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New approaches to the plant bioactive compound extraction, isolation, quantification, and characterization

Vinay Tiwari and D.N. Singh

Department of Chemistry, K.S. Saket P.G. College, Ayodhya-224001, India

Abstract

Introduction:The molecular diversity observed in natural products derived from medicinal plants, including both pure molecules and standardized extracts, offers vast potential for the discovery of novel therapeutic leads.

Methodology: An growing variety of procedures that combine chromatographic and spectroscopic or spectrometric techniques have been devised to elucidate the structures of both known and novel substances, obviating the necessity for separation. While the isolation of pure compounds from complex matrices such as organic matter continues to pose challenges, the development of more selective methods for extraction, fractionation, and purification holds promise for reducing the time required from the collection of biological materials to obtaining the final purified compound. However, it is important to note that the achievement of one-step isolation procedures is still a distant goal. In addition to the various procedures conducted on the plant, a significant deficiency exists in the isolation, identification, and quantification of plant extracts exhibiting notable antioxidant properties.

Results: Mass spectrometry is a very effective analytical methodology utilized for the purpose of identifying new compounds, quantifying known substances, and elucidating molecular structure and chemical characteristics. The utilization of an MS spectrum can facilitate the determination of a substance's molecular weight. This method is frequently employed in the field of organic compound structural elucidation, peptide or oligonucleotide sequencing, and monitoring the presence of known compounds in complex mixtures with a high level of specificity. It involves simultaneous determination of the molecular weight and identification of a diagnostic fragment of the molecule.

Conclusion: The primary objective of this study is to investigate the analytical methodology employed in the extraction, isolation, identification, quantification, and characterization of active components derived from plant extracts. The document provided a comprehensive description of the methodologies employed in the analysis of bioactive compounds present in plant extracts, encompassing spectroscopic, chromate-graphic, and traditional phytochemical screening techniques.

Keywords: Bioactive, Spectroscopic, Chromatographic, Screening, Plants

Addressfor correspondence: Vinay Tiwari, Department of Chemistry, K.S. Saket P.G. College, Ayodhya-224001, India. E-mail:vinaytiwari@gmail.com
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INTRODUCTION

The elegance of natural product chemistry is demonstrated by the enormous diversity of its sources. In large quantities, bioactive chemicals can be found in microorganisms, animals, and plants. Plants specifically provide cures for several anthropogenic disorders in numerous regions of the world, including Asia, Africa, and South America. As their main source of healthcare, traditional medicine is used by 80% of the world's population. The significance of compounds derived from natural sources in the process of creating new medications cannot be overstated. Artemisia has been used to successfully treat a wide range of conditions, including viruses, malaria, bacteria, hepatitis, fungi, cancer, and inflammation[1]. The Rosaceae plant

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family is typically divided into four subfamilies: Rosoideae, Prunoidae, Piraeoideae and Maloideae, depending on the type of fruit produced. Apples, almonds, cherries, pears, raspberries, strawberries, and other significant food crops belong to this plant family. According to research, Rosaceae plants contain bioactive compounds that have significant health benefits[2]. The majority of people in poor countries receive their primary medical care from traditional medicines, according to the World Health Organisation. India, which possesses a wealth of historically well-documented and frequently utilized herbal medicine knowledge, is one of the top producers of medicinal plants in the world and is appropriately referred to as the botanical garden of the world[3].

To better understand the quality of food, researchers have identified some essential dietary elements using a range of analytical approaches. The most often used techniques include chromatography, sensors, spectroscopy, spectrometry, and combinations of these. In the study of foods and beverages, mass spectrometry and gas chromatography are routinely coupled to detect individual molecules and search for contaminants or adulterations. Additional mass spectrometry (MS) techniques include proton transfer reaction (PTR)-MS, inductively coupled plasma (ICP)-MS, gas chromatography (GC)-MS, and high-performance liquid chromatography (HPLC)-MS. The quantitative PTR-MS approach is a simple-to-understand way to identify volatile organic compounds with less fragmentation. Tandem-MS (MSn) is useful for identifying compounds since it studies fragments of fragments[4]. It is without a doubt true that hybrid techniques like LC-NMR or LCMS permitted online structural elucidation and generated remarkable examples of NP identification without prior isolation, but in many circumstances, it is still required to have the purified compounds on hand[5]. The World Health Organisation (WHO) estimates that more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal treatments in Asia indicates a long history of human interactions with the environment. Both viral and chronic diseases can be treated with a variety of compounds that can be found in plants that are utilized in traditional medicine[6]. Numerous beneficial biological effects have been identified, including analgesic, anticancer, antibacterial, antioxidant, antidiarrheal, and wound healing properties. People commonly praise some organic or herbal products for their benefits. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive molecule and refute this conventional assertion[7].

Extraction techniques[8-14]

Traditional techniques

Extraction is the deliberate and selective extraction of medicinally active components of plants using a variety of methods. These include maceration, percolation, digestion, infusion, and decoction. Maceration involves placing solid plant components in a closed container with the full solvent and allowing them to stand for at least 3 days with frequent agitation. Percolation involves placing the plant matter into a percolation tube and allowing it to stand for 4 hours. Digestion involves light heat and stirring the mixture by hand. Infusion involves briefly macerating the plant material with either cold or hot water. Decoction involves distilling the extract from the plant material. The dried plant material is ground up and boiled in a certain amount of water for a certain amount of time. After cooling, the mixture is strained or filtered. Reflux is used to extract components that are heat- and water-stable. The tincture is an alcoholic extract of plant material. Pressurized Liquid Extraction (PLE) is an accelerated solvent extraction system. Soxhlet Extraction is the finest technique for the continuous extraction of a solid by a hot solvent and is named after Franz Ritter von Soxhlet, a German agricultural scientist. Steam Distillation is the process of distilling a liquid into a solid substance. Steam distillation is the standard method for extracting volatile oil from crude plant material. Hydro distillation is the most common method for isolating essential oils. Expression is a process of extracting citrus essential oils. Enfleurage is used to extract delicate fragrances from flowers. Supercritical Fluid Extraction (SFE) is the most sophisticated extraction technique available. Ultrasonic Extraction is the most advanced extraction technique available. Ultrasonic-assisted extraction (UAE) uses high-frequency sound to free natural chemicals from plant tissues. Microwave Assisted Extraction (MAE) combines microwave and standard solvent extraction methods. Solid Phase Extraction (SPE) is a fast, cheap, and sensitive approach that employs several cartridges and discs with various sorbents. Ionic liquids have been developed for analytical purposes with advantages in terms of quality and efficacy of extraction. Enzyme-assisted extraction is a modern and practical alternative to traditional solvent extraction techniques. It uses enzymes to catalyze reactions without subjecting them to harsh conditions. Pulsed-electric field-assisted extraction (PEFAE) is a unique extraction technology due to its purity, low energy demand, and solvent utilization. Electroporation is used to create nano- and micro-porations in the cell membrane to allow bioactive substances to escape.

Novel techniques

Extraction with ultrasound assistance

Ultrasound is a cutting-edge technology that increases yield for extraction by boosting mass transfer, bursting the cellular matrix, and releasing chemicals. High-power ultrasound is ultra-sonication with excessive intensities above 1 $W.cm^{-2,}$ while power ultrasound has frequencies of 20 kHz and 100 kHz and can cause cavitation. It is often used in the food industry.

Technology for instantaneous controlled pressure drops

The Regulated Pressure-Drop procedure (DIC) is a core-based concept that has led to the evolution of organic products. It involves a vacuum pump extraction vessel with a controlled pressure-drop valve, a vacuum machine with a capacity 50 times greater than the vessel to be treated, an extract collecting trap for condensate recovery, and hot air drying under ideal DIC conditions (0.35 MPa pressure for 10 seconds). DIC can prolong heat-sensitive food granule powder, such as apples and onions, and retain bioactive molecules and allow nutritional value.

Pulsed electric field (PEF)

PEF (electroporation or electro-permeabilization) is a non-thermal technique in which a bio cell is exposed to an external electrical field for a very short time. It consists of four distinct phases: post-treatment, extracellular compounds, changing the number or size of created holes, increasing the likelihood that the cytoplasmic membrane will cross, and creating tiny metastable

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hydrophilic holes if the trans-membrane possibility threshold is between 0.2 and 1.0 V.

Enzyme-assisted extraction (EAE)

Enzyme-assisted Extraction (EAE) is another cutting-edge method in which the extraction medium is supplemented with enzymes to enhance the recovery procedure. When derived from plant materials, the main enzyme activity is to weaken or crush the cell walls. This gives the active compounds access to the solvent. Bound phytochemicals (on cell walls or inside cells) are challenging to extract using a standard solvent extraction method. The surrounding items that can assist the release of these components were degraded by enzymes. However, the polyphenols bound to protein or carbohydrate extraction (inside or on cell walls) are thought to benefit from EAE. Enzymes for enzymatic extraction include lipase, -amylase, pectinase, amyloglucosidase, laccase, and protease.The particle size and the enzyme proportion to the sample are the main controlling variables for maximizing the polyphenol yield. A sample (a mixture of the enzyme and solvent) is incubated using the enzymatic hydrolysis extraction method at low temperatures (35–50°C) with a pH that has been adjusted. Low-temperature extraction uses less energy to prevent degradation because hydrolysis is halted while deactivating enzymes at a temperature of 80 to 90°C. The EAE is well known for its ability to be ecologically friendly. Water is employed as an organic solvent or as a chemical substitute since the enzyme functions best in an acidic environment. The biggest disadvantage of EAE is the lengthy extraction period (3 hours to 48 hours).

Pressurized liquid extraction (PLE)

High-pressure extraction techniques work well when dealing with polyphenols that are resistant to high temperatures. These techniques also improve polyphenol recovery. Pressurized liquid extraction (PLE) is based on the idea that pressure and boiling point temperature should be inversely related. The solution maintains its liquid condition when the extraction system's pressure is raised before the temperature is raised. The PLE ranges in temperature from 50 to 200 degrees Celsius. However, the solvent and the polyphenols have an impact on the maximum extraction temperature. Numerous researchers have found that PLE's chemical solubility (polyphenols in liquids) has increased.Higher polyphenol concentrations are recovered at higher temperatures. The procedure saves energy since a liquid's sensible heat is lower than the heat required for vaporization. Less heat is required to raise temperature than to create vapor. The main solvents used in PLE are aqueous alcohols and water. Since a large portion of solvents is water, they are cheap, nontoxic, and safe for the environment. The extractor and the setup that goes with it are the most important pieces of extraction equipment.

Combination of modern techniques for effective extraction

PEF and MAE

Pulsed electric field-assisted extraction (PEF) and microwave assistance (MAE) are two modern techniques used to extract biologically active compounds from plant sources. MAE uses water as an extraction solvent, while PEF uses organic solvents like ethanol. Both methods use little energy due to their quick processing times. MAE equipment is less expensive and easier to use than PEF.

MAE and SFE

Biologically active compounds are extracted from plant sources using supercritical fluid extraction technology. The most effective and environmentally friendly extraction methods are supercritical fluid extraction and microwave-assisted extraction. Compared to SFE, MAE operates at a cooler temperature. The SFE methodology requires expensive equipment that poses a safety risk to operational employees and the high-pressure method. Supercritical fluids can be recycled and used again, lowering waste production. Compared to SFE, the choice of extraction solvent for MAE is more specific. The polarity of various targeted chemicals can be used to determine the best extraction solvent.

EAE and MAE

The major focus of enzyme-assisted extraction (EAE) is the use of numerous enzymes that can catalyze processes with excellent specificity and region selectivity. The extraction methods used for enzyme pre-treatment include MAE, UAE, and supercritical fluid extraction. EAE and MAE are the two most recent, powerful extraction technologies. The cost of extraction is decreased by the use of fewer solvents and fewer extraction steps. Current enzyme preparations do not completely hydrolyze plant cell walls, which limits the generation of desired chemicals. Since enzymes are more expensive than solid reagents when processing large amounts of raw materials, microwave-assisted extraction costs less. However, other extraction methods, such as the MAE method, are frequently non-specific and can result in variation.Additionally, it is predicted that microwave-assisted extraction will have significantly higher upfront costs, including the high cost of the ball mill installation.

NPC and MAE

Modern extraction technology that is reliable and environmentally friendly is called negative pressure cavitation (NPC). Cavitation is a term used to describe a naturally occurring fluid mechanics phenomenon that can be further subdivided into hydrodynamic cavitation and auditory cavitation. NPC is a cost- and energy-efficient technology that can maintain appropriate intensities and low temperatures continuously. Negative pressure cavitation and microwave-assisted extraction techniques made it possible to effectively extract various physiologically active compounds from plant sources while requiring less time and energy. Temperature-sensitive chemicals that withstand lower operating temperatures can be efficiently removed using any of these two procedures. In contrast to a traditional organic solvent, water is typically employed as the primary solvent in the MAE process.Temperature-sensitive chemicals that withstand lower operating temperatures can be efficiently removed using any of these two procedures. In contrast to a traditional organic solvent, water is typically employed as the primary solvent in the MAE process. Negative pressure cavitation extraction techniques still call for organic solvents like ethanol, which are expensive and unfriendly to the environment. The NPC extraction technology gadget is generally not developed enough. Some researchers used the NPC apparatus they had developed in the lab. Because

Table 1 Methods of extraction of bio-active components

Method	Solvent	Temperature	Time	The volume of	The polarity of

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				organic solvent consumed	natural products extracted
Maceration	aqueous and non-aqueous solvents	Room temperature	Long	Large	Dependent on the extraction solvent
Percolation	aqueous and non-aqueous solvents	Room temperature, occasionally under heat	Long	Large	Dependent on the extraction solvent
Decoction	Water	Under heat	Moderate	None	Polar compounds
Reflux extraction	aqueous and non-aqueous solvents	Under heat	Moderate	Moderate	Depending on extracting solvent
Soxhlet extraction	Organic solvents	Under heat	Long	Moderate	Depending on extracting solvent
Pressurized liquid extraction*	aqueous and non-aqueous solvents	Under heat	Short	Small	Depending on extracting solvent
Supercritical fluid extraction*	Supercritical fluid (usually S-CO_2) sometimes with a modifier	Near room temperature	Short	None or small	Nonpolar to moderately polar compounds
Ultrasound-assisted extraction	aqueous and non-aqueous solvents	Near room temperature or under heat	Short	Moderate	Depending on extracting solvent
Microwave-assisted extraction	aqueous and non-aqueous solvents	Room temperature	Short	None of Moderate	Depending on extracting solvent
Pulsed electric field extraction	aqueous and non-aqueous solvents	Near room temperature or under heat	Short	Moderate	Depending on extracting solvent
Enzyme assisted extraction	aqueous and non-aqueous solvents	Room temperature, or under heat	Short	Moderate	Depending on extracting solvent
Hydro distillation and steam distillation	Water	Under heat	Long	None	Essential oil (usually on-polar)

*Extraction is done at high pressure

a negative pressure cavitation instrument is expensive, controlling the NPC process is more challenging. The simultaneous expansion of all parameters is not sufficient. Therefore, we should evaluate the requirements for the scale-up.

Isolation techniques[15-18]

The purification and isolation of bioactive compounds from plants have reached new heights in recent years. Chromatographic processes are often used to separate a mixture by passing it through a specified medium in which the components move at different rates. To isolate bioactive compounds, column chromatography techniques are often used. The greatest value for phytochemical separation is found in silica, alumina, cellulose, and polyamide. Plants include phytochemicals, which are bioactive substances derived from plant components such as leaves, barks, seeds, seed coats, flowers, roots, and pulps. Extracts have been demonstrated to be physiologically active in both in vitro and in vivo test systems in certain circumstances. Separation of chemicals is often followed by the assessment of the presence of specific compounds within plant extracts using a range of bioassays. Extraction of plant metabolites is essential for understanding their function in disease prevention and treatment, as well as their harmful consequences. Purification and separation of bioactive chemicals from plants is a technology that has shown considerable advancement, allowing for the simultaneous development and availability of various complex bioassays on the one hand, while also providing accurate isolation, separation, and purification procedures on the other. Animal studies are more costly, require more time, and are prone to ethical problems, so in vitro procedures are frequently used. Plant material selection and collection are essential processes in isolating and characterizing a bioactive phytochemical. Extracts may be prepared using a variety of solvents to separate and purify the active chemicals responsible for the bioactivity. Column chromatography methods may be used to isolate and purify bioactive substances, and the purified chemicals may be identified using a variety of spectroscopic methods. Thin layer chromatography (TLC) is a widely used method for separating mixtures. TLC analysis is a simple, efficient, and low-cost approach that gives the researcher with quick information on the amount of each component contained in the mixture. TLC analysis revealed the concentration of artemisinin in the standard and other root varieties tested. The hairy root extract had a greater content of artemisinin than the control roots, suggesting that stimulating the growth of hairy roots in the leaves of *A. annua* was beneficial for increasing artemisinin production. High-performance liquid chromatography (HPLC) is a widely used technology for isolating secondary metabolites. Table-top

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HPLC devices include a solvent supply pump, an auto-sampler or manual injection valve, a guard column, an analytical column, a detector, and a recorder or printer. HPLC may be used for chemical separations since the elements of the extract migrate at

Table 2 Isolation of bio-active components

Group	Example	Source	Methods
Glycosides	Cardenolide	*Nerium oleander*	IR, UV-Vis, HPLC-UV-Vis, NMR
Flavonoids	Silibinin	*Silybummarianum*	IR, UV-Vis, HPLC-UV-Vis, LC-MS
Proanthocyanidins	A- and B-type	Cranberry, bilberry, curry, cinnamo0n, tea	IR, NMR, FT-ICR-ESI-MS-MS, UPLC-IM-HR-MS
Stillbenoids	Resveratrol	Grape, apple, berries, pistachios, peanuts	HPLC-UV-Vis UPLC-MS-MS,NMR
Tannins	Ellagitannins	Sorghum, apple, grape	HPLC-UV-Vis, NMR, HPLC-ESI-MS-MS
Monoterpeniods	Limonene	*Quercus ilex*	FCG-PTR-MS, NMR
Sesquiterpenoids	Zerumbone	*Zingiberzerumbet*	GC-MS, IR, NMR, ESI-MS
Phenylpropanoids	Coumarins	*Artemisia annua*	HPLC-UV-Vis, LC-ESI-QTOF-MS-MS
Diterpenoids	Columbin	*Jateorhizacolumba*	NMR, LC-MS, UPLC-HR-MS
Tetraterpenoids	α- and β-caretone	Carrot, cantaloupe, tomato	HPLC-UV-Vis, HPLC-ESI-MS-MS, GC-MS
Resins	Stytonikinol A and B	*Styrax benzoin*	IR, UV-Vis, NMR
Lignans	Dibenzylbutane	*Phyllanthusniruri*	HPLC-DAD-ESI-QTOF-MS, NMR
Alkaloids	Triangularine	*Asteraceae family*	HPLC-UV-vis, LC-MS-MS, NMR
Furocoumarines	Bergamottin	*Grapefruit, orange*	UV-vis, UPLC-MS-MS
Naphthodianthrones	Hypericin	*Hypericum, triquetrifolium*	UPLC-ESI-MS-MS
Peptides	Sesquin	*Vignasesquipedalis*	IR, MALDESI-MS-MS
Proteins	Lectins	*Ricinuscommunis*	LC-QTOF-MS-MS

different rates and specific parameters are set. The main approach used for phytochemical separation and identification is an isocratic system, which uses a single mobile phase system. Gradient elution is ideal if the analytes have identical characteristics and retention periods. Purification in HPLC can be accomplished by separating the component of interest from other compounds or interferons. A good HPLC detector must be selected, configured to proper detection settings, and a technique for producing a clean peak on the chromatogram. UV detectors are popular among academics due to their excellent sensitivity. Other detectors, such as the diode array detector (DAD) linked with a mass spectrometer, are used to examine phytochemicals in addition to UV detectors. The current work is intended to extract, isolate, and identify bioactive chemicals from plants using various chromatographic and spectroscopic methods.

Identification, Quantification, and Characterisation of Bio-active Components[19-28]

Spectroscopic approaches have been used since the early 1960s to assess the structure and identify bioactive compounds in plant extracts. The basic idea behind spectroscopic analysis is to pass electromagnetic energy through an organic molecule and measure the amount of radiation absorbed. Spectra produced from three or four regions are used to determine the structure of an organic molecule. Column chromatography, TLC, HPLC, GC-MS, and LC-MS are also used to identify bioactive chemicals in plant extracts. Measurement of bioactive molecules is an important strategy as it can help relate the activity of the bioactive chemical to the quantity. Three primary techniques used to measure bioactive chemicals from plant extract are GC-MS, HPLC, and UV-spectrophotometry. UV-visible spectroscopy is used for qualitative investigation and for identifying certain kinds of compounds in pure and biological mixtures. It is less selective and capable of giving information on the composition of total polyphenol content. Data from spectroscopic techniques such as UV-Visible, Infrared (IR), Nuclear Magnetic Resonance (NMR), and Mass spectroscopy are used to determine the structure of natural goods. The basic principle behind spectroscopy is to send electromagnetic radiation through an organic material that absorbs some but not all of the energy. Scientists often use spectra obtained from three or four areas for structural clarity: ultraviolet (UV), visible, infrared (IR), radio frequency (FTIR), and electron beam. UV-visible spectroscopy is used for qualitative investigation and the identification of certain types of chemicals in pure and biological mixtures. Fourier-transform infrared spectroscopy is a high-resolution analytical tool used to identify chemical and structural constituents. Spectroscopy of Nuclear Magnetic Resonance is used to identify the molecular structure of solids. Mass spectrometry is a powerful analytical technique for discovering novel molecules, measuring existing substances, and deciphering molecular structure and chemical properties. Mass spectrometry is used to identify a material's molecular weight, which is commonly used for organic compound structural elucidation, peptide or oligonucleotide sequencing, and monitoring the presence of previously characterized compounds in complex mixtures with high specificity.

Conclusion

Several notable improvements in natural phytopharmaceutical

separation have been recognized in recent years. A rising number of methodologies based on the hyphenation of chromatographic and spectroscopic or spectrometric techniques have been developed to clarify the structures of known and novel compounds without the need for separation. Although pure compound isolation from difficult matrices like organic matter remains difficult, and we are still a long way from one-step isolation procedures, the use of more selective methods from extraction to fractionation and purification will shorten the time from biological material collection to final purified compound. Aside from the multiple operations performed on the plant, there is a considerable gap in the isolation, identification, and quantification of plant extracts with high antioxidant activity. Mass spectrometry is a powerful analytical technique for discovering novel molecules, measuring existing substances, and deciphering molecular structure and chemical properties. An MS spectrum may be used to identify a material's molecular weight. By defining both the molecular weight and a diagnostic fragment of the molecule at the same time, this method is commonly used for organic compound structural elucidation, peptide or oligonucleotide sequencing, and monitoring the presence of previously characterized compounds in complex mixtures with high specificity.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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