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Advanced Analytic Techniques for the Identification of Plant Derived Bioactive Compounds

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Submitted on: 15-MAR-2025, Revised on: 12-MAY-2025, Accepted and Published on: 13-MAY-2025 **REVIEW ARTICLE**

Abstract: This review takes a look at the latest ways to get and analyze plant-based compounds, with a focus on being eco-friendly and practical. Supercritical fluid extraction (SFE) uses CO_2, which helps increase yields while using less solvent and keeping the plant's molecules intact. Ultrasound-assisted extraction (UAE) uses sound waves to break down cell walls, making it easier to recover sensitive compounds quickly and with less solvent. Microwave-Assisted Extraction (MAE) heats the solvent directly, speeding up the process compared to traditional methods. For analyzing plant chemicals, high-resolution mass spectrometry gives detailed information about these substances and shows where they are found in plant tissues. Nuclear magnetic resonance (NMR) spectroscopy can identify compound structures easily, without needing complicated setups. Traditional methods like UV–vis and Fourier-transform infrared spectroscopy (FT-IR) are still valuable for quick measurements, especially when combined with data analysis. There are some challenges, too, like making sure extraction yields are consistent, keeping bioactive properties during isolation, and scaling up laboratory results for bigger production without changing the chemical makeup. New options like green solvents, including deep eutectic solvents and ionic liquids, are less toxic and biodegradable. In the future, using machine learning to model extraction processes might help move from small experiments to larger production more smoothly.

Keywords: *NMR, high-performance liquid chromatography (HPLC), GC, mass spectrometry (MS), FTIR, PC, UV–vis, LC-MS, UAE, natural Bioactive compounds (NBCs)*

UNSDG GOALS: UNSDG 3: Good Health and Well-being, UNSDG 9: Industry, Innovation and Infrastructure, UNSDG 12: Responsible Consumption and Production, UNSDG 15: Life on Land

1. INTRODUCTION

Recently, natural chemicals from plants have become popular as potential cancer treatments, catching the attention of both the public and researchers looking for new drugs. Many plant chemicals that were ignored in the past are now being studied for their health benefits (Atanasov et al., 2021). To get these compounds, you typically use solvents to pull the good stuff from plants or animals (Afzal et al., 2023). For a long time, people have been using these natural compounds in herbal medicine. This way can help extract different chemicals from plants, like tannins, terpenoids, and alkaloids. Comparatively speaking, the Natural Bioactive compounds (NBCs) made from medicinal plants are semisolids, liquids, or powders that are only meant to be applied topically or taken oral. Except when extraction thermosensitive chemicals, phytochemical extraction is a widely recognized as a universal technique that performs better than alternative conservative techniques. According to the World Health Organization, the greatest source of many medications are made from medicinal plants (Chaachouay & Zidane, 2024).

Traditional medicines, which contain substances made from therapeutic herbs, are employed in by almost 80 % of individuals in developed countries. Plants are large quantity of bioactive substances, like polyphenols, alkaloids, terpenes, flavonoid, and vital oils that have significant uses in medicine, food as well as cosmetics. The process of extracting these substances have a fundamental part in ensuring their efficacy and bioavailability (Altemim et al., 2017). Traditional extraction techniques like Soxhlet extraction and maceration have been extensively but require long extraction durations and excessive ingestion of solvents, leading to inefficiency and environmental concerns. Modern extraction techniques have been created to increase the process's efficiency while maintaining the stability of heat-sensitive compounds. Advances in extraction and various analytic methods have greatly enabled the discovery along with characterization of specific bioactive compounds (Usman et al., 2023). Because they enable the exact detection and profiling of complex plant extracts, techniques like spectroscopy using nuclear magnetic resonance, liquid chromatography-mass spectrometry, and high-performance liquid chromatography have become indispensable in phytochemical research. Furthermore, modern extraction procedures including supercritical fluid extraction (SFE) and ultrasound-assisted extraction (UAE), have improved efficiency of extracting while preserving their structural soundness, bioactive substances.

Medicinal herbs are widely used in poor countries. Becoming additional and more important at the primary medical care of both people and groups. Because they are very effective, inexpensive, reportedly side-effect-free, and used as an alternative to allopathic treatments, demand in international trade has increased (Ekor, 2014). Beneficial phytochemicals found in plants may complement the necessities of the human body functioning as organic antioxidants (Afzal et al., 2023). Numerous investigations have demonstrated that Antioxidants are abundant in a number of plants, for instance Natural antioxidants such as vitamins A,

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C, and E, as well as phenolic chemicals including flavonoids, tannins, and lignins, are abundant in plants. Providing a thorough overview of the sophisticated analytic methods used for the identification and characterization of plant derived chemicals produced from plants is the main goal of this review.

Table 1: **Features of Plants Derive Bioactive Substances**

Name of compounds	**Pharmacology properties**
Terpenoids	Antimicrobial, antibacterial, antiviral, anticancer, anthelmintic, anti-inflammatory, and antimalarial (Shakya, 2016).
Phenolcarboxylic acids	antimutagenic, anticarcinogenic, anti-inflammatory, and antiallergic (Anulika et al., 2016).
Alkaloids	local anesthetic, antimalarial, antihypertensive, antiasthma, diuretic, antibacterial, antispasmodic, analgesic, and diuretic properties (Chikezie et al., 2015)
Flavonoids	antioxidant action, hepatoprotective, antiviral, cardiovascular, antibacterial, and anti-inflammatory (Anulika et al., 2016)
Saponins	antioxidant, immunostimulant, antioxidant, hepatoprotective, anticancer, antifungal, anti-inflammatory, antiviral, antihyperglycemic, antihepatotoxic, anticoagulant, and neuroprotective (Kabera et al., 2014)
Tannins	metal ion-chelators, antioxidants, Hemostatic, diuretic, antiseptic, antimutagenic, and anticarcinogenic (Anulika et al., 2016)

METHOD

Phytochemical analysis involves a range of methodologies to extract, identify, and quantify bioactive compounds from plant materials. Each method is selected according to nature of the phytochemicals, the matrix of plants, and the intended application. The next section is comprehensive overview of these methodologies, including both traditional and advanced approaches.

Sample Preparation

Pretreatment of plant material is a crucial phase in guaranteeing the precision and dependability with phytochemical analysis. To prevent the breakdown of thermolabile chemicals, researcher's stress-dry plant material at low temperatures. To improve extraction, the dried plant sample is subsequently crushed to a fine powder by increasing its surface area (Sasidharan et al., 2011).

Choice and Types of Solvents

The physiologically significant NBCs in plant material are determined by the extraction method's solvent selection. Ncube et al. (2008) state that a suitable solvent should have the following structures. A highly selective solvent should be chosen, and it should extract the expected elements from the plant sample. To create a solvent-free extract, the solvent's boiling point needs to be as low as possible to make it easier to separate from the extract. The solvent should not easily break down and ought to be inactive chemically with the isolate (Canadas et al., 2020). Minimal viscosity in a good solvent results in good heat and mass transmission as well as a minimal pressure drop. The solvent must not be poisonous, flammable, or corrosive, and its elimination must none endanger the surroundings, the fluid ought to be inexpensive and widely available, to prevent solvent loss by evaporation, the solvent's vapor pressure should be low at working temperature (Clarke et al., 2018; Lee et al., 2024).

Water

The all-purpose solvent used extract the NBCs derived from plants using antibacterial effectiveness is water (H_2O). Traditional therapists primarily employ water, while organic solvent-derived plant extracts have the ability toward providing greater

dependable antimicrobial properties extract of water (azmir et al., 2013). Furthermore, hydrophilic phenolics are only significant as an antioxidant molecules, and hydrophilic flavonoids are unreliable against microorganisms.

Table 2 **Plant Derived Materials Extracted Using Various Solvent** (Azmir et al., 2013)

Methanol	**Ethanol**	**Water**	**Ether**	**Chloroform**	**Acetone**
Polyphenols	polyphenols	anthocyanins	terpenoids	flavonoids	flavonoids
Anthocyanins	alkaloids	saponins	alkaloids	terpenoids	
Tannins	tannins	terpenoids			
Flavons	flavonol	saponins			
Saponins	alkaloids				
Terpenoids					

Acetone

Both lipophilic and hydrophilic phytochemicals derived from two plants species employed can be dissolved by acetone, which is also volatile, miscible with water, and somewhat toxic to the biological examinatio. Acetone is a notably useful extraction solvent, particularly for antimicrobial testing when it's essential to isolate more phenolic chemicals derived from plant extracts (Khan and Abu-Reidah, 2023). Compared to aqueous-methanol, aqueous-acetone improved the isolation of tannins and other phenolic components. It was discovered that saponins with antimicrobial properties may be extracted from both acetone and methanol.

Ethyl alcohol and methanol (alcohol)

Higher concentrations derived from polyphenols in extracts of ethanol than in aqueous extracts are responsible for the more complex action of ethanol concentrates. It suggests that they are more active in the breakdown of seeds and cell walls, which are nonpolar characteristics together with release of polyphenols from cell extract (Dai & Mumper, 2010). The polyphenol oxidase enzyme, which causes water extracts' polyphenols to degrade although ethanol and methanol do nothing, offers a more practical justification for the decline within aqueous extract operation. Because ethanol, 70% has a higher polarity than pure ethanol, it has been observed to have more bioactive flavonoid components at advanced concentrations. To prepare 70% ethanol, water was added to the pure ethanol up to 30%, increasing the solvent's polarity.

Chloroform and ether

Chloroform, hexane, and methanol were used in sequential extractions of dried barks to produce the terpenoid lactones, with the chloroform fraction exhibiting the most biological activity. Terpenoids and tannins are more frequently formed by treating with less polar solvents, however they are occasionally observed in the aqueous phase. Fatty acids and coumarins are typically extracted selectively using ether.

Solvent Extraction Methods

Recently, solvent extraction has been accomplished using the System of Universal Extraction. A glass thimble containing dried powdered plant components is used to extract the material using a variety of solvents (Kapoor et al., 2020). Every extract goes through 10 rounds of the procedure, which modifies the temperature of the corresponding solvents below its boiling point. Filtered and concentrated in a vacuum concentrator, the resultant solvent extract is utilized to ascertain the plant bioactive compounds.

Decoction

Materials from hard plants like stems, bark, and roots can have their heat-stable, oil-soluble substances like flavonoids, tannins, and saponins extracted using a process called decoction. This procedure involves cooking plant material in enough water for 30–2 hr, after which is straining through muslin cloth layers-likely eight. To improve extraction, the process may be in conjunction with centrifugation, which collects supernatant and then evaporates the solven. According to Khajehei et al. (2017), extraction is often accomplishes at a temperature from 65 to 70 ℃ for nearly 2 hr, and the temperature is kept constant during the procedure. The amount of herbs, temperature, and pH each significantly affects the decoction. Several phytochemicals, including tannins, soluble polysaccharides, alkaloids, and flavonoids may become more soluble when employing the decoction technique rather than the maceration method. Glycosides cannot be changed into their aglycones because elevated temperature employed in the decoction procedure prevents the activity of glucuronidase. Higher concentrations of flavones, such as wogonin and baicalin are thus found in the decoctions that were extracted (Hidayat & Wulandari, 2021). The primary disadvantage of

crude extract is the high level of pollutants present in this technique, additionally, Volatile or heat-sensitive substances are not possible to be isolated using decoction components (Zhang et al., 2018).

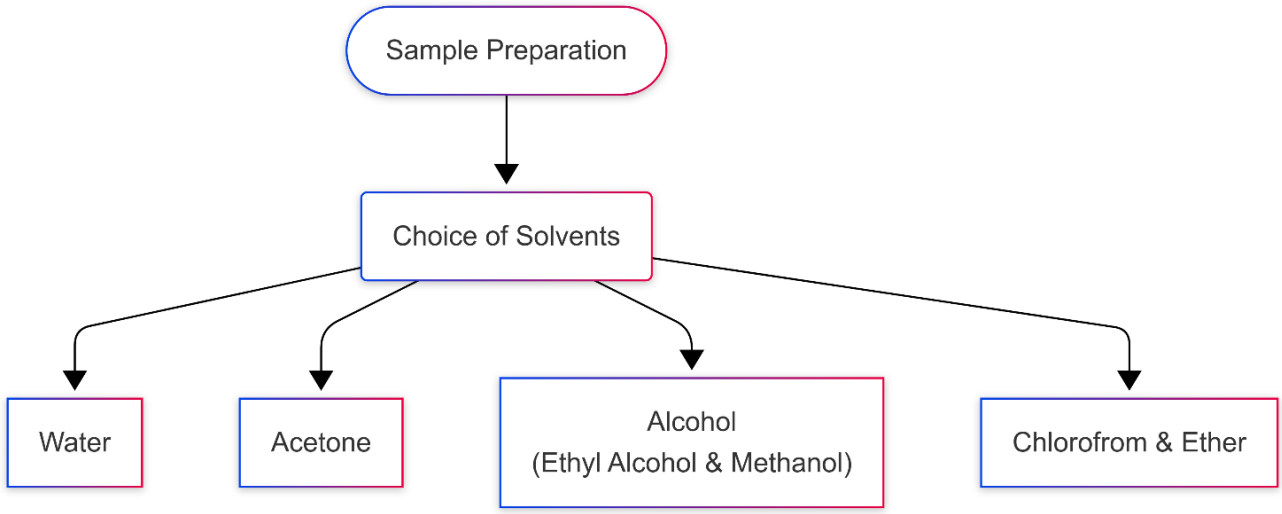

Figure 1. **Sample preparation and choice of solvents**

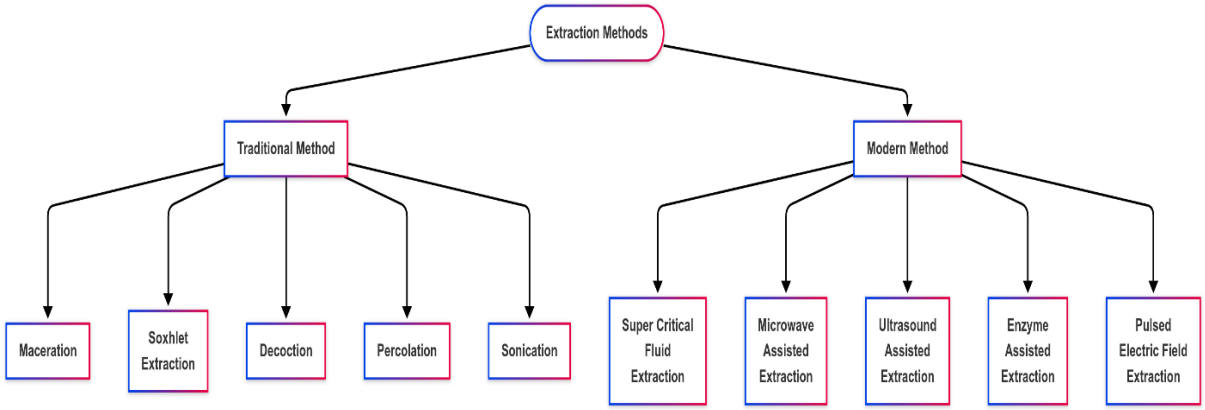

Figure 2. **shows traditional and modern methods of extraction**

Digestion

Digestion is one type of maceration that encourages extraction at a low temperature. When heated, the drug substance breaks down, increasing the solvent's effectiveness. For medications whose ingredients do not break down at relatively high temperatures, it is the preferable method (Pandey & Tripathi, 2014). During the process of extracting, the heat was applied to lessen the extraction viscosity of the solvent and make it easier to remove secondary metabolites. Plant-based goods which are readily Dissolvable lend themselves well to this technique.

Sonication

Ultrasound waves (20–2,000 kHz) are utilized in this sonochemistry-based technique to pierce the sample materials. It increases cell wall permeability and can be utilized at normal temperatures, but it is most commonly used on a hot plate at various raised temperatures. According to the vapor pressure, surface tension, polarity, and viscosity that affect the phenomenon of cavitation, the choice of solvents is crucial (De Monte et al., 2014). Ethanol, hexane and methanol are the most widely used solvents; water is occasionally included. Although the current technology become good to the components that are released, it has drawbacks, such as expensive installation and operating costs, the possibility of falsifying results due to the creation of free radicals in the samples, and the alteration of some active chemicals.

Maceration

Maceration refers to the process of softening. It is utilized to create concentrated infusions, tinctures, and extracts. To extract the phytochemicals, a solvent is soaked in the sample or eluent, including petroleum ether, ethanol, methanol and water for three to seven days, shaking occasionally. The fluid is filtered off after maceration, when the solid leftover material is compressed to

extract the maximum amount of liquid. The isolates are often clarified through filtration or decantation (Srivastava et al., 2021). Soft infusions come from parts of plants like flowers, leaves, and petals. Typically, you make these infusions by soaking the plant material in a water bath for about 15 min and then letting it cool to room temperature for 45 min (Shikov et al., 2022). This way can help extract different chemicals from plants, like tannins, terpenoids, and alkaloids.The Indian Pharmacopoeia considers it a safe way to create raw medicines. Factors like the type of food, temperature, time, and the solvent used can affect how well the extraction works. This approach helps to get the most out of the plants while using less solvent (Grdisa et al., 2020).

Percolation

When liquid moves through a material with tiny holes, like soil or some plants, that's called percolation. People often use this method to extract the good stuff from herbs for tinctures and drinks (Caldas et al., 2020). To start, you place the herbs in a jar that can be sealed and soak them in a liquid for about 4 hr (Hidayat and Wulandari (2021). After that, you put the herbs into a percolator, seal it up, and let the solvent flow through, pulling out the useful parts. You then let the mix sit for a day after adding more liquid to ensure everything blends well. Once you open the outlet, the liquid begins to drip out. The downsides of this method are that it uses a lot of solvent and takes quite a bit of time. It usually needs several extractions over a few days and a good amount of solvent compared to the herbs. There's a newer method called repercolation, where several percolators (Usually 3–12) are connected. In this set-up, the used plant material and fresh solvent move in opposite directions.

Soxhlet extraction

This method involves using a thimble with dry material in a distillation flask filled with solvents like petroleum ether, toluene, and hexane (Lopez et al., 2020). Soxhlet extraction is pretty effective compared to other methods like percolation or maceration. It's automated and runs continuously, which means you use less solvent and it speeds up the extraction process (Zhang et al., 2018). But using high heat for a long-time can lead to heat damage. The Soxhlet technique takes advantage of both percolation and reflux; usually, it operates at temperatures between 65 and 100 °C and takes about 24 hr. Petroleum ether is commonly used to get essential oils and steroids, remove fats, and extract chlorophyll from powdered leaves. Methanol can also be used to extract glycosides, flavonoids, steroids, and terpenoids. For isolating tannins, saponins, and carbohydrates, solvents like acetone, ethyl acetate, and chloroform work well. Just keep in mind that water can break down flavonoids, so this method is not ideal for watery samples. However, this procedure is expensive because it takes a long period and uses a high amount of solvents, and disposal of these solvents increase environmental issues lastly, the target compounds might break down due to the high extraction temperatures.

Ultrasonic assisted extraction (UAE)

This method uses ultrasonic vibrations to break down cell walls, making it easier for bioactive substances to blend into the solvent. It was reported that this technique is more efficient, uses less solvent, and helps keep delicate molecules safe (Chemat et al., 2012). UAE sends high-frequency sound waves (20 kHz–100 MHz) through the solvent, creating tiny bubbles. These bubbles produce shock waves that break the walls of plant cells. This method helps the solvent reach deeper by breaking apart the analytes and pulling out bioactive compounds from the cells. UAE is often used in the pharmaceutical industry to get polyphenols, alkaloids, and flavonoids. In food production, it's useful for extracting antioxidants and natural colorants. Some of the main perks of UAE are that it cuts down extraction times, uses less solvent, and does a better job of keeping heat-sensitive substances intact (Chemat et al., 2017). Although, excessive ultrasonic energy can cause free radicals to develop, this may break down the bioactive substances that have been recovere. Additionally, UAE may have limited penetration in dense plant matrices, reducing extraction efficiency. Various phytochemicals can be extracted in the United Arab Emirates, utilizing a range of solvents such as water, alcohols, acetone, and acidic aqueous solution. Acidic solution water is suitable for pectin extraction solvent. A temperature of 40 °C or lower is the ideal level for safety and to totally prevent phenolic thermo-degradation (Kentish & Ashokkumar, 2010). Due to inadequate power management, which causes inefficient energy transmission within the extraction medium, implementation of UAE is still not widely used to gather phytochemicals linked to complex matrixes like algae.

Supercritical fluid extraction (SFE)

Complex process called supercritical fluid extraction employs CO_2 as the solvent to extract bioactive substances that are non-polar at high temperatures and pressures (Lopez et al., 2023). The supercritical state of CO_2 provides high diffusivity and low viscosity, enabling it to enter the plant matrices effectively and dissolve target molecules specifically (Herrero et al., 2010). In SFE, nonpolar phytochemicals like essential oils and terpenoids are extracted using supercritical fluids, usually carbon dioxide, as a solvent. The stability and purity of the extracted chemicals are guaranteed by the controlled temperature and pressure used in this environmentally friendly process. SFE is often used to get essential oils from food and medicine, along with carotenoids and terpenoids. It's great because it's selective, does not require solvents, and is better for the environment. But there are some downsides too. It does not mix well with polar substances unless you throw in some solvents (Hasanov et al., 2023). After you extract, ensure to filter out the leftover plant parts. There are a few downsides, though. Enzymes can be expensive, making it tough to handle a lot of samples at once. This method is great for substances that can be affected by heat still, one of the issues is that you might get lower yields compared to using liquid solvents, and it requires high pressure (Khaw et al., 2017). The main extraction solvent used, SC-CO2, can also reduce the sample's polarity, which might limit its effectiveness.

Microwave-Assisted Extraction (MAE)

MAE is a method that uses microwave radiation to create heat quickly (López-Salazar et al., 2023). This heat warms up both the solvent and the plant material, helping to break down cell walls and release useful compounds into the solvent. MAE is commonly used in the food, natural dye, and pharmaceutical industries to extract things like essential oils, flavonoids, and alkaloids. The big advantages are that it works quickly, uses less solvent, and is more efficient. Just keep an eye on the microwave power levels to prevent overheating delicate compounds. Things like the type of solvents and plant materials can affect how well microwave-assisted extraction performs. At 120–140 °C, Kapoore et al. (2018) found that 30 s-20 min was the range of the extraction time. Water, ethanol, and methanol are examples of strong solvents that absorb microwave radiation, should be used in the procedures because they heat up quickly and reduce the amount of time needed to apply microwave radiation. MAE's drawback is that it can harm heat-sensitive polyphenols like anthocyanins as well as polyphenols with a lot of hydroxyl-type substituents.

Null Enzyme-Assisted Extraction (EAE)

EAE is a biotechnological technique that releases trapped bioactive substances via dissociation of plasma membrane of plant using hydrolytic enzymes such as cellulases, hemicellulases, and pectinases. This technique is commonly used in the food industry to increase the bioavailability of nutrients from plants and in the pharmaceutical industry to extract polysaccharides and flavonoids. EAE comes with some nice benefits, like being good for the environment, handling certain tasks effectively, and recovering useful compounds without needing tough conditions (Maric et al., 2018). But it's not all good there's the cost of the enzymes and the longer extraction times compared to regular solvent methods. Enzymes can be pricey, making it tough to process a lot of samples at once (Chemat et al., 2017). Plus, the enzyme mixes you find in stores often do not break down plant cell walls completely, so you might miss out on some of the phytochemicals you are looking for. Plus, extraction rates can change due to factors like temperature, oxygen levels in the water, and nutrients.

Pulse electric field extraction (PEFE)

PEFE is a method that uses strong electric currents for a short time, from nanoseconds to microseconds, to help get extracts from products (Beltran et al., 2021). Basically, you put the product between two electrodes and turn on the current. You can run the current continuously at levels between 20 and 80 kV/cm or in bursts at 100–300 V/cm (Martínez et al., 2020). This process helps the cell membranes open up and release their contents into a liquid (Sharma et al., 2022). The extraction time can vary from 5 to 48 hr and works well at temperatures from 20 to 50 °C, yielding about 76–85% (Lakka et al., 2021). The effectiveness of this method depends largely on things like the treatment medium, the pulse settings, and the physical properties of the cells involved. Factors such as water content, how ions move, thickness, size, and structure of the tissue also play a role in how well the extraction goes (Poojary et al., 2020). PEFE has been used to improve the extraction of sunflower seed oil too. After sunflower seeds were treated for 30 s with a frequency of 15 Hz and an electric field of 7.0 kV/cm, a solution with concentration of 40-wt percent, and a 30-s pulse width, 9.1% increase in oil extraction (Shortkii et al., 2017). PEFE can be used to extract proteins that are soluble in water (Gateau et al., 2021). When high-intensity therapy is used, chemical and electrochemical responses, such as electrode fouling and corrosion, are regarded as serious disadvantages.

Null Qualitative Analysis of Phytochemicals

Tests for alkaloids

Alkaloids content is frequently identified by the use of Wagner's and Mayer's reagents, which react with these substances to generate a precipitate. Alkaloids, for example, produce a reddish-brown or cream-colored precipitate when (2 ml) of either reagent is added to (1 ml) of a plant extract.

Tests for flavonoids

To check for flavonoids, we mix the extract with magnesium and strong hydrochloric acid in a test called the Shinoda test. If flavonoids are around, the extract will turn pink or scarlet. This test is pretty common for spotting flavonoids.

Tests for tannins

To check for tannins, mix a 5% ferric chloride solution with the plant extract. If it turns blue or greenish black, that means tannins are present. This test works for both hydrolysable and condensed tannins.

Phenolic test

For phenolic compounds, we use the Folin-Ciocalteu reagent. If the extract turns blue when we add this reagent, it means phenolics are present. We can measure how much is there using spectrophotometry.

ADVANCED CHROMATOGRAPHIC TECHNIQUES

Below are some of the advanced analytic techniques used to isolate, identified and measure these plant derived bioactive compounds from plant materials.

Gas–Liquid Chromatography

Hormones have relatively high molecular weights, so in gas-liquid chromatography, you need to use high temperatures, often above 200 °C, to get them to evaporate into the gas phase. A gas is used to carry the vaporized steroid through the column; helium is often chosen over nitrogen due to its greater separation properties, even though it is more expensive (Dmitrieva et al., 2022). Despite the fact that hydrogen can further enhance separation, safety considerations restrict its use. The study of gas chromatography was once thought to be the gold standard for high-resolution separation. Modern techniques mostly use capillary columns constructed of glass or fused silica, whereas earlier techniques depended on packed columns. Later developments include the use of gas-liquid chromatography and other methods to assess glucocorticoid readings in body fluids and the extension of bile-acid analysis in fecal samples to create thorough lipid profiles (Margolin et al., 2022).

High-Performance Liquid Chromatography (HPLC)

Complex extracts from plants are frequently separated and identified using HPLC-MS. The incorporation of high-resolution mass spectrometers: like Orbitrap or hybrid quadrupole-time of flight (Q-TOF, enables accurate determination of secondary metabolite molecular formulas. By saving time, preserving samples and solvents, and boosting peak capacity, UltraHigh-Performance Liquid chromatography has further enhanced analysis (Deepthi et al., 2023). Organic and inorganic solutes can be separated and identified using the analytic technique known as HPLC, in a variety of materials, particularly those related to biology, pharmacology, food, the environment, and industry. HPLC is commonly used to separate and measure plant chemicals. You put a plant extract into an HPLC system with a reverse-phase column and use detectors like UV–vis or fluorescence (Sanchez-Rangel et al., 2022). You can identify compounds by comparing their retention times to known standards.

Liquid Chromatography Coupled with Mass Spectrometry

Liquid chromatography with mass spectrometry, or LC-MS, is a method used to classify and measure different substances. It's really useful for studying different parts of plant extracts. Nowadays, the pharmaceutical industry uses LC-MS in various stages of drug development. With newer techniques like ion-spray, thermospray, and electrospray, LC-MS has become more accurate and sensitive. Furthermore, the combination of Mass spectroscopy of liquid secondary ions, more recently, laser mass spectroscopy running at 600 MHz enables the identification of isotope patterns and the accurate measurement of Protein and peptide molecular weights (Bianchi et al., 2018).

Nuclear Magnetic Resonance Spectroscopy (Nuclear Magnetic Resonance [NMR])

Nuclear magnetic resonance's main focus on the specific atomic nuclei magnetic properties, particularly proton (hydrogen) nucleus, carbon, and its isotopes. NMR offers a comprehensive map of these nuclei's locations within a molecule by identifying minute variations in their magnetic behavior (Gauto et al., 2023). The quantification of atoms in each confined environment is made possible by this information, which aids researchers in determining not only the spatial arrangement of atoms but also which atoms are clustered together. In the past, phenols have been isolated in a number of ways employing liquid chromatography, column chromatography, thin-layer chromatography, either preparative or semipreparative, and NMR for identify their structures off-line (Huang et al., 2024). NMR is a method of analysis employed to keep an eye about the composition of a mixture's constituents and determine the molecular structure of individual atomic nuclei by analyzing their magnetic characteristics. Moreover, molecule dynamics and interaction can be investigated using NMR (Emwas et al., 2024). The fundamental idea behind NMR spectroscopy is that radio waves are absorbed and emitted by nuclei because of other atoms or molecules being bound to them, detector then picks up the waves.

Liquid Chromatography-Nuclear Magnetic Resonance

Among the most efficient and successful methods for isolation and clarifying the structure of unknown substances is the combination of chromatographic separation technology with NMR spectroscopy, especially for clarification of the structure of substances that are susceptible to light and oxygen (Seger & Sturm, 2022). Electronic data collecting and evaluation in LC-NMR enhances detection sensitivity and speed, as well as the online LC-NMR method ongoing recording of time variations during the chromatographic run. The modern development of high resolution NMR using the pulsed field gradient approach and 3D technology has improved the molecular weight information and structure elucidation. These new methods can really help in toxicology testing, understanding how drugs work in the body, and discovering new medicines.

Mass Spectrometry (MS)

Mass spectrometry is a great tool for identifying new compounds, measuring chemicals, and figuring out the structure and properties of substances. This method helps figure out the molecular weight of different samples. You will commonly see it in peptide or oligonucleotide sequencing, as well as in studying the structures of organic molecules and identifying substances in complicated mixtures. It works by determining the molecular weight and examining a specific part of the molecule. It's particularly handy for analyzing extracts from plants and other items (Almajidi et al., 2024). It figures out the molecular weight and a certain part of the molecule. This approach is useful for identifying elements in plant extracts and other products. Using fragmentation of mass spectra to provide partial insights into partial structural make-up is also crucial. Depending on their developmental phases, mass spectroscopy can be separated into four types. Asserts that quadruple MS is employed for broad

purposes and that ion Ion trap Microsoft is a versatile tool. Because of its extreme sensitivity, triple quadruple mass spectrometry is typically employed toward specific samples. Since Fourier transform instruments provide information they are used for structural determination when extremely high-mass precision and resolution are needed, both with reference to the overall mass of the compound and the masses of different compound fragments (Marney et al., 2024).

Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared spectroscopy is useful for identifying both organic and inorganic materials. It is frequently used to measure specific elements in an unknown mixture and is applicable to solids, liquids, and gases (Workman, 2024). FTIR, which transforms interference patterns into spectra, is a contemporary method of data gathering. Analysis is quicker and more sensitive with modern computerized FTIR instruments than with outdated dispersive types. This method is frequently employed to find chemicals in situations like spills, paints, polymers, coatings, medications, and other pollutants (Kowalczuk & Pitucha, 2019). A helpful way to spot chemical bonds, especially functional groups, is by using Fourier-transform infrared Spectroscopy, or FTIR for short. A FTIR spectrum shows how different chemical bonds react to light at various wavelengths. By looking at the infrared absorption patterns, you can tell what types of bonds are in a molecule (Hazra et al., 2007). Pure chemicals typically have unique FTIR spectra, almost like a fingerprint, while inorganic materials usually display simpler patterns compared to the more complex ones found in organic compounds. You can usually identify many compounds by comparing your sample's spectrum to reference databases. To ensure you accurately identify some less common substances, FTIR is often used alongside other methods like X-ray diffraction, mass spectrometry, emission spectroscopy, and nuclear magnetic resonance.

Ultraviolet–Visible Spectroscopy

Spectroscopy is the scientific discipline that examines the interactions between matter and electromagnetic radiation. It comprises a number of sample analyses and is among the best resources for researching atomic and molecular structures (Thomas, 2024a; Thomas, 2024b). Wavelengths covered by optical spectroscopy range from 100 to 400 µm. UV–visible spectrophotometry is a widely used method in pharmaceutical analysis that quantifies the amount of visible or ultraviolet light taken up by a solution. Recent works talks about a method that measures UV–visible light from two sources. These tools help identify chemical molecules using basic information. It's a quick and accurate way to analyze small samples, figuring out how many molecules are there by looking at how much light they absorb (Kumar et al., 2021), based on the Beer–Lambert law.

ESTIMATION OF PLANT DERIVED BIOACTIVE COMPOUNDS

In phytochemical analysis, it's important to measure various bioactive compounds, like tannins, alkaloids, flavonoids, and total phenolics in plant extracts. Usually, colorimetric assays are the go-to for determining these compounds, and results are reported as standards per gram of extract.

Total Phenolic Content

For the total phenolic content, we used the Folin-Ciocalteu method, which is pretty common.The Folin-Ciocalteu reagent is reduced by phenolic chemicals in this test, resulting in a blue complex that is detected at 760 nm (Dai & Mumper, 2010). However, other reducing agents, such as ascorbic acid, may also disrupt the process, therefore this technique is not totally unique to phenolics.

$$TPC = \frac{CONCENTRATION \times VOLUME}{MASS} = \frac{CV}{M}$$

where:

Concentration of phenolics are expressed in milligram per milliliter

Volume express in milliliter

Mass express in gram.

To Find Total Tannin Content

Frequently ascertained by the vanillin Assay or the Folin-Ciocalteu reagent. Milligrams of tannic acid equivalent (mg TAE/g) per gram of extract is the unit of measurement.

Formula for calculation:

$$TTC = \frac{\textbf{concentration} \times \textbf{volume}}{\textbf{Mass}} = \frac{C \times V}{M}$$

where:

C is tannins concentration as determined in mg/ml,V is the volume in milliliter and M is the mass of extracts in gram (Rizvi et al., 2023).

Finding the Content of Flavonoids

The colorimetric method of aluminum chloride ($AlCl_3$) is commonly used to measure flavonoids. A golden coloring that may be measured between 410 and 423 nm is produced when flavonoids and $AlCl_3$ form complexes.

Formula for calculation:

$$TFC = \frac{\textbf{concentration} \times \textbf{volume}}{\textbf{Mass}} = \frac{cv}{\mathrm{M}}$$

where: C is the flavonoid concentration measured in mg/ml, V is the volume of the extracts in milliliters, and M is the mass of the extracts in grams.

Determination Alkaloids

Gravimetric analysis is a common way to measure alkaloids. We first extracted them using acidified ethanol, then added ammonium hydroxide to help them settle. After drying the solid, we weighed it. This lets us figure out the alkaloid concentration as a percentage of the dry weight

Formula for calculation:

$$\textbf{TAC} = \frac{\textbf{concentration} \times \textbf{volume}}{\textbf{Mass}} = \frac{cv}{\mathrm{M}}$$

where C is alkaloids concentration as determined in mg/ml, V is the extracts volume in milliliter and M is the mass express in gram (Wink, 2020).

Table 3: **Important Phytochemicals, Extraction Techniques and Uses**

Plant names	Phytochemicals presents	Extraction method	Applications	References
Terminalia catappaA	flavonoids, tannins, saponins, alkaloids	Soxhlet extractions with ethanol and methanol	antioxidant, anti-inflammatory, wound healing	Khan et al. (2014)
Syzygium cumini	anthocyanins, flavonoids, tannins, phenolics	U.A.E. with ethanol	antidiabetic, antimicrobial, hepatoprotective	da Rosa et al. (2024)
Bougainvillea glabra	betalains, flavonoids, alkaloids, phenolics	SFE with CO^2	anticancer, antihyperglycemic, anti-inflammatory	Lopez-Cruz et al. (2023)
Barringtonia racemosa	terpenoids, saponins, flavonoids, alkaloids	maceration with aqueous ethanol	antitumor, analgesic, antiarthritic	Das et al. (2023)
Azadirachta indica	azadirachtin, nimbin, tannins, terpenoids	Soxhlet extraction with acetone and hexane	antimicrobial, insecticidal, anticancer	Yang et al. (2025)
Moringa oleifera	quercetin, kaempferol, tannins, alkaloids	MAE	nutraceutical, water purification, antioxidant	(Nobossé et al., 2018)

Abbreviations: MAE Microwave-Assisted Extraction
SFE supercritical fluid extraction
U.A.E. ultrasound-assisted extraction.

CHALLENGES AND FUTURE DIRECTIONS

People are really interested in the bioactive compounds from natural sources because of their potential health benefits (Newman & Cragg, 2020). Yet, there are still many challenges in discovering and developing these compounds, even with modern extraction and analysis techniques.

Challenges

Extraction efficiency and selectivity

Traditional methods like maceration and Soxhlet extraction can take a lot of time and use a bunch of solvents that might damage sensitive chemicals. Even with newer techniques like pressurized liquid extraction and enzyme-assisted extraction, getting better results can still be tricky, especially when it comes to extracting rare bioactive compounds from complex natural mixtures (Azmir et al., 2013).

Stability and structural complexity

Many natural compounds like polyphenols, terpenoids, and alkaloids are sensitive to changes in light, temperature, and oxidation during processing. This makes extraction and storage tricky. Even advanced techniques like high-field NMR and MS struggle to fully explain the complex structures of these molecules (Pai et al., 2022).

Analytic instrumentation and data processing

Using combined methods like NMR, GC-MS, and LC-MS has really helped in identifying and measuring these bioactive compounds. But the high costs of these instruments and the need for specialized knowledge can be tough to manage, along with issues like data interpretation and standardization (Barri et al., 2024). To make sense of the large amounts of data generated, we need advanced data analysis and bioinformatics approaches.

Scalability and reproducibility

Keeping things consistent and under control can be tricky when moving from laboratory extraction techniques to large-scale production. Scalability is made more difficult by the requirement for sustainable and ecologically compatible extraction techniques, which must be both profitable and successful in maintaining the integrity of the compound (Capaldi et al., 2024).

Null Future Directions

Green and sustainable extraction technologies

The development of "green" extraction methods, which employ fewer solvents as well as the need of less energy is being strongly encouraged. Numerous disadvantages of traditional methods demands consideration by future developments in supercritical fluid extraction and hybrid approaches (like fusing enzyme-assisted techniques with ultrasound). Yield and reproducibility will probably be improved by process optimization utilizing response surface approaches and integration with process analytic technology (Chemat et al., 2012).

Integrated analytic platforms

Combining high-resolution analytic methods, extraction, and fractionation. High-throughput screening of complex natural matrices will be possible while reducing sample handling by combining techniques like LC-MS/MS with high-field NMR and even integrating microfluidic technologies for online extraction and analysis (Muhammad et al., 2024).

Advanced data analysis and machine learning

Improved chemo-metric and machine-learning techniques are crucial for better data processing because of the volume and complexity of spectral data. Artificial intelligence and pattern recognition have been used recently to help with structure elucidation and retention time prediction. Combining these computational methods with multiomics techniques (metabolomics, proteomics, and lipidomics, for example) might improve the discovery process and provide comprehensive insights into biological function (Maritha et al., 2022).

Standardization and scale-up strategies

Standardized procedures must be established for both extraction and analysis techniques to ensure laboratory reproducibility. The development of reliable, scalable, and sustainable procedures that can be consistently moved from the bench to industry without sacrificing the compounds bioactivity must be the main goal of future research (Dar et al., 2022).

CONCLUSION

In conclusion, sophisticated analytic methods for determining bioactive chemicals produced from plants have significantly changed in the last several years. New techniques like ultrasound-assisted extraction and microwave-assisted extraction are turning out to be more effective than the old ways. They save time and make it simpler to get what we want. Plus, they usually use greener solvents like ethanol, methanol, and water, based on what we are trying to extract. Still, there are some issues to tackle. We need to ensure we do not lose bioactivity, establish clear processes for consistency, and handle the varying quality of plant materials. Another big challenge is taking these methods from the laboratory to larger production without losing effectiveness. Future research should also look at combining these advanced extraction methods with high-quality testing techniques like NMR spectroscopy and liquid chromatography. This combo could make identifying bioactive compounds more sustainable, efficient, and reliable, leading to a smoother process from research to large-scale production while keeping the compounds active.

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Auwalu Abdullahi Shehu is currently pursuing his M.Sc in Chemistry at Kalinga University, Naya Raipur, Chhattisgarh, India. He has published a paper titled "Review on Biomolecules Detection Using Nanoparticles" and participated in an International Conference held at Maharana Pratap College of Pharmacy, Kanpur. He also earned a certificate from a one-day online workshop on "Article Publication in Reputable Journals" organized by Maryam Abacha American University of Nigeria. Recently, he authored a review paper titled "Polymeric Innovations Driving Sensitivity in Electrochemical Analysis", which highlights the role of conducting polymer composites in enhancing electrochemical sensor performance through improved sensitivity, selectivity, and stability.

Reena Kushwaha
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Reena Kushwaha is currently pursuing her Master of Science in Chemistry at Kalinga University, Naya Raipur, Chhattisgarh, India. Her research interests include functional materials, electrochemical sensing technologies, and the application of conducting polymer composites in analytical chemistry. She has recently authored a paper titled "Polymeric Innovations Driving Sensitivity in Electrochemical Analysis: A Review on Conducting Polymer Composites for Electrochemical Sensors." This review explores the synthesis, characterization, and sensing performance of various conducting polymer-based composites, particularly focusing on their sensitivity enhancements in electrochemical applications. The study highlights recent advances and emerging trends that contribute to the development of high-performance sensors for real-world analytical challenges. As part of her academic engagement, she presented this work at the First International Conference on Biologically Active Molecules 2025 (ICBAM'25), organized by the PG & Research Department of Chemistry, Saraswathi Narayanan College (affiliated to Madurai Kamaraj University), Perungudi, Madurai, on March 7, 2025, in hybrid mode. Her contribution was recognized with a certificate of participation and presentation.

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Dr. Preeti Pandey is an Assistant Professor in the Department of Chemistry, Faculty of Science, Kalinga University, Naya Raipur, Chhattisgarh, India. She received her Ph.D. in Chemistry from Jiwaji University, Gwalior, in 2015, with research focused on the electrochemical analysis of pharmaceuticals using chemically modified electrodes. She also holds an M.Sc. in Pharmaceutical Chemistry from the same institution. Dr. Pandey has over 11 years of experience in teaching and research. She has participated in five workshops and presented her work at more than 15 national and international conferences. Her academic contributions include over 12 research and review publications in reputed journals and two book chapters in national publications. She served as Co-Convener of the *International Interdisciplinary Conference on Science for Society (IICSS2022)* at Kalinga University. Her research on the electrocatalytic quantification of the antiviral drug valacyclovir earned her the Best Paper Award at the 2015 *Innovation and Research in Science, Management and Technology* conference in Bilaspur. She was also awarded a Fellowship for Training of Young Scientists (2013–2014) in Chemical Sciences by the M.P. Council of Science and Technology, Bhopal.

ALIGNMENT WITH UNITED NATIONS SUSTAINABLE DEVELOPMENT GOALS (UNSDGS)

This review supports several United Nations Sustainable Development Goals (UNSDGs) through its focus on green extraction methods, plant-based bioactive compounds, and eco-friendly analytical technologies. It contributes to **UNSDG 3 (Good Health and Well-being)** by promoting the identification and utilization of therapeutic plant compounds, **UNSDG 9 (Industry, Innovation and Infrastructure)** through advancements in analytical and extraction technologies, **UNSDG 12 (Responsible Consumption and Production)** by encouraging sustainable and less toxic methods, and **UNSDG 15 (Life on Land)** by highlighting the value of plant biodiversity in drug discovery and health sciences.