

INTRODUCTION OF ANALYTICAL TECHNIQUES USED IN HERBAL DRUG TECHNOLOGY

Abstract

By-products from plants are a great source of bio-active substances, such phenolic acid, carotenoids, and flavonoids (as phenolic compounds), peptide isolate, amino acids (bio-active proteins), fibres and fatty acids, and so on. Bio-active substances are defined as having a biological activity that causes metabolic changes linked to improvements in specific physiological processes or a decreased risk of developing various diseases. Plant sources typically have significant concentrations of bio-active chemicals, including a variety of molecules with various chemical and biological properties. Cancer, cataracts, Senile dementia, Parkinson disease, age-related ailments, and cardiovascular diseases are all at a lesser risk thanks to the bio-active components. Bio-active molecules include vitamins, minerals and other non-nutritious substances known as phytoconstituents, including glucosinolates, phytosterols, carotenoids, terpenoids, phenolic compounds, and folic acid, between others. In contrast to synthetic medications, medicines made from plants frequently have greater than one active ingredient, and the active ingredient or ingredients are frequently unknown. It can take a long time and be expensive to separate one or more chemicals from a raw extract or extract fractions. Many procedure that frequently calls for expertise, many separation processes, and various chromatographic techniques. Using information from several spectroscopic methods, it is possible to determine the structure of certain molecules including IR, mass spectroscopy, nuclear magnetic resonance (NMR), and UV-visible are used.

Keywords: Bio-active, phytoconstituents, separation, chromatographic, spectroscopy.

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I. INTRODUCTION

Extracts of natural products are a great source of physiologically useful secondary metabolites. The feasibility of using bio-active chemical screening to identify new therapeutic leads in natural products has been demonstrated. Additional problems arise from the variations in secondary metabolite concentrations. Unfortunately, it is difficult, costly and tedious to screening of natural goods for compounds that are physiologically active. As a result, high-throughput, efficient analytical techniques for identifying chemicals with the necessary biological effect have been developed. [1].

By-products from plants are a great source of bio-active substances, such phenolic acid, carotenoids, and flavonoids (as phenolic compounds), Isolated peptides and amino acids(bio-active proteins), fatty acids and fibres, and so on. Phytochemicals, phytosterols, and essential oils, for instance, can all be found in large quantities in fruit seeds. Similar to this, some fruits' peels have pectin, beneficial fibres, and minerals [2, 3]. Utilizing various technologies, these bio-active substances can be recovered from the by-products and utilised to create a variety of valorized compounds, including nutritious foods or nutritional supplements. Furthermore, this can limit the amount of garbage that is disposed into the environment. The effectiveness, affordability, and sustainability of the various extraction technologies are categorised. The recovery of bio-active chemicals from by-products of the fruit industry involves a number of extraction procedures. These substances can be distinguished, identified, and used by various the food, drug, cosmetic, or textile industries [2, 4].

1. Background and Rationale for the Topic

The background of the topic of understanding the bioactive molecules and modern analytical methods which were used in the identification of these bioactive molecules. We came to know that bio-active substances are defined as having a biological activity that causes metabolic changes linked to improvements in specific physiological processes or a decreased risk of developing various diseases. Plant sources typically have significant concentrations of bio-active chemicals, including a variety of molecules with various chemical and pharmacological properties. These include minerals, vitamins, and other non-nutritious substances known as phytoconstituents, including glucosinolates, phytosterols, folic acid, carotenoids, terpenoids, and phenolic substances, between others.[5, 6].

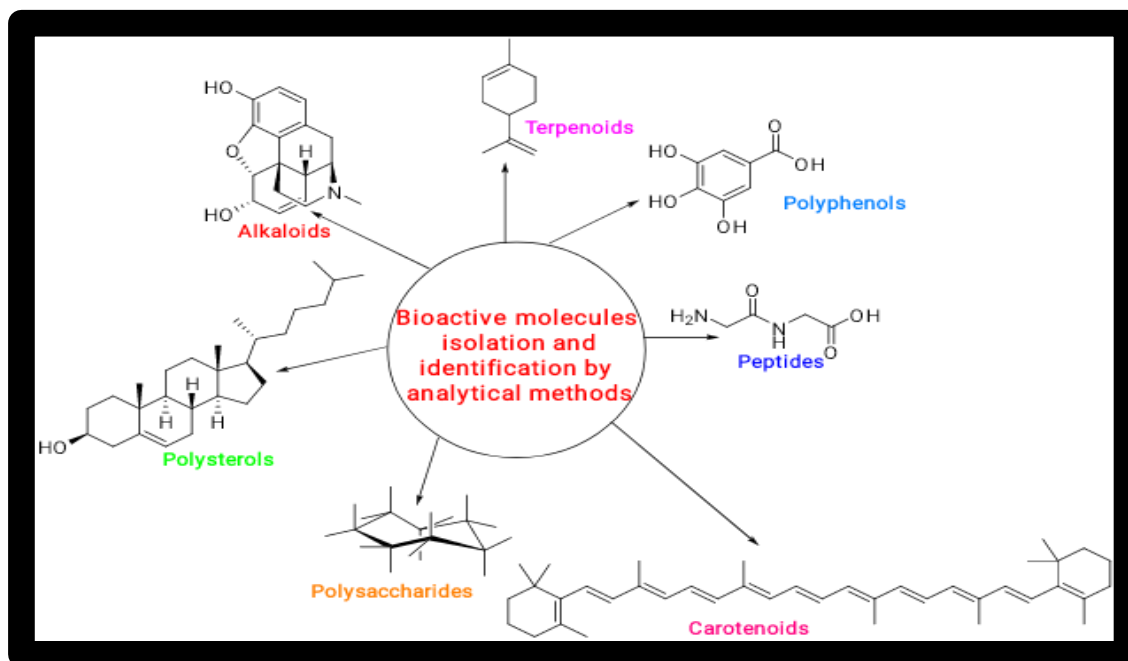


Figure 1: Bio-active molecules isolation and identification by analytical methods [7]

Cancer, cataracts, Senile dementia, Parkinson disorder, age-related ailments, and cardiovascular diseases are all at a lesser risk thanks to the bio-active components. These substances defend against chronic diseases, halt the generation of cancer-causing chemicals, and maintain immune system balance as a result of their high antioxidant and antibacterial activity. When used as a dietary supplement or as an ingredient in functional meals, these substances are advantageous. Natural anti-oxidants and colourants can be a superior substitute for manufactured antioxidants in addition to having nutraceutical benefits, making them suitable for usage in a variety of pharmaceutical and processing sectors [4, 8, 9]. New goods include cosmeceuticals, nutraceuticals, and foods with functional properties are being created as a result of advances in our understanding of bio-active compounds with the hope that their consumption will have a positive impact on our health as well as allow us to reevaluate potential agri-food byproducts [10, 11].

2. Objectives and Key Questions

This chapter's goal is to give a general review of the bioactive molecules and their identification with the help of modern analytical methods which includes chromatographic techniques as well as spectroscopy methods. Additionally, this chapter aims to explore the gap in knowledge of separation and identification of the biomolecules and their pharmacological action which were used in treatment of various disease. The analytical methods help in identification of the biomolecules and these biomolecules were used in future for the treatment of various disorders.

- **Key questions**

- Define bioactive molecules and their used in various disorder.
- Write the process of isolation, separation and identification of biomolecules.

- Classification of different chromatographic methods which were used in identification of biomolecules and explain them.
- Classification of different spectroscopic methods which were used in identification of biomolecules and explain them.

II. CLASSIFICATION OF ANALYTICAL TECHNIQUES

The bio-active chemicals are extracted, then they are separated, purified, and identified to find out if there are any particular compounds present in particular amounts. Functional activities of these substances, which are identified by various bioactivity assays, are also categorised. For the separating, identifying, and purifying of bio-active substances, a variety of analytical techniques are employed; however, the effectiveness, speed, and simplicity of each technique are compared [4, 9]. Separating and excluding the desired chemicals from a combination of extracts, various analytical techniques are applied. Based on factors including their boiling points, ionic strength, molecular size, adsorption characteristics, and other characteristics, the bio-active chemicals are isolated and purified [12]. The bio-active constituents are identified and characterized using a variety of spectroscopic methods [13].

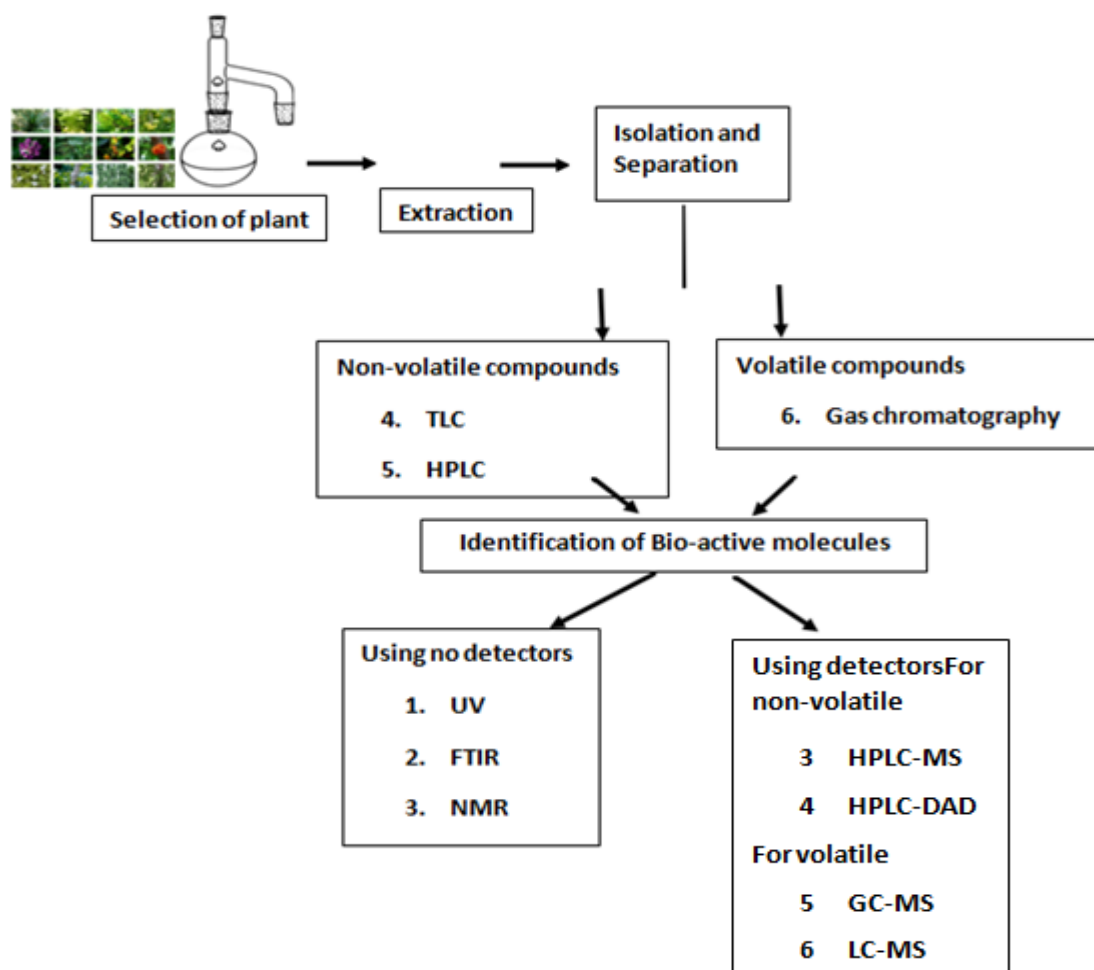


Figure 2: Isolation and separation of bio-active molecules using analytical methods [14]

In contrast to synthetic medications, drugs derived from the plant source frequently having greater than one active ingredient, and the active ingredient or ingredients are frequently unknown. The traditional medical system includes herbal medications inextricably [15,16]. The oldest traditional medical practise still in use today, Ayurveda, uses minerals and plant parts to promote human welfare. Along with biodiversity preservation, it is important to promote the rational using of these pharmacological herbs. Numerous herbal medicines are classified in the international pharmacopoeia as a result of research on a variety of medicinal plants. The WHO has established policies, directives, and standards for traditional medicines in recognition of their significance [17]. Adulteration or substitution is also heavily influenced due to improper recognition of the processed plant parts. As a result, it is critical to implement the necessary identification processes before moving on with the production of herbal formulations [18].

III. ISOLATION OF PHYTOCHEMICAL MARKER/S USING DIFFERENT CHROMATOGRAPHIC METHOD

The type of the markers needs to be known before moving forward with the isolation process. In general, the qualities of bioactive compounds that are useful for isolation include charge, molecular size, solubility, stability, & acid base characters. Separating an unknown substance is more challenging than isolating a known substance. The separation of either one or greater than one phytoconstituents in a mixture of extract fractions or crude extract can be a very time-consuming & costly procedure that frequently calls for expertise, many separation processes, and various chromatographic techniques. Broadly speaking, there are two types of chromatographic methods utilised for isolation [19, 20].

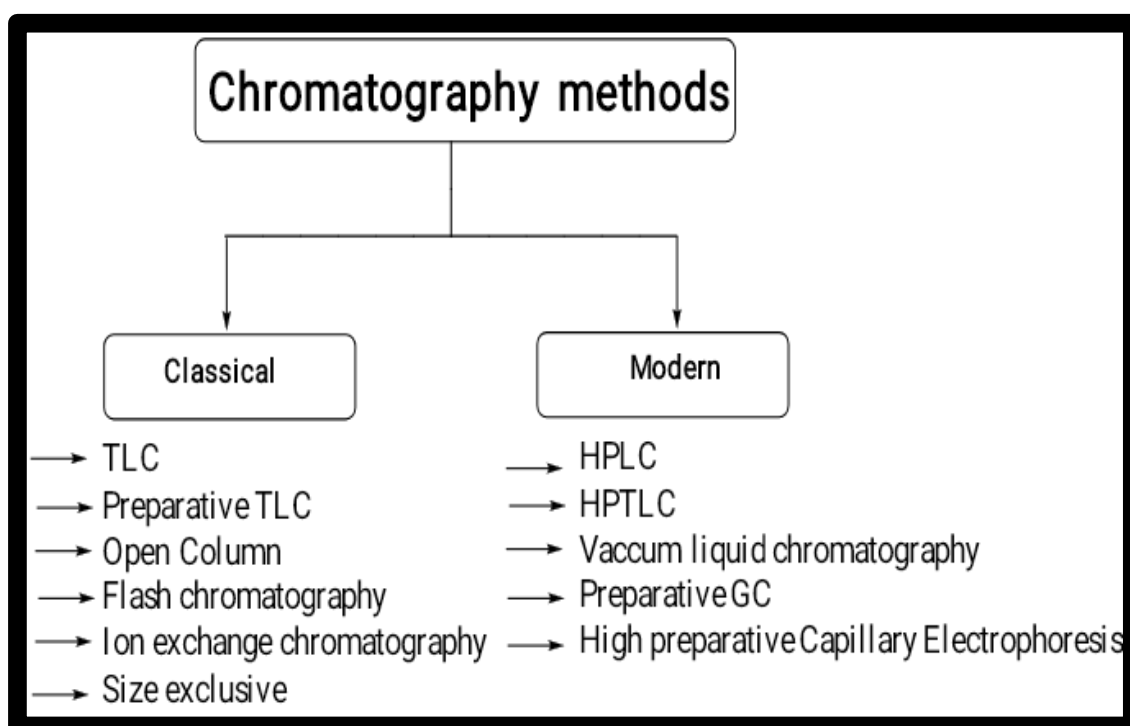


Figure 3: Different chromatographic methods are used for isolation of bio-active molecules [20]

1. Classical Techniques

- Thin Layer Chromatography (TLC):** Thin layer chromatography, often known as "planar" & "flat-bed" chromatography, that's the most straight forward to use of all the common chromatographic techniques. The stationary phase in TLC research is a thinly split solid coating with a rigid basis, like aluminium and glass, that permits the mobile phase, which can be either a pure solvent or a mixture of solvents, to flow through the stationary phase *via* capillary action [21, 22]. To determine how many components were present throughout the isolation process, TLC investigations are very helpful. The initial conditions for column chromatography are also decided using TLC. Compound isolation by TLC is simple and rapid based on the kind of compounds and the separation methods [23]. The molecular functional groups' varying binding energies, which result in the mixture's attachment to the silica gel, are used to separate the mixture's constituent parts. Higher polarity compounds are easily adsorbed by the silica gel, but lower polarity compounds are easily transported by the solvent's mobile phase, which is gathered at the bottom [24].

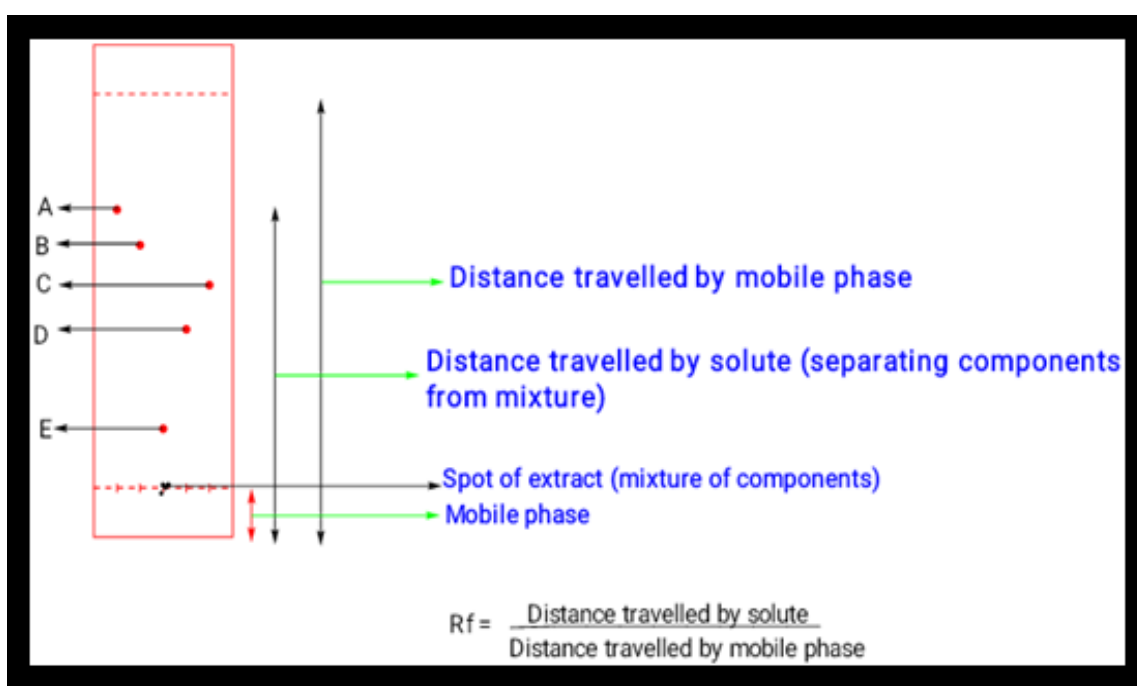


Figure 4: TLC representation of separation of biomolecules present in the extract [25]

- Preparative Thin Layer Chromatography (PTLC):** It is the simplest and mostly cost-effective technique for separating milligrammes from grammes. Silica gel is a popular adsorbent that is offered on pre-coated or manufactured plates. PTLC has a number of drawbacks. Recovery of chemicals from the plate can be challenging and manual sample application can be haphazard [26]. Before being characterised by spectroscopic methods, herbal components have been separated using PTLC [27, 28]. The least expensive separation method, preparative thin layer chromatography (prep-TLC), doesn't need high-tech equipment. Prep-TLC is essentially not advantageous for treating huge numbers of samples because it can only handle tiny sample sizes. A

TLC plate with silica slurry equally distributed on a glass plate, a plastic sheet, or a sheet of polymer is used to hold the sample or extract mixture. The mobile phase is frequently a combination of solvents, such as water and acetonitrile or acetone: benzene (1:3) v/v. The elements of the extract mixture are separated as the mobile phase moves along the silica plate in accordance with their polarity and the strength of the link between the organic molecule and Si atoms in the TLC. [29].

- **Open Column Chromatography (CC):** In traditional column chromatography, a column bed is let the mobile phase flow through the particles that range in size from 250 to 125 μ m using gravity. Column chromatography is a labor and solvent-intensive process. [30]. Additionally, there is a potential that substances will adhere to stationary phase surfaces in an irreversible manner [31].
- **Size Exclusion Chromatography:** The process, often referred to as molecular sieve chromatography, involves sorting the biological constituents of the extract mixture based on their sizes, which also determine their molecular weight. Enormous molecules with complicated structures, such as pectin, proteins, and fibres, are especially difficult to separate using this method. In essence, the materials used in this process are tiny, Dextran polymer-based pore-filled beads (Sephadex or BioGelP). These beads' pores catch molecules from the extract mixture in proportion to their sizes, making it simpler to gauge the sizes of macromolecules and produce a balanced molar mass distribution [32]. The technique is known as "gel-filtration chromatography" when a watery solution is used as the mobile phase, and as "gel permeation chromatography" when an organic solvent is used [33].

2. Modern Techniques

- **Prep-High Pressure Liquid Chromatography (Prep-HPLC):** In order to separate natural products, it is a flexible, reliable, and often applied method. Almost all kinds of natural compounds have been isolated using HPLC. A few decades ago, separations were thought to be very difficult. Now, they are very common. The most popular forms of separation in natural product isolation are reversed phase (stationary phase is non-polar exists & mobile phase is polar exists) & in normal phase (mobile phase is non-polar & stationary phase is polar exists) chromatography. Due to some of the utilised solvents in this method normal-phase chromatography significant to the UV light absorption, this technique has limited applicability in the isolation of organic substances. The majority of naturally occurring substances absorbs a little light between 190 and 210 nm. Therefore, using HPLC, the detecting area is typically used to separate and in a given mixture, identify the unknown ingredient.[34]. There are three types of preparative pressure liquid chromatography: Low pressure LC, (up to 5 bars pressure), and high-pressure LC all refers to pressures more than 20 bars & mediul pressureLC (up to 5 bar operating pressure) [35].
- **HPTLC (High Performance Thin Layer Chromatography):** In many analytical circumstances, HPTLC might be more advantageous than HPLC since it is a quick, affordable method of analysis when carried out correctly by skilled analysts. The key components of contemporary HPTLC methods include automated sample application,

automatic developing chambers, and densitometric scanning. The sensitive and 100 percent reliable HPTLC method is applicable to both qualitative and quantitative analysis [36].

- **Prep-High Performance Liquid Chromatography (Prep-HPLC):** Prep-HPLC is employed on an industrial scale, with kilogramme amounts, in order to isolate and purify of important bio-active chemicals. In this context, "preparative" refers to large columns that are running at high flow rates for the solvents used in elution. Comparatively speaking to preparative-HPLC, which is used for compound isolation and purification, analytical-HPLC is utilised for identifying chemicals qualitatively and calculating or estimating the quantity of a compound. This doesn't always refer to the instrument's size or the mobile phase's volume. In the first scenario, the sample leaves the detector after the operation and is wasted, but in the second scenario, the sample leaves the detector and enters the fraction collector. The 1950's and 1960's saw the development of prep-column chromatography, while the 1970's saw the introduction of prep-HPLC. The latter is superior to the former because it generates the flow using a high-pressure pump, which increases throughput and enables greater packing of the column with materials that having small particle size and better partition power. Currently, fully automatic preparative-HPLC is able to quickly purify a significant number of important molecules or compounds (daily over hundreds) in comparison to alternative purification methods. Because the results of a practise run are evaluated based on 3 characteristics—quality, yield, and throughput of the product and because these parameters are interdependent, optimization in column chromatography and prep-HPLC is exceedingly challenging or impossible. As a result, the purification procedure must be controlled to allow for the extraction of molecules despite having a lower throughput & yield, with excellent purity [37]. Prep-HPLC significantly increases the efficiency of the separation and salvation procedures when combined with a UV-Vis detector. Acetonitrile in water, at a linear gradient is one of the mobile phases that prep-HPLC scientists use the most frequently to separate flavanoids from citrus. Using prep-HPLC, the flavonoids are extracted within 5 to 30 minutes after diluting the crude sample with dimethyl-formamide (DMF). In the end, a rotator evaporator is used to mix and dry all of the fractions. For the intended usage, this can be reconstituted in an appropriate solvent [38].
- **Reverse-Phased HPLC (High-Performance Liquid Chromatography):** Reversed phased-HPLC frequently referred to as "hydrophobic chromatography" due to the employment of a hydrophobic stationary phase. Instead of the hydrophilic Si or Al resins that are used in conventional phase chromatography as stationary phases, it uses non-adsorption-inducing compounds. The less polar or hydrophobic molecules in the mixture are bound by the non-polar stationary phase in this type of chromatography, which is composed of covalently linked with alkyl-chains and transported by the mobile phase. C18-bonded (Octadecyl carbon) silica, Silica that is C8-bonded as well as cyano and phenyl-bounded are the most widely used column materials. These all serve as adequate packing for a successful separation procedure and the isolation of phytochemicals because they are all inert and polar compounds. Del Rio et al used reverse-phase HPLC with a C18 reverse-phase column and a mobile phase made of water, acetonitrile, and acetic acid in their analysis of the

related dihydro-chalcones and citrus flavanones to clarify the structures of the molecular which are separated [39].

- **Preparative Gas Chromatography (Prep-GC):** The basic purpose of prep-GC is to purify volatile substances. Both packed columns and capillary columns can be used to separate materials. Depending on the column size, it uses a greater carrier gas flow rate of 100 to 1000 ml/min. Only isothermal mode is used for separation utilising a packed column. Open tubular columns are preferred for small-scale compound isolation [40]. The isolation of volatile components from medicinal plants has been done using these methods. In their assessment of preparative gas chromatography, Yang et al. stated that technique can be used to separate volatile chemicals from essential oils [41]. From peppermint oil, menthol and menthone were produced, [42] and using same method, Curcumenone, Elemene, Curzerene, Curzerenone & Curcumenol were obtained from the methanol extract of Curcuma rhizome [43].

An emerging technology has emerged in recent years for the purification and separation of bio-active chemicals from plants [44, 45]. The capacity to parallel the creation and accessibility of several sophisticated bioassays and the availability of precise isolation, separation, and purification methods is provided by this contemporary approach. Identifying a technique to screening of the source material for bio-activity, includes anti-oxidant, antimicrobial, anticancer, while also being simple, specific, and quick aim when looking for bio-active substances [46]. Finding definitive methodologies or protocols to extract and characterise certain bio-active compounds is difficult due to a number of variables. This might be caused by a plant's various components (tissues), many of which create quite distinct molecules, as well as the bio-active phytochemicals' varying chemical structures and physicochemical characteristics [47]. For the purpose of isolating and identifying a bio-active phytochemical, selecting and gathering of plant materials are regarded as the first steps. Retrieved ethnobotanical data is then used to identify potential bio-active compounds in the following stage. Once the marker substances, which are accountable for the bio-activity have been isolated and purified, extract can be produced using various solvent types. For the separation and purification of bio-active substances, column chromatographic methods might be used. New techniques have been developed, such as HPLC, which expedites the purification of the bioactive chemicals. Numerous spectroscopic techniques, including as mass spectroscopy, NMR (nuclear magnetic resonance), infrared (IR), and UV-visible, can be used to determine which substances have been purified. [48]. Thin-layer chromatography and column chromatography are still the most popular techniques because of their accessibility, affordability, & accessibility in a range of stationary phases they are widely in used. The best substances for separating phytochemicals include cellulose, polyamide, alumina, and silica. Effective isolation is tough because botanical components contain considerable amounts of intricate phytoconstituents. As a result, by employing several mobile phases, polarity can be raised for separations that are quite beneficial. To analyse the chemical fractions in thin-layer chromatography & column chromatography has been widely used for a long time. Some analytical techniques have been utilised to distinguish bioactive compounds using TLC and Si-gel column chromatography [49].

Table 1: Enlisted the Separating and Identifying the Bioactive Substances found in Particular Plants [14]

Sl. No.	Name of the plant	Extraction method	Separation and Identification	Major bio-active compounds
1.	Orange (<i>Citrus sinensis</i>), lemon, (<i>Citrus limonia</i>), banana (<i>Musa acuminata</i>) peel	Conventional extraction	GC-MS analysis	Coumarine Tetrazene Mineral (Zn, Mg)
2.	<i>Myrciaria dubia</i> (Camu-camu) seed	Conventional extraction	HPLC–DAD	Vescalagin, Quercetin-3-Rutinoside, Castalagin, Gallic, and Ellagic Acids, as well as Malvidin-3,5-diglucoside and Cyanidin-3-O-glucoside
3.	<i>Punica granatum</i> (Pomegranate) seed	Enzyme-assisted extraction	MS	Leucine, Phenylalanine, Glutamic Acid, Punicic acid, Linolenic acid, & Linoleic acid.
4.	<i>Anana scomosus</i> (Pineapple) peel	Enzyme-assisted extraction	HPLC–MS	glucose and fructose
5.	<i>Butia catarinensis</i> (Jelly palm) seed	Supercritical fluid extraction	GC–MS	Epicatechin, Gallic acid, camphene, caprylic acids, and p-hydroxybenzoic acid
6.	<i>Vaccinium myrtillus</i> L (Bilberry) seed	Supercritical fluid extraction	HPLC	Linoleic acid, β -carotene, α -Linolenic acid, Lutein γ -tocopherol, β -cryptoxanthin, oleic acid,
7.	<i>Syzygium cumini</i> L (Jamun) seed	Microwave-assisted extraction	FTIR	Polysaccharide
8.	<i>Citrus sinensis</i> (Orange) peel	Ultrasound-assisted extraction	HPLC–DAD	Naringenin, Hesperin

IV. DIFFERENT SPECTROSCOPY TECHNIQUES FOR CLARIFYING THE STRUCTURAL PROPERTIES OF THE BIO-ACTIVE MOLECULES

To ascertain the structure of some compounds, data from a range of spectroscopic methods, including ultraviolet (UV), mass spectrometry, nuclear magnetic resonance (NMR), and infrared (IR) are used. The fundamental idea behind spectroscopy is to expose an organic molecule to electromagnetic radiation, some of which it absorbs but not all. A spectrum can be created by counting how much electromagnetic energy is absorbed. Specific chemical bonds give rise to specific spectral data. The plant-based molecule composition can be ascertained from these spectra. In order to clarify structural features, scientists typically utilise spectra produced from 3 or 4 regions: radio frequency, electron beam, visible, infrared (IR), and UV (ultraviolet) [48].

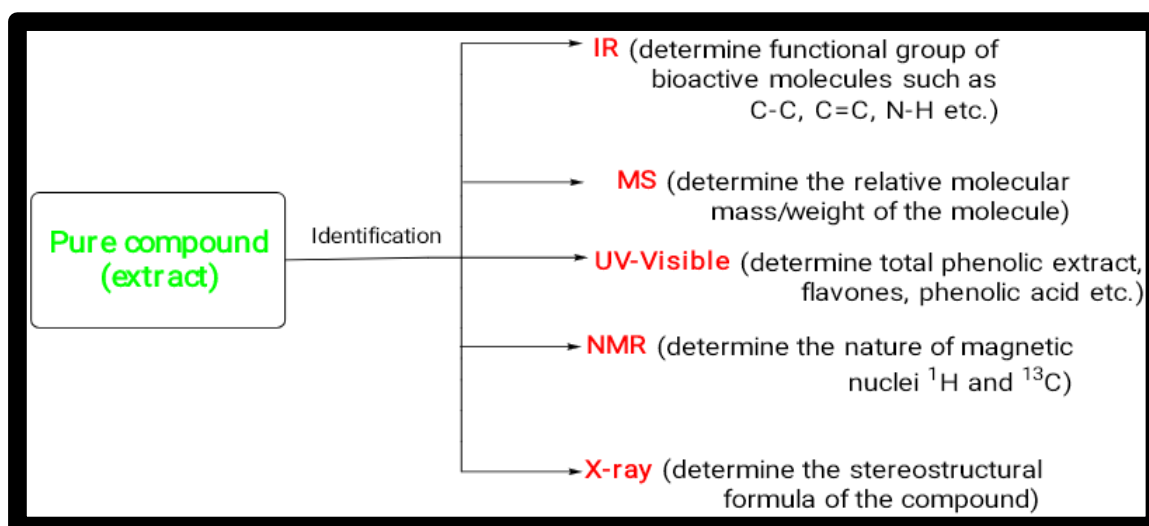


Figure 5: Different spectroscopic methods are used for identification of bio-active compounds [50]

1. Ultraviolet-Visible Spectroscopy

In both pure and pharmaceutical mixtures, UV-visible spectroscopy can be used for qualitative investigation and the detection of specific chemical classes. For quantitative research, UV-visible spectroscopy is preferred because aromatic chemicals have strong UV chromophores. Natural chemicals can be identified via UV-visible spectroscopy [51]. Iron forms complexes with hydroxybenzene includes phenols, polymer dyes, tannins, and anthocyanins, according to research using ultraviolet/visible (UV-Vis) spectroscopy [52]. The use of spectroscopic UV-Visible techniques, which are rarely used and reveal details on the composition of the overall polyphenol's concentration, was also found to be beneficial. Total phenolic extract was measured using UV-visible spectroscopy at wavelengths of 280, 320, and 360 nm, as well as total anthocyanins at 320 nm and above (520 nm). Compared to other methods, this one is quicker and cheaper [53].

2. IR (Infrared) Spectroscopy

Some infrared light frequencies are absorbed while others pass undetected through an organic molecule sample when infrared light passes through it. The vibrational alterations that take place when IR radiation enters in a molecule, which are connected to infra-red absorption. The type of vibrational shifts that a polar molecule experiences in response to IR radiation determines how well it absorbs that energy. As a result, one may broadly refer to infrared spectroscopy as a vibrational spectroscopy. Various bonds (N-H, O-H, C-H, C=C, C=O, C=O, and O-O) possess a range of vibrational frequencies. An organic molecule with these types of connections/bonds can be recognised by looking for the absorption band of distinctive frequency in the IR spectra [53]. FTIR (Fourier Transform Infrared Spectroscopy) is a high-resolution analytical technique used to determine the chemical ingredients and define the structural compositions. FTIR is time & money-effective, non-destructive examination approach that can provide fingerprints for the structural information of isolated biomolecules from herbals and powders.

3. NMR (Nuclear Magnetic Resonance) Spectroscopy

NMR generally focuses on the magnetic characteristics of a small number of atomic nuclei, namely the proton, isotopes of hydrogen ^1H , and a carbon isotope, ^{13}C . NMR spectroscopy, which records the variations between the various magnetic nuclei and so provides a clear picture of where these nuclei are positioned inside the molecule, has allowed a number of researchers to analyse molecules. Additionally, it will show which atoms are part of nearby groupings. The number of atoms in each of these environments can be calculated finally [51]. The separation of individual phenols, whose structures are subsequently determined by NMR off-line, has been attempted multiple utilising semi-preparative or preparatory methods TLC, CC & LC [52].

4. Mass Spectrometry for Chemical Compounds Identification

Organic molecules are exposed into charged ions with a high energy level when mass spectrometry is utilised, either by e- (electrons) or lasers. A mass spectrum is created by measuring the ions' mass/charge ratio (m/z) is plotted against the relative abundance of the broken-up ions. Mass spectrometry allows for highly accurate determination of the relative molecular mass (molecular weight) of molecules based on the sites where the molecule has been fragmented [54]. HPLC, column chromatography, and solvent extraction with bioactivity guidance were used in prior research to extract and purify bioactive chemicals from pith [55]. The structure of the bioactive molecule was described by spectroscopic methods such as IR, NMR, mass, and UV-visible. Hydrolyzed molecules can also be investigated, as well as their by-products. Tandem mass spectrometry (MS) is a method that employs mass spectrometry to generate a tonne of data for the molecular structures. When a pure standard is not available, HPLC and MS are used in order to swiftly and correctly identify chemical components in therapeutic herbs [56-59]. The study of phenolic compounds has recently made substantial use of LC/MS. As a result of its high efficiency of ionisation, electron-spray ionisation (ESI) is a better source for phenolic chemicals.

5. LC-MS (Liquid Chromatography-Mass Spectroscopy)

There are a number of issues arises with the extract of raw material in complex matrix analyses when the HPLC method is used exclusively without the use of additional methods in combination, an instance where the API concentration was before treated & purification is required for the procedure will be made simpler and gives better output. By greatly raising the sensitivity of detection, the HPLC-coupled mass spectrometry (MS) technology, also known as LC-MS solves this problem. The LC-MS spectroscopy capabilities include effective separation of analytical compounds, structural characterisation, molecular mass, fragmentation data, a long retention time and a wide detection range. The standard of the herbs in the extract of raw plant material and commercial goods can be identified, measured, and evaluated using the LC-MS combination approach [60,61].

6. GC-MS (Gas Chromatography-Mass Spectroscopy)

Gas Chromatography-Mass Spectroscopy, also known as GC-MS or gas chromatography plus mass spectrometry, is an analytical technique that combines GC (gas chromatography), which separates the various components of chemical compound mixtures, with MS (mass spectrometry), which analyses the different compounds that are being separated by the gas chromatography. The GC-MS approach can be used to determine the main component in the case of an analysis of a herbal product by looking at the extract. For the examination of the components of the drug, or more specifically the active medicinal ingredients, the fields of forensics, pharmaceuticals, cosmetics, food, and the environmental industries can all use GC-MS. The research of volatile, thermostable substances and their volatile derivatives is the most significant analysis performed by GC-MS. The GC-MS method is used to determine volatile oil both qualitatively and quantitatively. It can also be utilised to pinpoint a molecule's many constituent parts and metabolites of drugs. Comparatively, the LC-MS has greater sensitivity as compared to the GC-MS, however it can only analyse thermally unstable non-volatile molecules and cannot analyse the volatile components that are thermally stable. Both volatile and non-volatile chemicals can be quantified along with their qualitative identification and separation in a single analysis. The examination of many chemicals can be done simultaneously [60,61].

VI. CONCLUSION

Nowadays, the plant-based biomolecules are widely used for the treatment of various diseases. Due to their pharmacological properties, it is necessary to isolation, separation and identification of biomolecules. Separating an unknown substance is more challenging than isolating a known substance. The separation of either one or greater than one phytoconstituents in a mixture of extract fractions or crude extract can be a very time-consuming & costly procedure that frequently calls for expertise, many separation processes, and various chromatographic techniques. Mainly the separation and identification of biomolecules were performed by various analytical tools which includes chromatographic methods as well as spectroscopic methods. In this review chapter we explain the different chromatographic methods and spectrographic methods which were used in identification of the biomolecules.

Consent for Publication

None

Conflict of Interest

None

VII. ACKNOWLEDGEMENT

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