human diseases for thousands of years. Natural products are important sources for drug development. The amount of bioactive natural products in natural medicines is always relatively low. Today, it is very important to develop efficient and selective methodologies for the extraction and isolation of those bioactive natural products. This paper intends to provide a general overview of a variety of methodologies used in the extraction and isolation of natural products. This article also presents the advantage, disadvantage, and examples of traditional and modern methodologies pertained to in natural products research. Extraction is the first step to separate the desired natural developments from the natural materials. The methods of extraction include solvent extraction, distillation method, scraping method, and sublimation method.

The process of extracting natural harvests involves the following steps:
1)The Solvents Penetrate the Solid Matrix;
2)The Solute Dissolves in The Solvents.
3)The Solute Diffuses Out of The Solid Matrix;
4)The Solute Extracted Forms A Solution.
Any property that can improve the diffusivity and solubility in the above steps will promote the process of extraction. The chattels of the diluents, the extent of the raw material, the percentage of solvents to the raw material, the temperature, and the period will affect the efficiency of the extraction process.

Convectional Extraction Method use in Medical Plant, There Advantages and Disadvantages

Solvent used for active components extraction.

Water	Ethanol	Methanol	Chloroform	Acetone
Tannins	Tannins	Tannins	Flavonoids	Flavonoids
Anthocyanin	Terpenoids	Terpenoids	Terpenoids	
Terpenoids	Polyphenols	Polyphenols		
Saponins	Flavonoids	Saponins		
	Alkaloids	Anthocyanin		

IV. EXTRACTION OF PLANT MATERIAL

I. INTRODUCTION

The qualitative and quantifiable lessons of bioactive amalgams from plant materials mostly rely on the selection of proper extraction method [10, 11]. Plants materials are of growing curiosity for their submissions in medical, nutritive, and highlighting application. They are the source of useful bioactive ingredients, known for a long time ago by their traditional use for medical purposes [12]. The use of traditional medicine and medicinal plants in most of the developing countries has been widely observed, and about 80% of the world's population relies on herbal medicines rather than current modern medicine [13]. Plants contain many active compounds, such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, and flavonoids, deposited in their specific parts, such as leaves, flowers, bark, seeds, fruits, roots, and wood.[14] There are several extraction methods to obtain a compound from plants [15]. The extraction method can be named conventional (traditional using from a long ago) and new (modern and developed more recently). The conventional extraction method is those using solvent organic or water with atmospheric pressure. The new extraction method is those using pressure and /or high temperatures [16]. The extraction method used to obtain a compound from a plant is necessary in order to distinguish the originated components from the active components by using an appropriated solvent. During this process, the solvent penetrates the solid plant material and dissolves the components that have a similar polarity [17].

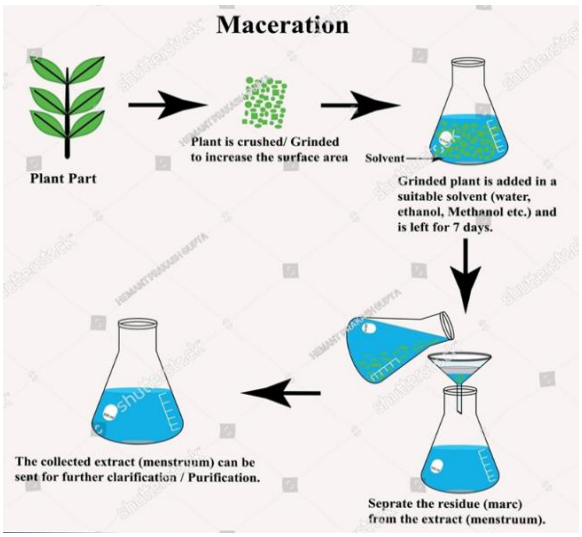
II. EXTRACTION METHODS

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active

compounds of plant or animal tissues from the inactive or inert components (desired and undesired) by the use of selective solvents in standard extraction procedures [18].

A. Maceration

It is an old technique used in medicinal preparation. It is contemplated as a broadly and low-priced way to get natural products from plant material. The maceration is a method of solid-liquid extraction. In this process, the powdered solid materials are kept in a closed vessel and solvent is added. It is allowed to stand for a long time, varying from hours to days, occasionally shaking. Adequate time is permitted for the flush to verbose through the cell wall to solubilize the constituent present in the plant. The process occurs only by molecular diffusion. After that, the liquid is strained off, and the solid residue is pressed to extract as much solvent as possible. If the solvent used is water and the period of maceration is long, then sometimes alcohol in small amounts is added to prevent microbial growth [19]. Maceration involves three principals' steps. Firstly, the materials are converted to powder form by grinding. After grinding, the solvent is added to the closed vessel. Next, the solution is strained off, and the solid matter from this process is pressed to obtain a large amount of solutions. While the process of maceration is going on, occasional shaking is used to facilitate the process of extraction. Concentrated solutions are removed from the sample surface to add fresh solutions to the menstruum for extraction.[20]



Advantages:

1. Maceration is an easy process that requires non-complicated utensil and equipment.
2. Skilled operator is not required.
3. Energy-saving process.
4. Some substances that are very less soluble in solvent and only require prolonged contact with solvent are best done by maceration.
5. Best method for less potent and cheaper drugs.

Disadvantage:

1. Unfortunately, the duration of extraction time is long and sometimes takes weeks.
2. Not exhaustively extract the drug.
3. Very slow process and time-consuming.
4. Solvent required is more.

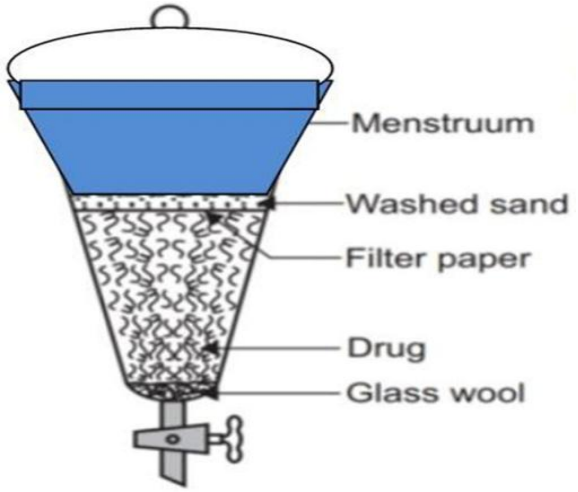
Compration of various Extraction method for natural products:

Method	Solvent	Temperature	Pressure	Time	Volume of Solvent
Maceration	Water aqueous and non-aqueous solvent	Room Temperature	Atmospheric pressure	Long	Large
Percolation	Water aqueous and non-aqueous solvent	Room Temperature, Occasionally Under Heat	Atmospheric pressure	Long	Large
Decoction	Water	Under Heat	Atmospheric pressure	Moderate	None
Soxhlet	Organic solvent	Under Heat	Atmospheric pressure	Long	Moderate
Hydro Distillation	Water	Under Heat	Atmospheric pressure	Long	None

B. Percolation

This method is the one which is most frequently used to obtain active ingredients in tinctures and fluid extracts. A percolator, which has a narrow shape with both ends open, is normally used for this purpose. The solid ingredients are moistened with an appropriate quantity of the solvent to be used, left to stand for approximately 4 hours in a well-closed container, after

which the mass is packed, and the top of the percolator is closed. Solvent is then added to the mixture to produce a shallow layer above the packed mass, which then macerates in the closed percolator for 24 hours. The percolator's passage is before unbolted, and the liquid collected in this part is left to drip slowly. Extra solvent whitethorn be further, if necessary, to produce three quarters of the volume of the final product required.[21] The extract is then pressed, and the liquid so formed is added to the percolate. Suitable amounts of the solvent are added to form the desired volume, and the liquid so formed is clarified by the process of filtration or by allowing the liquid to settle and then decanting. This process is repeated until a drop of the solvent, when allowed to evaporate from the percolator, does not leave a residue [22]



Advantages:

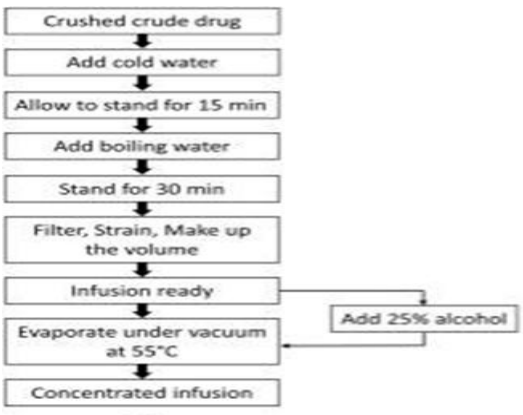
1. This process requires less time compared to the process of maceration.
2. This process might enable the extraction of thermolabile compounds.
3. This process is the right method for potent and expensive drugs.

Disadvantages:

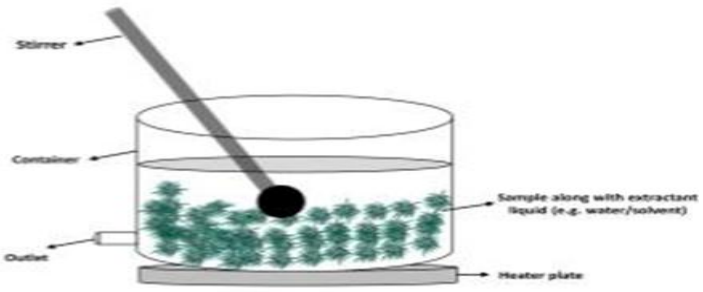
1. More time is required compared to soxhlation.
2. More solvent is required.
3. A skilled person is required.
4. Special attention should be paid on the particle size of the material and throughout the process.

C. Decoction

It is a suitable method for the extraction of the constituents which are soluble in water but cannot be destroyed with the effect of heat [23]. Decoction is a water-based preparation used to extract the active ingredients from the medicinal plant material. In this process, the liquid preparation is prepared by boiling the plant material with water Decoction is the method of choice when working with tough and fibrous plants, barks, and roots, as well as with plants that have water-soluble chemicals. The plant material is usually chopped into small pieces or powdered. Various techniques have been described for the preparation of decoctions. In the Ayurvedic method, traditionally called "Kwatha," the crude drug in the form of yavakuta (small pieces) is taken in earthen pots or tinned copper vessels with clay on the outside. Water is added to it, and the earthen pot is kept on fire. If the crude drug is soft, four times water is taken with 1 part crude drug; if it is moderately hard, eight times water is taken with 1 part crude drug; if it is very hard, then 16 times water is taken with 1 part crude drug. The mixture is boiled with low flame until it is reduced to 1/4th starting volume if the crude drug is soft, and 1/8th if it is moderately or very hard. It is then cooled, strained, and the filtrate is collected in clean vessels [21].



(a)



(b)

Advantages:

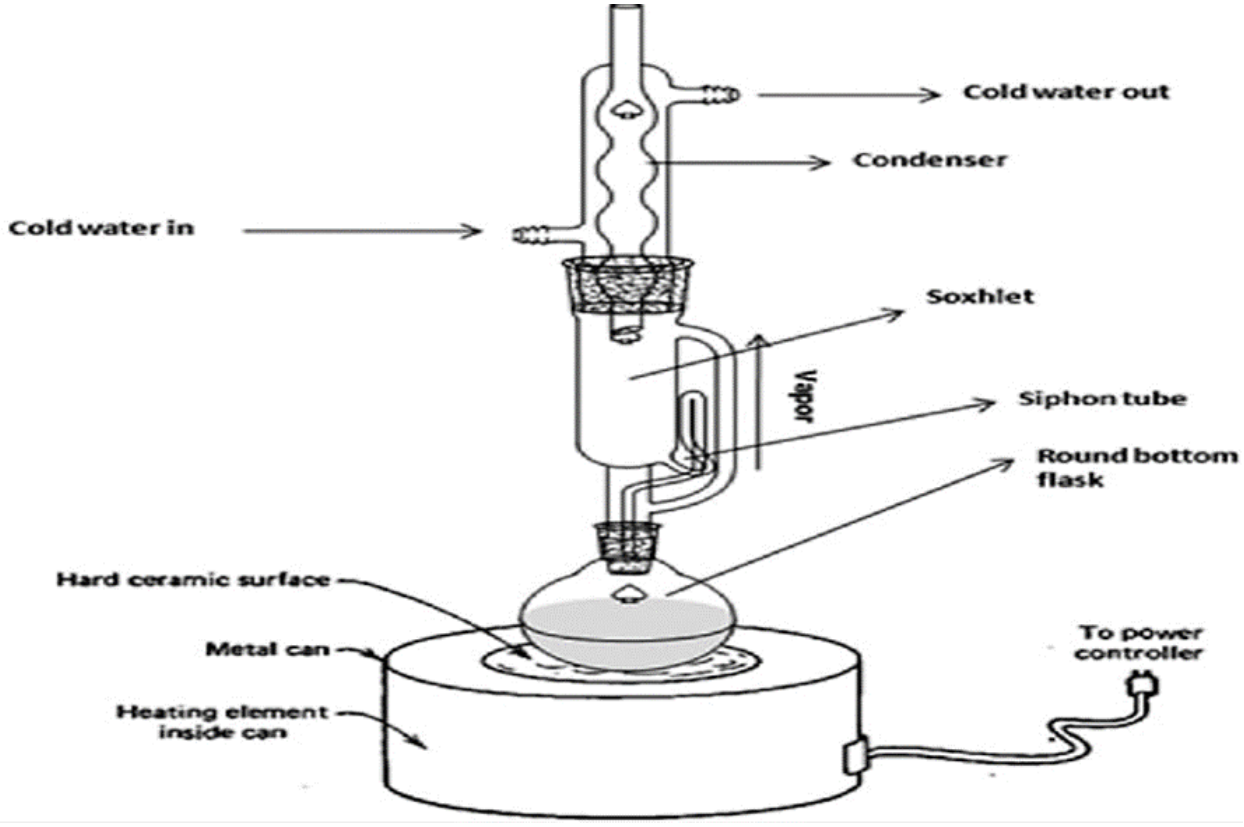
1. It can be used for the extraction of heat-stable substances.
2. This method does not require more and costly equipment.
3. It is easy to do.
4. No skilled person is required.

Disadvantage:

Tactlessly, it is not directed for the extraction of warmth sensitive residents.

D. Soxhlet Extraction

Soxhlet extraction method is best for the continuous extraction of a solid by a hot solvent. This method is named after 'Franz Ritter von Soxhlet', a German agricultural chemist.[24] Soxhlet apparatus is a specialized glassware used for refluxing. This method is mostly used for organic solvent extractions. Soxhlet extraction method is a general method, which is superior to other conventional extraction methods except for, to a limited extent, the extraction of thermolabile compounds. The powdered material to be extracted is put into a thimble made up of filter paper. The thimble is put inside the soxhlet apparatus. The soxhlet apparatus is connected to a round-bottomed (RB) flask containing the solvent and a reflux condenser. The solvent in the RB flask boils gently. The boiling solvent vapor rises up through the side tube and gets condensed by the condenser. The liquid then falls into the thimble comprising the factual and gradually seals the soxhlet . As the solvent touches the top of the attached tube, it siphons over to the flask, thereby removing the extracted portion of the substance. This process is repeated until the extraction process is complete [25].



Advantages:

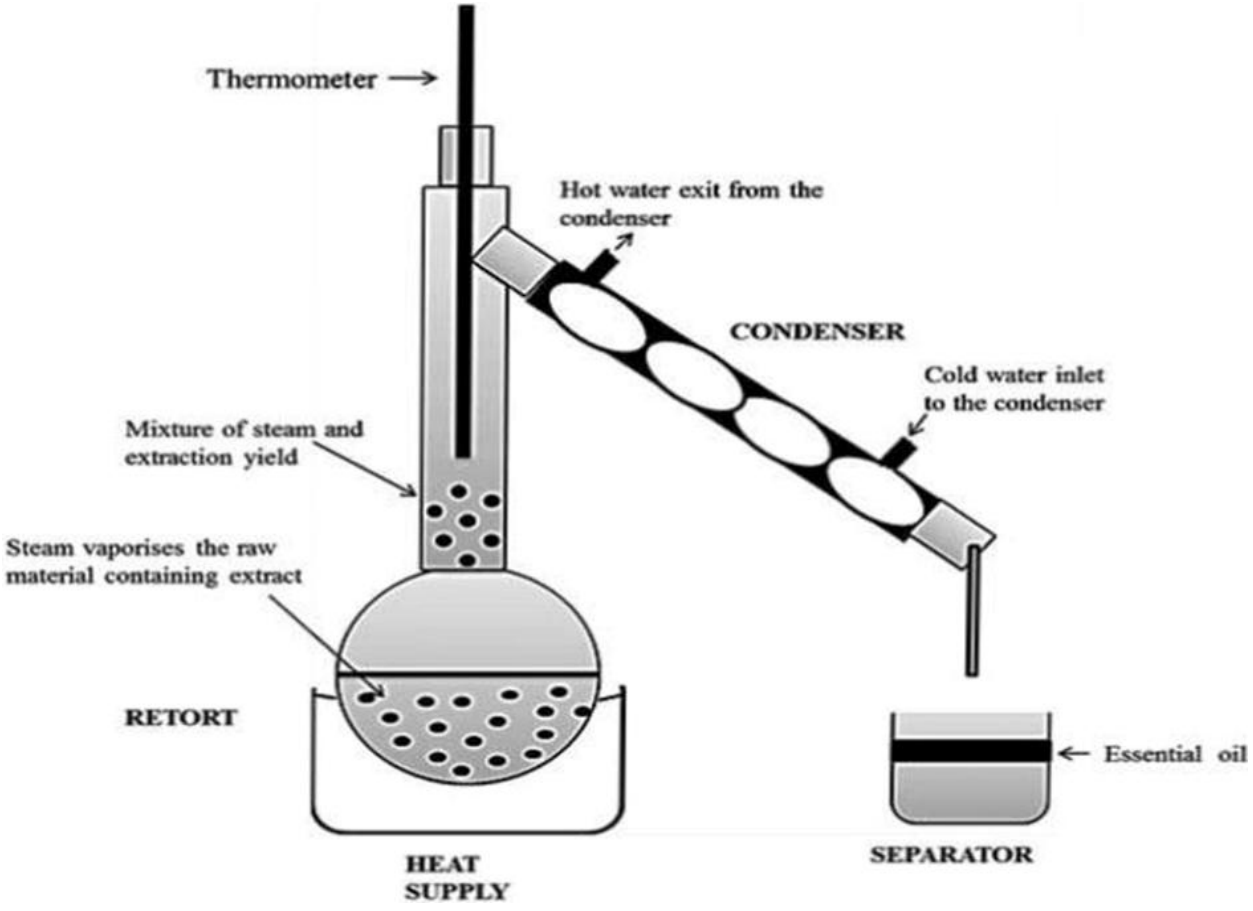
1. A large amount of plant materials can be extracted at a time.
2. The solvent used in this process can be used repeatedly.
3. This process does not require the use of a filtering agent after the extraction process.
4. This process does not require the selection of a particular matrix type.
5. This process is very simple.
6. Displacement of the transfer equilibrium by repeatedly bringing a fresh solvent in contact with the solid matrix.

Disadvantages:

1. The samples are subjected to high temperature at a relatively longer time. Therefore, the possibility of the thermal degradation of the sample under consideration cannot be ignored if the plant material is found to contain heat-sensitive material.
2. The time required is quite long, and the process is labor-intensive.
3. In the process, only a limited number of variables can be manipulated. Therefore, the process is subjected to wide criticism, and the Soxhlet method of extraction is not favored.[26]

E. Hydro distillation

Hydro distillation is a traditional method of plant material extraction without the use of organic solvents. In this method, the plant material is placed in the compartment of the distillation apparatus, and water is added to the sufficient amount. Then the water is heated to boiling point. Otherwise, the steam is straight inoculated into the plant sample. Hot water and steam play the key role as influencing factor to free the bioactive compounds from the plant material. Water vapor is condensed with the help of indirect cooling with the mixture of water and oil. Hydro distillation is potentially a very useful method to extract essential oil from the different plants and their different parts. The yield is affected by the parameters such as the weight of the raw material, volume of water, size of the raw material, and the nature of the raw material.[27]



Hydro distillation includes three physio-chemical processes, i.e., hydro diffusion, hydrolysis, and decomposition by heat. At tall temperature, around volatile complexes may be lost. This is the disadvantage of hydro distillation, and it cannot be used for thermolabile compounds. Hydro distillation apparatus Types of Hydro distillation: There are three types of hydro distillation used to separate essential oils from the plant material. They are as follows: Water distillation Water and steam distillation Direct steam distillation

a. Water Distillation:
In this process, the plant material is completely immersed in water. Then the water is boiled with the application of heat by the use of direct fire, steam jacket, closed steam jacket, closed steam coil, or open steam coil. In this process, the important feature is that the boiling water comes into direct contact with the plant material.

b. Water and Steam Distillation:
In this process, the steam may be generated with the use of a satellite boiler or the still itself but separated from the plant material. In this process, water and steam distillation is used as much as water distillation, especially in rural areas. In addition, this process does not require much more capital expenditure than water distillation. In this process, the apparatus used is the same as that used in water distillation, but the plant solid is maintained above the boiling water on a punctured grid. In this process, it is observed that the persons engaged in water distillation often shift to water and steam distillation.

c. Direct Steam Distillation:
As the name advises, direct steam distillation is the progression of distilling plant material with steam generated outside the still in a steam generator generally referred to as a boiler. Because steam is generated in a satellite boiler, the plant material is heated no higher than 100° C and, consequently, it should not undergo thermal degradation. Steam distillation is the most widely accepted process for the production of essential oils on large scale. Throughout the flavor and fragrance supply business, it is a standard practice.

Advantages:
1. More oil yields.
2. The tendency of components in volatile oil to undergo hydrolysis and polymerization is less (the control of wetness on the bottom of the still affects hydrolysis, whereas the thermal conductivity of the still walls affects polymerization).
3. If refluxing is controlled, then there is a minimum loss of polar components.
4. The quality of oil obtained in steam and water distillation is more reproducible.
5. No organic solvent is required in this method; hence, it is cheap and environment friendly.

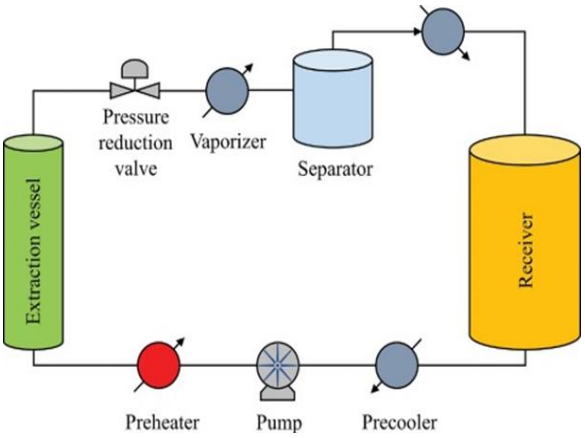
Disadvantages:
1. Complete extraction is not possible.
2. The plant material near the bottom of the still comes in direct contact with fire from furnace and hence may char and thus give an unpleasant odor to the essential oil obtained.
3. The hot water used in this process may cause hydrolysis of some components of essential oil like esters.
4. The process of controlling heat in this method is difficult; this may lead to variable rates of distillation.
5. This process requires a greater number of stills, space, and fuel; thus, it becomes uneconomical.

III. Advanced Extraction Techniques in Herbal Technology [28-30]

The process of extracting bioactive compounds from medicinal plants plays a vital role in herbal technology. Advanced techniques have been employed to overcome the limitations of traditional techniques, such as longer extraction times, lower yields, and excessive solvent use. These techniques have improved the efficiency, selectivity, and sustainability of the herbal extraction process.

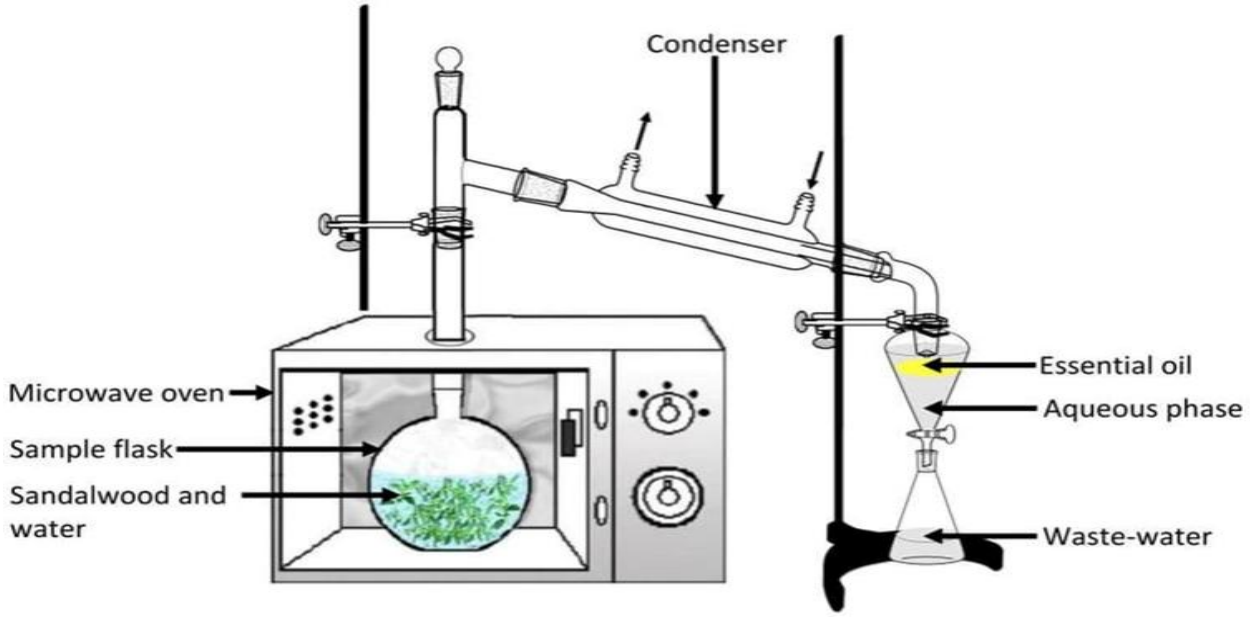
Importance of Advanced Extraction Techniques
a. Higher Efficiency: Improved efficiency in terms of yields and the quality of bioactive compounds.
b. Environmental Sustainability: Improved sustainability by minimizing solvent use and reducing the need for energy.
c. Preservation of Bioactivity: Minimized loss of thermolabile bioactive compounds.
d. Cost-Effectiveness: Improved efficiency by reducing the time required for the process.

Overview of Advanced Extraction Techniques:
A. Supercritical Fluid Extraction (SFE)
Principle:
This technique utilizes supercritical fluids as solvents. When fluids, e.g., carbon dioxide, are placed in a supercritical state, they exhibit properties of both liquids and gases, thus increasing the extraction efficiency of the solvents.



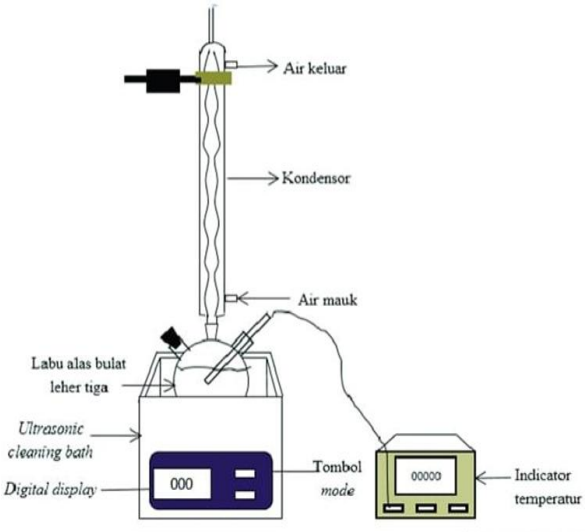
Advantages:
The technique is non-toxic and environmentally friendly, especially when using carbon dioxide as the fluid. The technique shows a high degree of selectivity for bioactive compounds. The technique results in a low level of residual solvents in the final product. Applications: This technique finds application in the extraction of essential oils, flavonoids, and alkaloids.

B. Microwave-Assisted Extraction (MAE)
Principle:
This technique involves the use of microwave energy to heat the solvents and the plant materials, thus disrupting the cell structures and releasing the bioactive compounds.



Advantages:
The technique accelerates the process of extraction by the use of microwave energy. The technique facilitates the extraction of thermolabile compounds.

C. Ultrasound-Assisted Extraction (UAE)
Principle:
This method uses ultrasonic waves to produce cavitation bubbles within the solvent. These bubbles collapse, breaking the cell walls of the plants and releasing the phytochemicals.



Advantages:
Shortens the time and temperature for extraction. Applicable for both large- and small-scale use.

Applications:
For the extraction of polyphenols, saponins, and flavonoids.

D. Solid Phase Extraction (SPE) Principle:
This method involves the adsorption of phytochemicals on a solid material. These phytochemicals are then eluted with a particular solvent.

Advantages:
For the purification and concentration of a particular compound. Reduces impurities present in the final product.

Applications:
For the isolation of alkaloids, glycosides, and secondary metabolites.

E. Microwave Ultrasound-Assisted Extraction (MUAE)
Principle:
This method combines the principles of both microwave and ultrasound assisted extraction for a synergistic effect. Maximizes the extraction process and minimizes time. Advantages: For temperature-sensitive compounds. Applications: For the extraction of volatile oils and antioxidants.

F. Enzyme-Assisted Extraction (EAE)
Principle:
This method involves the use of plant-specific enzymes, e.g., cellulase, pectinase, to hydrolyze the cell walls of plants to release bioactive compounds.
Advantages:
Environmentally friendly and non-toxic. Reduces the level of mechanical disruption involved.

Applications:
This method is used for the extraction of polysaccharides, flavonoids, and glycosides.

G. Supercritical Water Extraction (SCWE)
Principle:
This technique involves the use of water in its supercritical state, e.g., at a temperature of more than 374°C and 22 MPa, to dissolve the compounds.

Advantages:
Does not require the use of organic solvents. Efficient for the extraction of heat-stable compounds.

Applications:
This technique is used for the extraction of phenolic compounds and carbohydrates.

V. CHROMATOGRAPHY:

Chromatography is a technique for the separation of mixtures of molecules, e.g., the separation of the components of a mixture of liquids, dissolved solids, or gases. This technique is based on the principle of separating two or more fluids, a mobile phase, and a fluid stationary phase, separating from each other, moving along the surface or through the body of the other. The factors influencing the process of chromatography include the molecular characteristics related to adsorption, partition, and affinity or differences in their molecular weights. [31-32] Due to these differences, certain components will remain longer in the stationary phase; therefore, they will move slowly in the chromatography system, whereas certain components will pass rapidly to the mobile phase, moving out of the system quickly [33]. Based on this idea, three components are the foundation of the chromatography technique. Stationary Phase: In this type of chromatography, the stationary phase will always be a solid phase or a layer of a liquid adsorbed on the surface of a solid support.

Mobile Phase:
In this type of chromatography, the mobile phase will always be liquid or gaseous component. Separated molecules The type of interaction between the stationary phase, mobile phase, and substance in the mixture is the main component that affects the separation of the molecules from each other. The partition chromatography separation techniques are very effective in separation, particularly in the identification of small molecules such as amino acids, carbohydrates, fatty acids. However, in the separation of macromolecules such as nucleic acids and proteins, affinity chromatography techniques such as ion-exchange chromatography are more effective. Paper chromatography is used in separating proteins and in studies concerning protein synthesis.

Gas-liquid chromatography is used in separating alcohol, esther, lipid, amino groups, as well as in observing the interactions of enzymes. Molecular sieve chromatography is used in determining molecular weights of proteins. Agarose gel chromatography is used in purifying RNA, DNA particles, and viruses [34].

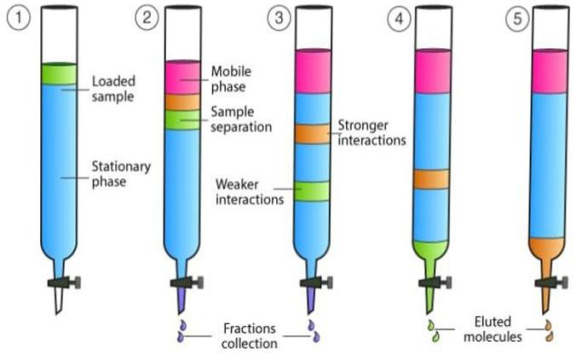
The stationary phase in chromatography, where a solid phase is combined with a liquid phase, is coated on the surface of the solid phase. On the other hand, the mobile phase that flows over the stationary phase is a gaseous phase or a liquid phase. If the mobile phase is a liquid, it is called Liquid Chromatography (LC), and if the mobile phase is a gaseous phase, then it is called Gas Chromatography (GC). Gas chromatography is used for the separation of gases, volatile liquids, and solid materials. Liquid chromatography is used for separating thermally unstable, non-volatile materials [35]. The objective of chromatography, apart from quantitative analysis, is to achieve a satisfactory separation within a reasonable time. Various chromatography techniques have been developed for this reason. Some of the chromatography techniques are column chromatography, thin layer chromatography, paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high pressure liquid chromatography, and affinity chromatography [36].

Types of Chromatography

A. Column Chromatography
B. Ion-exchange chromatography
C. Affinity chromatography
D. Paper chromatograph
E. Thin layer chromatography
F. Gas chromatography
G. High pressure Liquid Chromatography (HPLC)

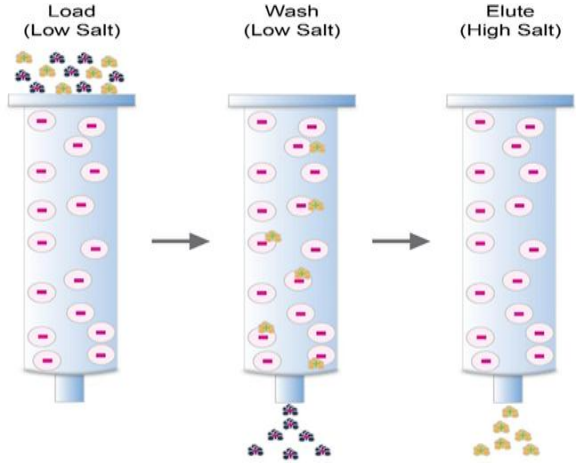
A. Column chromatography

Proteins possess distinctive characteristics, including size, shape, net charge, and affinity for specific ligands, all of which can be exploited for their separation. Chromatographic methods are commonly employed to purify biomolecules by leveraging these properties. Column chromatography, in particular, is a widely used technique in which the sample is passed through a column containing a stationary phase. The components of the mixture interact with this phase and are separated as a mobile phase (wash buffer) flow through the system.[37].



B. Ion-exchange chromatography

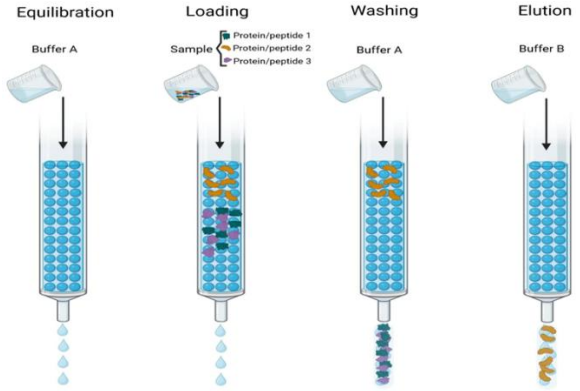
Ion-exchange chromatography separates proteins based on electrostatic interactions between charged groups on the protein surface and oppositely charged groups immobilized on a solid matrix. Proteins bind to the column through reversible ionic interactions. Elution of bound proteins is achieved by altering the properties of the mobile phase, such as adjusting the pH, increasing the salt concentration, or changing the ionic strength. Matrices with positively charged groups are termed anion exchangers and bind negatively charged proteins, while those with negatively charged groups are cation exchangers and bind positively charged proteins. [38].



C. Affinity chromatography

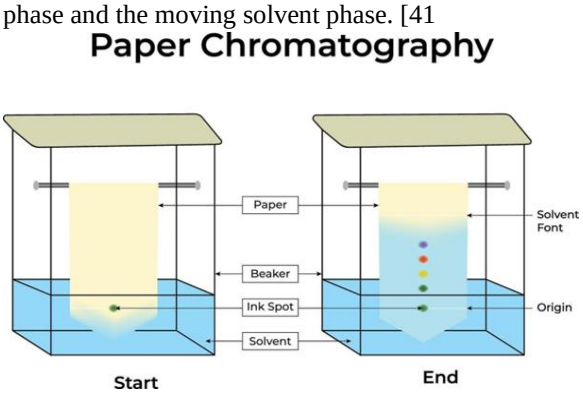
Affinity chromatography is a highly selective technique used for purifying specific biomolecules such as enzymes, hormones, antibodies, and nucleic acids. It relies on the reversible interaction between a target protein and a specific ligand covalently attached to the chromatography matrix. Common support

materials include dextran, polyacrylamide, and cellulose. When a complex mixture is passed through the column, the target protein binds to the ligand and is retained, while unbound proteins flow through. The bound protein can subsequently be eluted by disrupting the interaction, typically by modifying the buffer pH, ionic strength, or by introducing a competing agent. [40].



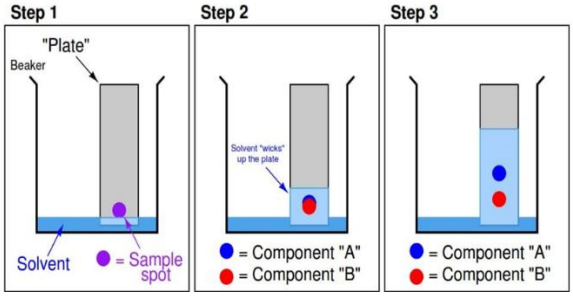
D. Paper chromatography

In paper chromatography, the stationary phase consists of a layer of water adsorbed onto cellulose fibers of a specialized filter paper. The mobile phase is a solvent that migrates through the paper by capillary action, carrying the sample components with it. This technique is considered a form of liquid-liquid chromatography, where separation occurs based on differential partitioning between the stationary water phase and the moving solvent phase. [41



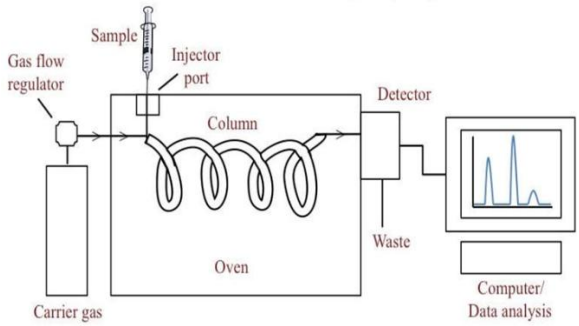
E. Thin layer chromatography

Thin layer chromatography is a solid-liquid adsorption chromatography technique. The stationary phase consists of a thin layer of adsorbent material, such as silica gel, alumina, or cellulose, coated onto a flat, inert support like a glass plate. Separation is achieved as the mobile phase solvent travels up the plate via capillary action, carrying the sample mixture deposited near the bottom. Components migrate at different rates depending on their affinity for the stationary phase and their solubility in the mobile phase. For colorless compounds, detection methods include fluorescence, radioactivity measurement, or chemical staining to visualize spots. The migration of each component is characterized by its retention factor (Rf), calculated as the ratio of the distance traveled by the compound to the distance traveled by the solvent front. Rf values serve as qualitative identifiers for different substances. [43].



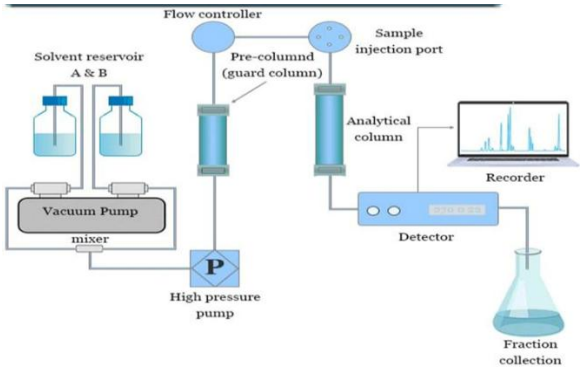
F. Gas chromatography

Gas chromatography is a separation technique where the mobile phase is an inert carrier gas, such as helium or nitrogen, which flows through a column containing a stationary phase. In gas-liquid chromatography, a common form of GC, the stationary phase is a non-volatile liquid immobilized on the inner surface of the column or on a solid support. The sample must be volatile or made volatile through derivatization; it is vaporized and carried through the column by the mobile phase. Components separate based on their differential partitioning between the mobile gas phase and the stationary liquid phase. GC is valued for its simplicity, high sensitivity, rapid analysis, and excellent resolution, making it suitable for separating and analyzing small, volatile molecules even in minute quantities [44]



G. High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography is an advanced form of column chromatography that enables rapid and efficient separation, identification, and purification of a wide range of molecules, including amino acids, carbohydrates, lipids, nucleic acids, proteins, and steroids. Its high resolution is achieved by forcing the mobile phase through the column under high pressure (typically 10–400 atmospheres), resulting in fast flow rates (0.1–5 cm/sec) and significantly reduced analysis times. A typical HPLC system comprises a solvent reservoir, a high-pressure pump, an injection port, a separation column, a detector, and a data recorder. Modern instruments are often computer-controlled, allowing for precise management of separation parameters and automated data collection and analysis [45]



VI. CONCLUSION

Many nations' healthcare systems heavily rely on herbal remedies. However, issues including inaccurate identification of herbal medications, adulteration, and a lack of standardization highlight the necessity of using appropriate authentication methods. The quality and effectiveness of herbal remedies are enhanced when traditional knowledge is combined with cutting-edge methods like molecular biology and contemporary extraction techniques. To produce high-quality bioactive chemicals, the right extraction and analysis methods and equipment must be chosen. Therefore, to guarantee the safety, effectiveness, and international acceptability of herbal medicines, standardization, authentication, and the application of contemporary technology in the analysis and extraction of herbal medicines are crucial.

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