J. Pharm. Res. 2025;24(2):64–77

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

REVIEW ARTICLE

Phytomedicine: A Promising Solution for Advancing Phytotherapy Applications - A Comprehensive Review

S Anusree Gangadharan[1], T Daniel Thangadurai[1,*], G Kausalya Sasikumar[1], Putrakumar Balla[2]

[1] *Centre for Nanoscience and Technology, KPR Institute of Engineering and Technology, Coimbatore, 641407, Tamil Nadu, India*
[2] *Department of Chemical Engineering and Applied Chemistry, Chungnam National University, Daejeon, 34134, South Korea*

ARTICLE INFO

Article history:
Received 03.02.2025
Accepted 17.03.2025
Published 18.04.2025

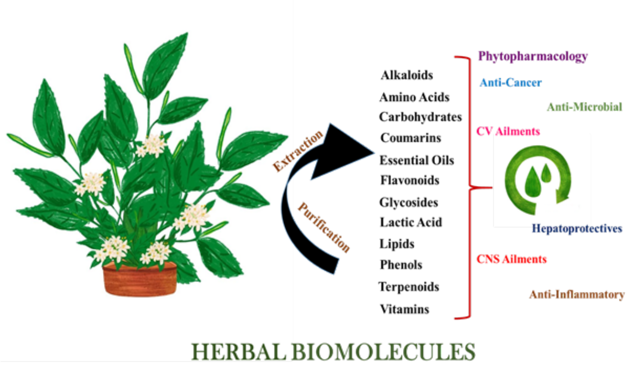
* *Corresponding author.*
T Daniel Thangadurai
danielthangadurai.t@kpriet.ac.in

https://doi.org/10.18579/jopcr/v24.i2.20

ABSTRACT

Over the last few decades, there has been a surge in the popularity of herbal medicine due to extensive research in traditional medicine. These organic bioactive compounds or agents include alkaloids, glycosides, coumarins, flavonoids, terpenoids, carbohydrates, and essential oils derived from plant sources that exhibit various biological functions. The phytomolecules obtained from herbal resources are widely used to treat multiple diseases and used as antioxidants, anticancer agents, agents for cardiovascular and central nervous system ailments, antidiabetic agents, antimicrobials, and so on. This review article offers a comprehensive overview of phytomolecules and their potential for healthcare. It explores the traditional use of medicinal plants and herbs for treating various ailments and the scientific rationale for their use as nutraceuticals. The article also presents the classification of herbal biomolecules, and the methods used for their extraction, purification, and characterization.

Graphical Abstract



Keywords: Herbal medicine; Phytomedicine; Bioactive compounds; Nutraceuticals; Chronic diseases

INTRODUCTION

In recent decades, there has been a growing interest in the field of phytomedicine due to its potential in advancing healthcare monitoring. Phytomedicine, also known as herbal medicine, is the use of plant-based products for medicinal purposes. The use of plants for medicinal purposes dates back to ancient times, and it continues to be widely used in many cultures around the world. Plants play a crucial role in the field of medicine and the maintenance of global well-being. Medicinal plants and herbs, traditionally believed to have medical or therapeutic benefits, have greatly influenced the medical industry worldwide. They are utilized to ensure well-being and optimal circumstances, as well as to treat various disorders. The significance of medicinal plants in the healthcare sector cannot be overstated, as they offer a natural, safe, and cost-effective alternative to synthetic drugs. Their integration into mainstream healthcare systems could potentially have far-reaching implications[1].

Research in phytomedicine has led to the development of several plant-based remedies that are effective in treating various ailments. The utilization of various plant components, including leaves, flowers, stems, seeds, buds, sprouts, and fruits, has been a traditional practice for treating various ailments. The use of plant-derived medicines or phytotherapies has been documented to have a long history of effectiveness in the treatment of various diseases[2,3]. The chemical composition of plant constituents exhibits variations that are dependent on their geographical origin[4]. Herbal biomolecules (HBs) have been employed in traditional medicinal systems for centuries for the treatment of various ailments. Current research on the ethnobotanical use of these herbal molecules provides a scientific justification for their use as potential nutraceuticals. The study of HBs has gained considerable interest in the scientific community due to their therapeutic potential and bioactive properties. The identification and extraction of active compounds from herbal sources have led to the development of novel and effective nutraceuticals. The use of HBs as nutraceuticals has several advantages, including their natural origin, low toxicity, and minimal side effects. These advantages make them an attractive alternative to synthetic nutraceuticals. The continued investigation of HBs as potential nutraceuticals holds great promise for the development of safe and effective therapeutic agents for the treatment of various ailments[5]. The initiation of a ligand-target interaction is a crucial event, which triggers a cascade of signalling steps that are essential for the normal functioning of the body. The molecular interaction between the ligand and target biomolecules initiates a series of signalling pathways that result in a diverse range of physiological responses. This intricate process is an integral part of the body's normal functioning and is crucial for maintaining homeostasis. Understanding this process is of utmost importance in the field of biomedicine, as it has significant implications for the development of novel therapeutic interventions and the treatment of various diseases[6].

The use of plant-based therapies for therapeutic purposes is still prevalent in developing countries. This is due to the easy accessibility of plants, their low production cost, their satisfying effects, and their minimal adverse effects. Such therapies have become a popular alternative to conventional pharmaceuticals. Despite the growing interest in plant-based therapies, there is still a need for further research to understand their mechanisms of action and efficacy[7]. The phytomolecules obtained from herbal resources are widely used to treat multiple diseases and used as antioxidants, anticancer agents, agents for cardiovascular and central nervous system ailments, antidiabetic agents, antimicrobials, and so on. As phytochemicals show a defensive mechanism of action against several chronic diseases, including cancer, cardiovascular diseases, diabetes, and neurodegenerative disease, developed countries are utilizing traditional medicine systems more and more. Plant biomolecules are also involved in antiviral as well as antimicrobial activity and also show efficacy in radioprotection[8].

The isolation, extraction, purification, and chemical characterization of biomolecules have been the focus of research since the BC century. Over time, it has been discovered that different plant parts contain a variety of biologically active compounds, including alkaloids, glycosides, phenolic compounds, terpenoids, flavonoids, tannins, carbohydrates, vitamins, organic acids, and more, which are used to treat various diseases. Herb-based biomolecules (HBs) are widely used in all aspects of healthcare[9]. This review article offers a comprehensive overview of phytomedicine and its potential for healthcare. It explores the traditional use of medicinal plants and herbs for treating various ailments and the scientific rationale for their use as nutraceuticals. The article also presents an in-depth analysis of the classification of herbal biomolecules and the methods used for their extraction, purification, and characterization. It concludes by highlighting the potential of plant-based therapeutic compounds in preventing and treating chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative diseases. Additionally, it highlights the effective utilization of herb-based biomolecules in phytotherapy. Overall, the review article emphasizes the importance of phytomedicine in healthcare and the need for further research in this area.

CLASSIFICATION OF HERBAL BIOMOLECULES (HBS)

HBs are a group of bioactive compounds derived from herbal plants, including, but not limited to, alkaloids, glycosides, coumarins, flavonoids, tannins, terpenoids, essential oils, fixed oils, phenol, and carbohydrates[10]. These compounds can be classified into two main groups of metabolites: primary and secondary as shown in Figure 1.

Primary metabolites and secondary metabolites

Primary metabolites, such as carbohydrates, amino acids, fatty acids, and organic acids, are essential for plant growth, development, and maintenance, playing critical roles in photosynthesis, respiration, protein synthesis, and other physiological processes. They serve as the building blocks for plant growth and development, providing energy and organic compounds necessary for plant survival[11]. In contrast, secondary metabolites, including flavonoids, alkaloids, phenolic acids, and others, are not essential for plant growth but play crucial roles in plant defence, stress response, and adaptation to environmental changes.

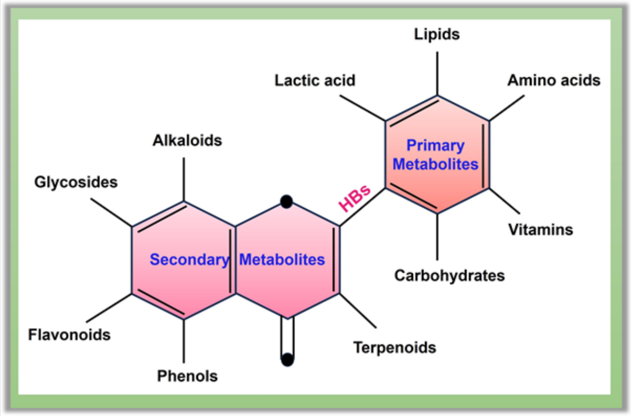


Fig. 1: Classification of Herbal Biomolecules (HBs) and its metabolites

These metabolites exhibit a range of biological activities, including antimicrobial, insecticidal, antioxidant, and signalling activities, which help plants to defend against pathogens and pests, protect against oxidative stress, and communicate with other plants and organisms. Under stress conditions, plants produce secondary metabolites to enhance their adaptability, and these metabolites play critical roles in defence against pathogens and pests, antioxidant activity, and signal molecule activities, ultimately contributing to plant survival and fitness[12]. Additionally, secondary metabolites can also play roles in plant-to-plant communication, attracting beneficial insects, and influencing soil microorganisms, highlighting their importance in plant ecology and interactions with the environment.

Alkaloids: Alkaloids are a diverse group of naturally occurring, organic compounds that contain nitrogen and are primarily found in plants, as well as some animals. One notable example is *Catharanthus roseus*, which produces an impressive array of around 120 alkaloids, with approximately 70 exhibiting pharmacological activity. The biological effects of alkaloids are wide-ranging, and their alkaline properties stem from the presence of a nitrogen atom. To date, approximately 10,000 alkaloids have been identified, with the majority being utilized for recreational and therapeutic purposes. Alkaloids are classified based on the type of nitrogen-containing ring they possess, which enables their categorization into distinct groups. Many alkaloids have been harnessed for their medicinal properties and are currently employed as drugs in modern medicine. Examples of these pharmacologically active alkaloids include morphine, nicotine, atropine, caffeine, quinine, and codeine. These compounds have been instrumental in the development of various treatments and therapies, underscoring the significant contribution of alkaloids to the field of medicine[13].

Glycosides: Glycosides are a diverse class of secondary metabolic products primarily derived from plants, characterized by their ability to break down into one or more sugars. The non-sugar portion of the glycoside is known as a glycone or genin, and glycosides play a vital role in the plant's life, regulating physiological processes, protecting against pathogens and pests, and promoting overall health. Various types of glycosides exist, including C-Glycosides, anthraquinone glycosides, saponin glycosides, cardiac glycosides (CGs), and isothiocyanate glycosides, each exhibiting unique biological activities such as anti-inflammatory, antioxidant, and antimicrobial properties[14–18]. Many medicinal plants contain significant levels of naturally occurring glycosides, including those with anticancer properties, and the use of glycosides derived from medicinal plants as alternative medications to treat various cancers has been recognized, highlighting their potential as valuable therapeutic agents. Furthermore, glycosides have been found to exhibit a range of biological activities, including anti-diabetic, anti-hypertensive, and anti-arthritic properties, making them a promising area of research for the development of new drugs. Additionally, glycosides have been used in traditional medicine for centuries, and their potential as lead compounds for the development of new pharmaceuticals is being increasingly recognized[8,19]. Overall, glycosides are an important class of compounds with a wide range of biological activities and potential therapeutic applications.

Terpenoids: Terpenes are naturally occurring molecules composed of several isoprene units, which are the building blocks of these compounds. Terpenoids, on the other hand, refer to the various types of terpenes that differ in the number of isoprene units they contain. Monoterpenoids and sesquiterpenoids are two examples of terpenoids, each with distinct properties and functions. Terpenoids are volatile substances responsible for the fragrances of plants and flowers and are widely distributed in the leaves and fruits of higher plants, conifers, citrus, and eucalyptus[20]. Herbal terpenoids, such as cadinene, caryophyllene, zingiberene, guaiazulene, and guaianolide, are widely used and characterized by their therapeutic and other effects. These compounds have been found to exhibit a range of biological activities, including anti-inflammatory, antioxidant, and antimicrobial properties, making them valuable for the development of new drugs and therapies[21]. One of the most well-known and widely used terpenoids is artemisinin, which is mainly isolated from *Artemisia annua*. Artemisinin is a potent antimalarial agent, and its derivatives are used for the treatment of malaria. In addition to its antimalarial properties, artemisinin has also been found to exhibit anticancer, anti-inflammatory, and antioxidant activities, highlighting its potential as a versatile therapeutic agent. Terpenoids have also been found to play important roles in plant defence, signalling, and communication[22]. They can act as repellents or attractants for insects and other animals and can also influence plant-plant interactions and soil microorganisms. Overall, terpenoids are an important class of compounds with a wide range of biological activities and potential therapeutic applications.

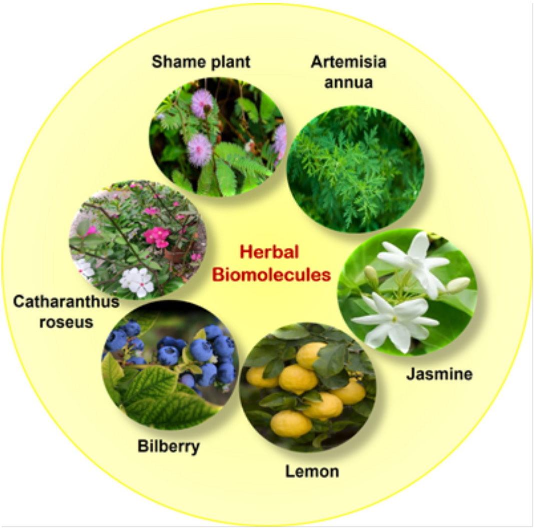


Fig. 2: An illustration depicting the presence of HBs in different plant species

Essential oil: Volatile oil, also known as ethereal oil or essential oil, is a fragrant and unstable substance found in plants and animals. These oils are characterized by their ability to evaporate quickly when exposed to air at room temperature, releasing their distinctive aromas. Essential oils are typically extracted from various parts of plants, including flowers, leaves, stems, bark, wood, roots, seeds, fruits, rhizomes, and gums or oleoresin exudations. The production and consumption of essential oils and perfumes are increasing worldwide, driven by their growing demand in aromatherapy, cosmetics, and pharmaceutical applications[23]. However, the quality and yield of essential oils can vary greatly depending on factors such as the plant material used, extraction methods, and production technologies. Therefore, advances in production technology are crucial for enhancing the overall quality and yield of essential oil. Most herbal volatile oils are used for therapeutic purposes, and their potential health benefits are being increasingly recognized[24]. These oils have been found to exhibit a range of biological activities, including anti-inflammatory, antimicrobial, and antioxidant properties, making them valuable for the development of new treatments and therapies. Some essential oils, such as tea tree oil and eucalyptus oil, are also used in traditional medicine for their antimicrobial and anti-inflammatory properties[25]. Overall, essential oils are a valuable resource with a wide range of applications from aromatherapy and perfumery to pharmaceuticals and traditional medicine.

Flavonoids: Flavonoids are a diverse group of phenolic substances extracted from various vascular plants, with approximately 8,000 distinct chemicals identified to date. These compounds serve multiple purposes in plants, including acting as antioxidants, antimicrobials, photoreceptors, visual attractants, feeding deterrents, and light filters[26]. The health benefits of flavonoids are numerous, and include vasodilating, anti-inflammatory, antiviral, and antiallergenic properties, making them a valuable component of a healthy diet. Flavonoids have received growing attention in recent years due to their potent antioxidant properties, which help prevent the production of free radicals and scavenge existing ones, thereby reducing oxidative stress and inflammation in the body[27]. These compounds are widely distributed in various plant-based foods, including vegetables, fruits, grains, roots, flowers, bark, stems, tea, and wine. Flavonoids can be classified into different types based on their chemical structure, which includes flavones, flavanones, flavonols, catechins, anthocyanins, and chalcones[28]. Each of these subclasses has unique properties and biological activities, and they have been found to have therapeutic significance in the prevention and treatment of various diseases, including cardiovascular disease, cancer, and neurodegenerative disorders.

The medical industry has widely adopted flavonoids due to their potential health benefits and therapeutic applications. Research has shown that flavonoids can interact with various cellular signalling pathways, influencing gene expression, enzyme activity, and cell signalling, which can lead to improved health outcomes. Overall, flavonoids are a valuable group of compounds with a wide range of health benefits and therapeutic applications.

Coumarins: Coumarins are naturally occurring biomolecules often containing an oxygen substituent at the C-7 position. These compounds are highly valued for their bacteriostatic, physiological, and anti-tumour properties, which make them an attractive target for further derivatization and screening as potential therapeutic agents, as shown in Figure 3. Coumarin can also be found in certain fruits (such as bilberry and cloudberry), green tea, and other foods, such as chicory. Most coumarins are found in higher plants, with the richest sources being Rutaceae and Umbelliferone. They are also in significant concentrations of essential oils, including cinnamon bark oil. These compounds exhibit various effective pharmacological actions, such as anti-inflammatory, anti-cancer, anti-bacterial, etc. Synthetic coumarins are primarily used as aroma chemicals due to their odour strength, alkali stability, and relatively low cost[29–31].

Biomolecules in various plant species

Numerous plants such as *Moringa oleifera, Duboisia species, Cannabis sativa, Ficus racemosa*, and members of the Amaryllidaceae family have been studied to gain a better

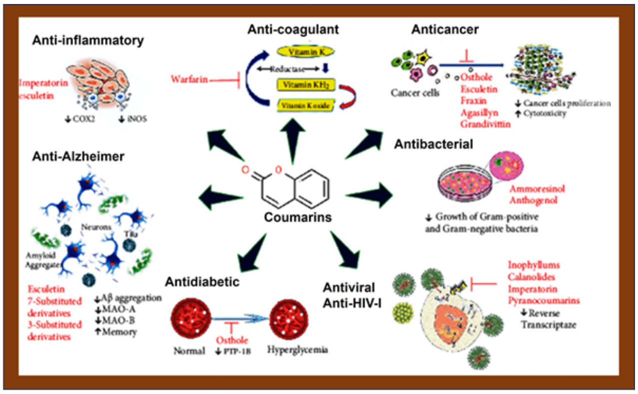


Fig. 3: Schematic representation of molecular mechanisms and signaling pathways of the most representative coumarins and their derivatives

understanding of the diverse biomolecules produced by both primary and secondary metabolism.

The variations in primary and secondary metabolites may arise from differences among species, within a species, in growing regions or environments, during processing and storage, as well as from other factors such as plant age, plant part, and analytical methodologies. Since plants grow in different locations and environments, they receive varying amounts of minerals and nutrients, which can be identified using spectroscopic and chromatographic techniques illustrated in Figure 4[32].

EXTRACTION OF BIOMOLECULES

Biomolecules are extensively employed in medical and non-medical fields, yet their widespread application has resulted in a limited availability of herbal plants. The extraction of biomolecules from herbal plants, which can alleviate the anxiety of the patients associated with synthetic drugs, is therefore imperative. It is crucial to purify biomolecules to develop therapeutic agents. Multiple approaches have been developed to establish the sustainability of medicinal plants, including good cultivation practices, proper collection methods, plant tissue culture, genetic modification, and more. Implementing these approaches can effectively ensure the longevity and abundance of medicinal plants[33].

HBs of phytomedicine can be extracted using either conventional or modern methods. The conventional extraction processes include maceration, percolation, decoction, infusion, soxhlet, and other classic miscellaneous conventional extraction techniques (MCET). There are two types of MCETs: infusion and decoction. Distillation, soxhlet extraction, and maceration are still frequently used processes.

Conventional route

- **Maceration:**

Maceration is a process for extracting both organized and unorganized plant drugs. Multiple macerations, such as double or triple, are often used to improve extraction efficiency. During maceration, plant material is soaked at slightly elevated temperatures called digestion. There are two types of maceration: circulatory extraction and multistage extraction techniques. Additionally, there is potential for improvement with this extraction technology, mostly due to efficiency, environmental concerns, and time constraints. Significantly, polarity and consequently solubility, groups involved in the study of natural products typically perform a series of extractions using solvents with varying polarities, ranging from apolar alkane-based solvents (such as n-heptane, n-hexane, and cyclohexane) to intermediate solvents (such as dichloromethane and ethyl acetate) and ultimately to polar solvents (alcohols and water)[34]. However, the maceration technique has significant disadvantages, such as excess solvent usage, time consumption, and inefficient extraction[35].

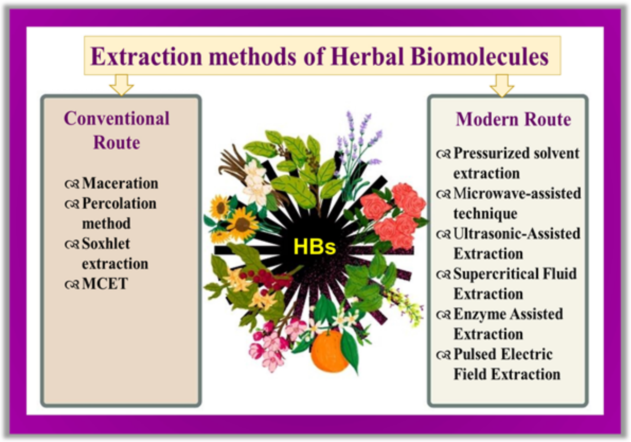


Fig. 4: Techniques for extracting herbal biomolecules (HBs) from phytomedicinal herbs

- **Percolation:**

The percolation method is a more efficient extraction technique compared to maceration, offering several advantages. During this process, an imbibition process is employed, where the solvent gradually penetrates the plant material, preventing the percolator - the vessel used for extraction - from getting clogged. This results in a more consistent and controlled extraction process. However, one of the primary disadvantages of the percolation method is the potential for dilution of the extract. As fresh solvents are added during the process, the extract can become diluted, leading to a longer evaporation time. This can be a significant drawback, particularly when working with thermolabile compounds or when a concentrated extract is required. Despite this limitation, the percolation method remains a popular choice for extracting bioactive compounds from plant materials, due to its efficiency and ability to produce high-quality extracts. By optimizing the extraction conditions and solvent composition, it is possible to minimize the disadvantages of the percolation method and obtain the desired extract[36].

- **Soxhlet extraction:**

Soxhlet extraction is a widely used and efficient method that combines the principles of percolation and maceration techniques. This hybrid approach allows for the optimal extraction of bioactive compounds from plant materials. The Soxhlet extraction process involves the use of high temperatures and a solvent recycling system, which increases the mass transfer rate and enhances the extraction efficiency. The Soxhlet apparatus consists of a distillation flask, a condenser, and a solvent reservoir. The plant material is placed in a thimble, and the solvent is heated, causing it to evaporate and rise into the condenser. The condensed solvent then drips into the thimble, extracting the bioactive compounds from the plant material. The extracted compounds are then collected in the solvent reservoir, and the process is repeated until the desired level of extraction is achieved. Soxhlet extraction offers several advantages over conventional methods, including increased efficiency, reduced solvent consumption, and improved extract quality. The high temperatures used in Soxhlet extraction can also help to break down complex plant matrices, releasing more bioactive compounds into the solvent[37]. The Soxhlet extraction is a reliable and efficient method for extracting bioactive compounds from plant materials, and is widely used in various industries, including pharmaceuticals, cosmetics, and food processing.

- **Miscellaneous conventional extraction techniques (MCET):**

The Multiple Component Extraction Technique (MCET) is a versatile method that combines reflux extraction, infusion, and decoction to extract bioactive compounds from plants. Reflux extraction, a key component of MCET, involves the repeated circulation of a solvent through the plant material, allowing for more efficient extraction of compounds compared to maceration and percolation. Although reflux extraction is less efficient than the Soxhlet method, its simplicity and ease of use make it a popular choice for plant extraction[38]. Decoctions, another component of MCET, are commonly used to prepare ayurvedic extracts known as "Kwaths." This traditional method involves boiling the plant material in water to extract the bioactive compounds. Decoctions are particularly useful for extracting water-soluble constituents, such as polysaccharides, glycosides, and alkaloids. Infusion, the third component of MCET, involves steeping the plant material in hot water to extract the bioactive compounds. This method is also used to extract water-soluble constituents and is commonly used to prepare herbal teas and tinctures[39]. Overall, the MCET method offers a flexible and efficient approach to plant extraction, allowing for the extraction of a wide range of bioactive compounds using different solvents and techniques. Its applications are diverse, ranging from the preparation of traditional herbal remedies to the extraction of high-value compounds for pharmaceutical and cosmetic applications.

Modern route

Various modern techniques are available for the extraction of HBs (Table 1), such as pressurized or accelerated solvent extraction (PSE or ASE), Microwave-assisted extraction (MAE), Ultrasonic-assisted extraction (UAE), Supercritical fluid extraction (SFE), Enzyme-assisted extraction (EAE), and Pulse electric field extraction (PEFE).

- **Pressurized or Accelerated Solvent Extraction (PSE or ASE):**

The Multiple Component Extraction Technique (MCET) is a highly efficient and versatile method that has gained widespread acceptance in the field of natural products extraction. Its flexibility in handling a diverse range of vegetative extraction samples, including wood, leaves, flowers, fruits, gums, and agricultural residues, makes it an ideal choice for extracting bioactive compounds from various plant materials. MCET is widely employed in the preparation of extracts rich in biologically active compounds, such as flavonoids, alkaloids, phenolic compounds, lipids, essential oils, and other valuable phytochemicals. The technique's ability to extract a broad spectrum of compounds has made it a valuable tool in the discovery and development of new natural products, including pharmaceuticals, nutraceuticals, and cosmeceuticals. The applications of MCET are diverse and continue to expand, with potential uses in the food, beverage, and pharmaceutical industries, as well as in the development of new sustainable products and technologies[40]. Overall, MCET is a powerful technique that offers a flexible and efficient approach to extracting bioactive compounds from plant materials, and its continued development and application are expected to have a significant impact on various industries and fields of research.

- **Microwave-assisted extraction (MAE):**

MAE involves microwaves that induce ionic conduction and dipole rotation in the solvent and the sample, producing heat that helps extract biomolecules from the plant matrix. MAE systems are broadly divided into multimode or closed systems and focused or mono-mode or open systems. In both systems, extraction is carried out at elevated atmospheric pressure. As a part of improving extraction efficiency, the MAE is further modified with nitrogen-protected MAE (NPMAE), vacuum-assisted MAE (VAMAE), ultrasonic MAE (UMAE), dynamic MAE, etc. Extraction efficiency mainly depends on factors like solvent selection, solvent ratio, extraction time, microwave power and temperature, nature of plant materials, etc.[41,42].

- **Ultrasonic-assisted extraction (UAE):**

The UAE technique, widely used in various healthcare applications, is a testament to the industry's commitment to safety and environmental friendliness. UAE uses both high-frequency and low-frequency ultrasound waves and has emerged as a useful, safer, and greener method of extraction. It depends on factors such as the frequency and intensity of the ultrasound waves, type of solvent, and temperature[43–45].

- **Supercritical fluid extraction (SFE):**

SFE involves extracting supercritical fluids, defined as any pure homogeneous substance above their critical points. To increase the efficiency of SFE, various strategies, such as the use of ionic liquid solvents combined with enzyme-assisted, sudden decompression, etc., are used. The efficiency of the SFE depends upon factors like particle size, moisture content, pressure and temperature, solvent-to-feed ratio, solvent flow rate, etc. It is being used to harvest a wide range of extracts, bioactive molecules, and single compounds[46].

- **Enzyme-assisted extraction (EAE):**

The EAE method is mainly used to overcome some demerits, like less efficiency and low quality of the extract, which is prepared through conventional methods. The basic principle of EAE is that enzymes are ideal for catalysis, which work with specificity and regioselectivity to extract bioactive compounds from the plant matrix. The efficiency of the EAE depends on various operational systems, extraction time, type, and concentration of enzyme, as well as substrate particle size. The EAE method mainly extracts different biomolecules and compounds responsible for flavour and colour, oils, juices, etc., from the plant matrix. The cost and availability of enzymes are the demerits of this method[47].

- **Pulse electric field extraction (PEFE):**

PEFE is a modern extraction technique largely used in the food industry to increase the shelf life of food products. It is efficient, non-thermal, and rapid. The basic principle of this technique is to increase the membrane permeability with the help of an electric current. The extraction yield of PEFE depends on various factors, such as the intensity of the electric field, duration of extraction, the shape of the pulse wave, type of solvent, and pH[48].

STAGES FOR PURIFYING THE HERBAL BIOMOLECULES OF PHYTOMEDICINE

Purification of phytomedicine is a crucial step in identifying potential lead molecules for therapeutic agents. This process involves various methods such as gas chromatography (GC), high-performance liquid chromatography-electrospray ionization tandem mass spectrometry analysis (HPLC-MS), isolation methods, and spectroscopic methods such as UV-visible spectroscopy, FTIR, etc. The purification process plays a vital role in removing inactive phytochemicals from the extract. Non-toxic solvents and methods can effectively purify the biomolecules from herbal products. However, the extraction, fractionation, and chemical structure identification processes are time-consuming and have slowed the use of herbal products in medicinal agents[49]. To overcome these challenges, automatic and rapid isolation techniques have been developed to purify the biomolecules from herbal products. As various extraction, fractionation, and chemical structure identification tools advance, these techniques may help the purification and structure identification processes depicted in Figure 5.

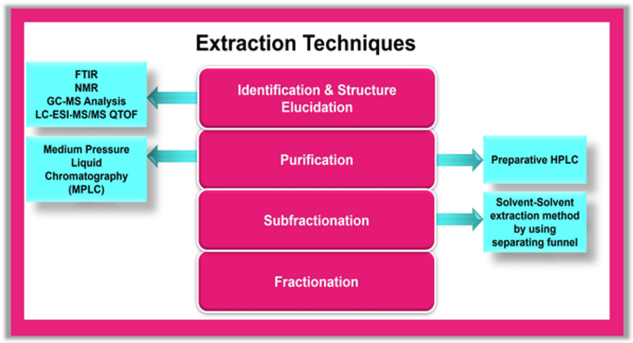


Fig. 5: Depiction of various stages involved in purifying herbal biomolecules of phytomedicine

TECHNIQUES FOR CHARACTERIZING THE HBS OF PHYTOMEDICINE

Various techniques are available for identifying and characterizing herbal biomolecules (HBs). These include ultraviolet-visible spectroscopy (UV-VIS), fluorescence, nuclear magnetic resonance spectroscopy (NMR), Fourier transform-infrared spectroscopy (FT-IR), single crystal X-ray diffraction (SC-XRD), mass spectrometry (MS), liquid chromatography (LC), gas chromatography (GC), polarimetry, thin layer chromatography (TLC), immunoassays (IA), gel and capillary electrophoresis (GCE) and DNA and protein sequencing (DNA-PS). These techniques aim to identify the different compositions and characterize the structure of various herbal biomolecules found in the leaves, roots, and stems of various herbal plants, which exhibit unique biochemical activity.

- **Spectroscopic techniques:**

Spectroscopic techniques are widely used to qualitatively and quantitatively measure biomolecules present in herbal plant extracts.

Table 1: Different modern routes for extraction HBs and their principle, applications, advantages, and disadvantages

Method	Principle	Applications	Advantages	Disadvantages	References
Pressurized or Accelerated Solvent Extraction (PSE or ASE)	Uses pressurized solvent to extract compound.	Extraction of flavonoids, alkaloids, phenolic compounds etc.	Low solvent usage, high extraction efficiency, and high selectivity.	Lack of equipment availability.	40
Microwave-assisted extraction (MAE)	Heating solvent by microwave and increase the solubility of molecule.	Extraction of polar and nonpolar bio active compounds.	Reduced solvent usage, low energy consumption.	Need high temperature, Degradation of molecules.	41
Ultrasonic-assisted extraction (UAE)	Using ultrasound waves to disrupt the cell wall and increase the solubility of target compounds.	Extraction of flavonoids, essential oils, and other bio active compounds.	High extraction yield, simple and low-cost method.	Low selectivity, Degradation of some compound.	45
Supercritical fluid extraction (SFE)	Using superficial fluids to extract the compounds.	Extraction of essential oils, flavours, pigments, etc.	Eco-friendly, low degradation of compounds, low energy consumption.	High cost, unsuitable molecules having high molecular weight.	46
Enzyme-assisted extraction (EAE)	Using enzyme to break the cell wall and release target compound.	Extraction of glycosides and polysaccharides, etc.	High selectivity and mild extraction condition.	Limited substate.	47
Pulse electric field extraction (PEFE)	Utilize high-voltage electrical pulses to rupture cell walls and release intercellular compounds.	Extraction of essential oil, anthocyanin, etc.	Green methodology, high extraction yield.	Complex process, limited scale process.	48

Spectrophotometry is used to identify even the smallest molecules and determine nucleic acid concentration. The fluorescence properties of molecules help identify the structure of various biomolecules. Polarimetry and Circular Dichroism (CD) are commonly used to characterize chiral molecules and proteins. Synchrotron radiation circular dichroism (SRCD) spectroscopy is a recent development that helps characterize protein conformation and interaction. FT-IR is used to describe organic functional groups and to confirm samples quickly. 1H and 13C NMRs are used to analyze simple molecules and to determine structural elucidation through the analysis of photon-photon coupling. High-resolution mass spectroscopy is mainly developed for analyzing, identifying, characterizing, and mapping biomolecules, using better mass analyzers such as time of flight (TOF) and orbitrap in addition to the quadrupole mass analyzer. Coupling a mass analyzer with modern chromatography, tandem MS, and the emergence of ambient desorption/ionization are recent developments in MS[50,51].

- **Chromatographic techniques:**

Chromatography is highly effective for separating and purifying biomolecules from their extracts. In the past, the bioautographic method relied on preventing microbial growth to identify antimicrobial elements in extracts chromatographed on a TLC layer. This method is the most effective assay for discovering antimicrobial agents[52]. One of the bioautographic techniques produces inhibition zones on TLC plates, which are used to determine the location of the bioactive molecule with antimicrobial activity in the TLC fingerprint concerning Rf values[53]. High-performance liquid chromatography (HPLC) is a versatile, dependable, and widely used method for isolating natural compounds[54]. It is increasingly becoming the preferred option for fingerprinting research for the quality control of herbal plants[55]. Affinity chromatography is a vital tool for separating biomolecules. It uses biological binding agents like antibodies, Ig-binding proteins, and lectins, as well as non-biological binding agents like immobilized metal-ion affinity chromatography and molecular imprinted polymers[56,57].

- **Immunoassay techniques:**

There are several biochemical methods used for analysis, including immunoassays, gel electrophoresis, capillary electrophoresis, sequencing nucleic acids, and sequencing proteins. Immunoassays rely on specific antigen-antibody reactions and encompass techniques such as enzyme-linked immunosorbent assays, human enzyme-linked immunosorbent assays, and electric trio chemiluminescence immunoassays. These methods are increasingly used to analyze bioactive substances, employing monoclonal antibodies against pharmaceuticals and low molecular weight natural bioactive molecules. The specificity and sensitivity of receptor binding investigations, enzyme tests, and qualitative and quantitative analytical procedures are greatly enhanced through

KRUPANIDHI COLLEGE OF PHARMACY

immunoassays. MAb-based enzyme-linked immunosorbent assays (ELISA) are often more sensitive than traditional HPLC techniques. Hybridoma technology is a specialized process for creating monoclonal antibodies[58]. Immunoassays are also commonly used in the detection of plant secondary metabolites.

- **Electrophoresis:**

Electrophoresis is widely used to separate DNA, RNA, or protein. Gel electrophoresis, in particular, separates molecules of a desired size range using different currents, various gel types, and different buffer pH. Polyacrylamide and agarose gels are the most commonly used supporting DNA or protein electrophoresis matrices. Capillary electrophoresis is similar to HPLC and uses various detectors depending on the types of molecules under separation[59].

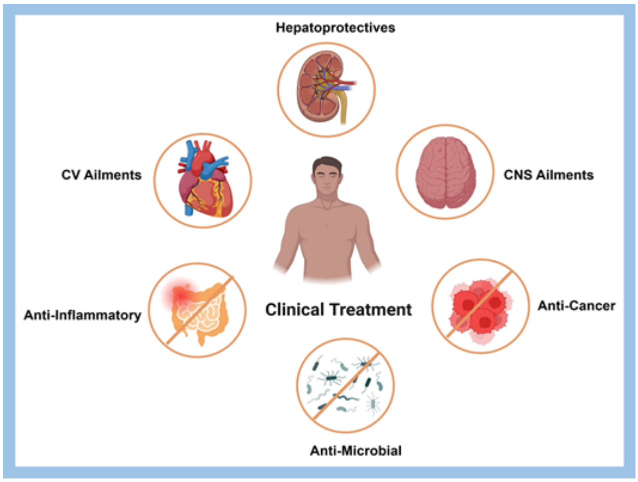


Fig. 6: Applications of HBs in various Phytotherapy treatments

PHYTOMEDICINES IN THE REALM OF PHYTOTHERAPY APPLICATIONS - A STUDY ON THE USE OF PLANTS FOR MEDICINAL PURPOSES

Herbal biomolecules possess various pharmacological potentials for treating various diseases. The application of the HBs for various phytotherapy treatments are visualized in Figure 6. These biomolecules are instrumental in overcoming unfavorable toxicities. As a viable alternative with fewer side effects, the scientific evidence, technological advances, and research trends support their development as a treatment.

Central Nervous System Ailments (CNSA): Schizophrenia, depression, Alzheimer's disease, and Parkinson's are debilitating conditions that affect the central nervous system. Several phytoconstituents have been identified as potential biomolecules for the treatment of these diseases. Natural nootropics, such as those found in HBs, stimulate brain health and functions. Additionally, saponins, alkaloids, and polyphenols, known for their anxiolytic and antidepressant-like effects, have been found to significantly contribute to brain health. Huperzine, a sesquiterpene alkaloid, has been found to improve cognitive function. Other effective compounds for brain health and function include curcumin (a phenol), ginkgolides, and resveratrol[60].

In their research, Vengalasetti et al. explained the role of neuroprotective herbs, such as Ashwagandha and Brahmi, which contain various bioactive compounds, including alkaloids, sterols, and betulinic acid. These herbs have shown promise in treating dementia associated with Alzheimer's disease[61].

Cardiovascular (CVA) Ailments: The World Health Organization (WHO) has reported that cardiovascular ailments are responsible for the majority of deaths worldwide. Various herbal plant-based drugs containing high levels of HBs have been employed as a preventive measure against various cardiovascular disorders. Digitalis, derived from the species *Digitalis purpurea*, is a vital source of cardenolides, primarily used to treat myocardial infarction, arterial hypertension, cardiac dysfunction, angina, and other related ailments[62,63].

Munawar et al. have conducted ex-vivo and in-vivo research highlighting the therapeutic potential of *Jasminum sambac* in cardiovascular diseases[64]. The antioxidants present in *Jasminum sambac* are known to help cardiomyocytes counteract oxidative stress resulting from adrenaline. The hydroalcoholic leaf extract of *Jasminum sambac* is believed to possess a cardioprotective effect owing to its various phytoconstituents. Phytochemical screening has revealed the presence of tannins, flavonoids, and cardiac glycosides, which are thought to play a crucial role in managing cardiovascular disorders, particularly hypertension-induced ventricular hypertrophy and myocardial infarction[65].

Phytomedicines as Antimicrobial Agents: The prevalence of bacterial, fungal, and viral diseases has affected humankind throughout the ages. In the medical industry, there has been an increasing use of HBs, which has led to a deeper exploration of the mechanisms of antibacterial activity of alkaloids. It has been found that the inhibition of cell division, DNA synthesis, and protein synthesis are some such mechanisms. Phytochemicals such as allicin, allin, ginsenosides, flavonoids, and leaching from various plants have also been identified for their antimicrobial properties[66].

In recent years, there has been a growing interest in exploring traditional medicinal practices for their scientific basis in treating microbial pathogens and infectious diseases. One such study by S. Gopika K. Prabu investigated the antibacterial and antifungal activity of *Ruellia tuberosa* (L.) leaf and stem. This study aimed to validate the use of *Ruellia tuberosa* in folk medicine for treating infectious diseases[67].

Another study conducted by Saeediye et al. compared the antibacterial activity of essential oil in both liquid and vapour phases against a panel of bacteria, including *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Shigella dysenteriae, Klebsiella pneumonia, Salmonella typhi*, and *Pseudomonas aeruginosa*[68]. The study employed the disc volatilization assay (DVA) and the broth dilution assays (BDA) to determine the inhibitory potential of HEOs.

Peter Cavazos et al. conducted a study to determine the presence of antibacterial activity, antioxidant activity, and secondary metabolites of sequential solvent extracts (acetone, methanol, and acetic acid) of *Acacia berlandieri* and *Acacia rigidula* leaves. The study found that rigidula leaf extracts can serve as a source of novel antimicrobial and antioxidant agents. The results of this study provide a basis for further investigations of the rigidula leaf extracts[69].

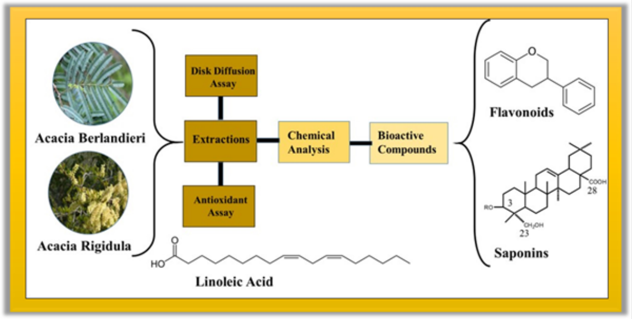


Fig. 7: Antibacterial and antioxidant properties of the leaf extracts of *Acacia rigidula* benth and *Acacia berlandieri* benth

As anti-inflammatory agents: Inflammation is a mediator that plays a role in various diseases. Consequently, developing improved anti-inflammatory drugs is essential. Herbal plants, with their superior safety profile and molecular diversity, have the potential to serve as alternative approaches in the field. Acheflan and Daflon, which consist of purified flavonoids, are marketed products used as anti-inflammatory drugs. Several isoquinoline alkaloids such as berbamine, berberine, cepharanthine, and tetrandrine have been examined for their anti-inflammatory activity. Alkaloids are present in flowering plants, with the most prevalent families being Papaveraceae (poppies), Papilionaceae (lupins), Ranunculaceae (aconites), and Solanaceae (tobacco and potatoes). They are also found in lower plants, insects, marine creatures, microbes, and animals. Plant-based biomolecules exhibit anti-inflammatory activities through multiple mechanisms, with low side effects and without toxicity. The mechanisms of anti-inflammatory activity are the downregulation of cytokines, adhesion molecules, and blockage of proinflammatory pathways. The most important plant constituents that exhibit anti-inflammatory activities are alkaloids, terpenes, and phenolic compounds such as tannins, lignins, coumarins, and saponins. Plant-based biomolecules with antioxidant characteristics can also be potential disease therapies[70,71].

As anti-cancer agents: Molecular therapy has the potential to be an effective and accessible means of treating cancer, particularly if it is affordable and widely available to the general population. Herbal remedies have emerged as a promising option for combating this deadly disease, as they offer numerous benefits and, when properly studied, can be among the most powerful tools available. Plant-based constituents, such as taxanes, vinca alkaloids, camptothecin derivatives, and colchicine, have already been investigated for their potential anticancer properties[72]. V. M. Patil and N. Masand have developed flavonoids and their synthetic analogues for cancer prevention and chemotherapy, highlighting their potential as an effective treatment method[6]. Additionally, parsley, oregano, celery, saffron, dill, fennel, and Tasmanian pepper have been found to have high concentrations of flavonoids. Flavonoids have been shown to have a broad range of anticancer effects, including the modulation of ROS-scavenging enzymes, participation in cell cycle arrest, induction of apoptosis and autophagy, and reduction in the proliferation and invasiveness of cancer cells[73–75].

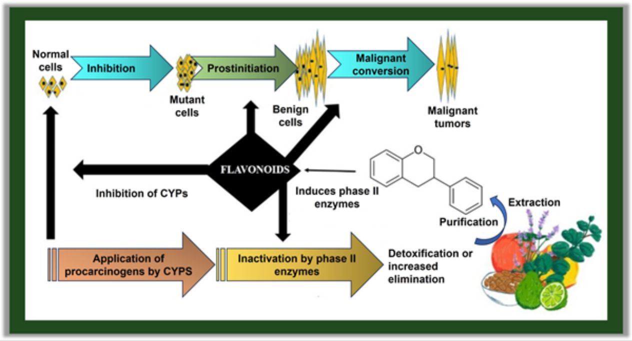


Fig. 8: Schematic representation of the anticancer activity of flavonoids

As hepatoprotectives: Hepatic diseases, such as liver cirrhosis, alcoholic liver disease, and nonalcoholic fatty liver disease, are a significant health concern. Numerous herbal remedies have been introduced to address these ailments, among which HBs have shown promising results. The hepatoprotective activity of phyto-molecular flavonoids, alkaloids, carotenoids, flavonolignans, and terpenes has been attributed to their antioxidant properties. In particular, Silymarin and glycyrrhizin are noteworthy herbal hepatoprotectives[76]. Recent research by N Huzio, A Grytsyk, et al. has demonstrated the potential of *A. eupatoria* L. herb extract for creating anti-inflammatory and hepatoprotective activity drugs[77]. The efficacy of *Glycyrrhiza glabra* extract in improving liver function in acute liver diseases has also been established[78]. Additionally, Asaad, Gihan Farag, et al. reported that Kaempferol glycosides isolated from *C. odorata* L. leaves exhibit antioxidant and antiapoptotic effects, ameliorating oxidative stress and inhibiting the Raf/MAPK pathway. These findings indicate the potential of herbal remedies in treating hepatic diseases and highlight the need for further research[79].

CONCLUSION AND FUTURE OUTLOOKS

The natural production of several biomolecules in plants is well documented. Different properties and functions are associated with the various biomolecules produced. Various conventional and modern methods extract these biomolecules from herbal plants. The phytopharmacological approach to natural biomolecules has the potential to cure several acute and chronic diseases. The identification of the different biomolecules derived from other plant species is made possible through several analytical methods. This review aims to explore the key biomolecules found in herbs, such as alkaloids, glycosides, flavonoids, anthraquinones, steroids, polysaccharides, tannins and polyphenolic chemicals, terpenes, lipids, waxes, proteins and peptides, and vitamins. The various bioactivities and pharmacological potentials attributed to these herbal biomolecules are also discussed. Additionally, this review provides a systematic understanding of how these herbal biomolecules are extracted, purified, characterized, and utilized in the healthcare industry. Understanding the types of biomolecules, analytical techniques, extraction and purification methods, and phytopharmacological approaches can help develop more advanced therapeutic methods, enhance therapeutic efficiency, and increase biomolecule bioavailability. Due to the increasing demand for greener and safer methods, expectations are high for future breakthroughs in the extraction, purification, and analysis of biomolecules. This review will particularly interest researchers studying bioactive natural products and biomolecules with nutraceutical properties. Experts in scientific fields, including biochemistry, pharmacology, analytical chemistry, organic chemistry, clinics, or engineering, focusing on bioactive natural compounds, can also benefit from this review[80–82].

Despite the many possibilities associated with herbal biomolecules, certain difficulties still need to be addressed, such as safety, effectiveness, suitable manufacturing equipment, quality assessment, and an insufficient regulatory framework. The regulatory status of herbal medicines and issues related to quality control of herbal medications are two significant obstacles in this regard. The abundance of accessible plants with therapeutic value is a blessing, and better healthcare can be guaranteed for many people worldwide through adequate cultivation, collection, storage, standardization for safety and efficiency, and other measures[83–86].

Declaration of competing interest

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

1. Oladeji O. The Characteristics and Roles of Medicinal Plants: Some Important Medicinal Plants in Nigeria. *Natural Products: An Indian Journal*. 2016;12(3):1–8. Available from: https://www.tsijournals.com/articles/the-characteristics-and-roles-of-medicinal-plants-some-important-medicinal-plants-in-nigeria.html.
2. Shin SA, Joo BJ, Lee JS, Ryu G, Han M, Kim WY, et al. Phytochemicals as anti-inflammatory agents in animal models of prevalent inflammatory disease. *Molecules*. 2020;25(24):1–27. Available from: https://doi.org/10.3390/molecules25245932.
3. Gurjar VK, Pal D. Natural Compounds Extracted from Medicinal Plants and Their Immunomodulatory Activities. In: Pal D, Nayak AK, editors. Bioactive Natural Products for Pharmaceutical Applications ;vol. 140 of Advanced Structured Materials. Cham. Springer. 2020;p. 197–261. Available from: https://doi.org/10.1007/978-3-030-54027-2_6.
4. Gabrani R, Naithani U, Jain J. Biomolecules and Pharmacology of Nepeta cataria L. (Family: Lamiaceae). In: Bioactives and Pharmacology of Medicinal Plants. New York.. Apple Academic Press. 2023;p. 369–378. Available from: https://www.taylorfrancis.com/chapters/edit/10.1201/9781003281658-29/biomolecules-pharmacology-nepeta-cataria-family-lamiaceae-reema-gabrani-ujjwala-naithani-jayshree-jain.
5. Pemmaraju DB, Ghosh A, Gangasani JK, Murthy USN, Naidu VGM, Rengan AK. Chapter 21 - Herbal biomolecules as nutraceuticals. In: Herbal Biomolecules in Healthcare Applications. Academic Press. 2022;p. 525–549. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00025-1.
6. Patil VM, Masand N. Chapter 12 - Anticancer Potential of Flavonoids: Chemistry, Biological Activities, and Future Perspectives. In: Studies in Natural Products Chemistry;vol. 59. 2018;p. 401–430. Available from: https://doi.org/10.1016/B978-0-444-64179-3.00012-8.
7. Hasan MK, Ara I, Mondal MSA, Kabir Y. Phytochemistry, pharmacologicalactivity, and potential health benefits of Glycyrrhiza glabra. *Heliyon*. 2021;7(6):1–10. Available from: https://doi.org/10.1016/j.heliyon.2021.e07240.
8. Mittal R, Kumar R, Chahal HS. Antimicrobial activity of Ocimum sanctum leaves extracts and oil. *Journal of Drug Delivery and Therapeutics*. 2018;8(6):201–204. Available from: https://jddtonline.info/index.php/jddt/article/view/2166.
9. Kandar CC. Secondary metabolites from plant sources. In: Pal D, Nayak AK, editors. Bioactive Natural Products for Pharmaceutical Applications;vol. 140 of Advanced Structured Materials. Springer, Cham. 2021;p. 329–377. Available from: https://doi.org/10.1007/978-3-030-54027-2_10.
10. Heller LE, Roepe PD. Artemisinin-based antimalarial drug therapy: molecular pharmacology and evolving resistance. *Tropical Medicine and Infectious Disease*. 2019;4(2):1–18. Available from: https://doi.org/10.3390/tropicalmed4020089.
11. Kennedy DO, and ELW. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Advances in nutrition*. 2011;2(1):32–50. Available from: https://doi.org/10.3945/an.110.000117.
12. Twaij BM, Hasan MN. Bioactive Secondary Metabolites from Plant Sources: Types, Synthesis, and Their Therapeutic Uses. *International Journal of Plant Biology*. 2022;13(1):4–14. Available from: https://doi.org/10.3390/ijpb13010003.
13. Kohnen-Johannsen KL, Kayser O. Tropane Alkaloids: chemistry, pharmacology, biosynthesis, and production. *Molecules*. 2019;24(4):1–23. Available from: https://doi.org/10.3390/molecules24040796.
14. Alamgir ANM. Secondary Metabolites: Secondary Metabolic Products Consisting of C and H; C, H, and O; N, S, and P Elements; and O/N Heterocycles. In: Therapeutic Use of Medicinal Plants and their Extracts: Volume 2 ;vol. 2 of Progress in Drug Research (PDR, volume 74). Cham. Springer. 2018;p. 165–309. Available from: https://doi.org/10.1007/978-3-319-92387-1_3.

15. Khan H, Saeedi M, Nabavi SM, Mubarak MS, Bishayee A. Glycosides from medicinal plants as potential anticancer agents: emerging trends towards future drugs. *Current Medicinal Chemistry*. 2019;26(13):2389–2406. Available from: https://doi.org/10.2174/0929867325666180403145137.
16. Khan H, Pervaiz A, Intagliata S, Das N, Venkata KC, Atanasov AG, et al. The analgesic potential of glycosides derived from medicinal plants. *DARU Journal of Pharmaceutical Sciences*. 2020;28(1):387–401. Available from: https://doi.org/10.1007/s40199-019-00319-7.
17. Kytidou K, Artola M, Overkleet HS, Alerts JMFG. Plant glycosides and glycosidases: a treasure-trove for therapeutics. *Frontiers in Plant Science*. 2020;11:1–21. Available from: https://doi.org/10.3389/fpls.2020.00357.
18. Whayne TF. Clinical use of digitalis: a state-of-the-art review. *American Journal of Cardiovascular Drugs*. 2018;18(6):427–440. Available from: https://doi.org/10.1007/s40256-018-0292-1.
19. Tauro S, Dhokchawle B, Mohite P, Nahar D, Nadar S, Coutinho E. Natural Anticancer Agents: Their Therapeutic Potential, Challenges and Promising Outcomes. *Current Medicinal Chemistry*. 2024;31(7):848–870. Available from: http://dx.doi.org/10.2174/0929867330666230502113150.
20. Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. Advances in pharmacological activities of terpenoids. *Natural Product Communications*. 2020;15(3):1–13. Available from: https://doi.org/10.1177/1934578X20903555.
21. Bergman ME, Davis B, Phillips MA. Medically useful plant terpenoids: biosynthesis, occurrence, and mechanism of action. *Molecules*. 2019;24(21):1–23. Available from: https://doi.org/10.3390/molecules24213961.
22. Cox-Georgian D, Ramadoss N, Dona C, Basu C. Therapeutic and Medicinal Uses of Terpenes. In: Joshee N, Dhekney S, Parajuli P, editors. Medicinal Plants . Springer, Cham. 2019;p. 333–359. Available from: https://doi.org/10.1007/978-3-030-31269-5_15.
23. Zhang Y, Long Y, Yu S, Li D, Yang M, Guan Y, et al. Natural volatile oils derived from herbal medicines: a promising therapy way for treating depressive disorder. *Pharmacological Research*. 2021;164:105376. Available from: https://doi.org/10.1016/j.phrs.2020.105376.
24. Jurado FR, Navarro-Cruz AR, Ochoa-Velasco CE, Palou E, Lopez-Malo A, Avila-Sosa R. Essential oils in vapor phase as alternative antimicrobials: A review. *Critical Reviews in Food Science and Nutrition*. 2020;60(10):1641–1650. Available from: https://doi.org/10.1080/10408398.2019.1586641.
25. Winska K, Maczka W, Lyczko J, Grabarczyk M, Czubaszek A, Szumny A. Essential oil as antimicrobial agents-myth or real alternative? *Molecules*. 2019;24(11):1–21. Available from: https://doi.org/10.3390/molecules24112130.
26. Pietta PG. Flavonoids as Antioxidants. *Journal of Natural Products*. 2000;63(7):1035–1042. Available from: https://doi.org/10.1021/np9904509.
27. Dhara AK, Nayak AK. Chapter 1 - Introduction to herbal biomolecules. In: Mandal SC, Dhara AK, Nayak AK, editors. Herbal biomolecules in healthcare applications. Elsevier. 2022;p. 1–19. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00005-6.
28. Alseekh S, Souza LP, Benina M, Fernie AR. The style and substance of plant flavonoid decoration; towards defining both structure and function. *Phytochemistry*. 2020;174:1–15. Available from: https://doi.org/10.1016/j.phytochem.2020.112347.
29. Sharifi-Rad J, Cruz-Martins N, López-Jornet P, Lopez EPF, Harun N, Yeskaliyeva B. Natural Coumarins: Exploring the Pharmacological Complexity and Underlying Molecular Mechanisms. *Oxidative Medicine and Cellular Longevity*. 2021;2021:1–19. Available from: https://doi.org/10.1155/2021/6492346.
30. Li RW, Smith PN, Lin GD. Chapter 5 - Variation of biomolecules in plant species. In: Herbal Biomolecules in Healthcare Applications. Academic Press. 2022;p. 81–99. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00028-7.
31. Stefanachi A, Leonetti F, Pisani L, Catto M, Carotti A. Coumarin: a natural, privileged and versatile scaffold for bioactive compounds. *Molecules*. 2018;23(2):1–34. Available from: https://doi.org/10.3390/molecules23020250.
32. Wen L, Zhang Z, Sun DW, Sivagnanam SP, Tiwari BK. Combination of emerging technologies for the extraction of bioactive compounds. *Critical Reviews in Food Science and Nutrition*. 2020;60(11):1826–1841. Available from: https://doi.org/10.1080/10408398.2019.1602823.
33. Malik J, Mandal SC. Chapter 2 - Extraction of herbal biomolecules. In: Herbal Biomolecules in Healthcare Applications. Elsevier. 2022;p. 21–46. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00015-9.
34. Gori A, Boucherle B, Rey A, Rome M, Fuzzati N, Peuchmaur M. Development of an innovative maceration technique to optimize extraction and phase partition of natural products. *Fitoterapia*. 2021;148. Available from: https://doi.org/10.1016/j.fitote.2020.104798.
35. Bilal M, Iqbal HMN. Biologically active macromolecules: Extraction strategies, therapeutic potential and biomedical perspective. *International Journal of Biological Macromolecules*. 2020;151:1–18. Available from: https://doi.org/10.1016/j.ijbiomac.2020.02.037.
36. Shireen S, Zarena AS. A Generalized Review on Extraction of Biomolecules. *Journal of Pharmacy and Nutrition Sciences*. 2022;12:175–187. Available from: https://doi.org/10.29169/1927-5951.2022.12.15.
37. Badgujar HF, Bora S, Kumar U. Eco-benevolent synthesis of ZnO nanoflowers using Oxalis corniculata leaf extract for potential antimicrobial application in agriculture and cosmeceutical. *Biocatalysis and Agricultural Biotechnology*. 2021;38:102216. Available from: https://doi.org/10.1016/j.bcab.2021.102216.
38. Bagatini L, Zandoná GP, Hoffmann JF, Cardoso JDS, Teixeira FC, Moroni LS, et al. Evaluation of Eugenia uniflora L. leaf extracts obtained by pressurized liquid extraction: Identification of chemical composition, antioxidant, antibacterial, and allelopathic activity. *Sustainable Chemistry and Pharmacy*. 2023;35:101214. Available from: https://doi.org/10.1016/j.scp.2023.101214.
39. Khalil N, Bishr M, Desouky S, Salma O. Ammi Visnaga L., a Potential Medicinal Plant: A Review. *Molecules*. 2020;25(2):1–18. Available from: https://doi.org/10.3390/molecules25020301.
40. Patil SJ, Sadashiv SO, Nandeshwarappa B. Purification of Herbal Biomolecules Using Chemical Biology Approaches. In: Advances in Chemical Biology-An Insight to New Applications and Developments;vol. 1. Shineeks Publishers. 2023. Available from: https://books.google.co.in/books/about/Advances_in_Chemical_Biology_An_Insight.html?id=3RLwEAAAQBAJ&redir_esc=y.
41. Bagade SB, Patil M. Recent advances in microwave assisted extraction of bioactive compounds from complex herbal samples: a review. *Critical Reviews in Analytical Chemistry*. 2021;51(2):138–149. Available from: https://doi.org/10.1080/10408347.2019.1686966.
42. Wang Y, Li R, Jiang ZT, Tan J, Tang SH, Li TT, et al. Green and solvent-free simultaneous ultrasonic-microwave assisted extraction of essential oil from white and black peppers. *Industrial Crops and Products*. 2018;114:164–172. Available from: https://doi.org/10.1016/j.indcrop.2018.02.002.
43. Sillero L, Prado R, Labidi J. Simultaneous microwave-ultrasound assisted extraction of bioactive compounds from bark. *Chemical Engineering and Processing - Process Intensification*. 2020;156:108100. Available from: https://doi.org/10.1016/j.cep.2020.108100.
44. Sumere BR, Souza MC, Santos MP, Benzerra RMN, Cunha DTD, Martinez J, et al. Combining pressurized liquids with ultrasound to improve the extraction of phenolic compounds from pomegranate peel (Punica granatum L.). *Ultrasonics Sonochemistry*. 2018;48:151–162. Available from: https://doi.org/10.1016/j.ultsonch.2018.05.028.
45. Wen C, Zhang J, Zhang H, Dzah CS, Zandle M, Duan Y, et al. Advances in ultrasound assisted extraction of bioactive compounds from cash crops-A review. *Ultrasonics Sonochemistry*. 2018;48:538–549. Available from: https://doi.org/10.1016/j.ultsonch.2018.07.018.
46. Corrêa PS, Júnior WGM, Martins AA, Caetano NS, Mata TM. Microalgae biomolecules: Extraction, separation and purification methods. *Processes*. 2020;9(1):1–43. Available from: https://doi.org/10.3390/pr9010010.

47. Bommakanti V, Ajikumar AP, Sivi CM, Prakash G, Mundanat AS, Ahmad F, et al. An Overview of Herbal Nutraceuticals, Their Extraction, Formulation, Therapeutic Effects and Potential Toxicity. *Separations*. 2023;10(3):1–28. Available from: https://doi.org/10.3390/separations10030177.
48. Liga S, Paul C, Péter F. Flavonoids: Overview of biosynthesis, biological activity, and current extraction techniques. *Plants*. 2023;12(14):1–25. Available from: https://doi.org/10.3390/plants12142732.
49. Nair KS, Shanmugapriya, Logeiswariy P, Sreeramanan S, Shakila R, Chen Y, et al. Chapter 3 - Purification of herbal biomolecules. In: Herbal Biomolecules in Healthcare Applications. Elsevier. 2022;p. 47–62. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00010-X.
50. Patil SS, Rathod VK. Extraction and purification of Curcuminoids from Curcumalonga using microwave assisted deep eutectic solvent-based system and cost estimation. *Process Chemistry*. 2023;126:61–71. Available from: https://doi.org/10.1016/j.procbio.2022.11.010.
51. Lin GD, Li RW. Chapter 7 - Analytical characterization of herbal biomolecules. In: Herbal Biomolecules in Healthcare Applications. 2022;p. 121–144. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00002-0.
52. Shahverdi AR, Abdolpour F, Monsef-Esfahani HR, Farsam H. TLC bioautographic assay for the detection of nitrofurantoin resistance reversal compound. *Journal of Chromatography B*. 2007;850(1-2):528–530. Available from: https://doi.org/10.1016/j.jchromb.2006.11.011.
53. Hamburger MO, Cordell GA. A direct bioautographic TLC assay for compounds possessing antibacterial activity. *Journal of Natural Products*. 1987;50(1):19–22. Available from: https://doi.org/10.1021/np50049a003.
54. Cannell RJP. Natural Products Isolation;vol. 4. 1st ed. New Jersey. Humana Totowa. 1998. Available from: https://doi.org/10.1007/978-1-59259-256-2.
55. Fan XH, Cheng YY, Ye ZL, Lin RC, Qian ZZ. Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines. *Analytica Chimica Acta*. 2006;555(2):217–224. Available from: https://doi.org/10.1016/j.aca.2005.09.037.
56. Donn P, Barciela P, Perez-Vazquez A, Cassani L, Simal-Gandara J, Prieto MA. Bioactive Compounds of Verbascum sinuatum L.: Health Benefits and Potential as New Ingredients for Industrial Applications. *Biomolecules*. 2023;13(3):1–21. Available from: https://doi.org/10.3390/biom13030427.
57. Goti D, Dasgupta S. A comprehensive review of conventional and non-conventional solvent extraction techniques. *Journal of Pharmacognosy and Phytochemistry*. 2023;12(3):202–211. Available from: https://doi.org/10.22271/phyto.2023.v12.i3c.14682.
58. Shoyama Y, Tanaka H, Fukuda N. Monoclonal antibodies against naturally occurring bioactive compounds. *Cytotechnology*. 1999;31(1-2):9–27. Available from: https://doi.org/10.1023/a:1008051618059.
59. Li Z, Wu Q, Zhang X, Chen G. Advances in the Applications of Capillary Electrophoresis to Tobacco Analysis. *Current Analytical Chemistry*. 2023;19(1):77–99. Available from: http://dx.doi.org/10.2174/1573411018666220927094137.
60. Oritsetimenyin SO. Chapter 20 - Herbal biomolecules acting on central nervous system. In: Herbal Biomolecules in Healthcare Applications. 2022;p. 475–523. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00030-5.
61. Gregory J, Vengalasetti YV, Bredesen DE, Rao RV. Neuroprotective Herbs for the Management of Alzheimer's Disease. *Biomolecules*. 2021;11(4):1–19. Available from: https://doi.org/10.3390/biom11040543.
62. Chaki R, Ghosh N, Mandal SC. Chapter 6 - Phytopharmacology of herbal biomolecules. In: Herbal Biomolecules in Healthcare Applications. 2022;p. 101–119. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00026-3.
63. Abdullahi A, Tijjani A, Abubakar AI, Mazmi A, Ismail MR. Chapter 17 - Plant biomolecule antimicrobials: an alternative control measures for food security and safety. In: Herbal Biomolecules in Healthcare Applications. 2022;p. 381–406. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00024-X.
64. Khan IA, Hussain M, Munawar SH, Iqbal MO, Arshad S, Manzoor A, et al. Jasminum sambac: A Potential Candidate for Drug Development to Cure Cardiovascular Ailments. *Molecules*. 2021;26(18):1–13. Available from: https://doi.org/10.3390/molecules26185664.
65. Adeosun WB, Loots DT. Medicinal Plants against Viral Infections: A Review of Metabolomics Evidence for the Antiviral Properties and Potentials in Plant Sources. *Viruses*. 2024;16(2):1–20. Available from: https://doi.org/10.3390/v16020218.
66. Milia EP, Sardellitti L, Eick S. Antimicrobial Efficiency of Pistacia lentiscus L. Derivates against Oral Biofilm-Associated Diseases-A Narrative Review. *Microorganisms*. 2023;11(6):1–17. Available from: https://doi.org/10.3390/microorganisms11061378.
67. Gopika S, Prabu K. Antibacterial and antifungal activities of Ruellia tuberosa (l.) leaf and stem extract. *Journal of Functional Materials and Biomolecules*. 2022;6(1):498–501. Available from: https://www.researchgate.net/publication/365759215_Antibacterial_and_antifungal_activities_of_Ruellia_tuberosa_l_leaf_and_stem_extract.
68. Saeediye M, Talebi AF, Morshedloo MR. Antimicrobial activity of Ferula gummosa and Artemisia sieber essential oils in gaseous phase. *Science Academique*. 2022;3(1):1–09. Available from: https://scienceacademique.com/wp-content/uploads/2022/06/SA2125-Galley-Proof.pdf.
69. Cavazos P, Gonzalez D, Lanorio J, Ynalvez R. Secondary metabolites, antibacterial and antioxidant properties of the leaf extracts of Acacia rigidula benth. and Acacia berlandieri benth. *SN Applied Sciences*. 2021;3:1–14. Available from: https://doi.org/10.1007/s42452-021-04513-8.
70. Li N, Wang M, Lyu Z, Shan K, Chen Z, Chen B, et al. Medicinal plant-based drug delivery system for inflammatory bowel disease. *Frontiers in Pharmacology*. 2023;14:1–13. Available from: https://doi.org/10.3389/fphar.2023.1158945.
71. Saleem A, Saleem M, Akhtar MF. Antioxidant, anti-inflammatory and anti-arthritic potential of Moringa Oleifera Lam: An ethnomedicinal plant of Moringaceae family. *South African Journal of Botany*. 2020;128:246–256. Available from: https://doi.org/10.1016/j.sajb.2019.11.023.
72. Desam NR, Al-Rajab AJ. Chapter 19 - Herbal biomolecules: anticancer agents. In: Herbal Biomolecules in Healthcare Applications. Elsevier. 2022;p. 435–474. Available from: https://doi.org/10.1016/B978-0-323-85852-6.00001-9.
73. Rodriguez-Garcia C, Sanchez-Quesada C, Gaforio JJ. Dietary Flavonoids as Cancer Chemopreventive Agents: An Updated Review of Human Studies. *Antioxidants*. 2019;8(5):1–23. Available from: https://doi.org/10.3390/antiox8050137.
74. Yahfoufi N, Alsadi N, Jambi M, Matar C. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. *Nutrients*. 2018;10(11):1–23. Available from: https://doi.org/10.3390/nu10111618.
75. Abotaleb M, Samuel SM, Varghese E, Varghese S, Kubatka P, Liskova A, et al. Flavonoids in Cancer and Apoptosis. *Cancers*. 2018;11(1):1–39. Available from: https://doi.org/10.3390/cancers11010028.
76. Saddick S, Ahmed D, Gul H. Isolation of biomedically important bioactive compounds from Debregeasia Salicifolia with extraordinary antioxidant potential hepato-protectivity. *Journal of King Saud University*. 2023;35(2):1–6. Available from: https://doi.org/10.1016/j.jksus.2022.102459.
77. Huzio N, Grytsyk A, Raal A, Grytsyk L, Koshovyi O. Phytochemical and Pharmacological Research in Agrimonia eupatoria L. Herb Extract with Anti-Inflammatory and Hepatoprotective Properties. *Plants*. 2022;11(18):1–16. Available from: https://doi.org/10.3390/plants11182371.
78. Goorani S, Morovvati H, Seydi N, Almasi M, Amiri-Paryan A, Nazari F, et al. Hepatoprotective and cytotoxicity properties of aqueous extract of Glycyrrhiza glabra in Wistar rats fed with high-fat diet. *Comparative Clinical Pathology*. 2019;28:1305–1312. Available from: https://doi.org/10.1007/s00580-019-02939-6.
79. Asaad GF, Abdallah HMI, Mohammed HS, Nomier YA. Hepatoprotective effect of kaempferol glycosides isolated from Cedrela odorata L. leaves in albino mice: involvement of Raf/MAPK pathway. *Research in Pharmaceutical Sciences*. 2021;16(4):370–380. Available from: https:

//doi.org/10.4103/1735-5362.319575.
80. Jayanti S, Vitek L, Verde CD, Llido JP, Sukowati C, Tiribelli C, et al. Role of Natural Compounds Modulating Heme Catabolic Pathway in Gut, Liver, Cardiovascular, and Brain Diseases. *Biomolecules*. 2024;14(1):1–23. Available from: https://doi.org/10.3390/biom14010063.
81. Kancherla N, Dhakshibnamoorthi A, Chithra K, Komaram RB. Preliminary analysis of phytoconstituents and evaluation of anthelminthic property of Cayratia auriculata (in vitro). *Maedica*. 2019;14(4):350–356. Available from: https://doi.org/10.26574/maedica.2019.14.4.350.
82. Pervaiz I, Saleem H, Sarfraz M, Tousif MI, Khurshid U, Ahmad S, et al. Multidirectional insights into the phytochemical, biological, and multivariate analysis of the famine food plant (Calligonum polygonoides L).: A novel source of bioactive phytocompounds. *Food Research International*. 2020;137:109606. Available from: https://doi.org/10.1016/j.foodres.2020.109606.
83. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*. 2018;13:1–26. Available from: https://doi.org/10.1186/s13020-018-0177-x.
84. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. *Molecules*. 2016;21(5):1–18. Available from: https://doi.org/10.3390/molecules21050559.
85. Araujo NMP, Arruda HS, Santos FN, De Morais NR, Pereira GA, Pastor GM. LC-MS/MS screening and identification of bioactive compounds in leaves, pulp and seed from Eugenia calycina Cambess. *Food Research International*. 2020;137:1–10. Available from: https://doi.org/10.1016/j.foodres.2020.109556.
86. Baran H, Pietryja MJ, Kepplinger B. Importance of Modulating Kynurenic Acid Metabolism-Approaches for the Treatment of Dementia. *Biomolecules*. 2025;15(1):1–27. Available from: https://doi.org/10.3390/biom15010074.