Chapter 9
Natural Plant-Derived Bioactive Compounds as Health Promoters

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Abstract Bioactive compounds in the form of primary and secondary metabolites were isolated and purified from plant products, including fungi, microbes, etc. In the literature, these metabolites are described as having immunobiological properties on organisms that may be used in the food and pharmaceutical industries. Identification, isolation, and finding novel properties of such molecules may be helpful in finding various applications in the fields of cosmetics, materials science, bioremediation, etc. So, these bioactive molecules were extracted and obtained from plant products, and this is the only option for preventing the burden of human diseases. These molecules are one of the ideal sources for drug development, and their concentration is very low, as reported in the literature. Nowadays, various methods are applied to develop some effective method or protocol for extraction along with isolation and characterization of bioactive molecules. Today, conventional methods are being replaced because they are time-consuming, and these are mainly through green solvents (ionic liquids, eutectic solvents, etc.) and nonconventional techniques (electric fields, microwaves, etc.). All these methods were characterized and optimized for their strategies, which mainly boost the commercial values of agrowastes along with organic residues, promoting a sustainable circular economy. Inspite of the development of microfluidics, nanoencapsulation, and metabolic engineering, which may be able to help improve the screening process along with the extraction, stability, and functionality of compounds. In this chapter, we focused on extraction and characterization methods of bioactive compounds and discuss about its promising health beneficial attributes of bioactive components.

Keywords Bioactive · Medicinal plant · Extraction · Isolation · Characterization

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9.1 Introduction

Extraction is one of the major methods for the extraction of molecules from medicinal plant products. It is crucial to isolate and extract the desired chemical component, which is available in crude form in plant material. Further standardization and characterization are required for getting the pure molecule and evaluating its biological properties. In this method, various steps were followed, i.e. prewashing, preparation of a homogenous sample from dried plant material, solvent system, etc. Proper precautions must be taken so as not to lose active constituents during plant extract preparation. The most important criteria for the selection of plant material are based on traditional uses. In addition, the selection of the solvent system (polar or nonpolar solvents) is also important and required for targeting the bioactive compounds (hydrophilic or lipophilic) [1, 2]. In hydrophilic extraction, it is preferable to use polar solvents like methanol, ethanol, ethyl acetate, etc., whereas in the case of lipophilic compounds, it is preferable to use dichloromethane or a mixture of dichloromethane and methanol in a ratio of 1:1. In contrast, hexane is also used to remove chlorophyll content. Depending on the suitability of the solvent system, we can easily target that compound in polar or nonpolar form using a suitable extraction method. In simple words, extraction efficiency is totally dependent on particle size because of the enhancement in solvent penetration and properly diffusing the solutes. If someone is working on fine particle size, in this case, we consume maximum absorption of solute in solids and face difficulty in subsequent filtration. If samples were exposed to high temperatures, which may ultimately increase the solubility rate, but somehow lost the extracts and obtained undesirable impurities, extraction efficiency is totally dependent on time duration, and it may increase within a certain time range. In contrast, if we enhance the time duration during extraction, it will not affect the sample at all until solute equilibrium is reached inside and outside the solid material. The extraction methods [3, 4] are totally dependent on two factors:

- Conventional methods (maceration, percolation, and decoction) preferably use organic solvents and require a large volume of solvent and a long extraction time. The simplest method is maceration, which is mainly applied to thermolabile components. This is a very simple extraction method, and its major disadvantage is its long extraction time and extremely low efficiency (Table 9.1).
- Some modern methods, such as supercritical fluid extraction, pressurized liquid extraction, and microwave-assisted extraction, have also been applied in natural product extraction, and they offer some advantages such as lower or minimized consumption of organic solvent, shorter or reduced extraction times, and higher selectivity. These modern methods have some advantages over conventional methods where they meet the concept of green processes. In short, these technologies are energy-efficient alternatives and produce maximum yield with less extraction time.

Table 9.1 Extraction methods for natural products

Types of method	Required solvent	Temperature	Time	Pressure	Consumption of organic solvent	Extraction of polar natural products
Maceration	Aqueous (water) and nonaqueous solvent	Room temperature	Extensive	Atmospheric	Huge	Dependent on extracting solvent
Percolation	Aqueous (water) and nonaqueous solvent	Occasionally under heat and normally, kept at room temperature	Extensive	Atmospheric	Huge	Dependent on extracting solvent
Decoction	Water	Under heat	Average	Atmospheric	None	Polar compounds

9.2 Plants' Major Types of Bioactive Compounds

9.2.1 Flavonoids

Flavonoids are the most abundant and diversified class of bioactive molecules, frequently referred to as phytonutrients or phytochemicals, and are the primary elements of polyphenols. They have a chemical structure that includes a double-bonded diphenylpropane structure characterized by two benzene rings (rings A and B) that are linked by a three carbon chain, which forms a closed pyran ring (heterocyclic ring with oxygen, the C ring) with the benzene A ring. Flavonoids are naturally found as glycosylated or esterified conjugates, although they can also exist as aglycones, particularly as the outcome of food. Flavonoids are found in all fruits and vegetables and, combined with carotenoids, are responsible for their distinct hues. Apples, oranges, carrots, onions, tomatoes, the latter, parsley, lemon, beans, and other fruits and vegetables are high in flavonoids. Flavonoids are compounds found in various plants that have been demonstrated to have beneficial effects on human health. These effects are mainly due to their antioxidant and anti-inflammatory properties, which help combat the damaging effects of free radicals on cells and tissues. Flavonoids have also been shown to possess anti-ageing and anti-carcinogenic properties. They can positively impact the nervous system and regulate the activities of certain enzymes and receptors. Proanthocyanidins, which are oligomers of flavonoids, have a similar structure and effects. Both flavonoids and proanthocyanidins are pigments that occur naturally in various plants [5, 6].

9.2.2 Terpenoids

Terpenoids represent a significant cluster of natural products, mainly coming from plants but also produced by other organisms like bacteria and yeast in primary or secondary metabolism. They are made up of two five-carbon units called isoprenoids. Terpenoids offer therapeutic benefits in the management of various types of diseases, including cancer, as well as displaying antimicrobial, antihyperglycaemic, and anti-inflammatory properties. Moreover, they have insecticidal properties and are anti-allergenic, antispasmodic, antiviral, and immune modulators. Monoterpenoids have two isoprene units, while sesquiterpenoids have three. They are considered lipophilic in nature and have a high volatilization rate. They have intense smells and tastes. Their activities vary significantly, and a variety of them have been used in herbal medicines. Alzheimer's patients have high acetylcholinesterase activity, which is decreased by some bicyclic monoterpenoids. Diterpenoids are made up of 4 isoprene units (each with 20 carbons). They are extremely lipophilic and have intense tastes, but they are not volatile and hence odourless. Diterpenoids are also often found in resins. The resins are complicated, lipid-soluble combinations that often contain both nonvolatile and volatile chemicals. Resins released by wood are the most common; however, resins are also found in herbaceous plants. All of them are sticky, and how fluid they are depending on how many volatile compounds they contain. They get harder when exposed to the air. The majority of resins have antibacterial and wound-healing properties. α-terpineol contains insecticidal and skin penetration enhancing effects. Sesquiterpenes have antiallergic and anti-inflammatory qualities [7, 8].

9.2.3 Alkaloids

Alkaloids contain nitrogen, which causes the alkalinity of the compound. They are bitter in taste and heterocyclic in nature. These substances are often generated by a wide variety of plant species, mostly blooming plants and certain animals. Like inorganic alkalis, alkaloids react with acids to produce salts. In acid-base methods, these nitrogen atoms have the ability to serve as bases. In their purest form, alkaloids are typically odourless, pigmentless crystalline solids; however, occasionally they can also be yellowish liquids. It demonstrated a wide range of therapeutic qualities. Although several of them have local anaesthetic characteristics, their usefulness for therapeutic purposes is constrained. Although morphine is a potent opioid used to treat pain, its utility is constrained by its propensity for addiction. Quinine is an effective antimalarial drug. The human diet includes several alkaloids in both food and beverage forms. Alkaloids are compounds found in plants that are consumed by humans, including tomatoes, potatoes, cacao seeds, coffee seeds, and tea leaves. Alkaloids can directly affect the human brain or stimulate human organs like the central nervous system. Nicotine is a powerful stimulant and an extremely addictive chemical that is derived from the tobacco plant (*Nicotiana tabacum*). The antiparasitic, antiplasmodial, anticorrosive, antioxidative, antibacterial, anti-HIV, and insecticidal actions of alkaloids are only a few of their numerous beneficial uses [9, 10].

9.2.4 Betalains

These are red and yellow in colour and contain nitrogen. The reddish-violet betacyanins and the yellow betaxanthins are two different types of indole-derived pigments known as betalains. The hues of these pigments are determined by the resonance of double bonds in the betalain structure. They can be included in an aqueous food system since they are water-soluble. Several cacti, pear, red and yellow beetroot, and amaranth are food sources of betalains. The health benefits of betalains are cumulative due to their antioxidant, anticancer, anti-lipidemic, and antibacterial properties. They are harmless when consumed, making them potentially useful as functional foods and a prospective replacement for supplemental medicines in diseases like cancer and hypertension that are examples of oxidative stress, inflammation, and dyslipidemia-related diseases [11].

9.2.5 Glucosinolates

The glucosinolates include sulphur-rich aglycones generated from amino acids. Certain pungent substances and their secondary metabolites, which include sulphur and nitrogen, naturally contain substances called glucosinolates. Sulphur and nitrogen are found in glucosinolates. They are produced from glucose and an amino acid. Cruciferous plants, such as wasabi, broccoli, cabbage, and kale, are the major sources of glucosinolate. The most physiologically active breakdown products of glucosinolates are isothiocyanates. Numerous glucosinolates and their physiologically active by-products, in particular the isothiocyanates, have been demonstrated in studies to have defensive properties against cancer and dementia and are well recognized for killing a variety of cancer cells without harming healthy cells. They lessen the chance of developing dementia and delay the elderly's rate of cognitive deterioration [12].

9.3 Bioactive Components and Epigenetic Modifications

In the literature, bioactive components used as nutrients in food may directly impact human health and also show effectiveness against intracellular pathogens. These bioactive compounds directly target the DNA expression in a cell or tissue and modify the genome, which indicates enhancing or declining the fabrication of specific proteins in a cell. So, these reorganizations were reported in DNA or histones and considered epigenetic marks, and the phenomenon is called epigenomics. In short, epigenetic modifications occur in chromatin without any effect on the nucleotide sequence. These changes modified the pattern of gene expression, but this expression may be progressive and somehow reversible. These epigenetic marks are transmitted from cell to cell or from one generation to another as cell division occurs. According to the literature, these epigenetic marks are totally dependent on two factors, i.e., DNA methylation and histone modification [13, 14]. In DNA methylation, proteins were chemically tagged with methyl groups of DNA bases, which make DNA more or less accessible to transcriptional apparatus and change the expression pattern of specific genes. Similarly, modifications in histones are also reported because DNA is always intact and shows its modifications chemically in histones. So, DNA is wrapped around histones, which directly affect the structure of DNA and also have an effect on proteins with reference to transcriptional activity.

- **Folates** (a water-soluble vitamin, foliate, and folic acid) are directly obtained from food and its supplements. The most familiar examples are lemons, oranges, and tomato juice, mushrooms, yeast, bananas, spinach, etc. In general, folate is available on the market in the form of capsules that are prevalent and enhance folate levels in the blood; more importantly, the use of these capsules in pregnant women has been linked to neural tube effects. This folate is mainly involved in the synthesis of DNA, including its repair, along with its methylation. During dietary digestion, folate undergoes a series of reactions, first being converted into tetrahydrofolate (remethylation of homocysteine to methionine, precursor of S-adenosyl-L-methionine). Thereafter, methyl group transfer occurred and converted S-adenosyl-L-methionine into S-adenosyl-L-homocysteine (SAH), an inhibitor of the methylation reactions [15]. This chemical reaction is having some significant impacts, especially in those patients with folate deficiency, and changes are occurring in specific proto-oncogenes because of DNA methylation patterns. In short, the recommendation of the uptake of folate capsules in the diet must be scrutinized and considered as one of the most active bioactive compounds pertaining to reducing the abnormal proto-oncogene expression in cellular events.
- **Vitamin A** (a fat-soluble vitamin; retinoids) is reported in green vegetables, fish, meat, etc. This vitamin plays an important role in cell growth, differentiation, reproduction, vision, and immune function. In addition, this vitamin is metabolized intracellularly in the form of retinal and retinoic acid (the active metabolite of vitamin A) and supports several physiological functions. In general, when this bioactive compound in the form of a vitamin is absorbed and migrated to the nucleus, it binds to receptors (nuclear retinoic acid receptors, RARs) and is then characterized in the form of RAR (α, β, and γ) which heterodimerize with retinoid X receptors (RXRs) [16]. Due to this complex, which ultimately binds with peculiar elements and declines at the gene level, our interest at the transcriptional level with reference to biological and pharmacological responses has declined in the case of disease conditions.
- **Vitamin D3** (cholecalciferol) is reported in eggs, fish, milk, etc. and, most importantly, is directly obtained from sun exposure. Contrasting appearances of vitamin D are available, e.g., in humans, the existence of two vitamins is reported, i.e., vitamin D2 (reported in plants) and vitamin D3 (reported in human skin when exposed to sunlight). In the literature, this vitamin D3 played an important role in calcium homeostasis and then converted vitamin D3 into calcitriol (the active form), which showed its importance as a bioactive component in human nutrition [17].

9.4 Promising Health Beneficial Attributes of Bioactive Components

Bioactive components were isolated, characterized, and purified from plant products using organic solvents, and they are called secondary metabolites. These metabolites have promising therapeutic applications, i.e., antioxidant properties. In the literature, phenolics from plant products may be considered one of the phytochemical or bioactive compounds that may help to maintain better human health. So, these phenolics, which are more commonly reported in fruits and vegetables (orange and yellow-coloured), may contain enough lipophilic molecules called carotenoids. These carotenoids are widely applied and used for various industrial purposes, especially food in the form of pigments, and promoted as health dietary agents. One of the most familiar examples is related to protection against cardiovascular diseases, sunburn, cataracts, etc. due to the usage of these bioactive compounds like zeaxanthin, β-cryptoxanthin, and lutein. In addition, carotenoids are gaining more interest because of their strong antioxidant properties, which may be helpful in reducing the burden of disease. Similarly, polyphenols are reported as natural antioxidants that are mainly derived from fruits, cereals, vegetables, spices, etc. Several classes of polyphenols (phenolic acids, flavonoids, and anthocyanins) are also reported, which show several immunobiological properties [18, 19].

In the literature, these phenolic components from plant products are called free radical inhibitory or antioxidant agents because of their ability to release electrons or hydrogen atoms. A large number of antioxidative compounds were reported from fruit and vegetables, which may be helpful in reducing the cases of disorders, especially in the heart, cancer, arthritis, etc. Similarly, bioactive compounds in the form of peptides were reported in wheat, cereals, and rice, which may show antihypertensive activity [18–20]. According to the literature, several plant-derived bioactive compounds have some biological properties, i.e., antidiabetic and anticancer activity. Some of the most common health-promoting attributes of bioactive components are:

Anticancer Medicinal plant-derived drugs play a paramount role in pharmaceutical or human health care. In nature, several prophylactic or therapeutic compounds with chemical diversity were reported in several species of plants, animals, and microorganisms. In the literature, plant-derived candidates were reported and claimed to have anticancer activity, but most of them are still in clinical trials. To date, researchers working on structural modifications of compounds derived from plants have shown chemical diversity, which may further improve bioactive molecules, which is helpful for the process of drug development. Numerous studies were conducted related to anticancer in order to identify some novel phytochemical or bioactive compounds from plant products. So, these bioactive molecules, alone or in combination with other drugs, showed additive or synergistic effects, giving some options or an ideal candidate against cancer therapies. The most familiar examples of phytochemicals, especially anthraquinone, reported in plants, e.g., *aloe vera*, have anticancer potential. *Aloe vera* leaves contain bioactive molecules, i.e., aloe-emodin and emodin, derivatives of anthraquinone, which have antiproliferative effects in cancer model studies. In addition, emodin showed effective results with reference to declining androgen receptors and prostate cancer growth. Both derivatives of anthraquinone that arrest cell cycle analysis (enhancement in p53 expression and upregulation of p21) played an important role in the induction of apoptosis and disrupted the membrane potential of mitochondria, cytochrome c release, and caspase activation [20–22].

Today, cancer is one of the serious health concerns that are reported in both developing and developed countries. This disease may be due to unwanted cell growth and irregular cell division. In this regard, medicinal plants containing bioactive molecules have been used by villagers since ancient times to treat cancer. The most familiar examples of phytochemicals from plants reported as having anticancer activity are curcumin, vinblastine, vincristine, camptothecin, etc. In contrast, terpenoids and flavonoids also showed promising results against cancer.

Antidiabetes One of the chronic diseases, diabetes mellitus, is one of the metabolic disorders seen in the pancreas, which is mainly seen through β-cells that have a condition called hyperglycaemia. This condition may arise due to a deficiency or decline in the level of insulin production by the pancreas, and several medications are required to control and lower the blood glucose level to a normal level. However, several drugs were available, but they showed several side effects and caused several serious consequences. In this regard, traditional methods were adopted using bioactive elements from plants and played an important role as alternative medicine. In the literature, bioactive compounds from plants were reported as antidiabetic agents and showed more promising activity in terms of their safety and efficacy. The most familiar example is seen in the case of *Momordica charantia*, i.e., bitter melon, which may have contained a number of bioactive compounds like momordin, momordicosides, polypeptide-p, saponins, etc., which are totally similar to insulin-like proteins and are responsible for declining blood glucose levels. These compounds were reported in the callus, seeds, and fruits of *Momordica charantia*, which are totally similar to human insulin and showed an antidiabetic effect in animal model studies. In addition, bitter melon may have enhanced the tolerance rate of glucose levels in diabetic mice and has also been reported in humans. Similarly, ginseng (family *Araliaceae*), a traditional plant, is reported as an antidiabetic plant because of a specific type of saponin called ginsenosides. In the literature, ginsenoside Rb2 was more effective in decreasing blood glucose levels, and these studies were conducted in streptozotocin-induced diabetic rats. The roots of this plant also showed antidiabetic properties because of bioactive compounds like ginsenosides (triterpene glycosides or saponins), panaxans, vanillic acid, salicylates, etc. All parts of the ginseng plant contained alkaloids, polypeptides, vitamins, proteins, phenols, etc. Moreover, ginseng is also available in red form, i.e., as red ginseng extracts, where ginsenoside (Rg2 and Rg3) concentrations are still higher than normal ginseng. This red ginseng significantly reduced blood glucose levels and enhanced the level of plasma insulin in streptozotocin-induced diabetic rats [22–

24]. Another antidiabetic plant like *Tinospora cordifolia* (family *Menispermaceae*) commonly known as Guduchi, reported polysaccharide content which is directly correlated with β-cell regenerative properties and claimed its antidiabetic medicine having some side effects. This activity is mainly due to its existence of glycosides, terpenoids, flavonoids, alkaloids, phenolic constituents, polysaccharides, etc.

Gut Health Food containing diverse varieties of bioactive components and bioactivators is reported in fruits, including phytochemicals. Diverse varieties of bioactive compounds were reported in different types of fruits, i.e., seed, peel, and fruit. Their concentrations of bioactive molecules may have varied within the same fruit due to geographical locations. All these variations are mainly due to some factors that are directly influenced by nutritional food components, i.e., environmental conditions, ripening stage, season, soil texture, etc. In the literature, fruit is rich in polyphenols, one of the largest groups of bioactive compounds, and their structure may have totally varied from one fruit to another. Most of the fruits claimed their antioxidant and anti-inflammatory potential and showcased their importance with reference to human health care. Several studies related to preclinical and clinical research were conducted related to the isolation and purification of bioactive compounds from fruits and showed their activity against human health disorders of digestive, reproductive, and cardiovascular disease. In short, these bioactive compounds from fruits may upregulate their antioxidant and anti-inflammatory potential to mitigate health ailments [25, 26].

Antithrombotic Bioactive substances have an impact on human health on a biological level. An example of this is an antithrombotic, which helps alleviate excessive bleeding. Platelet aggregation, which occurs when blood clots begin to develop as a result of platelets beginning to accumulate together in the circulation, may be prevented by polyphenols by slowing down the platelet aggregation process [24–27].

Manage Blood Pressure Bioactive chemicals found in a variety of fruits, vegetables, tea, and mineral water, according to epidemiological research, may help prevent high blood pressure. The inclusion of a variety of foods high in flavonoids in the diet may be a useful strategy for lowering blood pressure [26–28].

Anti-inflammatory Inflammation is a biological reaction to an infection, harm, or discomfort. Chronic inflammatory conditions, including arthritis, allergies, atherosclerosis, and possibly cancer, appear to be linked to one another. Nitric oxide (NO) is one of the main mediators of inflammation. The overproduction of NO is inhibited by bioactive elements such as polyphenols, bioactive peptides, etc. Consequently, bioactive substances can reduce inflammation [22–29].

Antioxidative The majority of the antioxidant properties of foods and plants are contributed by bioactive substances like polyphenols and carotenoids. Carotenoids' pigment has the capacity to function as antioxidants and thereby shielding cells from photooxidation. Additionally, research has been done on how carotenoids react with radical species, and they are well-known for their ability to quench singlet oxygen [22–30].

Eye Health Eye illnesses may be prevented or delayed in their course by bioactive substances found in plants and diets. According to recent research, consuming at least 6 milligrams of lutein (carotenoids) daily can reduce the prevalence of macular degeneration by 43%. Increasing lutein and zeaxanthin in the diet can also help decrease or stop present eye damage and prevent current issues from getting worse [30, 31].

Cardiovascular Disease Anthocyanins, polyphenols, flavonoids, and other plant-based bioactive chemicals may play a significant role in declining the rate of cardiovascular disease, according to epidemiological research. The likelihood of cardiovascular disease is reduced when people include foods with high anthocyanin and flavonoid content in their diet. The development of atherosclerosis and CVD is significantly influenced by oxidative stress and inflammation. The preventive effects of many bioactive substances against atherosclerosis and CVD may be related to their anti-inflammatory, antioxidative, and metabolic capabilities. It has been demonstrated in several studies that anthocyanin-rich berries are linked to improved heart health, substantial reductions in glucose metabolism, lipid peroxidation, LDL oxidation, dyslipidaemia, and total plasma antioxidant capacity. According to a number of cohort studies and randomized trials, flavonoids may reduce the incidence of CVD in part through improving LDL cholesterol levels, endothelial function, and sensitivity to insulin. Intake of six types of flavonoids, including anthocyanidins, proanthocyanidins, flavonols, flavanones, flavones, and flavan-3-ols, has also been linked to significant reductions in the risk of CVD [29–33].

Anticarcinogenic Numerous epidemiological studies that initially discovered a consistent link between a high intake of fruits and vegetables and a decreased risk of acquiring a variety of illnesses, including various types of cancer, have since revealed the health-promoting effects of bioactive compounds. These studies also discovered that bioactive compounds may inhibit the growth and progression of various cancer cells. Rich in phenolic compounds and with strong antioxidant qualities, eggplant might be useful in detoxifying free radicals. As a result, eggplants could potentially be used as a preventative food to lower the risk of cancer. Plant derivatives like polyphenols can restore adverse or unhealthy epigenetic alterations in cancer cells, obstruct carcinogenesis, stop the spread of metastatic cancer, or make tumour cells more sensitive to radiation and chemotherapy. The NF-κB pathway, which is known to control cell transformation, angiogenesis, cell proliferation, inflammation, invasion/metastasis, and survivability of cancer cells, is said to be inhibited by flavonoids found in fruits and vegetables. In addition, it has been demonstrated that the carotenoids and terpenoids found in plants have anti-inflammatory and anticancer properties. They hinder NF-κB signalling pathways, which play a crucial role in tumours and inflammatory diseases [31–34].

9.5 Characterization of Bioactive Molecules

Bioactive chemicals are compounds derived from natural sources that are not often found in food and are typically present in modest amounts. They have been recognized as essential elements associated with healthy living and illness prevention. These chemicals are the subject of significant research to determine their influence on health. They possess the power to influence one or more metabolic pathways, leading to improved health outcomes. Plants are the main source of bioactive substances. Natural bioactive compounds, commonly referred to as plant secondary metabolites, are vital for species competition, protection, attraction, and signalling but are not absolutely necessary for plant life. These substances are essential to animal life and hold considerable promise for improving human health, especially when it comes to their pharmacological or toxicological impacts on microbial infections and illnesses. Additionally, they have medicinal antioxidant qualities. Noncommunicable illnesses are spreading more widely as the population ages and becomes less active. As a result, consumers now have a greater need for natural goods as they look for long-term solutions to enhance their quality of life through customized nutrition. The necessity of a balanced intake is stressed since the quantity of bioactive compounds from plants consumed is frequently crucial in deciding whether the effect is beneficial or detrimental. These substances have a variety of effects, ranging from being very damaging to being beneficial or healing. In this regard, we explore characterization procedures for extracting bioactive compounds from plant products [4–8] (Fig. 9.1).

Nuclear Magnetic Resonance Spectroscopy (NMR Spectroscopy) It is a type of spectroscopy that relies on how atom nuclei absorb radiation from the electromagnetic spectrum in the range of 4 to 900 MHz. For figuring out the structure of bioactive (organic) molecules, NMR has emerged as the method of choice over the

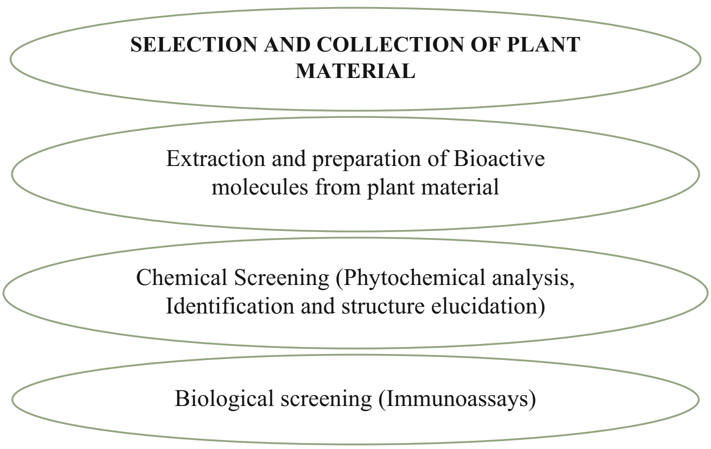

Fig. 9.1 Process of bioactive compounds from plant products

past 50 years. It is the only spectroscopic technique for which thorough investigation of the sample and deciphering of the full spectrum are often anticipated. Numerous nuclei have spin, and all nuclei have electrical charges, according to the basic principle of NMR. The base energy can move to a higher energy level (often a single energy gap) when an external magnetic field is supplied. Once the spin rebounds to its base level, energy is emitted at the exact same frequency that was used for the energy transfer, which occurs at a wavelength that is equivalent to radiofrequencies. To produce an NMR spectrum for the target nucleus, the signal that corresponds to this transfer is processed and measured in a variety of ways. The bioactive metabolite is put in a magnetic field, and the NMR signal is generated by radio waves excitation of the sample's nuclei, which is detected by sensitive radio receivers. A molecule's atom's intramolecular magnetic field can alter the resonance frequency, providing information about the molecule's electronic structure and its many functional groups. Proton and carbon-13 NMR spectroscopy are the two most commonly used forms of NMR; however, they may be used with any material that has nuclei with spin. The study of the manner in which electromagnetic radiation interacts with matter is known as spectroscopy. NMR spectroscopy uses NMR phenomena to investigate the material's physicochemical and biological characteristics [35, 36].

Mass Spectrometry One of the methods related to analytical chemistry for determining the bioactive compounds present is the mass-to-charge ratio and abundance of gas-phase ions. This technique involves bombarding the bioactive molecule (sample) with electrons; the bioactive molecules are mainly transformed into rapidly moving positive ions, including minor fragments, when they pass through a detector after moving through the electric and magnetic fields; and the charged particles are sorted according to their masses. Hence, they are detected, and the signals are recorded. A relative abundance in the form of a graph plotted against the mass/charge ratio (m/e) is called a mass spectrum. The masses of particles and molecules in a sample, their elemental or isotopic signatures, and the chemical structures of molecules and other chemical compounds may all be inferred from these spectra. A sample, which might be solid, liquid, or gas, is often ionized during a technique, such as by being bombarded with electrons. Some molecules in the sample might break into charged pieces as a result. Then, by accelerating them and exposing them to an electric or magnetic field, these ions are sorted based on their mass-to-charge ratio. Ions with the same mass-to-charge ratio will deflect to the same degree. A system that can detect charged particles, such as an electron multiplier, is used to find the ions [37, 38].

9.6 Chromatography Techniques

9.6.1 Thin-Layer Chromatography (TLC)

This technique conveniently delivers qualitative information, and quantitative data may be obtained by paying close attention to the little details. To separate and distinguish between different chemicals of interest, scientists use thin-layer chromatography [39]. A thin coating of silica is fixed to glass or aluminium to provide support in the construction of a TLC plate. The mobile phase is the solvent mixture, while the stationary phase is the silica gel. The compound of interest is, to varying degrees, soluble in the ideal solvent solution. The partition equilibrium of the mixture's constituent parts leads to separation. The simplest use of the method involves placing a small portion of the sample combination to be separated in a narrow zone or area towards one end of the TLC plate and then letting it dry. Making sure that the sample area is not submerged in the solvent, the strip or plate is next inserted into the solvent mixture, with this end sinking in. The test mixture divides into distinct components as the solvent goes towards the opposite end of the strip. This is called the development of TLC plates. Several things affect the separation.

- Solubility: A substance will travel along the plate more quickly; the more soluble it is in a solvent.
- Attractions between the elements and the silica; the compound travels less when it interacts with the silica.
- Compound size; bigger compounds travel along the plate more slowly.

9.6.2 HPLC (High-Performance Liquid Chromatography) and HPTLC (High-Performance Liquid Chromatography)

This is an analytical method that is applied for the separation and estimation of bioactive molecules from plant material. Utilizing the fundamentals or basic techniques of column chromatography, where bioactive compounds are easily separated, and using the spectroscopy method in order to identify and quantify them. The development of column chromatography from low-pressure compatible glass columns to high-pressure compatible metal columns occurred in the 1960s. In simple terms, HPLC is a greatly enhanced variation of column liquid chromatography. A solvent is pushed through a column at high pressures of up to 400 atmospheres rather than being permitted to slide through it under gravity. In a separation column with a stationary phase (granular substance with very tiny porous particles) and a mobile phase (solvent or solvent mix), it may proceed with the purification processes. The mobile phase is pushed along the separation column under high pressure. The sample is in the form of a bioactive metabolite, which may be delivered into the mobile phase flow from the pump to the separation column through a valve with a linked sample loop, such as a tiny tube or a stainless steel capillary [40]. So, due to interactions with the stationary phase, components of several bioactive molecules are maintained at variable degrees and then migrate at various speeds across the column. Following its exit from the column, each chemical or bioactive molecule is identified by an appropriate detector, which sends a signal to the computer's HPLC programme. A chromatogram is produced in the computer's HPLC software at the conclusion of this procedure or run. The various compounds may be recognized and measured using the chromatogram. An excellent substitute for high-performance liquid chromatography (HPLC) and gas chromatography (GC), the HPTLC technique, is an automated, high-tech variation of thin-layer chromatography with improved and enhanced separation efficiency and detection limits. High-performance thin-layer chromatography is often referred to as planar chromatography or flat-bed chromatography. High-performance thin-layer chromatography (HPTLC), an advancement of thin-layer chromatography (TLC), is a reliable, easy-to-use, quick, and effective technique for quantitative analysis of substances [41]. An analytical method termed HPTLC is based on traditional liquid chromatography (TLC), but it has been modified to allow for quantitative evaluation of the compounds and to improve the separation of the compounds' resolution. Some of the modifications allow for elevated resolution, such as the use of higher-quality TLC plates with smaller particles in the stationary phase. Repeated plate development utilizing a multiple development device can further enhance the separation. As a result, HPTLC provides a higher limit of detection (LOD) and improved resolution. Similar to the manner in which TLC separates matter by adsorption, HPTLC operates on the same principles. Through capillary action, the solvent or mobile phase flows. The adsorbent (stationary phase) is approached by the analytes with affinities that vary. Moving closer to the stationary phase is the component with stronger affinity. Towards the stationary phase, a low-affinity component moves quickly. So, the components are separated on a chromatographic plate.

9.6.3 Non-chromatographic Techniques

Phytochemical Screening Assay The word "phytochemicals", which refers to substances obtained from plants, is frequently used to refer to the numerous secondary metabolic products that are present in plants. A crucial tool in bioactive component analysis, the phytochemical screening assay, is a rapid, easy, and affordable process that provides the researcher with a quick answer regarding the different kinds of phytochemicals present in a combination. Following the extraction of the crude extract or active fraction from the plant material, phytochemical screening may be carried out using the proper assays to determine the types of phytochemicals present in the extract combination or fraction [42].

Immunoassay Analyses of bioactive substances are increasingly using immunoassays, which employ monoclonal antibodies against drugs as well as low molecular-weight natural bioactive molecules. For receptor-binding inquiries, enzyme tests, and analytical procedures, they exhibit great specificity and sensitivity. MAb-based enzyme-linked immunosorbent assays (ELISA) are frequently more sensitive than traditional HPLC techniques. Hybridoma technology is a process for producing monoclonal antibodies in specialized cells [43].

Fourier-Transform Infrared Spectroscopy (FTIR) For the characterization and identification of chemicals or functional groups (chemical bonds) found in an unknown mix of plants, FTIR has proven to be an invaluable instrument. A chemical "fingerprint" may be made from the FTIR spectra of pure substances since they are often so distinctive. By comparing an unknown compound's spectrum to a collection of known compounds, it is possible to determine the spectrum of the majority of common plant chemicals [37, 38]. There are several techniques to prepare samples for FTIR. The simplest method for liquid samples is to put one drop of the sample between two sodium chloride plates. A thin layer is created between the plates by the drop. An alternative is to dissolve solid samples in a solvent, such as methylene chloride, and then pour the resulting solution onto a single salt plate. A thin coating of the original substance is then left on the plate once the solvent has evaporated.

9.7 Future Prospects

Bioactive substances function as health promoters and are present in food, animal products, and nature. In vitro and in vivo are common laboratory techniques that are often used in order to demonstrate a bioactive substance. Bioactive compounds are the secondary metabolites produced by plants. They produce food that improves health by changing metabolic processes. Depending on the compound type, quantity, or bioavailability of the compound, the effects could be beneficial or detrimental. These substances have a variety of impacts, ranging from beneficial health maintenance to therapeutic benefits to being harmful or even lethal. The quantity of bioactive substances consumed frequently determines whether the impact is beneficial or harmful. They exhibit advantageous properties like antioxidative behaviour, antimicrobial impact, and organic pigmentation, among others. Recently, some indications have emerged regarding the involvement of specific bioactive substances in the postponement of illnesses like cancer and cardiovascular diseases. Although dietary nutrients are indispensable for survival, the necessity of bioactive compounds has not been established since the body can operate without them or their functions are concealed by nutrients that perform the same task. Numerous plant components that have the potential to have positive impacts on health are included in bioactive compounds. They are present in trace amounts and typically have pharmacological effects. These bioactive substances may have negative health impacts or positive health effects, depending on the dose. These effects have already been researched and studied using animal models and cell and tissue cultures. But in the present situation, an extensive amount of epidemiological evidence indicates that bioactive substances provide a wide range of health benefits for people, including the ability to prevent cancer, improve eye health, reduce the risk of cardiovascular disease, control blood pressure, and more.

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