An overview of Natural Medicinal Plant Extraction, Isolation and Characterization Methods in Improvement of Medicinal Research

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***Abstract*: Our plant biodiversity and conservation are greatly enhanced by the usage of medicinal herbs in traditional community practices. Natural products are essential components of traditional knowledge systems in nutraceuticals, food supplements, pharmacological bioactive metabolites of novel chemical entities, and complementary and alternative medicine. Due to the vast flora and fauna biodiversity that generates the requisite accessible chemical diversity, bioactive secondary metabolites from herbal plants of various kinds are key sources and offer major prospects for medication active pharmaceuticals. This paper is aimed to shed light on the fundamental principle of drug discovery from plants are procedure of extraction, isolation and characterization of molecules from plant tissue. These methods have been used in research, and publications on the subject are accessible. However, it is crucial to combine them and talk about their core definitions, including contrastive benefits and drawbacks. Students, researchers and project managers can choose the strategies to use in their projects by conducting a basic literature study of the techniques that are routinely used in drug discovery from natural sources.**

***Keywords*: Extraction, Isolation, Characterization, Drug discovery, Bioactive metabolites**

I. INTRODUCTION

Natural products, such as plants extract, open a new horizon for the discovery of new therapeutic agents (56). Research on plants that have medicinal properties and identifying the chemical components responsible for their activities have justified the ancient wisdom of traditional healing and have proven the long-term healing potential of many medicinal plants (54). Wild plants have always been imperative sources of primary health care and other everyday needs for local communities around the world, an indication that herbal remedies can provide the best alternative source for getting a variety of medications.

Naturally occurring drugs are now playing an increasingly important role in medical and health service because plant produced metabolites are an essential source of bioactive substances that can be used as an alternative to inexpensive and effective herbal drug against common infections (112). Over 80- 90% of the world's population depends on conventional drugs for their primary health care needs, most of which are related to the use of plant extract (111). Presently, plants are being explored broadly for their pharmacological reason as the source of data of major advance drugs. Extract of different parts of plant were being used to treat different type of contagious disease in ayurvedic system of medicines. Conventional medicine practices form an indispensable part of alternative medicines (118). In spite of the fact that their adequacy and mechanisms of action have not been tried experimentally in most cases these simple medicinal arrangements frequently dictate beneficial response due to their dynamic chemical constituents (122). Thus, the extraction of plants materials is crucial to confine biologically active compounds to their role in disease prevention and treatments and in knowing their harmful impacts as well. Moreover, the review compiles evidence of various bioactive compounds, serving as bibliographic support for researcher working in several fields (Biochemistry, biotechnology, pharmacology, microbiology, crop protection etc).

II. PLANT METABOLITES

Plant metabolites consist of compounds that promote the growth and development of plants; but the numbers of them are not required for the plant to survive. Primary metabolites are recognized critical or vital compounds and are at once worried within side of growth, development and reproduction of plants [35, 114]. Primary metabolites consist of fermentation products such as ethanol, acetic acid, citric acid and lactic acid [98].

Secondary metabolites are not specifically included in those processes and usually have a function but are not that vital for the living beings e.g. phenolics, steroids, lignans etc [108]. Secondary metabolites are created after the growing stage and are utilized to extend the ability of plants to outlive. Bioactive compounds are classified as terpenoids, alkaloids, phenolic compounds, nitrogen-containing compounds; organosulfur compounds.Bioactive compounds are detailed to have different bioactivities such as antioxidant, anticancer, antimalarial, antimicrobial, antiulcer and anti- inflammatory activities [115].

III. EXTRACTION TECHNIQUES

Extraction is the partition of medicinally active portions of plant utilizing specific and standard strategies. It is the crucial first step in the examination of medicinal plants, since it is necessary to extract the required chemical components from the plant materials for further separation and characterization (117). The selection of solvent system largely depends on the particular nature of the bioactive compound being focused on. Distinctive solvent systems are accessible to extract the bioactive compound from natural

products. The extractions of hydrophilic compounds use polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a blend of Dichloromethane / methanol in ratio of 1:1 are used (56). The modern extraction methods have more ever been connected in natural products extraction and they offer few advantages such as lower organic solvent consumption, shorter extraction time and improve extraction yield (69).
Various methods, such as sonification, maceration and soxhlet extraction are commonly used (101, 19).

LOW OR AMBIENT TEMPERATURE TECHNIQUES

A. Cold Extraction method

Literature (10,11) has provided descriptions of the technique. In a nutshell, dried plant part samples are placd in different solvents for seven days while being shaken every 24 hrs. the samples are then dried under vaccum at room tmperature on pre-weighed waatch glasses, filtered using a Whatman filer paper, and the mass of dried sample is determined. Maceration ia a typical instance of cold extraction. This process involves periodically shaking in contact with a finely powdered plant portion or complete plant material (128). This causes the solvent- dissolved soluble material to be released. The benefit of low cost temperature method of extraction are advantage since they are easy, affordable, and sustainable even out in the open. Their drawback is that they might not be able to extract all of the chemicals from the plant matrix (129).

B. Enzyme Assisted Extraction

This method makes use of solvents and a variety of enzymes that are chosen based on the conditions in which they function best and the pathway that the scientist desires the chemicals to be catalysed. Protease, lipase, and phospholipase are a few of the enzymes frequentl utilised in extraction, and they significantly cut down on the need for solvents (28). Pectinase and amlyase are the enzymes that are frequently employed with essential oils. Although the setup is expensive, the process does not degrade compounds. In terms of necessary nutrition, oxygen levels, and temperature optimizations, it is also excessively demanding.

C. Ionic liquid Extraction

This approach makes use of hydrophobic interactions, hydrogen bonding, stacking interactions, ion exchange, and ion exchange to selectively engage polar and nonpolar molecules with organic salts in liquid form (130). It is very effective approach that yield highly recovers both organic and inorganic ligands. The quality and effciency of the extraction are quite good because of the ionic interactions.

D. High temperature extraction techniques

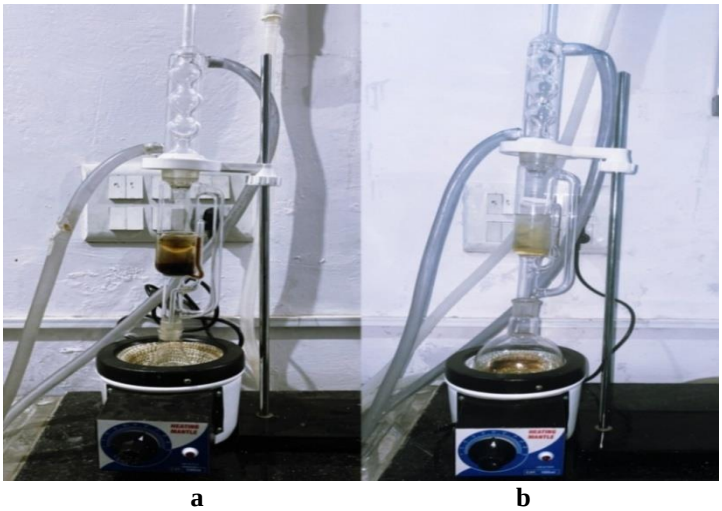
Known thermally stable compounds should be removed at high temperatures. Even though this is a common concern, extraction at high temperatures doesn't always result in the destruction of cruical compounds(64). Following extraction at 90ºC, research was done on the phenolics components and antioxidant qualities of plants. Understanding the composition of the pertiment compounds present in plant materials is therefore essential(42).

E. Decoction

This procedure requires 15 minutes of boiling in water, cooling, filtering, and adding the correct volume of cold water to the medication (64). This technique can be used to extract chemicals of interest from herbal plants that are heat stable and soluble in water (42). Due to the high temperatures used, this process produces more oil soluble chemicals than infusion and maceration (71).

F. Soxhlet extraction

The crude extract is finely pulverized and then placed in a porous bag or thimble composed of strong filter paper, which is then inserted in the Soxhlet apparatus chamber flask A's extracting is heated, and its vapor is released.The dripping concentrated extracting enters the little vial with the unrefined medication extract, and draws by direct contact, any time the level of the siphon tube top rises in the chamber's liquid from the compartment drains into flask A. This procedure is ongoing and continues till from the siphon tube, a drop of solvent to evaporate and leave behind residue. The benefits of this method over other extraction techniques are the ability to extract significant amounts of bioactive metabolites with a very tiny amount of solvent, which saves time, energy and money. On a medium or large scale, it is typically employed as a batch process, but when transformed in to a continuous extraction operation, it can become considerably more viable and affordable (36, 37, 30).



a **b**

Figure 1 a.The Soxhlet apparatus are set for the extraction b. After extraction is completed

G. Microwave assisted extraction

In essence, this is just a microwave- heated solvent and sample mixture extraction utilising a conventional solvent (71). Microwave energy is used in MAE to improve analyte partioning from the sample matrix into the solvent (19). Microwave radiation causes heating near the surface of the materials and heat transfer by conductio when it interacts with the dipoles of polar and polarizable materials, such as solvents and samples. The disruption of hydrogen bonds caused by microwave electromagnetic dipole rotation of the molecules faciliates the movement of dissolved ions and encourages solvent penetration into the matrix (20). Poor heating can happen in non-polar liquids because dielectric absorption is the primary method of energy transfer. The more polar compounds and solvents with greater dielectric constants are favoured by the selective MAE techniques (29).

Advantage and Disadvantage of MAE

It can shorten extraction times and use less solvent than more traditional techniques like maceraation and Soxhlet extraction. Due to the fact that only small molecule phenolics compounds can withstand microwave heating conditions of upto 100^0C for 20 minutes, including phenolic acids (gallic acid and ellagic acid), isoflavin, quercetin, and trans-resveratrol, this technique is restricted to these substances. Increased MAE cycles (e.g., from 2 to 3 s) can significantly reduce the yield of phenolics and flavanones, whih is mostly brought about by phytochemical oxidation (19). Tannins and anthocyanins are probably not appropriate for MAE since they are easily degraded at high temperatures (34).

H. The accelerated solvent extraction

Compared to maceration and Soxhlet extraction, ASE is a more effective method of liquid solvent extraction. A minimal amount of solvent is used in the procedure. In the stainless stell extraction cell, the sample is filled with inert material like sand to prevent the sample from aggregrating and further obstructing the system tubing (14, 2, 1). Between layers of cellulose filter paper and sand in a packed ASE are layers of sand sample mixture. It takes less than an hour to complete the extraction using this automated extraction system, which is perfectly suited to controlling the temperature and pressure for each sample. As with other solvent procedures, the kind of solvent is crucial for ASE. The maximum yield of bixin from Bixa or ellana with 68.16% purity has been demonstrated using a 6:4 v/v cyclohexane acetone solution and 5 minutes heating (50^0C) (15, 2).

I. Automated extraction technique using a solvent digester

The test materials are suspended in tiny glass jars filled with different extraction solvents in thimbles that have recently been used in universal extraction systems (such as BUCHI systems), where the temperature is kept slightly below the boiling point of the extraction solvent. Filtered and condensed using a vaccum concentrator, the extracted extract is prepared for phytochemical analysis (77).

J. Extraction of Liquid under Pressure (Pressurized Liquid Extraction)

This uses a set up that operates with pressure of 35 to 200 Bars and heat up to 200^0C. Samples are placed in a sample container with water often acting as the solvent. The solvent becomes less viscous as the temperature is raised, making it easier to permeate the plant matrix. The solvent is kept in the liquid phase by high pressure. The device design prevents the deterioration of oxygen and photosensitive chemicals. The process is costly yet environmentally beneficial. It works well for chemicals that are thermally stable specifically (28).

K. Pulsed electric field assisted extraction

The PEFAE can be regarded as an unique extraction technology for the extraction of bioactive compounds due to its purity, low energy demand, and solvent usage. Because the natural components are recovered at a low temperature without sacrificing quality and nutritional value, the procedure is also known as a non- thermal extraction (11, 13). In a study, the phenolic chemicals were extracted from apple peels using pulsed electric fields at various electric intensities and periods. The extraction is examined using confocal laser scanning microscopy and the disintegration index to measure electrical conductivity. According to the findings, the extraction was influenced by the electric field intensity and the cell integration index. The study also demonstrated that the soluble matter recovery increased with increasing intensity (1200V/cm) at constant cell integration constant (12).

OPTIMAL TEMPERATURE EXTRACTION

Depending on your understanding of the types of ingredients in the samples that are of interest to you, you may choose to use one or more of the following methods.

A. Ultrasound extraction method (Sonication)

This technique uses ultrasound to increase the permeability of cell walls and create cavitations. The ultrasonic frequencies used range from 20 kHz to 2000 kHz. Based on a factor including viscosity, polarity, surface tension and vapor pressure, choosing a solvent is important for this process (28). The most popular solvents are methanol, ethanol and hexane, occasionally with water added. Although the method can be used in particular situations, such as the extraction of rauwolfia root; their usage on a broad

scale is constrained by the associated expenses. This technique works well for releasing components. One drawback of this approach is the damaging impact of ultrasonic energy above 20 kHz on the bioactive metabolites of
Medicinal plants, which is brought on by the production of free radicals, which combine to produce unfavorable effects in the bioactive metabolites (47, 46, 63).

B. Supercritical fluid extraction

An alternate sample preparation technique known as supercritical fluid extraction aims to employ fewer organic solvents while increasing sample throughput. Pressure, sample volume, temperature, analyte collection, modifier or co-solvent addition, restrictors flow and pressure control are factors to be taken into account in the procedure (49, 51). The use of carbon dioxide as the extraction fluid has various benefits. In addition to its advantageous physical characteristics, safety and abundance, CO_2 is also inexpensive. Although carbon dioxide is the preferred fluid for SFE, it has a number of polarity restrictions. When extracting polar solutes and when there are significant analyte- matrix interactions, solvent polarity is essential. In order to overcome the issue of polarity restrictions, organic solvents are frequently added to the CO_2 extraction solution (57, 59, 123). Carbon dioxide has recently been replaced with argon because it is inert and less expensive. One particular benefit of the SFE technique is the ability to extract compounds at moderate temperatures, which minimizes damage from heat and some organic solvents. No solvent residues are left behind, and the extraction process is safe for the environment (29, 124).

C. Serial exhaustive extraction

In order to ensure the extraction of compounds across a wider range of polarities, this involves extraction in a sequence of solvents from the least polar (often n-hexane) to the most polar (methanol). It is one of the most used extraction techniques that can be used with either high temperatures (such as Soxhlet) or low temperatures, especially for chemicals that are thermo labile (61).

D. Hydro Distillation and Steam distillation

There are many techniques for extracting volatile oil, including HD and SD. Some organic substances are prone to breakdown in HD and SD. The primary and secondary essential oils from Mentha citrate have been proven to be strongly impacted by the distillation processes in terms of their chemical makeup and antibacterial properties. The yields of both main and secondary essential oils are greater by HD than by SD. It is crucial to keep in mind that phytochemical screening of secondary metabolites is crucial because bioactive metabolites reveal the kinds of biological activities that need to be investigated (25, 26).

IV. Separation and purification of plant ingredients using analytical methods

There is no one approach that may be universally suitable for the separation of all extracts due to the variety of phenolic compounds' nature (Polarity, chemical structure, glycosidic linkages and spectral properties), therefore the method that is used should be carefully chosen (97). The first step in developing drugs for conceivably novel therapeutic strategies against human diseases is the identification and extraction of bioactive components from plant extracts (98). Chromatographic techniques are used to separate materials after they have been purified using a variety of solvents with different polarity (55, 108) Liquid and gas chromatography are the two main categories of chromatography.
In contrast to gas chromatography, which uses a gas phase as the mobile phase, liquid chromatography uses a liquid as the mobile phase.

A. Liquid chromatographic techniques

According to their forms, sizes and charges, the molecules in test samples are sorted using this chromatographic method.
Phase states and mechanisms, phase polarities, separation zone geometries, gradients of experimental parameters and time, and column dimensions can all be used to categories chromatographic procedures. The methods based on phase states and mechanisms are discussed in this chapter (39).

B. Adsorption Chromatography

Based on solute interactions with active sites on the solid stationary phase, this is also known as
Liquid/solid or displacement chromatography. Specific functional groups in the mobile phase are specifically interacted with by the stationary phase via non-polar contacts, non-covalent bonds, hydrophobic interactions and Van-Der walls forces (113). According to similarities between the compounds in the mobile phase and those in the stationary phase, the compounds are separated, with molecules that are loosely bound eluting first

C. Partition Chromatography

The technique, often referred to as liquid/liquid chromatography, is based on how the molecules to be separated interact with two immiscible liquid phases in relation to their solubility, with the stationary liquid phase being adsorbed on a solid.
The components that are soluble in one are strongly held by it; if mobile phase, they are the first to be eluted, but if stationary phase is holding them more strongly, they will be delayed within the system (40).

D. Ion exchange chromatography

On the basis of their affinity to the ion exchange, it is a procedure that enables the separation of ions and polar molecule (50). Large proteins, tiny nucleotides, and amino acids are all types of charged molecules that it can function on. This procedure can be used to separate cations and anions. Extracts are added to the columns, where they interact with the ligands in the stationary phase. They are drawn to the stationary phase if they have a strong affinity for the ligands. If they have little to no affinity, they can be removed quickly from the system by employing buffers with a higher ionic strength or a different pH, leading to early elution. Typically, the ligands bind the desired components (77). In contrast, closely related ion chromatography divides polar molecules and ionic components in extracts according to their electrical characteristics. Ion resins are used in the stationary phase (72).

E. Size exclusion chromatography

This method, which is based on molecule size in relation to the diameters of the permeation
Spaces on the stationary phases, is also known as gel permeation, molecular sieve and gel filtration chromatography (22). By interacting with a porous structure, size exclusion chromatography separates molecules based on their hydrodynamic radius, or physical size in solution. The highest molecular weight molecules elute first because they are excluded from the pores, and the elution order normally follows molecular weight. Smaller molecules that can fit through holes in resin particles permeate a larger volume that is accessible within the column and are eluted later. Protein aggregates are expected to elute earlier since they are larger than native proteins (69). Phase equilibrium can be attained by partitioning or sieving. This method involves no chemical contact (63).

F. Ultra –High Performance Size exclusion chromatography

The resolution, sensitivity, and throughput offered by liquid chromatography were greatly enhanced by the introduction of small particle size packing materials (2m and less). Waters introduced the first liquid chromatography apparatus capable of handling decreased sub – 2m particles in 2004 (31). The apparatus was known as ultra performance or ultra- high performance liquid chromatography (UHPLC). The development of stationary phase materials with low dispersion UHPLC apparatus has also benefited modern SEC (22, 32,34).When screening several samples, such as when examining cell culture optimization or process variables on the level of aggregate, analytical speed is crucial.
The possibility of shear forces altering the sample composition or the column serving as a filter and trapping the multimers exists when SEC is used for the characterization and quantification of monomers, dimmers and multimers of proteins (65).

V. LIQUID CHROMATOGRAPHY EXAMPLES

Methods of planar chromatogrphy

These methods enable a solute in a solvent to flow on the surface of an adsorbent material that has been laid flat. Here, the stationary phase is either a solid containing silica gel or alumina (thin layer chromatography) or a liquid phase that flows through the stationary phase by capillary action or gravity (109).

A. Paper chromatography

In partition chromatography, the polar adsorbed water in the paper serves as the stationary phase in a 2D plate. Both the stationary phase and the mobile phase are liquids. The dissolving sample is placed in a tiny location on the filter paper, half an inch from the edge, and allowed to dry. The dry area will be kept at the front end in a sealed chamber filled with atmosphere, while the solvent will be in touch with the end closest to the sample and will travel up or down by capillary action (depending on the mode of action whether ascending means moves up along the paper or descending that moves down due to high viscosity of thus mobile phase). Upon reaching the end of the paper's mobile phase mixture, we divided the paper into separate colored and uncolored areas, and we measured each area separately using techniques called retention factor or rate flow (RF) (1).
The paper must have high porosity for rapid capillary action and be thick to hold a sizable number of samples more effectively (120). Paper chromatography has the benfit of being relatively more affordable and having very reproducible retention factor (RF) values right on the paper (121).

B. Thin- layer chromatography

This procedure moreover includes the use of adsorption instrument to isolate a compound from a mixture. Partition is depending on the interaction between the compounds in a mobile and, ljk, stationary phase. It is pertinent within the partition of compounds with low molecular weight (77). The stationary stage as a rule is 100g silica gel broken down in refined water to make slurry. In the mean time, in a few occasions Sephadex is applicable. The arrangement of silica gel poured into the glass plate with measurement 20cm× 20 cm to make a thickness of 1.5 mm. after that it kept for 1h at 105 ^{O}C to solidify (64). A short time later, 10ml of extricate is inoculate into the bottom of the plate and permitted to spread.

The plate is at that point carefully embedded into the separation chamber containing versatile stage and permitted to stand for 30 min. the compounds contained within the mixture will climb to different positions on the plate based on their dissolvability (111, 21). Each compound isolated is identified by calculating its impediment figure which is the ratio of separate traveled by the compound to the distance traveled by the dissolvable and compare it with that of a known compound (101, 42). The compounds spotted are scrapped at distinctive position utilizing spatula and finally re-extracted utilizing different solvents (77, 64). Advantage include less time consuming, creating clear spots, and steady to corrosive as dissolvable.

Silica gel (alkaloids, amino acids, lipids, sugars and fatty acids among others), Aluminum (phenols, alkaloids, carotenes, steroids and vitamins), celite (inorganic cations and steroids), starch (amino acid), and Sephadex are the most often used adsorbents with components they separate (proteins and amino acids) (77, 71).

Table 1 Different adsorbents used to separate various compounds (Rasul *et al.*, 2018)

Sr. No.	Adsorbent	Use to separate
1.	Silica gel	Amino acids, alkaloid, sugars, fatty acids, lipid etc.
2.	Aluminium	Alkaloids, phenols, steroids, vitamins and carotenes.
3.	Cellulose powder	Amino acids, food dyes, alkaloids
4.	Celite	Steroids and inorganic cations
5.	Starch	Amino acid
6.	Sephadex	Amino acid and protein

C. Column Chromatography (CC)

Column chromatography involves ion exchange, molecular sieves, and adsorption phenomenon. The conventional chromatography greatly dilutes the material, and the fractions usually require other steps. A newer method called displacement chromatography elute with some compounds that has great affinity for the adsorbent. Fractions of elute materials can be more concentrated than the original solution applied to column.

Silica gel (SiO2) and alumina (Al2O3) are the two adsorbents commonly used for column chromatography. These adsorbents have different mesh sizes, indicated by a number on the bottle label. The polarity of the solvent which is passed through the column affects the relative rates, at which compounds move through the column (125). Polar solvents can compete more effectively with the polar molecules of a mixture and will also solvate the polar constituents better. Consequently, a highly polar solvent will move more rapidly through the column. If a solvent is not polar enough, no compounds will elute from the column. A non-polar solvent is first used to elude the less-polar compounds. Once the less-polar compound is eluding, a more-polar solvent is added to the column to elude the more-polar compounds (81). Proper choice of an eluting solvent is thus crucial for the successful application of column chromatography as a separation technique.

D. Gas chromatography

It is an analytical technique for the division of volatile compounds. It's give both qualitative and quantitative data for individual compounds. It has two stages the gas stage is flowing and the liquid stage is stationary. The rate of migration for the chemical species is decided via its distribution within side the gas phase. For example, a species that distributes itself 100% in to gas phase will migrate on the identical fee because the flowing gas, whereas, a species will now no longer migrate at all (77, 64). Species that distribute themselves in part in each level will migrate at an intermediate rate. Gas chromatography includes a test being vaporized and infused onto the head of the chromatographic column. The sample is at that point transported through the column by the stream of inactive, vaporous versatile stage. The column itself contains a fluid stationary stage, which is adsorbed onto the surface of an inert solid.

Substances that are solvent within the gaseous phase will take off the fluid, move to the vaporous phase, and get isolated. Essentially, compounds that are soluble as it were in liquid form will stay within the stationary phase (66, 77). Inert helium gas was utilized as versatile phase, at a steady stream rate. The rough extricate to be analyzed was to begin with weakened with methanol and infused into the system (64). Advantage of this method incorporates ability to partitioned plant fabric sullied with volatile pesticides, moreover utilized in quality control testing.

Figure 2 Pharmaceutical analyses using supercritical chromatography (Desfontaine *et al.*, 2015)

Applications of Gas chromatography

In forensic toxicology, doping analysis, and normal bioanalytical laboratories, GC is frequently employed as an alternative to GC-MS/ MS, HPLC-MS/MS procedures due toits high precision and broad applicability in the study of numerous physiocochemical agents (93, 94). The majority of GC procedures employ open tubular capillary columns with a liquid film deposited on the column wall. Additionally, GC is employed in targeted analysis of a wide range of volatile chemicals for lipidomics and metabolomics. The substances that are commonly recognized in clinical CG protocols include fatty acids, steroids and other hormones, anaesthetics, analgesics, antidepressants, antipsychotics and antiepileptics, as well as narcotics like cocaine, cannabinoids, amphetamines, MeOH and breath volatile chemicals (126).
Biomarkers for diseases related to metabolic disorders, cancer, neurological diseases and tuberculosis are identified by clinical investigation. Within five minutes, this analysis is completed.

E. Supercritical Fluid Chromatography (SFC)

Similar to GC, supercritical fluid chromatography employs open tubular columns. The following are some benefits of this method over GC and HPLC:
1. Broad spectrum stationary phase
2. Eco- friendly CO_2 based mobile phase combined with organic solvents, enabling for quicker and more effective separation.
3. Greater dispersion and reduced viscosity.
These characteristics make supercritical fluid chromatography possible, enabling a quicker analysis with superior resolution. Additionally, this method complements the widely used GC and HPLC in clinical analysis. SFC thus has the potential to become a technique that receives a lot of attention in clinical analysis. Additionally, it is extensively employed in the development and discovery of drugs (25, 23).

F. High performance liquid chromatography

As of now this strategy is picking up ubiquity among deferent expository methods as the main choice for fingerprinting study about for the quality control of herbal plants (46, 111). High performance liquid chromatography is a particular shape of column chromatography usually used to separate, identify, and quantify the active compounds present in samples (91, 96). If a species interacts a lot of powerfully with the stationary introduce the column, it'll pay longer absorbable to the column's adsorbent and can, therefore, have a larger retention time. The column or stationary phase is that the core of any action system (89, 90). High-performance liquid chromatography (HPLC) has end up a favorite technique of herbal product isolation and purification.
Although preparative HPLC may be very much like analytical HPLC in place of injecting a small quantity of pattern to maximize the decision the quantity of feed may be very excessive that allow you to maximize the purification productiveness and decrease the quantity of solvent used (96). HPLC is valuable for compounds that cannot be vaporized that breakdown under high temperature and it gives a great complement to gas chromatography for detection of compounds (99). It is good for separation of component that are weekly or moderately polar and therefore the purification of amines.

G. High performance thin layer chromatography

High performance thin layer chromatography could be a planar chromatography where partition of sample component is accomplished on thin execution layers with discovery and information securing. This thin execution layers are precoated plates coated with absorbent of molecule estimate 5-7 microns and a layer thickness of 150-200 microns. The decrease in thickness of layer and molecule estimate comes about in expanding the plate proficiency as well as nature of partition. HPTLC gives a chromatogram i.e. isolated tests after chromatography can be assessed by the eyes as it were in case of HPTLC. The most contrast between HPLC and HPTLC are the molecule and pored estimate of sorbents (100, 97, 107, 128, 80).

Table 2 Difference between HPLC and HPTLC (Kathrivel *et al.*, 2012)

Feature	HPLC	HPTLC
Stationary phase	Liquid	Solid
Liquid		
Solid		
Mobile phase		
Liquid		
Liquid		
Conditioning phase		
None		
Stationary phase		
Mobile phase	Liquid	Liquid
Conditioning phase	None	Gas
Analysis	Online	Offline
Resolution	Very high	Moderate to high
Chromatography system	Closed	Open
Separating medium	Tubular column	Planar layer
No. of sample	Only 1 at a time	Up to 100 sample
Pressure	High	None
Time/ sample	2-60 min	1-3 min
Data obtained from chromatography	Limited to very high	High to very high
Cost/ analysis	Very high	Low

H. Lamina chromatography with maximum performance

This method combines TLC and HPLC principles and provides a preparative and analytical tool appropriate for both research and quality control laboratories. Pumping a liquid mobile phase through a column filled with bonded phase media or a solid stationary phase of silica (Amino, diol, cyano, ion exchange, C8 and C18) (77). To move the mobile phase through the planar columns at a consistent speed, a pressure of up to 50 bars is required. Purification may entail one or more of the processes listed above, depeding on paractically, ease of separation owing to the compounds involved, and accessibility of materials and instruments. Reverse phase HPLC and column chromatography were used to separate the phenolic compounds from *Tripodanthus acutifolius* leaves (16), however only using silica gel column chromatography, phenolics from piper betle roots were purified (17).

VI. NON- CHROMATOGRAPHIC TECHNIQUES

A. Immunoassay

Monoclonal antibodies are used in this technique to target low molecular mass bioactive substances found in nature and medications (110). They offer extremely high sensitivity for enzyme tests, qualitative and quantitative studies, and receptor binding assessments. Enzyme linked immunosorbent assays (ELISA) are typically more sensitive than chromatography. Although expensive for invitro cell line testing and requiring ethical approval for invivo assay, this method is effective (62).

B. Structure elucidation using spectroscopic techniques

Basically, structural elucidation includes characterizing the chemical components of samples using spectroscopic methods (104). Structure elucidation was defined as the comprehensive de novo identification of structures, fully translating into molecular linkages with appropriate stereo-chemical assignments. This is accomplished using a variety of spectroscopic approaches, each of which offers critical details that can be linked to the information offered by the others. The structures are determined by interpreting the spectra or by looking up and comparing existing, known information from spectral libraries (4, 3). A web-based search of formulae and masses of known molecular compounds with any of their available biological test results is offered by public chemical compound repositories as well as drug and metabolism databases (6, 7, 8). The method for identifying chemicals extracted from plant extracts' structures and names are presented in this section

C. Fourier-transform infrared spectroscopy

This method can be used to determine the functional groups that are present in isolates from plant extracts (38, 48). In order to create a thin film, a drop of liquid samples is put between two sodium chloride plates. For solid samples, they are homogenously combined with potassium bromide after being ground to the finest powder feasible (KBr). After being compressed into a pellet, the mixture is then set in a sample container and prepared for examination. Peaks at particular wave numbers are produced by the analysis and are indicative of the functional groups found in the samples (65).

D. Mass spectroscopy

This strategy is valuable within the identification of compounds based on chemical structure and molecular weight. The point is to arrangement and identify the unknown compounds I a mixture. The substances more often than not distinguished include oligonucleotides and peptides (62, 64, 41). The process starts by besieging a natural molecule with an electron and changes over it in to exceptionally energetic charged particles. The flag was to begin with recognized using electron ionization vitality of 70eV; moreover, the sample spectra are identified and recorded as percentage peak. Compounds are identified based on their relative atomic mass and atomic weight. This can be accomplished by plotting mass of the fragmented ions against the charges of individual ion (41, 77, 62). Notably, MS gives copious data on organic particles. Hence, one of the standard procedures in handling restorative plant is the combination of MS/HPLC.

E. Nuclear Magnetic Resonance

An NMR technology utilizes the paramagnetism of the nucleus and the applied magnetic field they connected (87). The strategy pays more consideration to the physical properties of the bioactive particle such as number and cluster of carbon molecule, presence of isotopes of carbon, hydrogen particle and protons (67, 68). Progresses within the innovation utilized to create the exceptionally expansive, superconducting magnet utilized these high determination thinks about has driven to progressively expanded unearthly determination, advancements which have permitted us to consider indeed bigger chemical frameworks (90) Be that as it may, with these advancements in sensitivity and determination come bigger and more strong rebellious requiring committed laboratories, with high buy and working costs, especially those including cry fluid recharging for the last mentioned; one major restriction of this approach is that such facility are inalienably inappropriate for field or bench top considers (76).

Table 3 Benefits, limitations and potential future applications of the NMR analysis of human or animal biofluids (Cosa *et al.*, 2016)

Criterion	Comments	Relevant References
Solvents	D_2O, H_2O + D_2O (9:1 in volume), acetonitril - d_3, acetone-d_6, benzene-d_6, toluene-d_8, DMSO-d_6, CD_2Cl_2.	Percival *et al.*, 2018, Cistola *et al.*, 2016
Sample preparation	The samples have to be dissolved in deuterated solvents. The NMR- tubes should to have a breadth of 5mm and least length of 17cm.Minimum filling stature is 6cm. The concentration of the sample should be at slightest 0.01mol/l for 1H. The sample should to be free from any sedimentation, conceivably requiring filtration of the sample prior to measurements.	Percival *et al.*, 2018, Cistola *et al.*, 2016
Selectivity	Constrained, but the foremost noticeable resonances. Which are clearly obvious and inordinate unaffected by minor possibly interferometer signals, may be spectroscopically clear locales are moreover available to measurement.	Percival *et al.*, 2018, Cistola *et al.*, 2016
Senstivity	Shockingly and excessively high because magnetic resonance viewpoint of NMR, which has powerless energies interaction involved. This could be advantageous in circumstances where it is alluring to keep the samples undisturbed, but it could be a noteworthy impediment when looking at complex responses, certain organic forms and metabolomics.	Percival *et al.*,2018, Cistola *et al.*, 2016
Potential	Compounds, citrate, creatinine/creatine, formate, 3 Dhydroxybutyrate, urea, indoxyl sulfate, hippuric acid and glucose in human pee. At slightest a few of these metabolites, at the side propionate, and encourage ones of moderately high concentration and with 1H NMR resonances with straightforward (first order) coupling designs such as glycine, methanol and succinate, are too quantifiable in human saliva.	Lee *et al.*, 2008

Reliability	Offers a really helpful, high- performance biomedical clinical chemistry NMR investigation framework at a taken a high which is much lower, than those field NMR spectrometers. Most offices utilize conventional 5 mm breadth NMR tubes.	Percival *et al.*, 2018, Cistola *et al.*, 2016
Versatility	Pertinent to most, in the event that not all, biofluids and fluid tissue biopsy extracts, but especially profitable for those containing small or no protein and other macromolecules, e.g. human pee and salivary supernatants. Both single pulse and Carr-Purcell Meiboom-Gill (CPMG) beat sequence filtered spectra may be obtained on human blood plasma samples.	Percival *et al.*, 2018, Lee *et al.*, 2008
Limitations	Elements within the ionic state don't react in NMR but the nearness of particles in a test contributes to unacceptable line broadening. Paramagnetic contaminants such as iron and dissolved oxygen also broaden NMR lines. It is restricted to the estimation of nuclei with magnetic moments. It may be less sensitive than other methods.	Percival *et al.*, 2018, Cistola *et al.*, 2016

I. 2D NMR

The resolution of NMR is improved by using two dimensions. When several metabolites are present, these techniques are crucial for metabolomics because they enable the identification of related peaks that are part of the same spin system on the same molecule. It helps increase signal dispersion in 1D NMR situations where there is significant spectrum overlap (130)**.** In comparison to one dimensional NMR spectra, 2D NMR provides more information about a molecule.

II. 3D NMR

Two 2D NMR experiments are combined to create a 3D NMR. Proteins can form flexible chains or clearly defined 3D structures, each of which exhibits distinctive NMR signals (131). Three types of atomic nuclei that makeup 3D NMR are commonly ^{1}H, ^{15}N, ^{13}C. It is common practice to use these tests to link particular resonance signals to protein atoms that have been isotopically enriched.

F. Infrared spectroscopy

This strategy tries to survey utilitarian groups present in a compound. Information of the functional group makes a difference in characterizing the physical and chemical properties of a given compound. Moreover, single, double, and numerous bonds are recognized through this process (56, 41, 99). The strategy includes passing an organic compound through infrared radiation, which is ingested in certain frequencies. Fluid samples are recognized utilizing sodium chloride plates, whereas solids tests are decided utilizing potassium bromide processed together and compressed into a thin pellet. The result is recorded as a range that is percentage transmittance (65).

G. Ultraviolet spectroscopy

This strategy is appropriate for subjective and quantitative examination of compounds display in the plants extricate. Different auxiliary metabolites such as phenols, anthocyanins, tannins, and polymer dyes can be recognized at certain frequencies. Total phenolic substance and other auxiliary metabolites can be set up utilizing this procedure. Specific frequencies were utilized to recognize flavonoids (320nm), phenolic compounds (280nm), anthocyanins (520nm), and phenolic acid (360nm) (41, 99)

H. Liquid chromatography-Mass spectrometry (LC-MS)

The hyphenated analytical technique known as liquid chromatography-mass spectrometry (LC-MS) combines liquid chromatography and Mass spectrometry. HPLC (LC) divides mixtures into their component parts by passing them through a chromatographic column. In most cases, LC alone cannot positively identify the separated components. Mass spectrometry is also used to clarify the structure, identify recognized and unknown molecules, and identify undiscovered substances (79). Because a mass spectrum mixture is essentially a complex of overlapping spectra from separated individual components, mass spectrometry is not particularly effective on its own for identifying mixtures. Connecting liquid chromatography (LC) and mass spectrometry is challenging (MS). The liquid eluents are moved from LC to the MS through an interface.

Studies on invite dissolution, bioavailability, bioequivalence and pharmacodynamics, employ LC-MS increasingly frequently. Rapid mass directed purification of individual chemicals from such combinations using preparative LC-MS systems is possible and useful in fundamental research, pharmaceutical, agrochemical, food and other industries (10, 9).

10. Conclusion

A constraining issue in extraction and isolation may be the cost of specific operational gear and equipment. This constraint might be overcome by combining many less complex and expensive techniques. A single method may not be the best way to extract and isolate them due to the range of components in plants that could be used to make drugs. Efficiency can occasionally be attained by combining two or more extraction and isolation procedures. In order to meaningfully interpret spectral data, structural elucidation is already performed in combination with a number of different approaches. To determine compound structures, a single sturdy piece of equipment is required that can perform all applicable approaches simultaneously. The methods given are unaffected by this restriction because even the short test methods utilizes the basic discussed in this review

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