Jorddy Neves Cruz *Editor*

Drug Discovery and Design Using Natural Products

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I would like to dedicate this book to my mother Carminda Neves da Cruz (in memoriam).

Preface

Drug design and drug discovery have transformed modern medicine, and natural products have played a pivotal role in this process. For thousands of years, various cultures around the world have recognized and utilized the medicinal properties of natural products. Many of the drugs currently used in medicine are derived from natural sources, highlighting the importance of natural products in drug discovery.

Natural products offer a rich source of drug candidates, with compounds obtained from plants, animals, and microorganisms, demonstrating promising therapeutic potential. These bioactive molecules have provided the foundation for the development of modern drugs. The drug discovery process involves identifying new drug candidates and optimizing them for use as medications. This book *Drug Discovery and Design Using Natural Products* aims to provide a comprehensive overview of this field, with a focus on the use of natural products as drug candidates.

The book covers various aspects of drug discovery and development, including the techniques used in drug design, such as 'Drug development projects guided by ethnobotany and ethnopharmacology', 'Artificial intelligence and discovery of microbial natural products', etc. Varieties of modern day tools have been utilized for natural product discovery, and I feel that this book is excellent to cover on such aspects. This book is divided into several sections, with each section covering a different aspect of drug discovery and design based on natural products. The introductory section provides an overview of the history of drug discovery and the role of natural products, while subsequent sections cover the isolation, characterization, and different classes of natural products used in drug discovery. This book also covers on the aspect of 'screening of compounds using molecular modeling approaches'. The third section of the book examines the future of drug discovery and design based on natural products with the help of in silico tools. The book also explores the sources of natural products with potential biological activities.

This book is a valuable resource for researchers, academics, and students in the fields of drug discovery, pharmacology, natural products, and medicinal chemistry, offering a comprehensive overview of this exciting and rapidly evolving area of research.

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Drug Development Projects Guided by Ethnobotany and Ethnopharmacology Studies

Sheikh Rezzak Ali, Shuby Kumari, Satyendra K. Prasad, Rupali S. Prasad, Saurabh K. Sinha, and Anshul Shakya

Abstract In order to determine the present state of knowledge on ethnobotany, ethnopharmacology, and its application to drug development, this work reviews a variety of texts and studies in these fields. Traditional medical systems based on plants continue to be crucial to healthcare since the majority of the world's population still relies on them as their primary form of treatment. Many significant development medications, including aspirin, digitoxin, vinblastine, reserpine, ephedrine, ergometrine, and atropine, have been discovered by following leads from traditional usage. The development of novel chemical entities for therapeutic use and potential lead compounds for the structural modification of current drugs to produce new and more powerful ones both rely heavily on natural products as a starting point. Ethnobotany has been crucial in the discovery of novel medicines for many years. In light of ethnopharmacological research, the development of modern treatment systems has benefited greatly from the discovery of drugs from natural sources. The application of current drug development concepts to the selection, authentication, extraction, biological screening, and analogue creation of plant-based natural products gives a thorough overview of the numerous methods employed in ethnobotanical and ethnopharmacological research.

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1 Introduction

The process of turning a molecule from a drug candidate to a product that has been authorized for sale by the relevant regulatory bodies is known as drug development. This occurs through early drug discovery, preclinical studies, clinical trial, regulatory review and post market monitoring. The speed of medication development is essential for its economic success, since development expenses make up roughly two-thirds of all research and development expenses and development time reduces the length of the drug‘s patent protection when it enters the market, development speed is a key factor in determining sales revenue. Even though the pharmaceutical industry is quite aware of the need to reduce the amount of time and money spent on development, both these parameters have risen significantly in the last 20 years. This is mostly due to external factors, especially the tougher standards employed by regulatory authorities to assess the usefulness and safety of new compounds (Rang and Hill 2013). Finding new chemical entities (NCEs) with the required properties of druggability and medicinal chemistry is the main component of new drug discovery. Both chemical synthesis and isolation from natural products are viable sources for these NCEs. According to study of the origins of new drugs from 1981 to 2007, majority of the drugs that have been approved since 1994 were based on natural products (Katiyar et al. 2012).

People have been utilizing material(s) existing in nature, derived from flora, fauna, and mineral sources to improve health and cure of diseases since ancient time (Lev and Amar 2000). According to their historic uses, many new medications have been derived from natural sources. Since around 80% of the world’s population still predominantly relies on traditional medicines for their primary treatment, these plant-based traditional medicinal systems continue to play a significant role in healthcare. Following leads from traditional applications has led to the discovery of several important modern pharmaceuticals, such as aspirin, digitoxin, vinblastine, reserpine, ephedrine, ergometrine, atropine, and tubocurarine (Anyinam, 1995). Natural products serve as a crucial starting point for the discovery of new chemical entities for therapeutic application as well as prospective lead compounds for the structural alteration of existing medications to create new and more potent ones. Although natural products include a wide range of complicated chemical structures, secondary metabolites from plants appear to be more biologically friendly and drug-like than drugs made entirely of synthetic materials (Balunas and Kinghorn 2005). The majority of the common human diseases, such as peptic ulcers, infectious diseases, cancers, as well as diseases of the digestive, cardiovascular, and respiratory systems, are treated with or prevented by using natural products and related medications (Newman et al. 2003). The use of modern medicine as a method

of treating human diseases has surpassed the use of traditional medicine (Yuan et al. 2016). But, in many nations, even rich nations, the use of medicinal plants for illness prevention and treatment has increased during the past few decades (Yatoo et al. 2017). Indeed, a wide variety of developed nations, including the China, the United Kingdom, France, and Germany, currently use a variety of medicinal plant extracts as prescription medications (Ji et al. 2017; Ruhsam and Hollingsworth 2018).

2 Ethnobotany

The study of the interaction between humans and plants is known as ethnobotany, which combines the "ethno" study of people with the "botany" study of plants (Martin 2004). The study of how people and plants interact over time and location is known as ethnobotany (Ur et al. 2016). Dr. John William Hershberger, an American botanist, used the term "ethnobotany"for the first time in 1895 and one year after he coined the term and proposed "ethnobotany"as a discipline that clarifies the cultural significance of the plants' trunks, which are used for clothes, construction materials, medicine, ornamentation, fences, hunting, fuel, food, agricultural equipment, and religious rituals (Ahmad et al. 2006). Because plants play a significant role in almost every aspect of human activity, ethnobotany encompasses a wide range of academic disciplines, including pharmacognosy, botany, biochemistry, agriculture, toxicology, medicine, nutrition, ecology, cognitive studies, comparative religion, sociology, anthropology, linguistics, history, and archaeology. Numerous scientists may now explore the many uses of plants because of ethnobotany's complex nature, which also opens the door to a broad range of approaches and applications (Alexiades and Sheldon 1996). It should be emphasized that this scientific discipline allows for the rebuilding of ethnic traditions, from small human tribes to vast civilizations, through the analysis of historical applications for food, wood, magic, and religion, as well as for medical and textile purposes (Norton 1981).

The globe and its environment have seen a significant transformation as a result of the quick development and advancements in science, technology, and the global economy. A growing market for natural products and phytomedicine has diverted research and development efforts into the production of new drugs in light of the striking advancements in human healthcare on the one hand and the degradation of the environment on the other. Many research organizations and businesses in this field are now focusing on traditional medicine, particularly the use of plants as a source for novel medications. To determine which plants are the best prospects for additional screening and chemical analysis, researchers employ ethnobotanical knowledge. Along with developing nations in Asia, this development tendency is not exclusive to Western nations (Farnsworth 1993). Often, chemical screening of new drug development is guided by ethnobotanical knowledge of medicinal plants. The first candidates for screening were conventional herbs with established clinical efficacy and safety. After that, in conjunction with local herbal medicine users, plant materials that had been collected and identified using ethnobotanical data and

phytochemical analysis were tested. Pharmaceutics, animal testing, and clinical studies are used to validate the identified lead ingredients of herbal remedies (Sheng-Ji 2001).

The most well-known classical medications produced from ethnobotanical sources include aspirin, vinblastine and vincristine, codeine and papaverine, colchicine, digoxin and digitoxin, tetrahydrocannabinol, and cannabidiol from *Filipendula ulmaria*, *Catharanthus roseus*, *Papaver somniferum*, *Colchicum autumnale*, *Digitalis purpurea*, and *Cannabis sativa*, respectively (Chadwick and Marsh 2008). The effectiveness of the anticancer drug paclitaxel serves as an encouraging example of the potential of plant-based components in pharmaceutical development. Based on ethnobotanical data from Chinese traditional medicine, oseltamivir was successfully created from *Illicium verum* Hook.f. during the avian flu outbreak. Additionally, ethnobotanical records helped isolate and produce the potent antimalarial medicine artemisinin from the plant *Artemisia annua* (Tu 2011). Drug discovery in Africa based on ethnobotanical leads has taken two directions: the traditional route of identifying single plant species with physiologically active chemicals and the standardization and characterization of traditional recipes for development as medications. The first method resulted in the identification of several biologically active molecules and the medical use of numerous African plants. Examples include the physostigmine obtained from *Physostigma venenosum* is used to treat glaucoma, and the recently discovered antiviral agents from *Ancistrocladus abbreviatus*. The second strategy aims to increase the use of blended medications in formulated dosage forms, may be more applicable to the needs of the underdeveloped rural regions, but it has received little attention (Iwu 2002).

2.1 Ethnobotany and Natural Products

Pharmaceuticals are often developed in the following order: discovery of active lead compounds, thorough biological testing, formulation of dosage forms, and multiple rounds of clinical research to determine the drug‘s safety, effectiveness, and pharmacokinetics profile. Clinical studies may reveal potential interactions with food and other medicines. Herbal medicines used in traditional medicine have long been an important part of the healthcare systems in many nations. Native Indians use a number of herbal remedies to effectively treat a range of illnesses. Although some of the traditional healers are still successfully using herbs to cure people, the expertise of herbal remedies is slowly fading. Local people in the region commonly utilize these plants to treat a variety of ailments. The communities freely share, care for, and sustain the traditional knowledge, skill, and traditions as their common property (Patwardhan et al. 2005; Rego et al. 2022). Studies into the traditional utilization of local flora have shown that, there is a wealth of local knowledge about many plant species’ physical and chemical qualities as well as their phenological and ecological characteristics, especially in the context of domesticated species.

Despite collaboration between ethnobotanists and pharmacologists has numerous advantages for both parties, there are obstacles which needs to be addressed in order to create effective relationships. The disparity in perspective that underpins the research goals of the two areas is complicated. While some pharmacologists still have bioprospecting as their goal, the field of ethnobotany is typically more interested in the cultural significance of the relationship between humans and plants than in prospecting for plant pharmaceuticals and knowledge about plants. In the past, bioprospecting conducted direct ethnobotanical research, but today the focus is on comprehending the human study population and examining the significance of the link between people and plants across a wide range of cultural contexts. Benefiting human study communities and frequently their related ecosystems has emerged as a key goal, significantly altering the kinds of hypotheses being investigated in contemporary ethnobotanical research as opposed to those conducted in the colonial past (Salick et al. 2003; Cunningham 2008). The ideas of sickness and healing are often seen differently by pharmacologists and ethnobotanists. When studying healthcare systems and medicinal plants, ethnobotanists operate within both a medical anthropological and an ethnopharmacological research framework, in contrast to most pharmacologists who only consider one model of health, which is a biomedical approach (Etkin 1993; Hahn 1995).

2.2 Ethnobotanical Approaches and Omic Techniques in Conjunction

For determining the most promising plant taxa or genes within those taxa among plants with well-known ethnobotanical applications for food and medicine, in addition to the phylogenetic approach, large data sets acquired using omic techniques (metabolomics, proteomics, genomics, transcriptomics) and their analyses using bioinformatic tools are helpful. These techniques and the resulting data sets help to better understand the evolutionary background of culinary and medicinal plants (Hao and Xiao 2015). The usage of metabolomics is expanding as a result of the fast-paced development of the primary analytical methods for metabolites such as high-performance liquid chromatography (HPLC), gas chromatography, and nuclear magnetic resonance. Monitoring the geographical and temporal distribution of relevant phytochemicals impacted by plant environmental and developmental signals is made possible by metabolomics, which aims to offer overall qualitative and quantitative descriptions of metabolites in organisms exposed to various contexts (Penuelas and Sardans 2009). It is becoming easier to identify new biosynthetic routes for specialized bioactive metabolites because to the integration of this metabolomic technique with genome-based functional characteristic of candidate genes from significant ethnobotanical plants. As a result, this integration has significantly increased the possibility of discovering and producing pharmaceutical and food items. For instance, traditional breeding techniques are being used to increase

the production of the antimalarial drug artemisinin, along with new high-yielding hybrids to transform *A. annua* into a strong cropping system and the renewal of the artemisinin biosynthetic pathway in a modified microbial host (Hale et al. 2007). The finding of several FAD2 phytoconstituents in a nonplant recombinant host system following correlation of the transcriptomes and metabolomes of developing seeds that accumulate unusual fatty acids is another illustration of the successful blending of omic techniques with ethnobotanical approaches (Sumner et al. 2015; Lima et al. 2022).

3 Ethnopharmacology

Efron and colleagues originally used the term "ethnopharmacology"in 1967 as the title of a book on hallucinogens—"Ethnopharmacological Search for Psychoactive Drugs" (Efron et al. 1967; Holmstedt 1967). Ethnopharmacology, defined by Rivier and Bruhn (1979), is a multidisciplinary field of study focused on the observation, description, and experimental exploration of indigenous substances and their biological actions (Rivier and Bruhn, 1979). Ethnopharmacology is a scientific approach to investigating the biological effects of any human-use product that can, in a very broad sense, either be useful or poisonous or have other immediate pharmacological consequences. Studies outlining the usage of beneficial plants are typically included in this definition; however, these studies are typically carried out with the intention of advancing an experimental investigation of botanical medicines (Heinrich et al. 2009). The areas of ethnopharmacology and ethnobotany are closely related. Ethnobotany is the study of the multifaceted interactions between cultural plant practices, with a particular emphasis on how diverse human societies manage, make use of, and perceive plants. On the contrary, ethnopharmacology is the interdisciplinary study of biologically active substances and traditionally prescribed indigenous medications (Soejarto et al. 2005). In order to explore physiologically active substances from plants, minerals, animals, fungi, and microorganisms, ethnopharmacology has a bigger range. Without examining any potential causal relationships with the substances or molecules contained, the initial step in these domains is to present the usage of extracts in a specific ailment (Sargin 2015).

In emerging nations in Asia, Africa, and South America where there is a legacy of indigenous medical knowledge, ethnopharmacology research methodology is frequently used. Additionally, ethnopharmacological research have proliferated considerably throughout Europe over the past ten years, concentrating particularly on the Mediterranean world, which includes Italy, Turkey, and Spain (Pieroni and Privitera 2014). People have connected with diverse plants since past civilizations in attempt to understand their biological impacts. Information about particular plants and how to use them to treat particular ailments has been verbally transmitted through various generations. The knowledge about medicinal plants was eventually scientifically documented in ethnobotanical field research (Hamburger and

Hostettmann 1991). Documentation states that a taxonomist collects and identifies the plant material. For botanical documentation, the species must be identified using its most recent, taxonomically valid Latin binomial, and voucher specimens must be deposited in an internationally accessible herbarium. The plant parts that are known to be utilized for medicine, such as the flowers, leaves, stems, barks, seeds, fruits, roots, or the entire plant, should be the subject of ethnopharmacological studies (Bambhole and Jiddewar 1985). Preclinical research is then conducted on these plant sections. After a proper experimental setup has been constructed in experimental animals, plant extracts are applied to animals. In order to identify the most efficacious fractions and identify the bioactive molecules in the way of bioactivity-guided fractionation and isolation studies, the fractions obtained by the phytochemical separation studies are subjected to the activity evaluation process at each step. Since all-natural products start out as combinations of chemically similar substances from which the active ingredient is separated and purified using additional extraction, chromatography, and crystallization techniques. Following purification, examinations on chemical structural characterization and different chemical synthesis are conducted to evaluate the structure–activity relation (Khalid et al. 2013; Almeida et al. 2022).

3.1 Ethnopharmacology and Drug Development

A botanist, ethnobotanist, ethnopharmacologist, or plant ecologist gathers and identifies the plant(s) of interest as the first step in the process of developing a medication from ethnomedicine or ethnopharmacology. Although the ethnopharmacology approach is based on pharmacology, chemistry, and botany other fields have also significantly contributed. To preserve and record significant cultural heritage before it is lost, as well as to look into and assess the agents used, are the goals of ethnopharmacology. As a result, it is extremely important in the evaluation of natural products, especially herbal medicines from traditional and folkloric sources. Ethnopharmacology includes field observations, descriptions of the use and results of conventional treatments, identification of plants, and phytochemical and pharmacological research (Cordell and Colvard 2005; Patwardhan 2005). Ethnopharmacology is much more than a discredited science with antiquated methods. It continues to serve as the scientific foundation for the creation of active therapeutics based on folk remedies from different ethnic groups. Its ultimate goal is to validate conventional remedies through the isolation of active ingredients or various pharmacological discoveries (Mukherjee 2005). In many regions across India, the use of Ayurvedic medicines and formulations has long been a crucial component in the treatment of various illnesses. Ayurvedic drug leads have produced a large number of drug candidates that are now widely used in commercial markets.

Along with many regulatory measures, the Indian government has made considerable efforts to advance the safety, stability, effectiveness, and application of herbal medicine. In fact, numerous contemporary medications that are used to treat serious

illnesses have been created using Indian medicinal herbs. These include medications such as psoralen, reserpine withanolide, sennoside, glycyrrhizin, and curcumin quinine (Mukherjee et al. 2010; Alves et al. 2023). Additionally, a number of lead molecules have been isolated including betulinic acid (an immunomodulatory agent), β-Asarone, and mahanimbine (AChE inhibitor, tilianin (a hepatoprotective)), and their reported pharmacological activities have been confirmed (Mukherjee et al. 2009).

Recently, a significant amount of ethnobotanical and ethnopharmacological research have been started in an effort to find novel pharmaceuticals. For many centuries, ethnobotany and ethnopharmacology have been significant contributor to the development of novel medicines. Table 1 lists drugs derived from ethnobotanical and ethnopharmacological studies and their applications.

4 Process of Ethnobotanical and Ethnopharmacological Drug Development

4.1 Plant Selection Guided by Ethnopharmacological Knowledge

The strategy is based on the tradition of plant use in ethnomedicine. For instance, andrographolide was obtained from the herb *Andrographis paniculata* that was utilized in ethnomedicine to cure dysentery. Similarly, this method was used to isolate several bioactive molecules from *Berberis aristata, Papaver somniferum*, and *Picrorhiza kurroa*. The potential plants are chosen using this method based on observation, description, and sometimes even some experimental evaluation (Katiyar et al. 2012). Traditional medicines from nations such as China and India have a long history with well-documented records that are based on a codified system of medications from botanical sources. Codified systems were built on strong intellectual foundations of pharmacology and human physiology, whereas the ethnomedicinal practice mostly rely on actual experiences. In contrast to ethnomedical traditions, where materials were mostly employed as crude extracts such as decoction and juices, the notion of pharmaceutical formulations was more established in the classical codified system. While the conventional system is heavily institutionalized, the ethnomedical practices are often confined and controlled by a small portion of the population. Bacosides, boswellic acid, artemisinin, and reserpine are some of the significant examples of natural products discovered by utilizing the methodology based on the codified system of medicine. These substances are used as memory enhancers, anti-inflammatory, antimalarials, and antihypertensive agents, respectively (Katiyar et al. 2012).

Table 1 Drugs developed from ethnobotanical and ethnopharmacological studies

Drug	Source	Pharmacological action
Acetyldigoxin	*Digitalis lanata*	Cardiotonic
Adoniside	*Adonis vernalis*	Cardiotonic
Aescin	*Aesculus hippocastanum*	Anti-inflammatory
Aesculetin	*Fraxinus rhynchophylla*	Antidysentery
Agrimophol	*Agrimonia eupatoria*	Anthelmintic
Ajmalicine, serpentine	*Rauvolfia serpentina*	Treatment for circulatory disorders
Allyl isothiocyanate	*Brassica nigra*	Rubefacient
Andrographolide	*Andrographis paniculata*	Treatment for bacillary dysentery, hepatoprotective
Anisodamine	*Anisodus tanguticus*	Anticholinergic
Anisodine	*Anisodus tanguticus*	Anticholinergic
Arecoline	*Areca catechu*	Anthelmintic
Asiaticoside	*Centella asiatica*	Vulnerary
Berberine	*Berberis vulgaris*	Treatment for bacillary dysentery
Bergenin	*Ardisia japonica*	Antitussive
Betulinic acid	*Betula alba*	Anticancerous
Bromelain	*Ananas comosus*	Anti-inflammatory, proteolytic
Caffeine	*Camellia sinensis*	CNS stimulant
(+)-catechin	*Potentilla fragarioides*	Hemostatic
Chymopapain	*Carica papaya*	Proteolytic, mucolytic
Cocaine	*Erythroxylum coca*	Local anesthetic
Codeine	*Papaver somniferum*	Analgesic, antitussive
Colchicine	*Colchicum autumnale*	Antitumor, antigout
Convallatoxin	*Convallaria majalis*	Cardiotonic
Curcumin	*Curcuma longa*	Choleretic
Cynarin	*Cynara scolymus*	Choleretic
Danthron	*Cassia species*	Laxative
Deserpidine	*Rauvolfia canescens*	Antihypertensive, tranquilizer
Deslanoside	*Digitalis lanata*	Cardiotonic
Digitalin	*Digitalis purpurea*	Cardiotonic
Digitoxin	*Digitalis purpurea*	Cardiotonic
Digoxin	*Digitalis purpurea*	Cardiotonic
Emetine	*Cephaelis ipecacuanha*	Amoebicide, emetic
Ephedrine	*Ephedra sinica*	Sympathomimetic, antihistamine
Etoposide	*Podophyllum peltatum*	Antitumor agent
Gitalin	*Digitalis purpurea*	Cardiotonic
Glaucarubin	*Simarouba glauca*	Amoebicide
Glycyrrhizin	*Glycyrrhiza glabra*	Sweetener, treatment for Addison's disease
Gossypol	*Gossypium species*	Male contraceptive
Hemsleyadin	*Hemsleya amabilis*	Treatment for bacillary dysentery
Hydrastine	*Hydrastis canadensis*	Hemostatic, astringent

(continued)

Table 1 (continued)

Drug	Source	Pharmacological action
Hyoscyamine	*Hyoscyamus niger*	Anticholinergic
Irinotecan	*Camptotheca acuminata*	Anticancer, antitumor agent
Kainic acid	*Digenea simplex*	Acaricide
Kawain	*Piper methysticum*	Tranquilizer
Kheltin	*Ammi visnaga*	Bronchodilator
Lanatosides A, B, C	*Digitalis lanata*	Cardiotonic
Lapachol	*Tabebuia species*	Anticancer, antitumor
a-Lobeline	*Lobelia inflate*	Smoking deterrent, respiratory stimulant
Monocrotaline	*Crotalaria sessiliflora*	Topical antitumor agent
Morphine	*Papaver somniferum*	Analgesic
Neoandrographolide	*Andrographis paniculata*	Treatment of dysentery
Noscapine	*Papaver somniferum*	Antitussive
Ouabain	*Strophanthus gratus*	Cardiotonic
Papain	*Carica papaya*	Proteolytic, mucolytic
Phyllodulcin	*Hydrangea macrophylla*	Sweetener
Physostigmine	*Cholinesterase inhibitor*	Cholinesterase inhibitor
Picrotoxin	*Anamirta cocculus*	Analeptic
Pilocarpine	*Pilocarpus jaborandi*	Parasympathomimetic
Podophyllotoxin	*Podophyllum peltatum*	Antitumor, anticancer agent
Protoveratrines A, B	*Veratrum album*	Antihypertensives
Pseudoephedrine	*Ephedra sinica*	Sympathomimetic
Nor-pseudoephedrine	*Ephedra sinica*	Sympathomimetic
Quinine	*Cinchona ledgeriana*	Antimalarial, antipyretic
Quisqualic acid	*Quisqualis indica*	Anthelmintic
Rescinnamine	*Rauvolfia serpentina*	Antihypertensive, tranquilizer
Reserpine	*Rauvolfia serpentina*	Antihypertensive, tranquilizer
Rhomitoxin	*Rhododendron molle*	Antihypertensive, tranquilizer
Rorifone	*Rorippa indica*	Antitussive
Rotenone	*Lonchocarpus nicou*	Piscicide, insecticide
Rotundine	*Stephania sinica*	Analgesic, sedative, tranquilizer
Salicin	*Salix alba*	Analgesic
Santonin	*Artemisia maritima*	Acaricide
Scillaren A	*Urginea maritime*	Cardiotonic
Scopolamine	Datura species	Sedative
Sennosides A, B	Cassia species	Laxative
Silymarin	*Silybum marianum*	Antihepatotoxic
Stevioside	*Stevia rebaudiana*	Sweetener
Strychnine	*Strychnos nux-vomica*	CNS stimulant
Teniposide	*Podophyllum peltatum*	Antitumor agent
Tetrahydropalmatine	*Corydalis ambigua*	Analgesic, sedative, tranquilizer
Theobromine	*Theobroma cacao*	Diuretic, vasodilator

(continued)

Table 1 (continued)

Drug	Source	Pharmacological action
Theophylline	*Theobroma cacao and others*	Diuretic, bronchodilator
Thymol	*Thymus vulgaris*	Antimicrobial
Trichosanthin	*Thymus vulgaris*	Abortifacient
Topotecan	*Camptotheca acuminata*	Antitumor, anticancer agent
Trichosanthin	*Trichosanthes kirilowii*	Abortifacient
Tubocurarine	*Chondrodendron tomentosum*	Skeletal muscle relaxant
Valepotriates	*Valeriana officinalis*	Sedative
Vasicine	*Adhatoda vasica* Nees	Respiratory stimulant
Vincamine	*Vinca minor*	Nootropic
Xanthotoxin	*Ammi majus*	Antivitiligo
Yohimbine	*Pausinystalia yohimbe*	Aphrodisiac
Yuanhuacine	*Daphne genkwa*	Abortifacient
Yuanhuadine	*Daphne genkwa*	Abortifacient

4.2 Authentication of Plant

Identification of the botanical origin and determination of the scientific name constitute very first part in the authentication of plant species. Macroscopic characterization is accomplished by comparing the plant material's organoleptic qualities (taste, color, odor, size, shape, texture, surface properties, and fracture characteristics) with accepted reference material (Smillie and Khan 2010). To distinguish and identify highly similar medicinal plants, the microscopic technique is typically used. This quick and easy procedure uses a microscope to identify intrinsic structural characteristics at the tissue and cellular levels. Ordinary light microscopes are normally sufficient for this purpose; however, polarization and fluorescence microscopes can also be employed to improve the detection's reliability (Lau et al. 2004; Liang et al. 2006). For the qualitative and quantitative examination of natural products, chromatographic methods such as capillary electrophoresis, high-performance liquid chromatography, thin-layer chromatography, and high-performance thin-layer chromatography are very helpful. Gas chromatographic technology is used to test herbal medications that include volatile principles (Liang et al. 2004; Muzammil et al. 2023). Thin-layer chromatography offers a preliminary fingerprinting of the natural product, and it is useful since it is straightforward and can analyze several samples in a single run. Natural medicine's volatile components offer the necessary fingerprints that may be used to identify plants. Due to its high separation efficiency, the need for just a little amount of material, and quick analysis, capillary electrophoresis is useful (Liang et al. 2004).

DNA barcoding may provide trustworthy information for the authentication and quality control of medicinal plants because each plant species' genetic composition is unique and untouched by situations such as environment, age, and others. This

approach covers a variety of concerns pertaining to taxonomy and population genetics and is commonly utilized in research and industry for molecular identification, the avoidance of illegal wildlife trade and collection, and the assurance of the quality of food and pharmaceutical products (Raclariu et al. 2018). British Pharmacopoeia has unveiled the first universal DNA-based technique of identification. The technique focused on plant sampling, DNA extraction, barcode region, purification, amplification, and sequence reference databases and utilized *Ocimum tenuiflorum* L. as an example (Sgamma et al. 2017).

4.3 Extraction and Isolation of Natural Compounds

Recent years have seen a large-scale adoption of chromatographic separation techniques combined with physiological activity-guided fractionation and isolation. In this method, the fractionation of the plant extract involves a stage process separation of the plant extract and is based on bioactivity rather than a class of component of interest. Further fractionation and testing are performed based on physicochemical parameters and bioactivity. All fractions are initially tested for bioactivity, and only those fractions with notable bioactivity are then processed more until they yield the pure isolate that is in charge of the desired biological activity. After identifying the active isolates, the chemical characterization and structural elucidation are carried out (Katiyar et al. 2012; Nothias et al. 2018). Using this method, several plant-derived natural chemicals have been identified, including the anticancer medicines paclitaxel and camptothecin from *Taxus brevifolia* and *Camptotheca acuminate*, respectively (Kinghorn 1994). Other natural remedies or modified versions of natural products have included the apomorphine derived from morphine; tiotropium, which is used to treat chronic obstructive pulmonary disease; galantamine, obtained from *Galanthus nivalis*; and arteether, derived from artemisinin; these are all natural products or modified versions of natural products.

4.4 Structure Elucidation of Isolated Components

The primary method used today to figure out the structural integrity of phytochemicals is spectroscopic analysis. After extracts are first biologically screened, high-performance liquid chromatography can quickly separate out the bioactive ones, and then nuclear magnetic resonance (NMR) spectroscopic analysis and liquid chromatography-mass spectrometric (LC-MS) are used to characterize the chemical makeup of the fractions. After LC-MS analysis of the isolates, the previously recognized compounds are first separated from the novel chemicals by comparing the MS data with those compounds, which are easily accessible in the web databases. Similarly, HPLC may be used to swiftly extract large quantities of pure compounds utilizing automated extract injection, followed by the collection of fractions, and

their structural elucidation can be achieved by MS and NMR analysis (Harvey 1999; Lawrence 1999). The emergence of analytical techniques such as mass spectrometry and NMR spectroscopy to isolate, purify, and elucidate the structure of natural products, as well as the development of effective fractionation methods such as counter-current chromatography, have now made it possible for natural product screening to fit within the timeframe of high-throughput screening (Wu et al. 2008). Crude extracts are also chemically characterized using analytical techniques, primarily utilizing LC-MS/MS and GC-MS/MS methods. This is done in addition to characterizing pure isolates. Even with a complicated composition, preliminary NMR spectroscopic analysis can be done to investigate the chemical components of the crude extract. The functional groups and substances, such as sugars, phenolics, steroids, terpenoids, and fatty acid esters, may be identified using the NMR spectrum data. It is possible to determine which chemical compounds or classes of chemical compounds are present in the extract, which aids in selecting the best separation technique for subsequent fractionation, such as reverse or normal phase chromatography (Gray et al. 2012).

4.5 Bioscreening of Extracts, Fractions, and Isolates

According to their stated ethnopharmacological and traditional usage, natural materials are typically examined for their biological activity. For instance, after a very excellent "hit" molecule has been attained, the traditional use of a medicinal plant for the treatment of diabetic complications may be investigated for its ability to lower blood sugar levels, and this traditional use is supported by scientific evidence. In vitro screening does not frequently duplicate the activity, though. Since most natural products have low yields, they can be biologically screened using a variety of bioassay techniques that produce quick, accurate findings. Numerous animals or human cell lines and microorganisms are used in these tests. In this regard, a number of precise and effective instruments have been created (Hamid et al. 2004; Coats et al. 2008; Freshney 2010). Animal models are still employed for biological screening of extract and pure substances from natural sources, despite the fact that this approach has limitations such the necessity for a significant number of samples, laborious and difficult experimental methods, limited sensitivity, and ethical concern. It is quite difficult to provide bioactive pure compounds in the quantities needed for animal testing since the practical yield of these substances is often fairly low. On the contrary, potential hits could be judged risky based on toxic side effects discovered during cell-based screening, which might have showed favorable safety profiles in the animal's body as a result of liver detoxification (Liska 1998).

The growth of research in the field of life sciences, which has revealed a range of pathophysiological processes and mechanisms of pharmacological activities, has enabled the development of several cellular and molecular bioassay techniques. Many of these bioassays meet the HTS method's timeframe criterion. The HTS approaches could greatly reduce the microgram-required test sample amounts for

screening, allowing the assessment of pure chemicals isolated in very small concentrations. Furthermore, by making it easy to quickly run bioassays on hundreds of samples (Sittampalam et al. 1997; Kell 1999).

4.6 Molecular Modeling and Natural Product Database

The bioactive natural products that are found can be utilized as lead compounds for the modification of structural characteristics to create new and more effective analogues by utilizing modern medicinal chemistry approaches, such as molecular modeling and combinatorial chemistry. Furthermore, because natural products and other compounds comprise a family of structurally related molecules, it is possible to collect several homologues from a single source that can reveal information about SAR. Molecular modeling and SAR studies are used in the current drug development of natural products to create analogues with higher potency, fewer harmful side effects, and better pharmacokinetic patterns. These analogues are created from isolated novel compounds with acceptable bioactivity. In vitro and in vivo biological assays can be used to assess the analogues with the best druggability after they have been generated in the lab (Kitchen et al. 2004).

Procedures for molecular modeling demand PDB-formatted, optimized three-dimensional (3D) structures of the ligands. The structures of recognized natural compounds may be retrieved from natural product databases and other databases such as PUBCHEM and ZINC in a variety of acceptable forms (Sorokina and Steinbeck 2020). To have the least amount of energy, the shape of the structures must be tuned. Energy reduction can be done before docking in independent structure construction and optimization tools such as Chimera, Chem 3D Ultra, and Avogadro or docking software such as AutoDock Vina and Discovery Studio (Chen et al. 2020). Using docking software, the 3D structures of natural compounds are docked to the target structure and rated by the binding energy. AutoDock, AutoDock Vina, FlexX, Discovery Studio, and MDock are common docking tools (Chen et al. 2020). Through the preparation of various analogues of the hits, the optimization process of hits is carried out by observing better affinity toward the target. The hits showing the best affinity are then developed, and various drug-like properties, including stability, pharmacokinetics, and pharmacodynamic properties, can be studied using QSAR software (Sullivan et al. 2014) (Fig. 1).

5 Conclusions

Numerous advancements in pharmaceutical research have been influenced by natural ingredients. There are numerous instances in history where the natural product served as both a therapeutic and a contribution to the discovery of a novel element of drug isolation. It is still appealing to screen mixtures of molecules to separate and

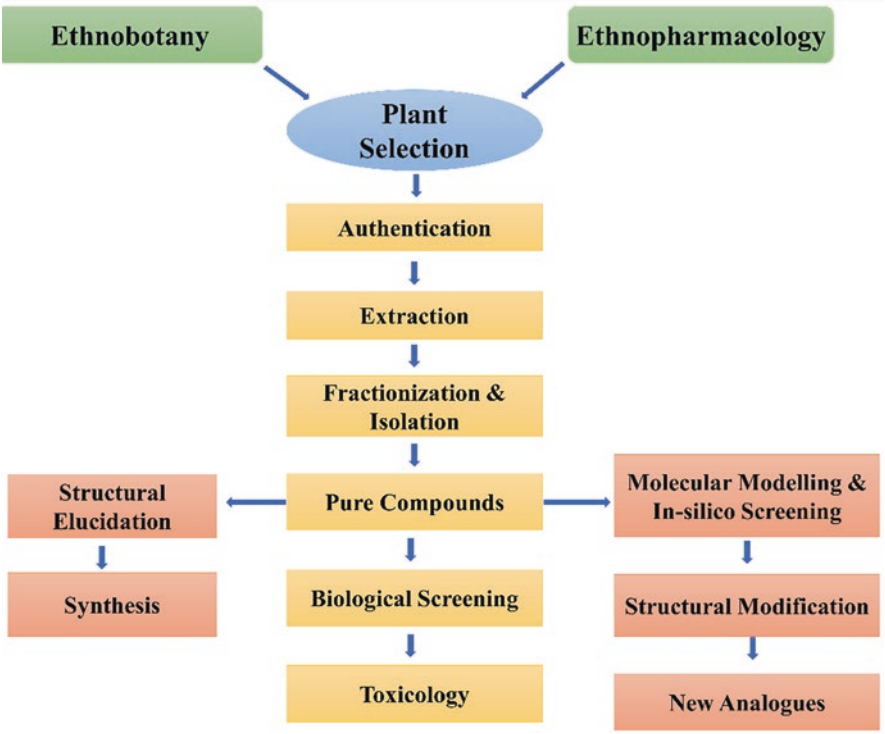

Fig. 1 The general methods used in the process of ethno-directed drug development

identify the active lead, not only from the plant extracts but also from microorganisms, because it is very time-consuming and expensive to generate huge libraries of isolated and structurally defined natural products. For many years, the primary goal of the pharma industry's research and development has been the creation of novel medications using components from medicinal herbs. Choosing plants at random or using knowledge-based criteria could possibly yield valuable chemicals for the pharmaceutical business. Traditional medical knowledge is crucial for people's healthcare in the past, present, and future. Information repositories on traditional medicine and ethnobotanical knowledge have contributed to medication development in China and many other nations in different ways, and they will do so going forward. There is also the incontrovertible fact that, at this time, no single medical system, whether it be Western, Eastern, allopathic, or homoeopathic, is perfect and comprehensive in its ability to treat all forms of illness and disease. Therefore, it is crucial for healthcare professionals everywhere to comprehend and assess the medical traditions that are used in every nation. Traditional Asian remedies have a significant role to play in this regard.

The most effective combinatorial chemists are plants, and they continue to offer undiscovered secrets of their therapeutic abilities to protect people from fatal diseases. The knowledge that is currently available about ethnic medicines has

generated various leads in both medication and healthcare research as well as served as a model for discovery. A significant amount of ethnic data used in healthcare is in danger of being lost due to the speed of modern industrialization. Therefore, it is the perfect time to create and record traditional knowledge and medicine in order to aid in the future development of effective medications for a variety of ailments.

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Natural Biopolymers as Scaffold

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Abstract Earlier, natural compounds and their structural analogs have significantly influenced pharmacology, particularly for the treatment of cancer and infectious disorders. However, natural products also pose difficulties for the development of new drugs, such as technological obstacles to screening, isolation, characterization, and optimization, which led to a drop in the pursuit of these substances. In this chapter, the therapeutic use of natural products and scaffold based on natural products have been discussed briefly.

Keywords Drugs · Scaffold · Natural products · Molecular target

1 Introduction

Scaffolds for tissue engineering are support systems created to promote cellular proliferation and growth after being implanted into a patient (Gomillion and Burg 2011). These scaffolds should have high biocompatibility which is determined by the important aspects such as biomaterial synthesis procedure, the scaffold manufacturing process, and the sterilizing conditions, and all have an impact on the material chemistry, scaffold structure, and morphology (Nardo et al. 2017).

Various scaffolds are created by employing a variety of biomaterials and the finest biological and material science concepts. Regardless of the tissue type, a variety

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of elements are important in the scaffold‘s creation. Some of the internal and external elements are crucial for the proper operation of a scaffold (Deb et al. 2018). Porous scaffolds can be created using naturally occurring biomaterials that have been extracted from their original sources. The ECM formed from allografts and xenografts is one example of a substance that can exist in its natural condition. Other examples include organic polymers such as proteins, polysaccharides, lipids, and polynucleotides, as well as inorganic ceramics such as calcium phosphates. Since cells may adhere and grow with great viability on natural biomaterials, they typically have excellent biocompatibility (Chan and Leong 2008).

Over the years, numerous scaffolds found in the structures of natural products have produced a sizable number of approved medicines and therapeutic candidates for a variety of ailments. Natural product scaffolds, or the basic structure from a natural product that is used or altered by direct substitution and/or isosteric alterations, are numerous and have led to or are currently being researched as leads to medications in numerous pharmacologic fields (Newman and Cragg 2009). Natural product scaffold diversity can be a powerful but difficult tool for exploring the larger chemical space and finding possible therapeutic leads (Wang et al. 2015). No screening library of synthetic compounds can compare to the biological activity and structural variety of natural products. Therefore, in order to create chemical libraries to find new therapeutic candidates, these privileged scaffolds act as significant, biologically prevalidated platforms (Davison and Brimble 2019).

In this chapter, various natural product-based scaffold and their applications have been discussed briefly.

2 Therapeutic Uses of Natural Products

2.1 Terpenes and Terpenoid

The largest and most diversified collection of naturally occurring substances is comprised of terpenes, sometimes referred to as terpenoids (Cox-Georgian et al. 2019). Terpenoids are oxygen-containing hydrocarbons, classified as a modified class of terpenes with various functional groups and oxidized methyl groups moved or removed at various positions, in contrast to terpenes which are described as compounds with simple hydrocarbon structures (Masyita et al. 2022). Isopentenyl Pyrophosphate (IPP) and dimethylallyl pyrophosphate are the precursors of terpenes and terpenoids which are produced through the 2C-methyl-d-erythritol-4-phosphate pathway in the plastid and the mevalonic acid pathway in the cytosol, respectively (Oldfield and Lin 2012).

They are mostly present in plants and make up the bulk of essential oils made from plants. Terpenoids are essential for a plant’s physiology, reactivity to the environment, and growth and development. It performs a significant and wide range of roles among the natural products that offer medical benefits to an organism. They are typically found in plants such as tea, thyme, cannabis, Spanish sage, and citrus fruits such as orange, lemon, and mandarin. These have several medical applications such as anticancer, antiviral, antimalarial, pro-antibacterial, transdermal

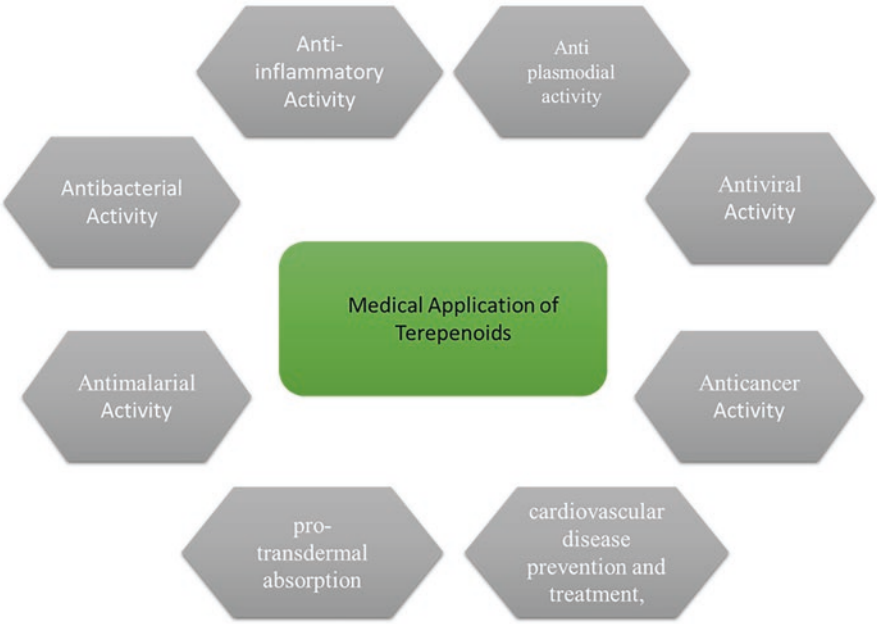

Fig. 1 Medical application of terpenoids

absorption, anti-inflammatory cardiovascular disease prevention and treatment, and hypoglycemic properties, and out of them, antiplasmodial activity stands out because of how similar their mechanism of action is to that of the widely used antimalarial medication chloroquine (Cox-Georgian et al. 2019) (Fig. 1).

With their specific structural characteristics and strong antitumor activity, terpenoids have caught the attention of many medicinal chemists and have the potential to serve as the basis for effective and secure anticancer drug development (Yang et al. 2020). Studies have identified paclitaxel, geraniol, and perillyl alcohol as terpenoids that exhibit significant antitumor effects (Chen et al. 2015; Galle et al. 2014; Kim et al. 2011; Yang et al. 2020).

Terpenes plays role in lessening of the symptoms of inflammation, by reducing the secretion of proinflammatory cytokines such nuclear transcription factor-κB, interleukin 1, and tumor necrosis factor-α. Most investigations have found that terpenes generally have the effect of decreasing the expression of proinflammatory cytokines. For instance, in the RAW 264.7 macrophage cell line, certain terpenes such as borneol, α-phellandrene, triterpene glycosides, terpinolene, and D-limonene can lower the production of tumor necrosis factor, interleukin-1 (IL-1), and interleukin-6 (IL-6) (Liang et al. 2010; Prado-Audelo et al. 2021).

2.2 Alkaloids

Alkaloids make for around 20% of all known secondary metabolites found in plants (Kaur and Arora 2015). Alkaloids are naturally occurring substances with the main sources of plants, particularly specific blooming plants and also produced by

animals, bacteria, and fungi typically containing carbon, hydrogen, nitrogen, and oxygen (Hussain et al. 2018; Perviz et al. 2016). They are produced in response to environmental changes and biotic or abiotic stress, which gives them a variety of structural characteristics and important biological functions.

Alkaloids in plants control growth and shield them from predators. Both human treatment and natural defense mechanism of an organism depend heavily on alkaloids. Alkaloids are particularly well known for their therapeutic uses as anesthetics, cardioprotectants, and anti-inflammatory drugs. Numerous well-known alkaloids are employed in clinical contexts, including nicotine, ephedrine, strychnine, quinine, and morphine (Kurek 2019). Interest in bioactive natural compounds has recently increased due to both their potential for drug discovery and a very aggressive development in the study of traditional remedies (Heinrich et al. 2021). They are also best therapeutic and management tools for reducing the key symptoms of neurodegenerative disorders such Alzheimer's disease, stroke, schizophrenia, and Parkinson's disease (Hussain et al. 2018).

It has also been demonstrated that alkaloids interact with a variety of biological targets and in antiviral activity (Rao and Venkatachalam 2000). The primary antiviral activities of many widely used phytochemical substances include antioxidant qualities, scavenging aptitudes, inhibition of DNA and RNA synthesis, and viral replication suppression, which may be attributed to the synergistic effects of more than one mechanism. Even though the cell membrane is not the intended target, several natural alkaloids successfully interfere with it and indirectly work by attaching to the viral glycoprotein. The amphiphilic nature of alkaloids probably explains why they have antiviral properties, especially as an entrance inhibitor that prevents viral attachment (Abookleesh et al. 2022). These natural alkaloids can also be used to make brand-new potent medications such as inflammatory bowel disease (Peng et al. 2019).

2.3 Phenylpropanoid

Plant phenylpropanoids are a large and structurally varied class of metabolites produced from phenylalanine that are essential in the interaction of plants with other living things (Soledade et al. 2010). Plants undergo biosynthesis to convert phenylalanine and tyrosine, which possess an aromatic ring and three carbons, into phenylpropanoids (Kawaguchi et al. 2017).

All plants have the general phenylpropanoid pathway, which is responsible for producing a range of secondary metabolites, such as phytoalexins that prevent pathogen invasion and precursors for the lignin biosynthesis, production of metabolites involved in mediating plant–microbe interactions as well as numerous flavonoids such as flavones and isoflavones. (Stacey 2007). They are widely used and serve important roles in plant development by providing as vital elements of cell

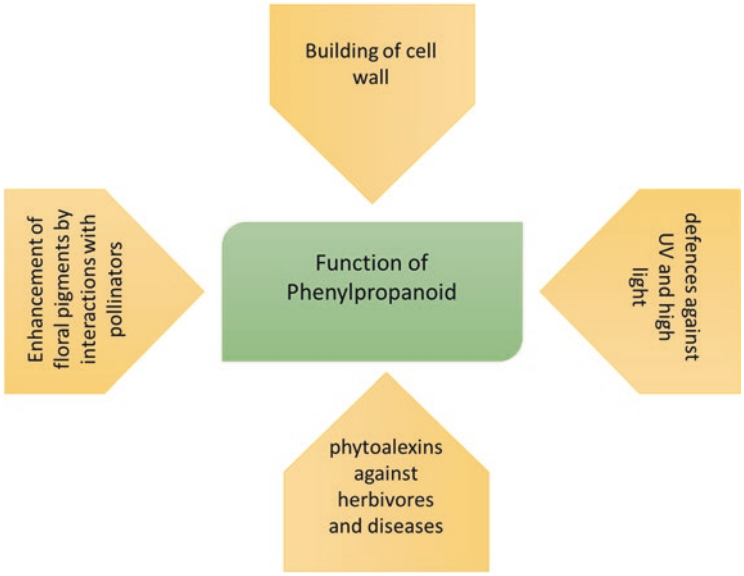

Fig. 2 Function of phenylpropanoid

walls, defense against UV and high light, act as phytoalexins against herbivores and diseases, and enhance floral pigments to mediate interactions with pollinators (Fig. 2). Phenylpropanoids also have a variety of biological properties that are advantageous to human health (Agar and Cankaya 2020).

Since 2007, natural and manufactured phenylpropanoids have garnered a lot of attention because to their potential medical applications as antibacterial agents, UV screens, antioxidants, antiviral, anti-inflammatory, anticancer, and wound healing,. They are highly used as active natural ingredients in the cosmetic industries and perfumery (Korkina 2007).

Naringin, a flavonoid, is a metabolic product of phenylpropanoid pathway possessing significant therapeutic use. It has been reported that exposure to naringin in vivo and in vitro in a variety of test animals and cell lines has activities that may be used to treat tumors, hyperthyroidism, hyperlipidemia, asthma, diabetes, and osteoclastogenesis. According to the study, naringin may be an effective natural medication for treating human metabolic diseases (Sharma et al. 2019). Also, esters of phenylpropanoid such as phenylpropanoid sucrose esters are naturally occurring substances structurally distinguished by a sucrose core that is linked to one or more Ph-CH=CH-CO- moieties via an ester bond isolated from different plants. These substances have been widely utilized in traditional medicine, and it has been discovered that they have a variety of biological properties, including glycosidase inhibitory actions antibacterial, antioxidant, antiviral, anti-inflammatory, neuroprotective, and anticancer activity (Panda et al. 2011).

2.4 *Evodiamine*

Evodiamine, a quinolone alkaloid, is extracted from *Fructus evodia* and *Evodia rutaecarpa* (Li et al. 2022a, 2022b). It has a variety of biological effects, including those on the antinociceptive, release of testosterone, uterotonic effects, antiobesity, vasodilatory, thermoregulatory, catecholamine, and anti-inflammatory. They has the strongest cytotoxic action against human colon and hepatoblastoma cell lines and inhibitory activity on human colon carcinoma cell migration, according to studies on the cytotoxicity or inhibitory activity on cancer cell migration screening of alkaloids (Jiang and Hu 2009). These are also a strong inducer of apoptosis in human nonsmall cell lung cancer A549 cells (Zou et al. 2015), and it has been demonstrated that evodiamine-induced apoptosis occurs downstream of mitotic arrest and subsequent mitotic slippage (Luo et al. 2021).

In mice, evodiamine appears to be the most effective treatment in terms of reducing tumor volume and weight, which boosts our faith in the findings and their applicability in the real world. This is due to the ability of evodiamine to inhibit proliferation, prevent invasion, and trigger apoptosis in animal tests (Yin et al. 2021).

Evodiamine also showed strong antiproliferative effects on human lung cancer A549 cells. The suppression of cyclin A, cdk2, p-cdc2, and cyclin B1 and the increase of p-chk1 and p-chk2 were well linked with the cell cycle arrest. Additionally, evodiamine dramatically lowered procaspase-3 and raised the ratio of Bax/Bcl-2, indicating that it promoted apoptosis through the intrinsic apoptotic mechanism (Hong et al. 2014). It also inhibited ovarian cancer cells from growing by inducing intrinsic and extrinsic apoptosis, as well as G2/M arrest. Additionally, cell death may be influenced by evodiamine-induced PI3K/Akt, ERK1/2, MAPK, and activation of p38 MAPK pathway (Lijuan et al. 2016). Additionally, breast cancer MDA-MB-231 cell migration has been inhibited by evodiamine, and lung metastasis has been significantly decreased. Additionally, it activated caspase to cause apoptosis in cancer cells. When compared to the control group, xenografted mice treated with evodiamine displayed nearly 50% reduction in lung metastasis (Koltai 2018).

Evodiamine also plays role in upregulation of $CD8^+$ T cells and downregulating the MUC1-C/PD-L1 axis, thus inhibiting nonsmall cell lung cancer (Jiang et al. 2020). It has also been showed that evodiamine therapy might stop the cell cycle from progressing and suppression of carcinogenesis, and cause caspase-dependent cell death in human urothelial cell carcinoma cells. After being exposed to evodiamine, human urothelial cell carcinoma cells displayed an inherent caspase-dependent apoptosis with or without an extrinsic caspase-dependent apoptosis pathway (Shi et al. 2017).

2.5 Tetracyclines

Tetracyclines are organic compounds produced by *actinomycetes* during fermentation. Ben Duggar at Lederle Laboratories initially reported chlortetracycline, which is synthesized by *Streptomyces aureofaciens* and sold as aureomycin, in 1948. That same year, it was authorized for clinical usage (Duggar 1948). Soon after, researchers at Pfizer, New York discovered oxytetracycline, which was later given FDA approval in 1950 and was sold as Terramycin (Finlay et al. 1950). Over the subsequent two decades, more tetracyclines were also naturally occurring substances created by streptomycetes (Grossman 2016).

Tetracyclines are broad-spectrum antibiotics that work effectively against both Gram-positive and Gram-negative bacteria, as well as uncommon microorganisms such *rickettsiae, mycoplasmas,* and *chlamydiae,* as well as protozoan parasites (Chopra and Roberts 2001). By attaching to the ribosomal complex and blocking the interaction of aminoacyl-tRNA with the bacterial ribosome, tetracycline reversibly suppresses bacterial protein production. Tetracyclines are transported through membranes via porin channels in gram-negative bacteria and build up in the periplasm. Tetracycline molecules attach reversibly to the prokaryotic 30S ribosomal subunit once they have entered the bacterial cell, halting protein synthesis (Eliopoulos et al. 2003).

These drugs are widely used in the treatment of infections in both humans and animals because to their effective antibacterial capabilities and lack of significant unfavorable side effects. Additionally, they are utilized as a preventative measure against mefloquine-resistant *Plasmodium falciparum* malaria (Chopra and Roberts 2001).

Tetracycline has found to be a strong affinity for hard tissues and can adhere to the surfaces of teeth. With positive clinical and bacteriological outcomes, it is utilized locally in periodontics, and the derivative doxycycline serves as the active ingredient in antibiotics (Ørstavik 2010). Early-seropositive rheumatoid arthritis has been treated using tetracycline's action on matrix proteins as well as its immunomodulatory effects, which include upregulating interleukin-10 and regulating T-cell and B-cell function (Gaur and Bal 2022).

3 Natural Product-Based Scaffold

3.1 Alkaloid-Based Scaffold

The scaffolds of alkaloid structures in drugs and leads are derived from those found in nature. In addition to pyridine and piperidine, scaffolds such as quinolinone, quinazoline, and isoquinoline are also available, as well as indoles, indolinones, isoindoles, isoxazoles, imidazoles, indazoles, thiazoles, pyrazoles, oxazolidinones, oxadiazoles, and benzazepine (Kittakoop et al. 2014). Through a quick, adaptable,

three-step modular synthesis using easily and readily available indole derivatives, the complicated tetracyclic scaffolds are created with high yields and surplus enantiomers (Rossi-Ashton et al. 2020).

3.2 Phenylpropanoid-Based Scaffold

Plant secondary cell walls include lignin, a complex and amorphous biopolymer, made up of phenylpropanoid units such as cumaryl, coniferyl, and sinapyl alcohol that are randomly crosslinked. The lignin building components p-hydroxyphenyl, guaiacyl, and syringyl are created based on these monolignol units. There are various kinds of links that connect these building blocks (Witzler et al. 2018). Due to the synergistic interactions between organic lignin and inorganic nanocomposites, the resulting lignin-based nanomaterials can be exploited as high-value-added materials for prospective applications in several biological domains, particularly in drug/gene delivery and tissue engineering. Lignin can bind to nanomaterials or other multivalent metal ions due to its active functional groups (phenol, hydroxyl, and carboxyl groups), which act as both chelating and reducing agents to the center of the metal (Kumar et al. 2021).

Numerous studies have demonstrated the neuroprotective properties of salidroside, a phenylpropanoid glycoside produced from *Rhodiola rosea L*, which may be promising for nerve rehabilitation. The findings demonstrated that salidroside significantly improved Schwann cells proliferation and functionality. The underlying process may be because salidroside modifies neurotrophic factors, which then impacts Schwann cells growth. With a 12 mm gap of sciatic nerve damage, salidroside-PLGA/Schwann cells produced satisfactory results for nerve regeneration 12 weeks after implantation (Liu et al. 2017).

A guaiacol replaced with an allyl chain, eugenol is a phenylpropene belonging to the group of chemical substances known as phenylpropanoids. It is an oily liquid that is colorless to pale yellow and is obtained from several essential oils, particularly clove oil (Fadilah et al. 2017). In a study, phenylpropanoids 7 and 5-fluorouracil were combined to treat the human cervical cancer (HeLa) cell line with anticancer drugs. Compared to the separate treatments, there were a noticeably higher percentage of apoptotic cells in the combination. Compared to control, treatment with 5-fluorouracil and eugenol enhanced the proportion of cells in the G0/G1 and G2/M phases. Additionally, there was a rise in cells that were in the sub-G1 phase (Hemaiswarya and Doble 2013).

The natural products containing phenylpropanoids demonstrated the strongest antiplatelet effectiveness against ADP, arachidonic acid, and the thromboxane A2 agonist U46619 as well as a good ability to disrupt clot retraction. A strong relationship between antiplatelet potency and phenylpropanoids content (54%–86%) was discovered, pointing to the crucial function that this moiety plays in preventing blood clot formation (Tognolini et al. 2006).

Due to their characteristics that damage cell membranes, a variety of phenylpropanoids and their derivatives have been shown to have broad-spectrum antibacterial activity (Lima et al. 2016; Engels et al. 2012; Hemaiswarya et al. 2011; Khatkar et al. 2015). Additionally, phenylpropanoids have potent antioxidant properties, which are principally attributable to their structure's prolonged side-chain conjugation, hydroxyl function, and methoxyl group (Jia et al. 2018). Additionally, natural phenylpropanoids have been found to exhibit antityrosinase activity. This is because they share structural similarities with the natural substrates of tyrosinase, l-tyrosine, and l-DOPA (Takahashi and Miyazawa 2010).

In food industry, to lessen undesirable browning of food caused by tyrosinase and oxidative spoilage, novel compounds with natural scaffolds such phenylpropanoids C6-C3 backbone were created. Compared to kojic acid, the majority of the substances showed greater mushroom tyrosinase inhibition. In comparison to the reference compounds, kojic acid, and ascorbic acid, compound CE48 has shown superior antityrosinase and antioxidant action (Ahlawat et al. 2021).

3.3 Evodiamine-Based Scaffold

A highly effective indolopyrazinoquinazolinone derivative of evodiamine with low nanomolar inhibitory activity against the HCT116 cell line was found through systematic structural optimization and SAR investigations. Additional mechanistic investigations showed that it operated by simultaneously inhibiting Top1 and tubulin. It is found to a promising lead molecule for the creation of novel antitumor medications, demonstrated remarkable in vivo antitumor efficacy. Additionally, it has been demonstrated that scaffold hopping is a successful strategy for increasing the druggability of evodiamine (Wang et al. 2019).

The majority of the time, the hit-to-lead and lead-to-candidate processes will result in an increase in molecular weight and hydrophobicity. It is more likely that an initial structure with a low molecular weight will be optimized to produce drug-like candidates (Dong et al. 2010).

The free amine group in evodiamine has a moderate molecular weight and is easily converted into active derivatives with drug-like characteristics. We could learn anything about hit selection from this case after conducting SBVS structure-based virtual screening studies. The next SBVS study might incorporate such a structural requirement, a scaffold with a free N-H group and a molecular weight of roughly 350 (Dong et al. 2010).

In the study, the synthesis of anticancer compounds and the synthesis of novel evodiamine analogs, bearing a carboxyl group at position 5 of the evodiamine skeleton, have been carried. According to research on the antiproliferative activity of substances tested on the H460, MCF-7, and HepG2 cell lines, they were not very effective. Also, it has been reported that the activity of the discovered evodiamine derivatives as prospective topoisomerase I inhibitors based on recent observations that had been written about in the literature (Fig. 3). On the other hand, SIRT2 was

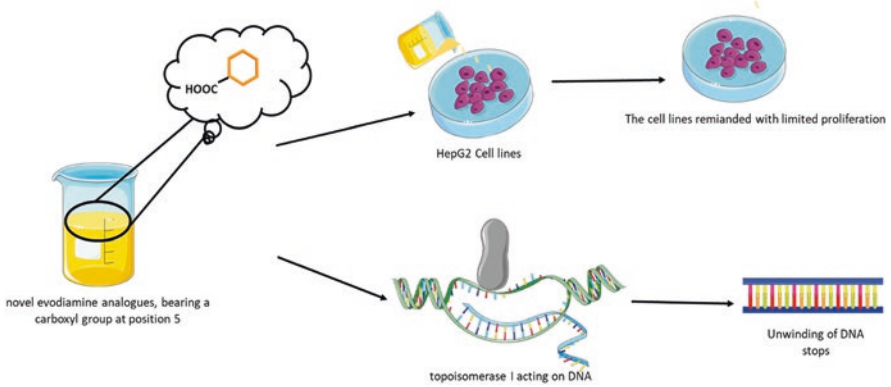

Fig. 3 Applications of novel evodiamine analogs

effectively inhibited by five of the identified compounds. S enantiomers appear to be marginally more advantageous. Three of them exhibit strong preference for sirtuins 2 over sirtuins 1 and sirtuins 3.

3.4 Tetracyclines-Based Scaffold

Tetracycline hydrochloride and Aloe vera both have considerable anti-inflammatory, antioxidant, and antibacterial qualities that help with skin tissue engineering. According to the release study, tetracycline hydrochloride was released initially in a burst and then continued over time. The fibroblasts' proliferation, adhesion, and spreading along the nanofiber orientation were encouraged by the release of tetracycline hydrochloride from the poly-ε-caprolactone/Aloe vera containing curcumin, and tetracycline hydrochloride-loaded hybrid nanofibrous scaffold, which also promoted the improved deposition of collagen. A wide range of antibacterial activity was present in the poly-ε-caprolactone/Aloe vera containing curcumin, and tetracycline hydrochloride-loaded hybrid nanofibrous scaffold against both Gram-positive and Gram-negative bacteria. In compared to poly-ε-caprolactone/Aloe vera and tetracycline hydrochloride-loaded hybrid nanofibrous scaffold loaded with curcumin, the tetracycline hydrochloride-laden poly-ε-caprolactone/Aloe vera scaffold showed higher biocompatibility, enhanced mechanical property, greater surface wettability, and antibacterial activity, and they can be employed for diabetic wound healing applications (Ezhilarasu et al. 2019).

For bone tissue engineering, bioglass(®)-based scaffolds that can also act as drug delivery systems have been developed. To do this, tetracycline-loaded P(3HB) microspheres were created and immobilized on the scaffold surfaces using a modified slurry dipping method. In simulated bodily fluid, it was discovered to have the capacity for prolonged drug delivery. The minimal cytotoxicity of the

tetracycline-loaded microspheres created in this study was demonstrated by the MTT experiment employing mouse fibroblast cells (Meng et al. 2013).

Dayaghi et al. (2019) have demonstrated that the presence of apatite layers produced on the surface scaffolds after 7 days was granted to support the bioactivity of magnesium-zinc and magnesium-zinc composite scaffolds with varying tetracycline concentrations scaffolds. Tetracycline's inclusion in the scaffolds'makeup ensures their antibacterial properties because it causes the inhibition zone to expand as tetracycline concentration rises. They are now prospective options for scaffolds used in bone tissue creation since they have developed biodegradable, bioactive, and drug delivery capabilities (Dayaghi et al. 2019).

4 Conclusion

Over the years, numerous "scaffolds"found in the structures of natural products have produced a sizable number of approved medications and therapeutic candidates for a variety of ailments. Unexplored molecular frameworks in natural goods are available for the creation of novel chemical leads and pharmaceuticals. The utilization of natural product-based scaffold offers biocompatible source of drug discovery and drug lead and can also be used in the treatment of disease and in tissue engineering.

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Artificial Intelligence and Discovery of Microbial Natural Products

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Abstract Natural products (NPs) have a variety of potential medicinal applications as drug or lead molecules for drug development. NPs are subset of small chemical molecules known as secondary metabolites that are produced by living organisms, including microorganisms. Artificial intelligence (AI) is the fourth industrial revolution that significantly reduces the cost and time of drug discovery. Over the past 10 years, the tremendous advancement in AI and its applications has improved the NPs discovery through the extensive analysis of large collected computerized, experimental genomics, transcriptomics, and metabolomics data. By using machine learning (ML) algorithms, AI can identify patterns and relationships within the data that are difficult or impossible to discern through manual analysis. AI can be employed in the discovery and elucidation of NPs' chemical structure, mechanism of action, toxicity, and phenotypic activity. AI can also assist in the design of experiments to screen for new microbial metabolites. By using optimization algorithms, AI can identify the most promising conditions for screening experiments based on a variety of factors, such as the characteristics of the microorganism, the type of metabolite being produced, and the growth condition. AI can aid in mining the microbial genome for the discovery and development of novel bioactive microbial metabolite. AI can assess a train model for virtual screening of NPs' chemical space database for biological active lead and subsequent inspiration of de novo design of

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NPs mimetics compounds with improved attributes. Taken together, AI has the potential to transform the future of NPs discovery and development.

Keywords Natural products · Artificial intelligence · Microbial metabolites · Condition optimization · Chemometrics · Pathways · Deorphanization

1 Introduction

Natural products (NPs) including microbial metabolites are characterized by their complex structures and unique chemical features (Newman and Cragg 2012). Identification of new molecules within the massive biodiversity of nature requires time, human resources, and technical equipment. Most of recently approved pharmaceutical drugs by the Food and Drug Administration (FDA) are NPs or NPs-inspired compounds (Schneider et al. 2022). For instance, the discovery of NPs has made a leap forward for the development of NP-inspired chemical entities (Hamoda et al. 2021). Despite the incomparable appeal of NPs as a source of inspiration for drug discovery, they have shown limitation of supply, partially drawn-out an expensive total synthesis, and their intricate structures (chiral centers, fused ring system, rotatable bonds and large molecular weight) (Yao et al. 2017). Therefore, the use of AI as a tool in discovery, improvement of the production, and optimization of structure and activity of NPs has emerged. In this chapter, details about the fundamental knowledge, tools, and application of AI in NP drug discovery are described.

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2 Machine Learning (ML) Algorithms in Microbial Drug Discovery

Machine learning (ML) algorithms have stumbled their way in NPs research as a reliable source of discovery of potential small molecule drugs (Battina 2017). First, an in silico generative method is used to generate several potential chemical possibilities. This is guided by topological similarity matching between the candidates and NP template, which is followed by the selection of top-ranking structures for the synthesis and biochemical validation. Finally, the identification of its biological target's and ADMET (absorption, distribution, metabolism, excretion, and toxicity) are carried out (Real et al. 2020) (Fig. 1).

The process of ML includes encoding NPs into molecular representations, molecular descriptors, similarity scores, chemical space, retrosynthesis, predicting biological roles, deorphanizing, and creating de novo compounds inspired by NPs (Zhang et al. 2021) (Fig. 2). Digitization of chemical information encodes the NP molecular representation for machine reading. Recently, the most advanced optical chemical entity recognition software DECIMER has been generated, to reveal chemical structures from published articles (Rajan et al. 2021).

Molecular representation includes international chemical identifier (InChI), simplified input line entry system (SMILES), SMILES arbitrary target specification (SMARTS), DeepSMILES, and SELFIEs (Krenn et al. 2022). They are developed to store and retrieve molecular information and to identify shared molecular features or substructures from databases (Raghunathan and Priyakumar 2022). Neural networks were trained with various chemical large datasets to capture the intricate connections between input information (topological fingerprint and molecular

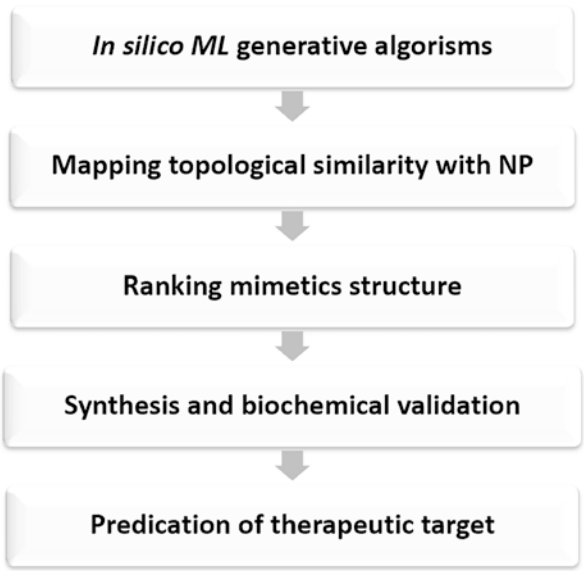

Fig. 1 ML generative model for in silico design of NP-inspired compounds

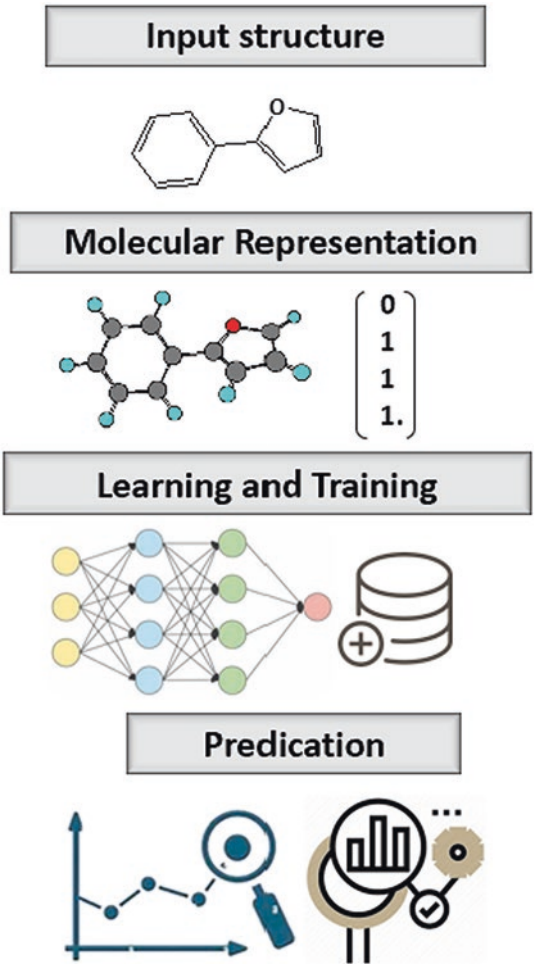

Fig. 2 General overview of ML model

descriptors) and decisions (prediction of biological activity). Most neural networks handled NP databases are COCONUT and its upgraded version LOTUS (Rutz et al. 2021) or dataset from the National Cancer Institute (NCI) (Kim and Chung 2022), datasets from the European Molecular Biology Laboratory such as ChEMBL (Mayr et al. 2018), MEGx NPdata base (Moret et al. 2020), SIDER (Jamal et al. 2017), and the NP bioactive fragment database that provided sufficient knowledge for combinatorial drug design (Zhang et al. 2021).

ML tools integrate dimensionality reduction techniques to map the NPs chemical space and the scanning of organic molecules to forecast their biological roles. Mapping the structural similarity of synthetic chemical space for NPs mimic bioactive compounds is firstly achieved by the NPs molecular fingerprint (NC-MFP) (Seo et al. 2020). Other metrics benchmarks, used to scoring the functions, are rapid overlay of chemical structures (ROCS) for spatial shape similarity and topological

fingerprints (Kearnes and Pande 2016; Hert et al. 2004). Recently, QSAR model described the use of three-dimensional (3D) fingerprints to rank and predict biological activity (Myint et al. 2012). LEMONS algorism used several techniques to compare NPs' molecular similarity with NP chemotypes for the potential identification of configurable NPs (Skinnider et al. 2017). Successfully, researchers have begun to use generative models and neural network topologies for molecular design of bioactive compounds (Friedrich 2019) such as potential kinase inhibitors of the discoidin domain receptor 1 (DD1), which were effectively created by deep learning (DL) model called Generative Tensorial Reinforcement Learning (GENTRL) (Zhavoronkov et al. 2019).

2.1 ML in Microbial Drug Discovery

The creation of predictive models using ML approaches is a powerful tool for virtual screening campaigns for the discovery of novel antibiotics (Diéguez-Santana and González-Díaz 2023). ML technology could leverage whole genome sequencing datasets to identify novel diagnostics agent (Smith et al. 2020), microbial resistance mechanisms (Chen et al. 2019), ultimately assisting in the identification of molecular targets, and the development of novel antibacterial drugs (Wang et al. 2022a). This is, in addition to its role to improve the efficacy of potential antibiotics, and to evaluate their pharmacokinetics and toxicity properties (Vamathevan et al. 2019). ML techniques have demonstrated tremendous promise for the accurate prediction of quorum-sensing peptides (QSPs) that successfully capture the sequence determinants to represent the feature descriptors of QSPs, hence improving predictive performance (Wei et al. 2020). QSPred-FL is a powerful bioinformatics tool used for the detection of putative QSPs in massive amounts of proteomic data by wrapping this feature representation learning technique and speed up the investigation of their functional processes (Wei et al. 2020). Dias and colleagues highlighted the growth of ML models in the innovative identification of antibiotics against methicillin-resistant bacteria (Dias et al. 2018). Furthermore, predicting the phenotypic properties of bacterial isolates from their genomic sequences has numerous potential impacts (Aun et al. 2018).

3 Genome Mining in Relation to Microbial Drug Discovery

Genome mining using AI has made it possible to find cryptic biosynthetic gene clusters (BGCs) in the microbial genomes and hence the experimental discovery of new bioactive NPs (Kim et al. 2021). Genes encoding enzymes involved in NPs biosynthesis can be readily identified in sequenced genomes by the use of computational sequence comparison tools of microbial DNA sequence databases (Zerikly and Challis 2009). Recent developments in genome sequencing have uncovered the

genetic and metabolic underpinnings of microbial NPs (Wambo 2022). Microbes have become biofactories to produce extracellular metabolites, peptides, and proteins through recombinant DNA technology, in addition to discovery of novel chemical entities (Pham et al. 2019). These secondary specialized metabolites are produced by enzymatic complexes such as polyketide synthases (PKSs), nonribosomal peptide synthases (NRPSs), or ribosomal synthesized and posttranslationally modified peptides (RiPPs) (Velásquez and Van der Donk 2011). Novel BGCs and predicted chemical structures have been examined by ML algorithms and pattern-recognition techniques (Gore 2020). Recurrent neural networks (RNN) were employed by deep learning (DL) algorithm Deep BGC's to discover novel BGC classes; afterward, Random Forest (RF) classifiers were applied to forecast the biological activities of those classes (Hannigan et al. 2019). Later on, an emerged combinatorial approach PRediction Informatics for Secondary Metabolomes (PRISM) trained and predicted NPs from bacterial BGCs such as Lincosamides, β-lactams, alkaloids, and aminoglycosides (Skinnider et al. 2015). Therefore, metabolite engineering by directly hacking the BGC biofactories such as streptomyces can provide innovative intricate NPs in easily sustainable manner (Aware and Jadhav 2022).

4 Computer-Assisted Prediction of Conditional Production of Microbial Natural Products

Computer-assisted prediction of conditional production of microbial products refers to the application of various computational tools such as ML algorithms, statistical modeling, and bioinformatics tools to forecast the production of microbial products in a controlled environment (Fig. 3). These computational tools are applied to estimate conditions such as temperature, pH, light, and nutrient media. These conditions affect the growth and metabolic activity of a microbe to optimize the production of desired microbial products. This can help to improve the efficiency and yield of microbial fermentation processes used in the production of various products such as biofuels, pharmaceuticals, and industrial enzymes.

4.1 ML Algorithms and Conditional Production of Microbial Products

Various ML algorithms are used to predict the optimum condition for microbial production. These include Artificial Neural Networks (ANN), Support Vector Machines (SVM), Random Forest, k-Nearest Neighbors (k-NN), and Gradient Boosting (Dutta et al. 2022). The criteria of algorithm selection depend on the data characteristics, the complexity of the system, and the research question. Table 1 provides a brief overview of some of the commonly used ML algorithms in the

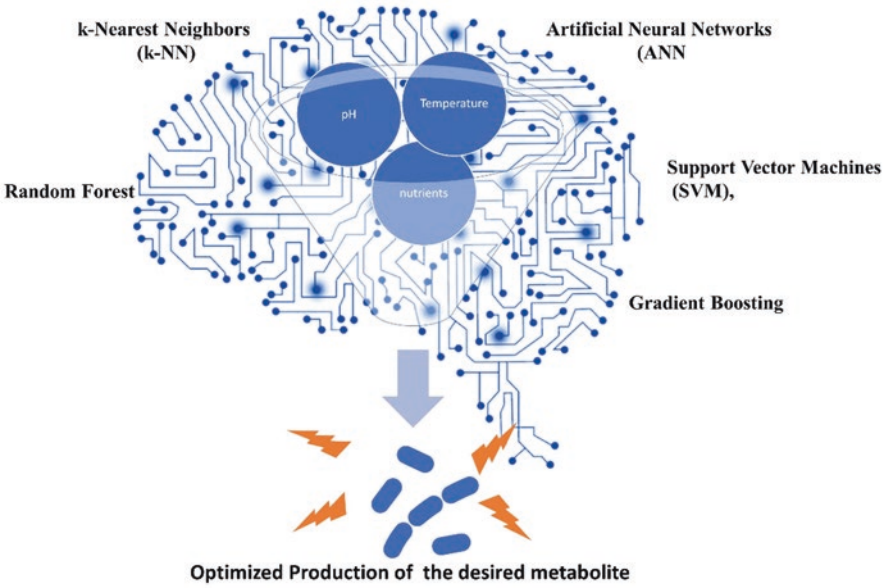

Fig. 3 The framework of computer-assisted prediction of conditional production of microbial natural products

prediction of microbial products, but other algorithms may also be considered depending on the specific application and dataset (Oyetunde et al. 2018).

4.1.1 Artificial Neural Networks (ANN)

Artificial Neural Networks (ANN) are a type of ML algorithm that models a complex relationship between inputs and outputs, making them well-suited for predicting microbial products. ANN was applied in the novel utilization of deoiled cake from *Guizotia abyssinica* (niger) for the cost-effective production of the potential anticancer drug L-asparaginase. ANN model estimated the influential process parameters, namely, autoclaving time, moisture, temperature, and pH, leading to a 1.36-fold improvement in enzyme activity (Sharma and Mishra 2022). Another application was reported to investigate the effect of incubation time and aspartic acid concentration on the predicted biomass concentration, *Bacillus* sporulation, and antifungal activity of compound AFA produced by *Bacillus amyloliquefaciens* CCMI-1 (Teresa Caldeira et al. 2011). Rafigh et al. applied response surface methodology (RSM) and ANN to optimize the culture medium and modeling of curdlan production from *Paenibacillus polymyxa (*Rafigh et al. 2014*).* Curdlan is a polysaccharide that is used in a variety of medical and industrial applications, including wound healing and as a thickening agent in food production. RSM and ANN were used to model cultural conditions of curdlan production; the maximum yield of curdlan production was predicted to be 6.68 and 6.85 g/L. ANN model was more

Table 1 Overview of common ML algorithms and their application in conditional production of microbial products

Algorithm	Description	Application	Reference
Artificial neural networks (ANN)	A type of ML algorithm modeled after the structure and function of the human brain.	Model complex relationships between inputs and outputs, making them well-suited for predicting microbial products.	Sharma and Mishra (2022) Teresa Caldeira et al. (2011) Rafigh et al. (2014)
Support vector machines (SVM)	A type of supervised learning algorithm that can be used for both classification and regression.	Particularly useful for predicting microbial products when there are many features or when the relationship between inputs and outputs is nonlinear.	Kumar et al. (2015) Packiam et al. (2022)
Random Forest	An ensemble learning method for classification and regression.	Random Forest is designed as an ensemble of decision trees that are constructed during the training phase. Each decision tree uses a subset of the data and features to make predictions. The output of the random Forest algorithm is either the mode of the classes (in classification) or the average prediction of the individual trees (in regression).	Packiam et al. (2022)
k-nearest neighbors (k-NN)	A nonparametric method is used for classification and regression.	A simple algorithm that stores all available cases and classifies new cases based on a similarity measure (e.g., distance functions).	Patel et al. (2021)
Gradient boosting	Boosting algorithm that builds a model in a forward stage-wise fashion	Allows for the optimization of arbitrary differentiable loss functions.	Grafskaia et al. (2022)

accurate compared to RSM, and the predicted production of curdlan was similar to the commercial curdlan production with an average molecular weight of 170 kDa as determined by gel permeation chromatography (Rafigh et al. 2014).

ANN and genetic algorithm (GA) were applied recently to optimize the production of carboxymethylcelluloses (CMCase) by *Trichoderma stromaticum* AM7 using peach-palm waste as a substrate in solid-state fermentation (SSF) (Singhal et al. 2022). The optimal influence of nitrogen source concentration, time, and temperature on cellulase production was determined using ANN-GA with 98% prediction efficiency of endoglucanase activity. The optimized parameters led to a three-fold increase in CMCase activity compared to initial fermentation, and the treatment of waste with *T. stromaticum* AM7 endoglucanase showed positive effects on fiber degradation and sugar release. This study highlights the potential use of ANN-GA and agro-industrial waste for cellulase production. Cellulase has the potential use in treating celiac disease, constipation, promoting insulin production,

and wound healing; however, most of these applications are still in the research phase, and further studies are needed to confirm their effectiveness, and safety.

4.1.2 Support Vector Machines (SVMs)

Support Vector Machines (SVMs) are a type of supervised learning algorithm that can be used for both classification and regression. They are particularly useful for predicting microbial products when there are many features or when the relationship between inputs and outputs is nonlinear. Kumar et al. developed an SVM-based two-level method to predict the β-lactamases protein responsible for bacterial resistance against β-lactam antibiotics. This method differentiated between β-lactamases and non-β-lactamases (Kumar et al. 2015). A web server, PredLactamase, was also developed to make the method available to the scientific community. This predictive tool might aid in both basic research and drug development.

4.1.3 Random Forest

Random Forest is an ensemble learning method for classification and regression. Random Forest is designed as an ensemble of decision trees that are constructed during the training phase. Each decision tree uses a subset of data and features to make predictions. The output of the Random Forest algorithm is either the mode of the classes (in classification) or the average prediction of the individual trees (in regression). Packiam et al. combine ML approaches with fermentation process conditions and amino acid sequence to predict the optimal protein yields and corresponding fermentation conditions for the expression of recombinant proteins in *E. coli (*Packiam et al. 2022*)*. Two sets of XGBoost classifiers were used in the first stage to classify the expression levels of the target protein, and a second-stage framework, consisting of three regression models involving Support Vector Machines and Random Forest, were used to predict the expression yields. The predictor achieved an overall average accuracy of 75% and a Pearson coefficient correlation of 0.91 for correctly classified instances. Such models can be used as a substitute for numerous trial-and-error experiments in identifying optimal fermentation conditions and yield for recombinant protein production.

4.1.4 K-Nearest-Neighbor (K-NN)

k-NN is a nonparametric method used for classification and regression. It is a simple algorithm that stores all available cases and classifies new cases based on a similarity measure (e.g., distance functions). Patel et al. applied the k-nearest-neighbor (k-NN) algorithm to optimize the production of mycophenolic acid (MPA) titer from *Penicillium brevicompactum* with respect to ultrasonic stimulation (Patel et al. 2021). During the ultrasonic treatment, different independent factors such as

ultrasound power, irradiation duration, treatment frequency, and duty cycle were studied to determine their ability to enhance the MPA titer value. The use of k-NN algorithm to optimize these factors increases the production of MPA by 1.64-fold compared to nonoptimized condition.

4.1.5 Gradient Boosting

Gradient Boosting is a boosting algorithm that builds a model in a forward stage-wise fashion; it allows for the optimization of arbitrary differentiable loss functions (Bentéjac et al. 2021). Grafskaia et al. focus on using the gradient-boosting approach (CatBoost algorithm) to design new antimicrobial peptides (AMPs) to combat antibiotic-resistant pathogens (Grafskaia et al. 2022). The researchers used a database of leech metagenome proteins and utilized the gradient-boosting approach to identify peptides with antimicrobial activity and reduced toxicity. Among the peptides identified, Hm-AMP2 was found to be the most promising, with strong antibacterial potential against both Gram-positive and Gram-negative bacteria, with minimal toxic and hemolytic effects (Grafskaia et al. 2022). The peptide can disrupt the bacterial membranes at low concentrations and adopts an α-helical structure in a membrane environment. The research also found that Hm-AMP2 interacts with lipopolysaccharides of different bacteria and can play a role in the defense against bacterial invasion (Grafskaia et al. 2022). The employed gradient-boosting approach was effective in identifying promising AMPs and could be useful for the rational design of effective, nontoxic peptide antibiotics (Bentéjac et al. 2021).

The future of computer-assisted prediction of conditioned production of microbial products is likely to continue to evolve and improve as a computational tool. Some potential areas of development could include the use of more advanced ML algorithms such as DL or reinforcement learning to improve the accuracy and efficiency of predictions. Additionally, the integration of big data and cloud computing technologies may enable the processing and analysis of larger and more diverse sets of data, which can further enhance the performance of computer-assisted prediction methods.

5 Chemometrics and Automated Microbial Drug Discovery in Dereplication Process

Dereplication is currently utilized to speed up NP screening and overcome the new drug discovery challenges. The initial definition of dereplication in 1990 was “the process of quickly identifying known chemotypes” (Beutler et al. 1990). Dereplication is applied nowadays for the discovery of new drugs. There are five dereplication categories (DEREP1-DEREP5), which are involved in the search for novel natural bioactive compounds; however, each category follows certain workflow according to different separation procedures, different starting materials, and

different structural elucidation techniques (Hubert et al. 2017; Nahar and Sarker 2018). Recently, a huge improvement has been made in the field of separation and identification of compounds in pure form and within a mixture such as microbial culture or plant extracts by employing a variety of advanced analytical techniques (Atanasov et al. 2021). These analytical techniques include high-performance liquid chromatography (HPLC) (Wolfender 2009), high-performance thin-layer chromatography (HPTLC) (Srivastava 2010), gas chromatography (GC) (Stavri et al. 2004), nuclear magnetic resonance (NMR) spectroscopy (Johansen et al. 2013), mass spectrometry (MS), combinations thereof, and MALDI-TOF MS (Tarfeen et al. 2022). Dereplication procedures have been applied in microbiological studies, mainly to identify the producer microbes by either morphology or gene analysis, and is used in combination with various chemometric techniques (Fiorini et al. 2022). Dereplication process performed in 5 main steps including (i) detection of the producer microbial colonies (Demarque et al. 2020), (ii) construction of metabolite library by UHPLC-MS profiling (Genilloud et al. 2011; Ito and Masubuchi 2014), (iii) identification of the active peak by micro-fractionation (Harris et al. 2011), (iv) comparative quantification of small-amount compounds (Khoury et al. 2018; Spina et al. 2021), and (v) identification of structures of small amounts (Sugiki et al. 2018).

Chemometrics utilize multivariate analysis of data derived from mathematical, statistical, and optical radiation for the quick separation of known and unidentified bioactive NPs from natural crude extract (Gaudêncio and Pereira 2015). The most typical statistical techniques employed to study NPs were listed by Cornejo-Baez and colleagues (Saldívar-González et al. 2022). These include supervised ML methods such as orthogonal projection to latent structures and partial least squares, in addition to unsupervised featured as principal component analysis (PCA), hierarchical cluster analysis and discriminant analysis (Granato et al. 2018). New biological insights were developed by using ML algorithms to extract information from metabolomic data (Liebal et al. 2020). Particularly, due to their capacity to make quantitative predictions, supervised machine learning algorithms such as SVM, Random Forest, ANN, and genetic algorithms have demonstrated significant promise in metabolomics research (Rafferty et al. 2020). The use of these algorithms has sped up the processing of analytical data, integrated omics information, and spurred biological applications. The overview of the application of AI approach in chemometrics discovery is shown in Fig. 4.

Chemical profiles are conducted through bioassay-guided isolation and utilization of dereplication process. Metabolomics analysis facilitates the bioassay-guided isolation by applying the multivariate data analysis to shorten the isolation path of an active compound, mainly in the identification and dereplication stages (Ho et al. 2021). Multivariate data resulted from measuring several variables exist in the same sample. The main steps involved in a chemometric analysis are experimental designing, data processing, classification, and calibration (Hanrahan and Gomez 2009). Modeling with chemometrics involves the utilization of instruments and software to interpret data patterns. Among methods included in chemometrics are PCA and prediction analysis; partial least square-discriminant analysis (PLS-DA). A multivariate analysis of data represents the statistical weights of the significant variables

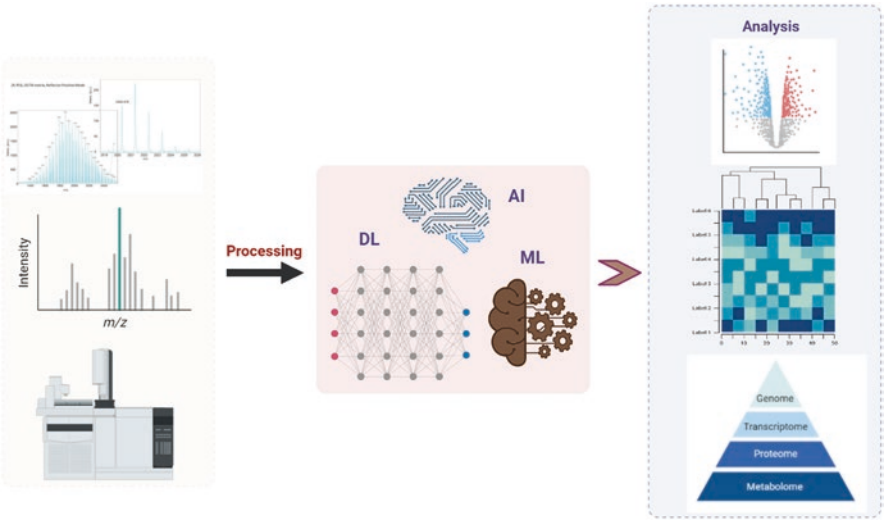

Fig. 4 Overview of application of AI approach in chemometrics automated discovery

distributed among individuals based on their respective biochemical contents (Berrueta et al. 2007). The purpose of PCA is to decrease the large dimensions of the data space to smaller dimensions to simplify data description, reduce the number of variables in a matrix, and to identify new variables. Interpretation of PCA can be performed through a correlation between the original variable and the new variable (Samirana et al. 2022).

For the aim of identification and dereplication, the metabolomic application is conducted through data reduction approach. The use of various analytical techniques coupled with multivariate analytical data can be performed to fasten the isolation route by applying bioassays together with data reduction approaches, especially in both identification and dereplication phases (Cheng et al. 2015). A study has reported that PCA was used to analyze ^{1}H-NMR data obtained from *Fusarium solani* and *F. oxysporum* isolated from *Senna spectabilis's* rhizosphere. The algorithm used loading values to choose the important peaks that differentiate between the species in PCA, leading to compound dereplication (Selegato et al. 2016).

5.1 Metabolite Dereplication Using MS and NMR Data

Dereplication of a metabolite by employing AI requires a careful comparative analysis of an extract and blank. LC-UV-MS is usually employed to extract comparable data in collision-induced dissociation (CID) process. Slicing the raw data into two datasets according to the ionization mode can be performed by MassConvert tool from ProteoWizard. The sliced datasets can be then imported into MZmine

(differential analysis framework for mass spectrometric data) where the high-resolution mass spectral datasets can be deconvoluted and deisotoped. Chromatogram builder is used for the peak detection in both the sample and blank. Individual peaks can then be detected by performing chromatogram deconvolution. This is followed by isotopes identification using the isotopic peak grouper, reduction of the inter-batch variation by the retention time normalizer, peak lists alignment (achieved by join aligner parameters) and gap filling peak finder is used to detect the missing peaks (Abdelmohsen et al. 2014).

Prediction of molecular formula and identification of a peak from the processed datasets are performed through a library creation by employing an algorithm from Antibase® or Marinlit® to recalculate the exact masses (Ricart 2020). Coupling the created library to MZmine using this custom database led to peak identification and dereplication. Xcalibur software is used to double check the hits and the unidentified peaks against the MS raw data (Li et al. 2020). Currently, several techniques are accessible for increasing the diversity and production of microbial secondary metabolites (Berdy 2005). HPLC has been used as a method to improve media, evaluate NP libraries, increase chemical diversity of collections, and even find relationships between species (Hubert et al. 2017). ML algorithms were used for the construction of NP collections and the identification of links between strains with various ancestries (Chen and Kirchmair 2020). It employed automated metabolite profiles identification and creating libraries of NP for drug development. Candidates that presented a clear MS/MS fragmentation pattern and concentrated on those with complex MS/MS profiles were ruled out, resulted in pinpointing of 10 promising strains that may be producing novel cyclic peptides. *Paramyrothecium* sp. was discovered to biosynthesize xylomyrocins A–C, which were confirmed by 2 D NMR analysis (Wang et al. 2022b).

The Global Natural Products Social Molecular Networking (GNPS) created visual molecular networks from enormous tandem MS datasets. Nodes in molecular networking (MN) are used to display high-resolution spectra, while edges are used to describe alignments between spectra. Tandem MS records cannot be aligned, and the molecules cannot be recognized as long as the reference spectra are absent in molecular databases. As an alternative, researchers have created programs that connect tandem MS spectra to specialized chemical databases to discover NP substructures such as CSI: FingerID (Blaženović et al. 2018), SIRIUS 4, and MS2LDA for small molecules (Qin et al. 2023), and VarQuest for peptides. ML methods (multiple kernel learning, and SVM) were used to build fragmentation trees from MS spectra and predict the presence or absence of large chemical fingerprints in unidentified chemicals such as ChemDistiller, MetFID (Fan et al. 2020), and the Critical Assessment of Small Molecule Identification (CASMI) challenge (Shen et al. 2013). Platt probabilities are used to assess and rank each molecular fingerprint. ML tools applied for chemometric and dereplication process are listed in Table 2.

Computer-assisted structural elucidation (CASE) tool facilitates the identification of NPs by comparing and integrating their 1D and 2D NMR spectral characteristics to potential matches of multitechnique databases (Castaing-Cordier et al. 2022). Automated CASE program with residual chemical shift anisotropy (RCSA)

and residual dipolar couplings (RDCs) were employed for the determination of relative configurations in molecules (Pereira and Aires-de-Sousa 2018). Recently, the proposed chemical structure of the aquatolide was revised based on RDC/RCSA data for the model structure revealed the unusual core structure, which was subsequently confirmed by X-ray crystallography (Pereira and Aires-de-Sousa 2018) (Fig. 5).

Small Molecule Accurate Recognition Technology (SMART 2.0) was the first ML-driven method described by Reher and his colleagues for the quick identification of JEOL database NPs (Reher et al. 2020). CNNs were trained on a set of 2D-NMR spectra from Heteronuclear Single Quantum Coherence spectroscopy (HSQC) of NCEs and ACD Labs Predictor (Zhang et al. 2017). SMART technology was employed for analysis of the filamentous marine *Cyanobacterium symploca* sp. mixture that revealed a novel cytotoxic swinholide known as symplocolide A (Zhang et al. 2017). CIM-ID as ML-based techniques established by Allen et al. increases the precision of computing molecular structure (Wang et al. 2021).

Among the recent examples is the discovery of polyol cyclodepsipeptides by applying HRMS-guided chemometrics (Wang et al. 2022b). Extraction of a loopful of collected mycelia was carried out from agar media, the extract was subjected to direct MALDI-TOF MS analysis. Around 182 strains out of 1748 were detected to produce a minimum of one set peptide-like metabolites. HCA was applied using imageGP platform to deconvolute the 182 peptide-containing extracts, resulting in 61 clades where each clade shares a characteristic fingerprint of peptides in MALDI-TOF MS profiles. A representative strain was selected from each of the 61 clades to perform a solid-state fermentation, and the production of the metabolites was monitored by LC-HRMS/MS.

6 Prediction of Biological Function and Deorphanization of Microbial Natural Products

6.1 AI in the Prediction of Biological Function of Microbial Metabolites

A metabolic pathway is a step-by-step series of interconnected biochemical reactions that use variable precursors through a series of metabolic intermediates to yield a final product molecule (LibreTexts Project 2023). Researchers have developed many public repositories for microbial metabolites according to their resources and the identification process (Table 3). FAIRsharing is a curated, informative, and educational resource that is employing metadata standards to inter-relate databases and data policies (FAIRsharing n.d.). FAIRsharing provided 1985 databases as registry of knowledge bases and repositories of data and other digital assets (FAIRsharing n.d.). In the same context, Integbio Database Catalog that was developed by the University of Tokyo (Integbio Database Catalog University of Tokyo

Table 2 Chemometric and dereplication software combined ML tools for NPs discovery process

Software/Website tools	ML and chemometric tools features	Reference
SWATH-MS	Identification of peptide biomarkers-based proteomic and chemometric analysis using ML models	Hu et al. (2018)
Mclust	R program chemometric analysis model-based clustering and associated techniques for density estimation and discriminant analysis	Fraley and Raftery (2007)
ChemDistiller	Mass spectrometry fingerprint and ML metabolite annotation	Laponogov et al. (2018)
MetFID	MetFID predicts compound fingerprints for metabolite annotation using ANN	Fan et al. (2020)
Deep kernel learning	Deep kernel learning optimizes the ability to predict chemical fingerprints from NP multiple spectral data	Dührkop (2022)
CASE	CASE speeded up novel NP identification by comparing and merging the 1D and 2D NMR benchtops of the NPs	Castaing-Cordier et al. (2022)
CASMI	First small molecule metabolite identification tool of the computational MS community	Shen et al. (2013)
PLSR	Combined with DL model for quick identification process	Divyanth et al. (2022)
SIMCA P	Chemometric software programming tools that can accommodate ML algorithm for secondary metabolite	Solihin et al. (2021), Shin et al. (2020)
MetaboAnalyst 5	Growing data, a platform for metabolomic analysis	Fernandes et al. (2019)
CSI: FingerID and SIRIUS 4	Discover novel NP substructure molecules by integrating ML algorithms for matching fragmentation ions with molecular substructures	www.csi-fingerid.org
MS2LDA	MS2LDA framework used unsupervised technique of latent Dirichlet allocation (LDA) to break down tandem MS data (MS2-) into groups of co-occurring fragments (called Mass2Motifs)	Blaženović et al. (2018) http://ms2lda.org/
VarQuest	Dereplicator tool for discovery and identification of novel peptides	Qin et al. (2023)
SMART	First ML-driven method employing CNN for the quick identification of novel NPs utilizing 2D-NMR spectra	Castaing-Cordier et al. (2022), Zhang et al. (2017)
CFM-ID	Hybrid ML and rule based for annotation, spectrum prediction, and metabolite identification from combinatorial mass spectra	Allen et al. (2014), Wang et al. (2021)

Library System n.d.) collectively includes the international life science databases and the associated basic metadata in Japan and all over the World. This catalog provided 2147 different databases with a description of these databases and information about different organisms. Users can identify many databases according to the selective species and the identified targets of metabolites, proteins, carbohydrates, and lipids (Integbio Database Catalog University of Tokyo Library System n.d.).

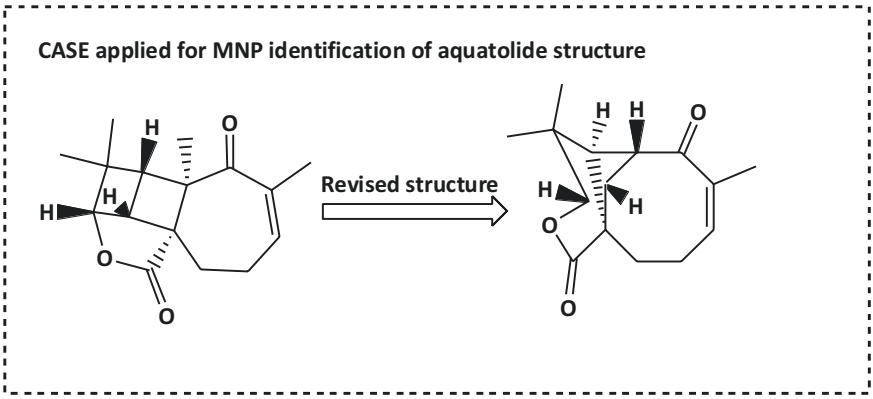

Fig. 5 Identification of MNP structure utilizing CASE tool

Integbio Database Catalog also provided batches of downloadable databases and a primitive map of correlated Japanese databases relying on the description of each database and other scientific terminologies to calculate the similarity between these databases (https://integbio.jp/dbcatalog/files/relation-map.pdf). This promising future correlation of different databases facilitate the use of a batch of databases that can unravel new unpredicted knowledge.

6.2 AI in the Identification of Microbial Metabolic Pathways

In a cell, metabolic pathways are linked together in a series of chemical reactions. Enzymes catalyze these reactions, where the product of one enzyme acts as a substrate for the next enzyme. ML is used to analyze metabolic pathways in three pipelines: prediction (Faust et al. 2011), design or reconstruction (Qi et al. 2014), and optimization (Planes and Beasley 2009) (Fig. 6). ML prediction refers to the output of an algorithm after it is trained on a historical dataset and applied to new data, hidden from the training data, and used for testing the designed model. The algorithm will generate probable values for an unknown variable for each record in the new data, allowing the scientist to identify the exact value (DataRobot n.d.). In MS, metabolites identification is performed based on ion fragmentation for accurate identification and quantification (Xiao et al. 2012). The prediction of the metabolites is based on the similarity of spectra in the database and the compound under investigation (Hufsky et al. 2014). Furthermore, the metabolite's retention time provides another ML angle that allows more insight into the true structure of that compound in comparison to the available database (de Cripan et al. 2022; Domingo-Almenara et al. 2019).

MS/MS machines accompanied with ML can provide direct identification of the microbial metabolites from biological samples. However, the reference database is

Table 3 Microbial software for compound/metabolite identification

Software/database	Microorganisms	Target	Web link
Automatic molecular interaction predictions: InteroPorc	Bacteria	Proteins	http://biodev.cea.fr/interoporc/
Autophagy database	Fungi	Proteins	http://www.tanpaku.org/autophagy/
BacMap	Archaea/ bacteria	Proteins	http://bacmap.wishartlab.com/
Bacterial protein interaction database: Bacteriome.org	Bacteria	Proteins	https://www.compsysbio.org/bacteriome/
BACTIBASE: Database dedicated to bacteriocins: BACTIBASE	Bacteria	Proteins	https://www.re3data.org/repository/r3d100012755
BCCM/IHEM biomedical fungi and yeasts collection: BCCM/IHEM	Fungi	Metabolites	https://bccm.belspo.be/
BioCyc	Bacteria	Proteins	https://biocyc.org/
Biological general repository for interaction datasets: BioGRID	Fungi	Proteins	https://thebiogrid.org/
CGD: Candida genome database	Fungi	Proteins	http://www.candidagenome.org/
ChIP-atlas	Fungi	Proteins	https://chip-atlas.org/
CollecTF	Bacteria	Proteins	http://www.collectf.org/browse/home/
Compartmentalized protein-protein interaction: ComPPI	Fungi	Proteins	https://comppi.linkgroup.hu/
CPLM: Compendium of protein lysine modifications	Fungi	Proteins	http://www.biocuckoo.org/
CSDB: Carbohydrate structure database	Archaea/ bacteria/ fungi	Carbohydrates	http://csdb.glycoscience.ru/database/
Database of bacterial exotoxins for human: DBETH	Bacteria	Proteins	http://www.hpppi.iicb.res.in/btox/
Database of protein disorder: DisProt	Archaea/ bacteria/ virus	Proteins	https://www.disprot.org/

(continued)

Table 3 (continued)

Software/database	Microorganisms	Target	Web link
DIP: Database of interacting proteins	Bacteria/ fungi	Proteins	https://dip.doe-mbi.ucla.edu/dip/Main.cgi
DoBISCUIT: Database of BIoSynthesis clusters CUrated and InTegrated	Bacteria	Metabolites	https://www.nite.go.jp/nbrc/pks/
EAWAG-BBD: University of Minnesota Biocatalysis/biodegradation database	Archaea/ bacteria	Metabolites/ proteins	http://eawag-bbd.ethz.ch/
EcoCyc	Bacteria	Metabolites/ proteins	https://ecocyc.org/
EffectiveDB	Bacteria	Proteins	https://effectors.csb.univie.ac.at/
Enzyme portal	Bacteria/ fungi	Proteins	https://www.ebi.ac.uk/enzymeportal/
eSOL: Solubility database of all E. coli proteins	Bacteria	Proteins	http://www.tanpaku.org/tp-esol/index.php?lang=en
Fluorome: The cyanobacterial chlorophyll fluorescence database	Bacteria	Metabolites	http://www.photosynthesis.jp/fluorome.html
Gclust server	Fungi	Proteins	http://gclust.c.u-tokyo.ac.jp/
GermOnline	Fungi	Proteins	http://www.germonline.org/index.html
GWIPS-viz	Bacteria/ fungi/ virus	Proteins	https://gwips.ucc.ie/
GyDB: Gypsy database 2.0	Virus	Proteins	https://gydb.org/index.php?title=Main_Page
Hepatitis virus database	Virus	Proteins	https://hbvdb.lyon.inserm.fr/HBVdb/
HitPredict: A database of high confidence protein-protein interactions	Bacteria/ fungi	Proteins	http://www.hitpredict.org/
HIV DATABASES	Virus	Proteins	https://actgnetwork.org/hiv-databases/
HIV-1, human protein interaction database	Virus	Proteins	https://www.ncbi.nlm.nih.gov/genome/viruses/retroviruses/hiv-1/interactions/
HomoloGene	Fungi	Proteins	https://www.ncbi.nlm.nih.gov/homologene?Db=homologene&Cmd=DetailsSearch&Term=Ubxn1%5BAll+Fields%5D
IDEAL: Intrinsically disordered proteins with extensive annotations and literatures	Archaea/ bacteria/ fungi/ virus	Proteins	https://www.ideal-db.org/
Influenza research database: IRD	Virus	Proteins	https://legacy.fludb.org/brc/home.spg?decorator=influenza

Influenza virus resource	Virus	Proteins	https://www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-select.cgi?go=database
IRESite	Virus	Proteins	http://iresite.org/
Isobase	Fungi	Proteins	https://worksafetech.com/iso-base/
KEGG: Kyoto encyclopedia of genes and genomes	Archaea/ bacteria/ fungi/ virus	Proteins/ carbohydrates/ metabolites/ lipid	https://www.genome.jp/kegg/kegg2.html
KNApSAck family	Bacteria	Metabolites	http://www.knapsackfamily.com/KNApSAcK_Family/
KNApSAcK motorcycle: Motorcycle metabolic pathway	Bacteria/ fungi	Proteins	http://www.knapsackfamily.com/motorcycle/top.php
KomicMarket: Kazusa Omics data market	Bacteria/ fungi	Metabolites	http://www.kazusa.or.jp/komics/en/
LfDB: Lectin frontier database	Bacteria/ fungi/ virus	Proteins/ carbohydrates	https://acgg.asia/lfdb2/index
MassBase: A comprehensive mass spectral tags archive for plant metabolomics	Bacteria/ fungi	Metabolites	http://webs2.kazusa.or.jp/massbase/index.php/
MetaNetX: MNXref	Archaea/ bacteria/ fungi	Metabolites	https://www.metanetx.org/
MiCroKiTS	Fungi	Proteins	http://microkit.biocuckoo.org/
MiFuP safety	Bacteria	Metabolites	https://www.nite.go.jp/nbrc/mrinda/mifup_safety/
MODOMICS	Archaea/ bacteria	Proteins	https://genesilico.pl/modomics/
MushPlant	Fungi	Metabolites	http://www.nihs.go.jp/dnfi/Mush-en.html
mVOC 3.0: Microbial volatile organic compound database	Bacteria/ fungi	Metabolites	https://bioinformatics.charite.de/mvoc/
NASA GeneLab: Genelab	Bacteria/ fungi/ virus	Metabolites proteins	https://genelab.nasa.gov/
Networks of functional coupling of proteins: FunCoup	Fungi	Proteins	https://funcoup5.scilifelab.se/search/
Universal PBM resource for oligonucleotide binding evaluation: UniPROBE	Bacteria	Proteins	http://thebrain.bwh.harvard.edu/uniprobe/index.php

(continued)

Table 3 (continued)

Software/database	Microorganisms	Target	Web link
Pathguide: Pathway resource list	Bacteria/ virus	Metabolites proteins	http://www.pathguide.org/
PCoM: Protein co-migration database for photosynthetic organisms	Bacteria	Proteins	http://pcomdb.lowtem.hokudai.ac.jp/proteins/top
PeptideAtlas	Fungi	Proteins	http://www.peptideatlas.org/
ProteoRed	Archaea/ bacteria	Proteins	https://es.datarooms.org/proteored/
Pseudomonas genome database V2	Bacteria	Proteins	https://www.pseudomonas.com/
PTMcode	Fungi	Proteins	https://ptmcode.embl.de/
REACTOME	Fungi/ bacteria	Proteins	https://reactome.org/
REBASE: The restriction enzyme database	Archaea/ bacteria/ virus	Proteins	https://www.re3data.org/
RefSeq: Reference sequence	Virus	Proteins	https://www.ncbi.nlm.nih.gov/refseq/
REPAIRtoire: A database of DNA repair pathways	Bacteria/ fungi	Proteins	https://repairtoire.genesilico.pl/
SGD: Saccharomyces genome database	Fungi	Proteins	https://www.yeastgenome.org/
SigMol	Archaea/ bacteria	Metabolites	https://bioinfo.imtech.res.in/manojk/sigmol/index.php
SMART: Simple modular architecture research tool	Archaea/ bacteria/ fungi	Proteins	http://smart.embl-heidelberg.de/
SRPDB: Signal recognition particle database	Archaea/ bacteria	Proteins	https://rth.dk/resources/rnp/SRPDB/
STRING: Search tool for the retrieval of interacting genes/proteins	Archaea/ bacteria/ virus	Proteins	https://string-db.org/
SubtiWiki	Bacteria	Metabolites/ proteins	http://subtiwiki.uni-goettingen.de/
SWISS-2DPAGE: SWISS-two-dimensional polyacrylamide gel electrophoresis database	Fungi	Proteins	https://world-2dpage.expasy.org/swiss-2dpage
Swiss-Czech proteomics server: SWICZ	Bacteria	Proteins	http://proteom.biomed.cas.cz/
SwissLipids	Bacteria/ fungi	Proteins/ lipids	https://www.swisslipids.org/#/
TADB 2.0: An updated database of bacterial type II toxin-antitoxin loci	Archaea/ bacteria	Metabolites	https://bioinfo-mml.sjtu.edu.cn/TADB2/index.php

Termini-oriented protein function INferred database: TopFIND	Fungi	Proteins	https://topfind.clip.msl.ubc.ca/
The yeast metabolome DataBase: YMDB	Fungi	Metabolites/ proteins	http://www.ymdb.ca/
TMFunction (archive): Functional database of membrane proteins	Virus	Proteins	http://togodb.biosciencedbc.jp/togodb/view/tmfunction#en
tmRDB	Bacteria	Proteins	https://rth.dk/resources/rnp/tmRDB/tmRDB.html
Universal PBM resource for oligonucleotide binding evaluation: UniPROBE	Bacteria/ fungi	Proteins	http://thebrain.bwh.harvard.edu/uniprobe/index.php
VIPERdb	Virus	Proteins	https://viperdb.org/
VirHostNet 3.0	Virus	Proteins	https://virhostnet.prabi.fr/
VMH: Virtual metabolic human	Bacteria	Metabolites	https://www.vmh.life/
WikiPathways	Bacteria/ fungi	Proteins	https://www.wikipathways.org/index.php/WikiPathways
Yeast interacting proteins database	Fungi	Proteins	http://itolab.med.kyushu-u.ac.jp/Y2H/
Yeast resource center public data repository	Fungi	Proteins	https://www.yeastrc.org/pdr/pages/front.jsp
YEASTNET: A consensus reconstruction of yeast metabolism	Fungi	Metabolites	http://www.comp-sys-bio.org/yeastnet/
YeastRGB	Fungi	Proteins	https://shmoo.weizmann.ac.il/elevy/YeastRGB/HTML/YeastRGB.html

sometimes incomplete and leading to unreliable matching results if the reference spectrum of the targeted metabolite is not contained within the database. Therefore, predicting the metabolic profiling from metagenomic sequencing may cover the shortcoming of MS/MS approaches. The development of MelonnPan is a computational method used to predict metabolite features from metagenomic sequencing data by incorporating biological knowledge in the form of either taxonomic or functional profiles. MelonnPan uses elastic net model for prediction (Mallick et al. 2019). Later, Xie et al. improved elastic net model by adding extra step to consider variable importance scores and thus achieved better prediction power for the metabolites (Xie et al. 2021). The continuous improvement and novelty in AI models and biomedical ML are coming side by side with the importance of in vitro and in vivo assay for the functional identification of a metabolite or a bunch of metabolites (Fig. 6).

Although, there is a great development in constructing large datasets for metabolic pathways represented mostly in KEGG (Okuda et al. 2008), BioCyc (Karp et al. 2019), and MetaCyc (Caspi et al. 2006), there are several metabolic pathways that remain unknown, and many reactions are still missing even in pathways that are well known. It is therefore necessary to identify these missing reactions during the reconstruction of metabolic pathways (Kotera and Goto 2016). Large datasets such as KEGG and MetaCyc are using the Enzyme List to reconstruct the incomplete metabolic pathways. The Enzyme List belongs to the Nomenclature Committee of IUBMB (NC-IUBMB) (McDonald and Tipton 2014). In this system, each enzyme is given a unique four-digit code called the Enzyme Commission (EC) number, in which the first three digits represent a hierarchical classification of the enzyme as class, subclass, and subsubclass (Kotera and Goto 2016). Recently, the Enzyme List (McDonald et al. 2009) was updated to be an online database "ExplorEnz" and publicly available at https://www.enzyme-database.org/newenz.php. By using the

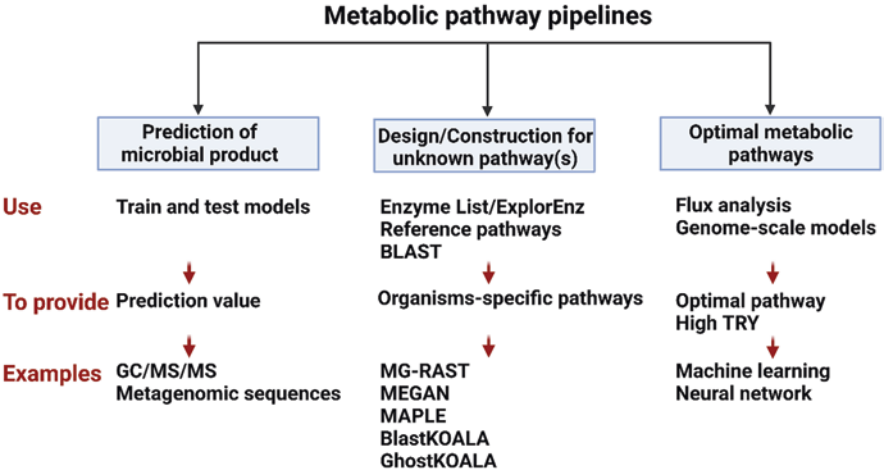

Fig. 6 Summarized ML methods in the identification of metabolic pathways

Enzyme List collectively, metabolic pathways can be reconstructed (Fig. 6). This Enzyme List contains enzymes that have been experimentally characterized in full. Several enzymes have already been described in the literature but may not yet be listed. Some enzymes that catalyze alternative or spontaneous reactions, may not be included in the Enzyme List (McDonald et al. 2009).

In MetaCyc (Caspi et al. 2006) and KEGG (Okuda et al. 2008), a metabolic pathway reconstruction is conducted using the Enzyme List and complemented with additional reactions to fill in the gaps for unknown compounds. These reconstructions are combined pathways that describe the chemical transformations without distinguishing between closely related organisms. These combined pathways are called "reference pathways" (Kotera et al. 2014). To overcome this challenge, their reconstruction processes should use sequence similarity programs such as BLAST and manual curation to assign orthologs based on the strongest hits to the genomes of various organisms (Kanehisa et al. 2016a; Caspi et al. 2016) (Fig. 6). KEGG provides reconstructed pathways based on BioCyc, a collection of organism-specific pathways (Caspi et al. 2016), and for complete genomes, KEGG provides pathways specific to each organism. It also provides a web-based tool called KEGG Automatic Annotation Server (KAAS) that enables reference-based metabolic pathway reconstruction on demand (http://www.genome.jp/tools/kaas/) (Moriya et al. 2007). In the model SEED, functionally related enzyme genes, called subsystems, are represented in a table-like manner to simplify reconstruction from a genome sequence (Henry et al. 2010). In recent years, improved tools have been developed with greater efficiency and interpretability including MG-RAST and MEGAN for reconstructing pathways and analyzing species distributions in large metagenomic datasets (Meyer et al. 2008; Huson et al. 2011), MAPLE for easier interpretation of the available metabolic functions (Takami et al. 2012), and BlastKOALA and GhostKOALA for efficient ortholog assignments using reduced sets of reference genome datasets (Kanehisa et al. 2016b). These programs can be used for gaining insight into the metabolic potential in various environments (Fig. 6).

Most of the naturally produced metabolites or enzymes belong to a specific subset of microorganisms, in this situation, the reconstruction of reference-based metabolic pathway is not possible (Kotera and Goto 2016). Researchers are depending on two frameworks to solve this issue; compound-filling framework and the reaction-filling framework (Kotera and Goto 2016). Software programs in compound-filling framework predict pathways by hypothesizing intermediate compounds between source and target compounds (Kotera et al. 2013). This framework's prediction systems are freely available at PathPred (Moriya et al. 2010) and at the University of Minnesota Pathway Prediction System (UMPPS) (Gao et al. 2011). Due to its prohibitive computational cost, the compound-filling framework is not suitable for predicting pathways for many compounds at once (Kotera et al. 2013). In the reaction-filling framework, chemical compounds are predefined, and pathways are predicted by filling in the reactions across them. The availability of databases containing chemical compounds with identified structures is enabling this framework to be adopted more widely (Aharoni et al. 2002; Kind and Fiehn 2006).

Optimizing metabolic pathways is the third pipeline of ML, which involves finding or generating the optimal pathways to maximize product titers, rates, and yields (TRY)or minimize reaction numbers (Shah et al. 2021; Lawson et al. 2021) (Fig. 6). In this attempt, ML provides an orthogonal approach to computational approaches to improve the flux analysis and genome-scale models, which have been successfully used in the past to increase TRY (Maia 2018). Combining both approaches can be more effective than using them separately. TRY can also be increased by fine-tuning gene expression by modifying the promoter and ribosome binding site (RBS) sequences (Lawson et al. 2021). Predicting gene expression requires a comprehensive understanding of transcription and translation (Leveau and Lindow 2001; Salis et al. 2009; Rhodius and Mutalik 2010). It is often difficult to obtain this knowledge, especially for nonmodel organisms. Thus, the majority of gene expression optimization efforts rely on trial-and-error experimental approaches based on the promoter and RBS library screening (Choi et al. 2019). Neural network of ML guided the design of promoter and RBS sequences in a ML-driven pathway to improve the gene expression (Kotopka and Smolke 2020) (Fig. 6). Recently, Neural Network ensembles were implemented to improve a 5-step pathway for violacein production by selecting promoter combinations to tune the gene expression. They used only 24 strains in their training set and obtained a new strain that improved violacein titer by 2.42-fold after only 1 design-build-test-learn iteration (Zhou et al. 2018). The impact of systemically leveraging high-throughput strain construction, testing, and ML to optimize multistep pathway expression can improve the product of TRY.

6.3 Deorphanization

Deorphanization of a NP is used to identify the native target protein (Civelli et al. 2013). Most biologically active NPs were discovered through phenotypic studies, which rarely reveal the targets of their protein binding (Civelli et al. 2013). Emerging trends in the identification of an action mechanism, termed as "target fishing," include ML algorithm for predicting the ligand-target proteins and to provide NP deorphanization (Jenkins et al. 2006). Deorphanizing predictors for NP drug targets utilize ML algorithms that have been trained with a combination of features such as structural representations as well as pharmacophoric descriptors necessary for the target interaction (Nisius et al. 2012). The major method used by ML servers for ligand-based target fishing is chemical similarity searches. The first used server was Prediction of Activity Spectra for Substances (PASS) (Parasuraman 2011), ChEMBL (Mayr et al. 2018), and similarity ensemble approach (SEA) (Wang et al. 2016) to predict NPs biological activities from 2D chemical structures using molecular fragment descriptors. SPiDER strategy used self-organizing maps (SOMs), a clustering technique that maps the links between chemical compounds using pharmacophore correlations and physicochemical attributes (Chau et al. 2001). SPiDER was successfully employed to predict the target of NP intricate structure such as the macrocyclic archazolid A, and (-)-englerin-A by deconvoluting structures into fragments

Fig. 7 Deorphanization of (±)-marinopyrrole A using TIGER software

that store the bioactivity fingerprint (Schneider and Schneider 2018). Target Inference GEneratoR (TIGER) is a computational chemocentric (ligand-based) target prediction tool using scoring approach (Schneider and Schneider 2018), where higher score values indicate a greater agreement in the prediction (Rodrigues and Bernardes 2020). Drug-Target Relationship Predictor (DEcRyPT) was utilized to accurately identify β-lapachone target as an allosteric modulator of 5-lipoxygenase (Akhtar et al. 2020). It was also successfully applied to deorphanize marine natural anticancer (±)-marinopyrrole A (Schneider and Schneider 2017) (Fig. 7).

7 Perspective and De Novo Generation of NP-Inspired Compounds

NPs contain privileged scaffold with pharmacophoric features required for interaction with biological target and can be used for the de novo design of mimicry structures (Welsch et al. 2010) (Fig. 8). Therefore, NPs with a successful track record in the era of evolutionary drug development offer benchmark data for exploring innovative molecular frameworks for synthetically accessible therapeutics drugs (Chen and Kirchmair 2020).

7.1 NPs-Based De Novo Drug Design Using AI

De novo drug design is a computational tool used to design novel bioactive structures entirely from scratch using chemical building blocks (Mouchlis et al. 2021). It transferred relevant attributes and activity of pharmacologically active NPs to synthetic small molecule drug. De novo design had become popular in studies of natural drugs (Popova et al. 2018). It includes ligand-based and structure-based drug design (SBDD) that depend on the recognition of pharmacophoric features of the ligand or the characterization of the biological target's active site, respectively. The availability of 3D crystal structure of a biological target provides an excellent opportunity for SBDD (Hamdy et al. 2022). Ligand-based de novo design of

Fig. 8 De novo design and generation process of NP-inspired compounds

distinctive molecular cores with drug-like properties that are inspired by NPs offers a promising solution to minimize the NPs synthetic burden (Perron et al. 2022).

De novo design algorithms previously relied on the selection of appropriate scaffold and the subsequent hybridization to create novel bioactive compounds (Hartenfeller and Schneider 2011). Recently, the emergence of AI approach including ML provide excellent opportunities for the de novo design by training neuronal networks to generate innovative molecules (Grebner et al. 2020). Artificial Neural Networks of DL and reinforcement learning (RL) architectures were combined in deep reinforcement learning (DRL), a subset of ML that is used in de novo drug design (Popova et al. 2018; Wang et al. 2022c) (Fig. 9).

Drug design approaches applied a variety of artificial networks including recurrent neural networks (RNN) with long-short term memory (LSTM), generative adversarial networks (GAN) (Martinelli 2022), convolutional neural networks (CNN) (Xiong et al. 2021), and autoencoders (AE) (Blaschke et al. 2018). ML algorithms were implemented in every stage of process of developing innovative NP-inspired drug candidates with inherited bioactivities (Button et al. 2019). DL model was trained with NPs structure libraries to design, enumerate, and explore novel small molecule with synthetic accessibility (Martinelli 2022). Reinforcement Learning for Structural Evolution (ReLeaSE) applied DRL algorithm to develop chemical libraries with the appropriate physicochemical and pharmacological activity (Mouchlis et al. 2021). Mapping and selection of relevant chemical candidate from the pool of enumerated compounds followed by deorphanizing techniques were employed to identify the target of interest (Mouchlis et al. 2021). Muller and his colleagues recently adjusted LSTM-RNNs to develop unique peptide sequences from natural antimicrobial peptides that have been void of repeated cysteine and proline residues (Saldívar-González et al. 2022). The model was established to implicitly capture pertinent structural properties for the targets of interest, only a minimal collection of known bioactive template structures were required (Gallego et al. 2021).

The discovery and development of NP mimetics therapeutic candidates have considerably improved due to advancements in high-throughput screening of in-house or commercially available libraries (Mishra et al. 2008). The primary hurdle in de novo drug design is the limited available chemical space to explore with synthetic accessibility (Chen and Kirchmair 2020). Therefore, respective advancements have been made to generate as many libraries as feasible (Friedrich 2019). Exploring

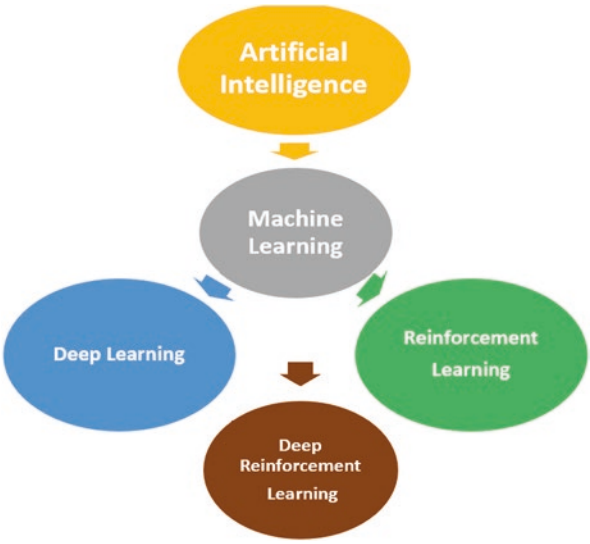

Fig. 9 Employing artificial intelligence in de novo drug design

novel chemical entities that preserve the privilege structures of NPs, several combinatorial de novo design strategies have been employed for developing synthetically accessible NP mimetics hits such as Diversity-Oriented Synthesis (DOS), Complexity to Diversity (CtD), Biology-Oriented Synthesis (BIOS), and Functionally Oriented Synthesis (FOS) (Saldívar-González et al. 2022) (Fig. 10).

7.2 Biology-Oriented Synthesis (BIOS)

BIOS uses NPs as templates to create derivatives with synthetic accessibility (Wilk et al. 2010). Most small molecule inhibitors and medications are built on ring systems, cyclization typically causes the overall molecular structure to tighten, which increases the target affinity because less entropy is lost during the binding (Zimmermann 2012). The underlying NPs framework is broken down into smaller scaffold based on rings, linkers chains, and ring-based double bonds (Zimmermann 2012). The BIOS concept depends on that the scaffold's fundamental structural underpinnings with biological relevance and prevalidation are used to generate compound collections with targeted biological activity (Cremosnik et al. 2020). Waldmann and his coworker have employed cheminformatic approach to visualize the chemical space occupied by NPs and their scaffolds, as a consequence, a new class of mycobacterium tuberculosis protein tyrosine phosphatase B inhibitors was discovered (Nören-Müller et al. 2008).

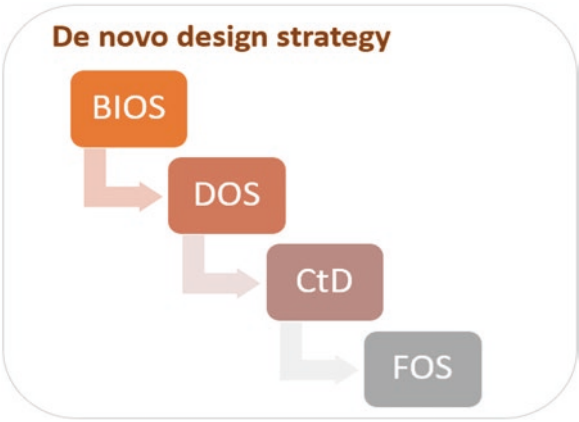

Fig. 10 Combinatorial de novo design strategies

The reductionistic scaffold tree's hierarchy of scaffolds shows many chemical entities and substructures of the original NPs (Bon and Waldmann 2010). Thus, the tree offers the opportunity to categorize structural variety to logical and chemically significant (Wilk et al. 2010). Consequently, the scaffold tree enables the comparison of increasingly complicated scaffolds and provides a tool for the reduction of molecular intricately to simpler frameworks (Kaiser et al. 2008). Recently, alternative scaffold trees have been produced using bioactivity data and a technique known as "brachiation" as intuitive leading criteria to choose hierarchically arranged scaffold sequences (Wetzel et al. 2011). For example, application of BIOS strategy revealed a highly selective protein phosphatase 2A inhibitor with improved characteristics from cytostatin, a naturally occurring molecule with an unsaturated d-lactone motif (Umarye et al. 2007) (Fig. 11). Finally, additional cheminformatic studies showed great potential to increase the applicability of compound libraries derived from BIOS (Bon and Waldmann 2010). It is reasonable to conclude that continued advancement of alternative cheminformatic techniques in this direction would be extremely beneficial to the field of biological science and chemical engineering (Wilk et al. 2010).

7.3 Diversity-Oriented Synthesis Strategy

Diversity-oriented synthesis (DOS) aimed to discover novel unexplored chemical space with NP-like pharmacophores and certain degree of chemical diversity. DOS library compounds have been found to alter protein-protein interactions, function of transcription factor, and multidrug resistance (Galloway et al. 2009). Wyatt and colleagues explored DOS libraries for first in class antibiotics with distinctive biological properties (Galloway et al. 2009). The study resulted in the discovery of the previously unexplored structure of emmacin antibiotic, which emphasizes the value of DOS as a tool in the de novo drug development process (Galloway et al. 2009).

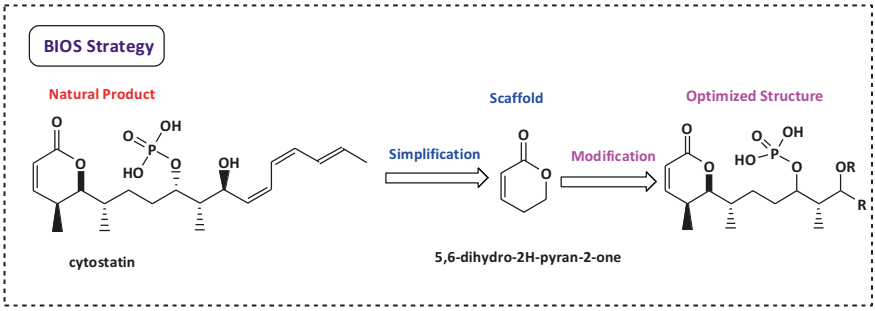

Fig. 11 Biology-oriented synthesis of cytostatin-inspired compound

7.4 Complexity-to-Diversity Strategy

The complexity-to-diversity technique (CtD) chemically functionalizes and distorts NPs to produce structurally varied compound collections, simulating enzyme processes in a synthetic manner (Srinivasulu et al. 2022). The diversification of pleuromutilin and ring contraction using CtD, along with screening in a phenotypic assay, revealed the biological relevance of CtD and led to the discovery of ferroptocide, which has anticancer potential by blocking redox proteins called thioredoxin (Llabani et al. 2019) (Fig. 12).

7.5 Functionally Oriented Synthesis Strategy

FOS represents an increasingly significant direction in synthesis that is centered on achieving function and has inspired the development of innovative methodologies with improved or entirely new functions (Wender et al. 2008). FOS-inspired strategy further expanded the BIOS concept by recapitulating or fine-tuning the function of a biologically active lead structure to produce more straight forward scaffolds and make them easier to synthesize (Wender et al. 2008).

7.6 Pseudonatural Products

Pseudonatural products (Pseudo-NPs), which exhibit biological function unrelated to the guiding NPs, have been developed recently by combining NP-derived fragments (NPDFs) (Saldívar-González et al. 2022; Grigalunas et al. 2020). It discovered through cheminformatic research of the Dictionary of Natural Products (DNP). Strategy of pseudo-NPs creation facilitates the chemical innovation and incorporation of new scaffolds fragments, that were assembled in arrangements irrelevant to the biosynthetic pathway; this is leading to the development of innovative libraries

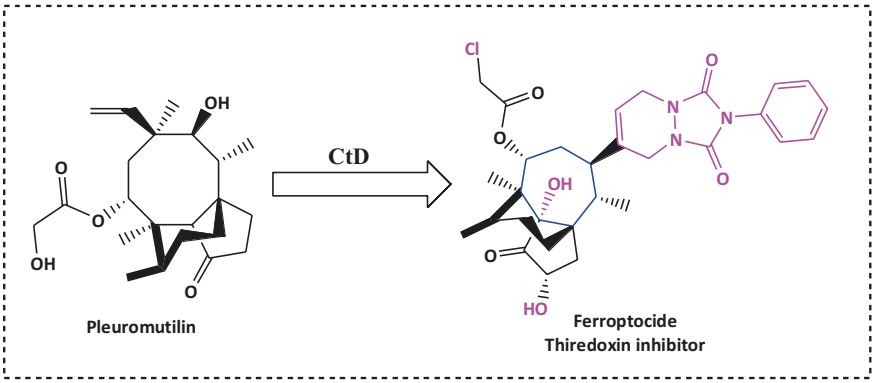

Fig. 12 CtD strategy of pleuromutilin involved ring contraction in blue and diversification in purple

with drug-like features such as sizes, shapes, and lipophilicity (Wilk et al. 2010). It is worth noting that chromopynone, the first example of pseudo-NPs provides a novel bioactivity (Yildirim 2021). It was created by a combination of biosynthetic unrelated fragments of chromane and a tetrahydro-pyrimidine (Karageorgis et al. 2018), which revealed a selective inhibition of glucose transporters GLUT-1 and GLUT-3 (Fig. 13).

7.7 Scaffold Hopping with the Design of Genuine Structures (DOGS)

Molecular scaffold hopping aims to identify molecules with distinctly different chemical structures that share a desirable function by binding to the same biological target (Mauser and Guba 2008). De novo structure development enumerates a structurally diverse compounds that share specific pharmacophoric features by computationally screen an unlimited chemical space (Zhao 2007). De novo fragment-base design using scaffold hopping from NPs is a validated approach for the discovery of isofunctional hit and lead compounds (Krueger et al. 2009), with unique structural properties in absence of the biological target information (Lloyd et al. 2004). DOGS is one of the most implemented software for the de novo molecular design and utilization of unexplored molecular building blocks and chemical reactions (Merk et al. 2018a). It was firstly implemented for independent scaffold hopping design of valerenic acid, isopimaric acid, and dehydroabietic acid as template to generate and test NP mimetic chemotypes that resulted in the de novo generated tetrahydroindole molecule (Schneider et al. 2022) (Fig. 14). Retinoid X receptor (RXR) agonism was predicted by target prediction using SPiDER software for both NPs and the de novo designed compound (Rodrigues and Bernardes 2020). On all three RXR subtypes, screening confirmed the de novo compound potent micromolar potency (Schneider et al. 2022).

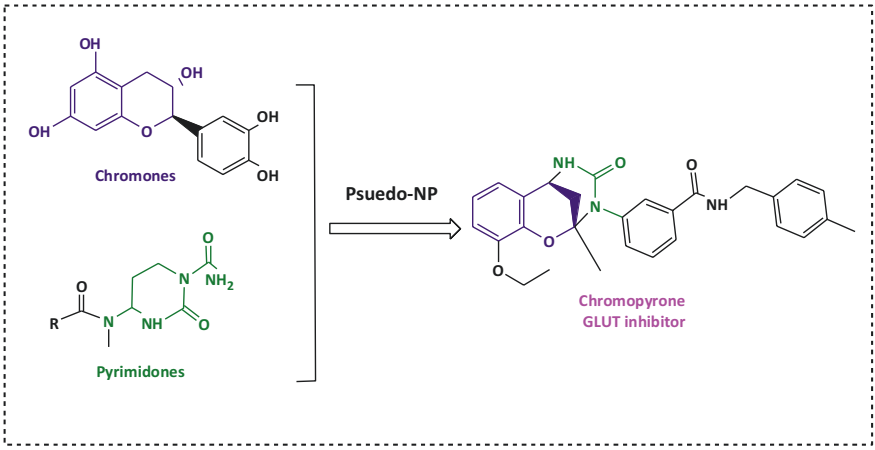

Fig. 13 Pseudo-NPs derived from unrelated chromones in purple and 4-H-pyrimidines NPDFs resulted in chromopyrone selective inhibitor of GLUT-1 and 3

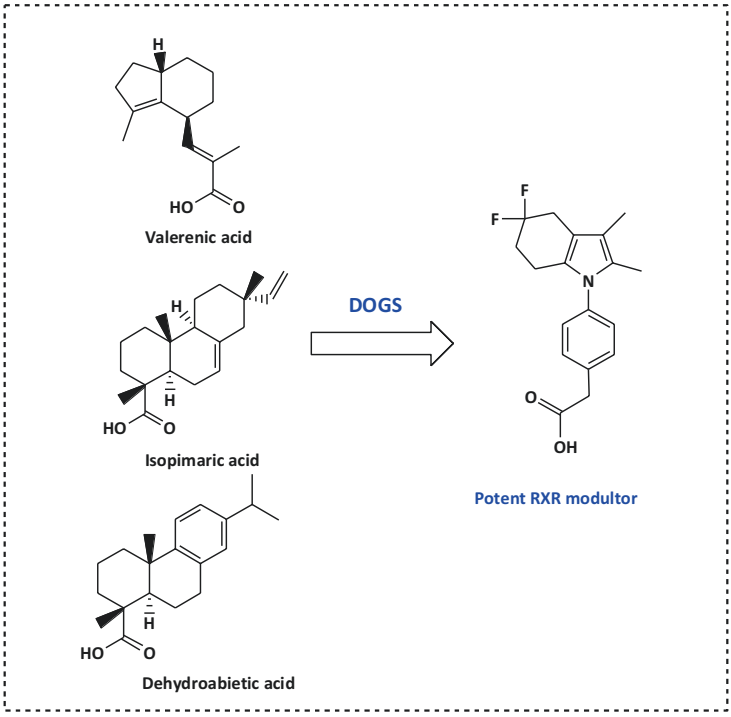

Fig. 14 DOGS software was used to build NP mimetic compounds using the scaffold-hops de novo design technique

7.8 Shape-Based De Novo Design

The shape of a molecule shapes its binding to a biological target from alignment-based approaches (Desaphy et al. 2012). Prominent strategies maximize possible overlap and rank against NP template (Ballester and Richards 2007). Shape analysis offers a quick filtering of large compound libraries based on similarity-based virtual screening of fixed set reference descriptors considering the geometrical distance distribution and its Connolly surface. Weighted holistic atom localization and entity shape (WHALES) descriptors was carried out by Grisoni and colleagues as ranking criterion for shape-based virtual screening using the four most prevalent phytocannabinoid NPs, that led to prospective potent cannabinoid receptor modulators with novel scaffold (Grisoni et al. 2018). Consequently, it is apparent that the WHALES technique is effective at retrieving isofunctional synthetic characteristics of bioactive natural compounds (Skalic et al. 2019).

ML model framework that has been trained to automatically generate small synthetic molecules that mimic NPs characteristics features based on shape similarity search (Merk et al. 2018b). NP mimetic molecules of the intricate natural anticancer drug (−)- englerin was computationally designed by Friedrich and colleagues in 2016. ML tool using SPiDER software was employed for the target predication of a potential calcium channel subfamily M (melastatin) member 8 (TRPM8) (Friedrich et al. 2016, 2020) (Fig. 15).

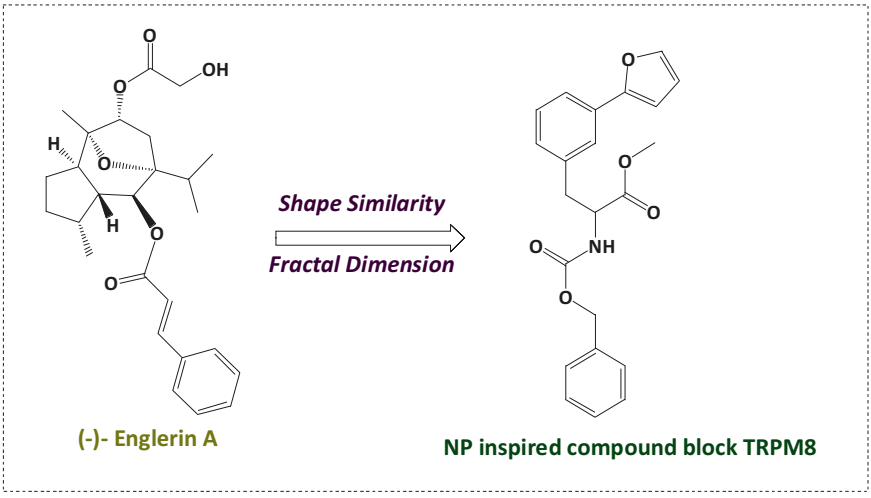

Fig. 15 Shape-based de novo design of potential NP-inspired TRPM8 inhibitor using (-)- Englerin A as a template

8 Limitation of AI Application in NP Drug Discovery

Like any emerging technologies, AI is still developing and still has a lot to achieve (Sethuraman 2020). One restriction is the requirement for considerable training data that relies on human judgment, which might be incorrect (Pareek et al. 2022). In the de novo drug discovery process, AI can forecast models to predict structures that are not readily accessible by chemical synthesis (Pareek et al. 2022).

9 Conclusion

NPs are the continuous source of numerous successful medication discovery tales. AI approach has gradually integrated diverse stages of NP drug research. ML algorithms assisted the discovery and elucidation of bioactive structures to capture the molecular patterns of these favored structures for molecular design and target selectivity. ML can also identify the most promising conditions for compound production based on a variety of factors including the characteristics of the organism and the type of NP being targeted.

ML-generated techniques for chemometric analysis and sequential use of dimensionality reduction techniques have provided the means to compare NP-privileged properties with those of pharmaceuticals interpreting of the free accessible datasets. The development of ML models to predict the biological activity of NPs has pushed candidates into more advanced stages of drug development. Deorphanizing models and de novo design work together to create novel isofunctional chemotypes, or "NP mimetics." Eventually, the synthetic accessibility, potency, and drug-likeness resemblance of NP-inspired compounds are being improved by these techniques. Overall, AI has the potential to enhance the discovery of new NP-based medication from microorganisms.

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Drug Development from Essential Oils: New Discoveries and Perspectives

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Abstract Essential oils are secondary metabolites biosynthesized by aromatic plants, composed of terpene derivatives (monoterpenes, sesquiterpenes), phenylpropanoids, aldehydes, ketones, esters, furans, and lactones. They have biological properties, including bactericide, fungicidal, cytotoxic, insecticide, antiparasitic, anti-inflammatory, analgesic, and antioxidant. Thus, they have potential in the discovery and development of new drugs. This work shows several studies involving pharmacological properties associated with the bioactive constituents of essential oils, aiming to elucidate and encourage their application in developing new phytotherapies. Thus, the oils have shown promising antimicrobial, antiviral, anti-inflammatory, antioxidant, and anticancer agents against in vitro and in vivo assays. In addition, many essential oils are considered safe with low toxicity, so they can be incorporated into different pharmaceutical forms to improve their bioavailability. However, developing research that evaluates essential oils'pharmacokinetic mechanisms and quality control is fundamental.

Keywords Biological properties · Antimicrobial · Antiviral · Anti-inflammatory · Antioxidant · Anticancer · Toxicity · Nanoparticle · Nanocapsule · Nanoemulsion

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1 Introduction

Since ancient civilizations, essential oils have been used as cosmetics, medicine, religious rituals, or cooking. The interest in this natural product is growing due to its aroma and biological properties. Therefore, essential oils research has been developed to evaluate their biological activities once they can treat diseases (Tariq et al. 2019; Wani et al. 2021).

The essential oils are volatile compounds of secondary metabolites composed of monoterpenes, sesquiterpenes, and phenylpropanoids, which are biosynthesized by different parts of the plant such as leaves, seeds, bark, and fruits (Chamorro et al. 2012; Tariq et al. 2019). In addition, they have a lipophilic character and low molecular weight, which allow them to cross cell membranes easily (Sharifi-Rad et al. 2017; Tariq et al. 2019).

However, for the essential oil's application, it is important to carry out their chemical characterization, as the composition may change between individuals of the same botanical species. These variations are due to the occurrence of different chemotypes, according to the adaptation of the plant to the environment, genetic variation, or its development (Chamorro et al. 2012). Therefore, the extracted volatile can vary quantitatively and qualitatively due to climate, soil characteristics, plant part, and age (de Sousa Peixoto Barros et al. 2022).

In this way, the phytochemical constituents of essential oils can be effective as antimicrobial agents against neurodegenerative diseases, heart disease, lung disorders, and cancer. Therefore, essential oils have the potential for use as new drugs. However, studies related to safety, efficacy, dosage, formulation, and drug interaction must be considered to avoid unwanted responses such as hypersensitivity and inflammation (Osuntokun 2017).

2 Essential Oils: General Aspects

Essential oils are volatile, liquid, natural substances with a strong odor. They are secondary metabolites synthesized by aromatic plants and responsible for flavor and aroma. In the plant, they have important functions such as protection against bacteria, fungi, and viruses, and they can also attract insects to favor pollination or repel undesirable ones (Bakkali et al. 2008; Tisserand and Young 2014).

In addition, essential oils have other physicochemical characteristics such as low stability in the air presence, light, heat, and humidity, optical properties, and most of them are yellowish or colorless. The chemical composition of this natural product is complex, and its main constituents are terpene derivatives and phenylpropanoids. Among the different chemical classes present in EOs are hydrocarbons (monoterpenes, sesquiterpenes), aldehydes, ketones, esters, furans, and lactones (Sangwan et al. 2001; Simões and Spitzer 2007).

Essential oils can be extracted from various plants part such as flowers, leaves, fruits, roots, stems, and seeds. The excretory plant structures of these volatiles vary according to the botanical family, such as glandular hairs (Lamiaceae), parenchyma cells (Lauraceae, Piperaceae, Poaceae), oil channels (Apiaceae), or in lysigenous or schizolysigenous pockets (Pinaceae, Rutaceae) (Simões and Spitzer 2007; Tisserand and Young 2014).

Regarding the extraction methods, the most used are enfloration, expression (pressing), solvent extraction, supercritical CO_2, steam distillation, and hydrodistillation (Buckle 2003). However, regardless of the type of extraction, the essential oil yield at the end of the extraction is generally very low quantitatively (Silveira et al. 2012).

Several factors influence the chemical composition and yield of essential oils as the plant organ, environmental factors (climate, soil, temperature), water stress, and collection period. These are said to be intrinsic parameters. Other parameters that can affect the oil qualitatively and quantitatively are the extrinsic ones, which include the method and time of extraction and storage of the botanical material (Li et al. 2014; Duarte et al. 2018).

The essential oils application is becoming increasingly widespread as an alternative to synthetic substances in pharmaceutical, food, agronomic, cosmetic, and sanitary products. This fact is due to the biological properties, some of which have been known since antiquity, such as bactericide, fungicide, cytotoxic, insecticide, antiparasitic, anti-inflammatory, analgesic, and antioxidant (Bakkali et al. 2008; Schmidt 2010; Bhardwaj et al. 2013).

3 Biological Activities

3.1 Antimicrobial Activity

The infections caused by microorganisms represent a significant health concern and are responsible for most deaths worldwide (Nair et al. 2022). In addition, the emergence of new drug-resistant strains commonly used in clinical practice has hampered therapeutic success. In this context, essential oils can be an alternative for treating these pathogens (Tariq et al. 2019; Nair et al. 2022).

Several bacteria species transmit antimicrobial resistance through the gene exchange cycle's translation, conjugation, and transformation processes (Nair et al. 2022). Thus, the growth of infections by bacterial pathogens occurs, resulting in high rates of tolerance to multidrug worldwide, in addition to the adverse effects due to the use of antimicrobials in higher doses (Hou et al. 2022).

Essential oils can act on microorganisms according to the strain and agent. Gram-positive bacteria are more vulnerable to the action of this natural product, as they do not have a thick layer of lipopolysaccharides, unlike gram-negative bacteria. Thus, the active components of essential oils can bind to the cell surface and

permeate the phospholipid bilayer of the membrane, which causes metabolic damage and apoptosis (Nair et al. 2022).

The phenolic monoterpene carvacrol is a lipophilic compound that interferes with the membrane structure modifying the fatty acid profile and causing adenosine triphosphate (ATP) depletion in microbial agents. The oxygenated monoterpenes carveol and carvone contribute to the leakage of potassium ions. In addition, other compounds such as methyl carvacrol, menthol, citronellol, and thymol can increase passive ionic movement between phospholipids and expand the cell wall (Nair et al. 2022; Hou et al. 2022).

Furthermore, some oils, such as those from *Alpinia galanga*, *Elwendia persica*, *Litsea cubeba,* and *Homalomena pineodora*, and their components, such as ethyl cinnamate, methyl cinnamate, α-terpinene, cuminaldehyde, and α-phellandrene, have antimicrobial activities through cell membrane disruption, cytolytic swelling, and loss of membrane function (Nair et al. 2022; Hou et al. 2022). These constituents are illustrated in Fig. 1.

The antibacterial action of essential oils can inhibit the proliferation of bacteria or kill them. Therefore, several methods, such as microdilution wells and disc diffusion, are applied to determine the minimum inhibitory concentration (MIC) of microbial agents. In this sense, the most appropriate bioassays to indicate the MIC value are dilution methods, tests in agar medium or broth (Chouhan et al. 2017). In

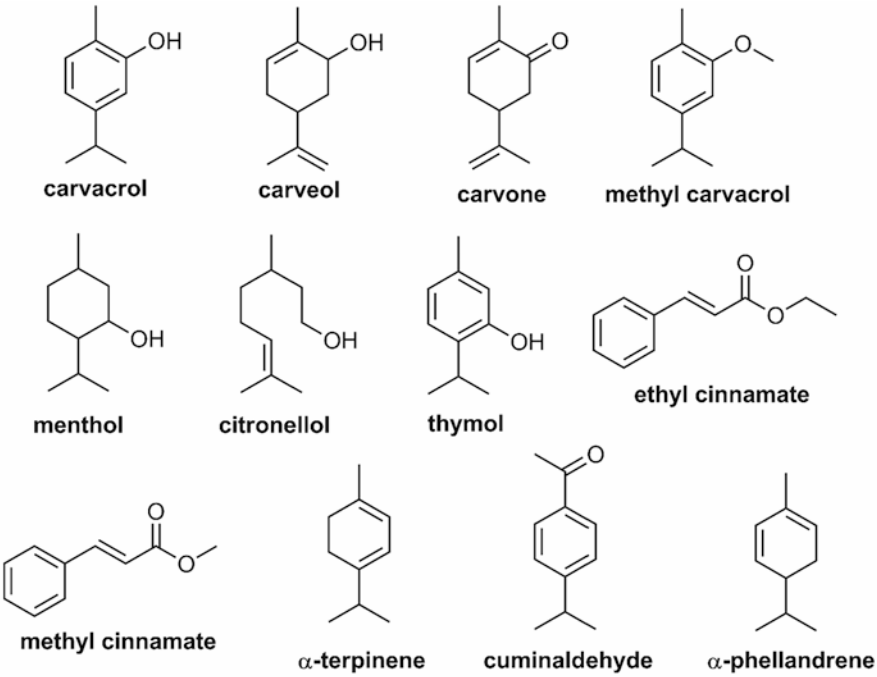

Fig. 1 Essential oil constituents with antimicrobial properties

comparison, the minimum bactericidal concentration (MBC) evaluates the ability to eliminate 99.9% of the initial inoculum, where agar diffusion is used (Tariq et al. 2019).

In vitro MIC values show that essential oils with strong antimicrobial action should be up to 500 μg/mL, while moderate activity is between 600 and 1500 μg/mL, and values greater than 1600 μg/mL have poor activity (Nair et al. 2022). Table 1 shows some essential oils and the spectrum of action achieved.

Thus, *Eryngium campestre* essential oil has shown strong activity (MIC 250 μg/mL) against *Bacillus cereus* and *Staphylococcus aureus* bacteria (Medbouhi et al. 2019). Moreover, *Origanum majorana* oil showed MIC of 156 μg/mL and 312 μg/mL against *Staphylococcus epidermidis* and *Cryptococcus neoformans* strains, respectively (Paudel et al. 2022). Furthermore, the essential oil extracted from *Solanum nigrum* exhibited MIC of 200 μg/mL combating the gram-negative bacteria *Proteus mirabilis* (Khaled et al. 2021).

A study carried out by Da Silva et al. (2016) evaluated the antimicrobial activity of essential oils from leaves and branches of *Endlicheria arenosa* from the Brazilian Amazon. The oils showed high antibacterial potential against *Escherichia coli*, with MIC of 0.020 and 0.156 mg/mL, for leaves and branches, respectively. Furthermore, both oils showed strong activity against *Bacillus cereus* (MIC 0.156 mg/mL).

Table 1 Antimicrobial activities of essential oils and their main chemical components

Specie	Plant part	Strain	Assay methods	Minimum inhibitory concentration (MIC) (μg/mL)	Reference
Endlicheria arenosa	Leaves	*Escherichia coli*	Micro-broth dilution method	20	da Silva et al. (2016)
		Bacillus cereus		156	
	Twigs	*Bacillus cereus*		156	
Origanum majorana	Aerial parts	*Staphylococcus epidermidis*	Micro-broth dilution method	312	Paudel et al. (2022)
		Candida albicans		156	
		Cryptococcus neoformans		312	
Eryngium campestre	Aerial parts	*Bacillus cereus*	Dilution agar assays	250	Medbouhi et al. (2019)
		Enterococcus faecalis		125	
		Staphylococcus aureus		125	
Syzygium aromaticum	Flowers	*Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Salmonella typhimurium*	Micro-broth dilution	304	Radünz et al. (2019)
Solanum nigrum	Seeds	*Proteus mirabilis*	Micro-broth dilution	200	Khaled et al. (2021)

In vivo studies performed using the essential oil of *Zingiber officinale* report increased survival of mice infected with *Klebsiella pneumonia*. During the test, the animals treated with the oil received 300 mg/kg; this dosage was responsible for the decrease in the number of colony-forming units in the pleural fluid, where there was a significant reduction in the bacteremia of those infected. This points to the therapeutic efficiency of *Z. officinale* oil compared to polymyxin antibiotics (Vaz et al. 2022).

The antimicrobial properties of essential oils can help treat or kill microbial cell, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella* spp., *Staphylococcus* Coagulase-negative, *Shigella* sp., *Enterococcus* sp., and *Escherichia coli*, which are more resistant and are commonly acquired in the community and hospitals. Therefore, essential oils can be applied to control resistant pathogens (Tariq et al. 2019; Mangalagiri et al. 2021; Hou et al. 2022).

3.1.1 Essential Oils in Combination with Antibiotics

The combination of essential oils and antimicrobials can lead to an antagonistic, additive, or synergistic effect. The antagonistic effect occurs when there is a decrease in antimicrobial activity. The additive effect happens when two antimicrobial agents' results equal the sum of the individual effects. On the other hand, synergism occurs when this combination potentiates and increases antimicrobial activity (Chouhan et al. 2017). Therefore, antimicrobial activity is determined by its chemical content, concentration, interactions between the main active components, and the vulnerability of microorganisms (Wani et al. 2021).

Chemical constituents such as carvacrol, cinnamaldehyde, cinnamic acid, eugenol, and thymol present in essential oils can synergistically affect antimicrobials. Studies show that essential oils synergize with beta-lactam antibiotics acting on the cell membrane. This effect was observed between penicillin and thymol, a constituent present in the oil of *Origanum vulgare*, against *Escherichia coli* (Rosato et al. 2010; Langeveld et al. 2014).

The association of aminoglycoside antibiotics with *Melaleuca alternifolia* oil was analyzed. In combination with gentamicin, there was synergism against strains of *Escherichia coli*, *Yersinia enterocolitica*, *Serratia marcescens,* and *Staphylococcus aureus*. Furthermore, *Melaleuca alternifolia* oil, in conjunction with tobramycin exhibited synergism against *E. coli* and *S. aureus*. The interaction between beta-lactams and eugenol against *E. coli* strains is diversified. The combination of eugenol with penicillin showed a synergistic effect for unspecified strains. However, ampicillin, penicillin, or erythromycin in association with eugenol demonstrated an additive action against *E. coli* (Langeveld et al. 2014).

3.2 Antiviral

The incidence of viral pathogens, such as herpes simplex virus (HSV), human immunodeficiency virus (HIV), swine flu (H1N1), and coronavirus (COVID-19), constantly affects society and causes irreversible damage to public health. Through their capsule proteins, viruses have an accelerated replication that favors entry and compromises the body's immune defense (Ghosh et al. 2022).

Few antiviral drugs promote effective therapy due to resistance acquired by some viruses to drugs and difficulty in the treatment of viral diseases. Thus, essential oils are considered a new therapeutic option due to their phytochemical constituents, but studies are mainly focused on in vitro assays (Reichling et al. 2009; Strub et al. 2022).

The most suitable antiviral drugs for clinical practice are substances that act on specific stages of viral synthesis. As a result, they hinder specific procedures in the viral replication phase to minimize or prevent the virus's progeny. Antivirals should be used in low concentrations without influencing the host cell mechanism and impairing viral dissemination. In this way, antivirals denature proteins or glycoproteins, thereby eliminating the ability to infect (Reichling et al. 2009). Essential oils are efficient in combating a wide variety of viruses, such as influenza virus, human immunodeficiency, herpes, avian influenza, and yellow fever, which are the ones that most lead to death in immunocompromised patients (Asif et al. 2020).

Among the essential oil compounds, illustrated in Fig. 2, with antiviral properties eugenol, carvacrol, thymol, 1,8-cineole, pulegone, piperitenone oxide, methyl salicylate, germacrone, α-thujone, terpinen-4-ol, terpinolene, α-terpineol, cinnamaldehyde, patchouli alcohol, *p*-cymene, and β-caryophyllene stand out in the literature (Reichling 2022).

The different polarity of the thymol-derived influences their activities against herpes simplex virus (Ma and Yao 2020). Moreover, cytopathogenic cell assays evidenced about 221 compounds of essential oils have activity against severe acute respiratory syndrome (SARS-CoV) (Wani et al. 2021).

In vitro assays were performed to determine the properties of essential oils and their isolated constituents against viral pathogens such as HSV, influenza, HIV, and COVID-19. Therefore, a study showed that the treatment with *Syzygium aromaticum* essential oil increased the primary and secondary humoral response and also showed antiviral activities against the herpes simplex virus, using a set of primary human cell systems, which simulates the environment of the herpes simplex virus and cellular biomarkers (Panda et al. 2022).

The essential oils of *Cinnamomum cassia*, *Cymbopogon citratus*, *Citrus bergamia*, *Thymus vulgaris*, and *Lavandula angustifolia* have high antiviral activity against the type A influenza virus, while the oils extracted from *Lippia* spp. showed efficacy against the yellow fever virus at a concentration of 11.1 μg/mL (Wani et al. 2021).

In in vivo assay, animals treated with 1,8-cineole could prolong survival by five days after virus infection. In addition to these results, it was also reported that the constituent was able to reduce nuclear factor-κB by 60 and 120 mg/kg, linked to the

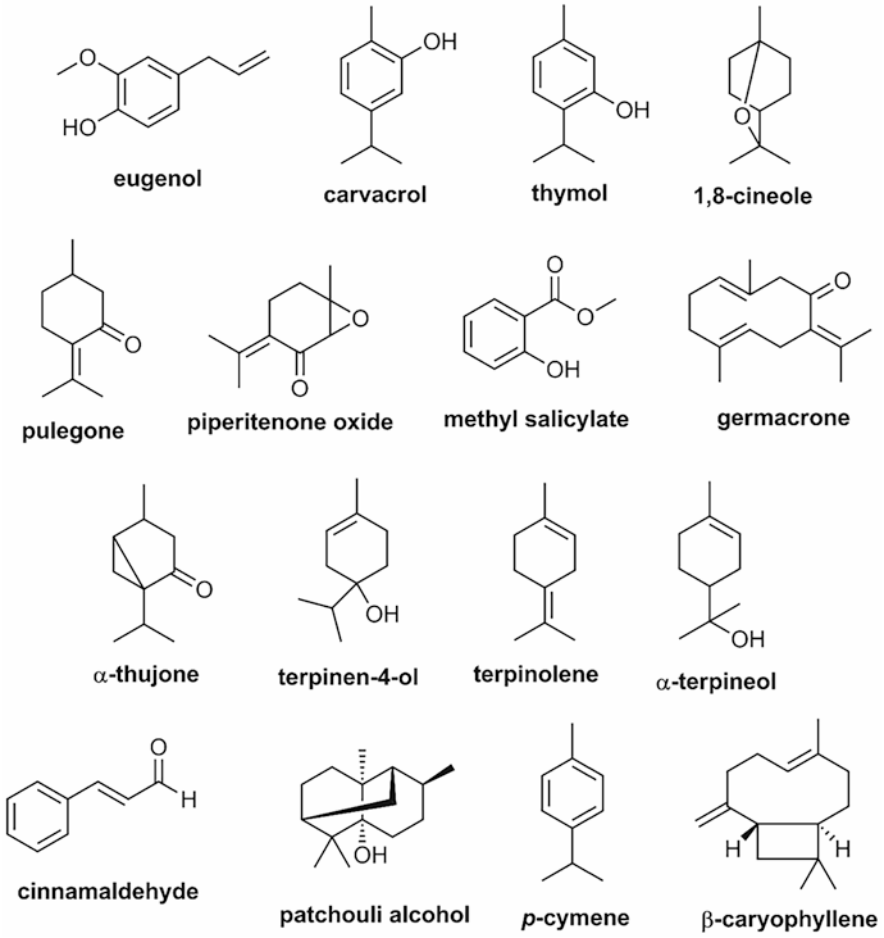

Fig. 2 Essential oil constituents with antiviral properties

inflammatory response, in lung tissues of mice, proving the reduction of lung inflammation (Reichling 2022).

In addition, the use of essential oils in the form of steam can help in the treatment of the flu. A study evaluated the anti-influenza properties of liquid and vapor forms of various plant species' essential oils. Oil vapors extracted from *Citrus bergamia*, *Eucalyptus globulus,* and their constituents, such as citronellol and eugenol, exhibited rapid action against the influenza virus. On the other hand, in liquid form, oils from *Cinnamomum zeylanicum*, *Citrus bergamia*, *Cymbopogon flexuosus,* and *Thymus vulgaris* obtained better results against the virus, with 100% inhibitory activity at 3.1 μL/mL (Asif et al. 2020).

Moreover, in vivo studies of plants compounds such as decanoyl acetaldehyde, myrcene, lauric aldehyde, α-pinene, D-limonene, and methyl *n*-nonyl ketone

demonstrate positive effects against HIV infection by interfering with the virus envelope. Furthermore, carvacrol and its thymol isomer, compounds identified in *Origanum vulgare* oil, blocked viral entry into the host organism from the depletion of cholesterol in the HIV-1 envelope (Ghosh et al. 2022).

3.2.1 Activity Against COVID-19 Virus

Coronaviruses are covered with a lipid layer, that is, enveloped, the main target of the hosts is the animal kingdom. The viruses commonly cause milder, common colds are HCoV-HKU1, HCoV-229E, HCoV-NL63, and HCoV-OC43, while MERS-CoV and SARS-CoV can progress to a more severe respiratory infection that can lead to death (Strub et al. 2022; Ghosh et al. 2022).

COVID-19 virus is the seventh coronavirus known to infect humans, which triggered the current pandemic as a severe respiratory syndrome. Combating this recent virus is a complex problem due to its high mutation rate, which forms more virulent strains. Current treatments with corticosteroids provide only symptomatic relief in mild cases and support of vital function in severe cases. Thus, existing vaccines remain viable to address this global health issue (Valussi et al. 2021; Strub et al. 2022; Ghosh et al. 2022).

In this context, international efforts have been made to find an adequate drug therapy to suppress the virus. However, the effectiveness is still insufficient. To avoid indiscriminate use and collateral damage, bioactive phytochemicals have been considered to guarantee the safety of the treatment, being a viable alternative to synthetic drugs (Strub et al. 2022). In view of this, numerous essential oils are currently being studied as options for the symptomatic treatment of respiratory diseases in adequate doses; they can be used orally, inhaled, or topically due to their therapeutic effects on the respiratory system described in the literature (Valussi et al. 2021).

The viral protease that acts in the replication process of SARS CoV-2 is the main therapeutic target of phytochemical compounds. In recent studies, *Eucalyptus globulus*, *Eucalyptus jensenii*, *Origanum vulgare*, and *Allium sativum* oils have been reported to be effective in combating COVID-19 (Reichling 2022). Furthermore, *Eucalyptus* spp. essential oil can expand the ciliary performance of human nasal epithelial cells, which enhances the natural protections of the upper airways. On the other hand, the monoterpenes eucalyptol (1,8-cineole) and menthol are the two components most analyzed in oils (Valussi et al. 2021). So, these oils have been used in treating respiratory disorders and have shown positive results (Panikar et al. 2021).

3.3 Anti-Inflammatory

The inflammation is part of innate immunity acting in response to the pathogenic invasion, infection, tissue injury, and other external agents. The inflammatory process includes increased blood flow, endothelial cell permeability, immune cell influx, and release of inflammatory mediators, in addition to the action of enzymes, such as oxygenases, nitric oxide synthases, and peroxidases that will affect tissue function and structure (Miguel 2010; Korinek et al. 2021; Pandur et al. 2021).

Neutrophils constitute the body's first line of recognition and defense, the most abundant and essential innate immune cells at the beginning of the inflammatory process since they activate the other immune system cells and eliminate invading microorganisms. While monocytes differentiate into M1 with proinflammatory and phagocytic activity, or M2 stimulating proliferation and tissue repair. Acute inflammation can evolve into chronic inflammation, when not properly cured, which contributes to the emergence of other diseases, such as autoimmune, pulmonary, neurodegenerative and cancerous diseases (Miguel 2010; Korinek et al. 2021; Pandur et al. 2021).

Studies show that some essential oils have anti-inflammatory activity. *Melaleuca alternifolia* and *Lavandula angustifolia* oils have been reported to have immunomodulatory activity, which is related to inflammatory processes. Therefore, they may be a potential alternative treating of numerous infectious or immunological diseases (Sandner et al. 2020; Pandur et al. 2021). Moreover, Sandner et al. (2020) reported that the essential oils of *Eucalyptus globulus*, *Melaleuca alternifolia, Lavandula angustifolia,* and *Syzygium aromaticum* promoted the reduction of cytokines, such as interleukins (IL-1α, IL-1β, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13), tumor necrosis factor (TNFα), nitric oxide, and interferon (IFNγ), measured in activated monocytes and macrophages.

In vitro assays using myristicin, a phenylpropanoid present in the essential oil of *Myristica fragrans*, evaluated the anti-inflammatory activity of this constituent in mouse macrophages stimulated by double-stranded RNA. Thus, it was observed that myristicin stopped the production of calcium, nitric oxide, interleukins, interferon-10 inducible protein, monocyte chemotactic protein, granulocyte-macrophage colony stimulating factor, macrophage inflammatory proteins, and leukemia inhibitory factor, all directly related to inflammation. Therefore, these results corroborate the anti-inflammatory action of the essential oil (de Cássia da Silveira E Sá et al. 2014). In addition, the in vitro anti-inflammatory activities of *Hyssopus officinalis* oil were also evaluated, demonstrating an inhibitory effect on the activity of cyclooxygenase enzymes (COX-1 and -2) at a concentration of 20 μg/mL (Mićović et al. 2022).

The in vivo study performed by (Wong et al. 2022) demonstrated that *Houttuynia cordata* essential oil reduced edema growth in mice. During the analysis, 66 μg of the aerosol formulation developed with the oil were applied. This average dose showed a 66.6% reduction in ear edema, presenting an inhibitory effect with peritoneal capillary permeability.

Another research carried out in mice with the essential oils of *Boswellia serrata*, *Commiphora myrrha*, *Aucklandia costus*, *Matricaria chamomilla*, *Jasminum sambac,* and *Syzygium aromaticum* evaluated the inflammatory responses through the inhibition of the activation of cyclooxygenase 2 (COX-2), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and nuclear factor-κB (NF-κB) revealed that *Commiphora myrrha*, *A. costus,* and *M. chamomilla* oils showed better anti-inflammatory activity compared to commercial anti-inflammatory drugs (ibuprofen) (Zhang et al. 2020).

In another assay carried out in mice, it was observed that *Hyptis crenata* oil, rich in 1,8-cineol, camphor, α-pinene, and β-pinene, showed significant anti-inflammatory activity, with peripheral antinociceptive action and without central antinociceptive action. Abdominal writhing decreased at 300 mg/kg (44.4%) and 100 mg/kg (79.5%). While for ear edema, the oil showed an inhibitory effect in all doses tested, but at a dose of 30 mg/kg, the best result was shown with 64% of inhibition. Thus, these results show a real perspective on the use of *H. crenata* oil in the development of herbal product (de Lima et al. 2022).

Furthermore, the isolated compounds safrole, dillapiole, and dihydrodylapiol, present in *Piper aduncum* oil, significantly reduced paw edema, exhibiting anti-inflammatory action. Since dillapiol and dihydrodilapiol caused edema suppression, while safrole had the lowest inhibitory activity than the positive control. Constituents such as eugenol, o-cresol, and guaiacol from *Croton geayi* oil, when applied topically, inhibited the formation of edema in mice at doses of 0.2 and 0.5 mg per site for 15 minutes, or 1.0 and 2 0.0 mg for 60 minutes after oil application (de Cássia da Silveira E Sá et al. 2014).

3.4 Antioxidant

Antioxidants are substances that react with radicals or mitigate oxidative stress caused by reactive oxygen species. Many chemical assays are developed to identify the antioxidant capacity of isolated phytochemicals or natural extracts based on the reaction of this potential with a radical, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) or 2,2′-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS+); or nonradical oxidizing substances, such as Fe^{3+} ions in the iron reduction method (FRAP) (Amorati et al. 2013).

The DPPH in methanol is a stable free radical in its reduced form in the presence of radical scavengers as they supply hydrogen radicals or release electrons. While ABTS+ is obtained through 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, in these methods, the antioxidant captures radicals causing a decrease in absorbance. On the other hand, FRAP comprises the formation of Fe^{2+} ions through the reduction of Fe^{3+} ions, detected in the 2,4,6-tripyridyl-s-triazine complex (TPTZ) and indicates the ability of antioxidant compounds to donate electrons and reduce the products oxidized in the process of lipid peroxidation (Locatelli et al. 2017).

The inhibitory concentration (IC_{50}) represents the necessary capacity of an antioxidant to sequester by 50% the free radicals present in the DPPH or ABTS solution (Caldwell et al. 2012).

Synergistic, additive, and antagonistic interactions are parameters used to identify the type of antioxidant interaction, when there is a combination of two or more antioxidant agents, being verified from the antioxidant combination index (CI), in which CI greater than 1, given as antagonist; CI equal to 1, additive; and CI less than 1, synergistic (Purkait et al. 2020).

A study evaluated the antioxidant capacity by the DPPH method of *Syzygium aromaticum* and *Cinnamomum verum* oils. *S. aromaticum* oil had the highest antioxidant potential compared to *C. verum*, due to the higher concentration of the phenolic compound such as eugenol. On the other hand, in that same research, the combination of these oils resulted in an IC equal to 0.82, which indicates a synergistic antioxidant activity (Purkait et al. 2020).

In the studies by Ge et al. (2019), ABTS and DPPH assays were used to evaluate the antioxidant activities of *Camellia euphlebia*, *C. petelotii,* and *C. tunghinensis* oils that presented IC_{50} in the ABTS method equal to 102.56, 198.34, and 321.7 μg/mL, respectively. While the results for the DPPH assay were 42.8, 90.9, and 164.8 μg/mL. In this sense, the essential oil of *C. euphlebia* showed greater efficiency in eliminating radicals than other oils. Furthermore, *C. tunghinensis* exhibited moderate antioxidant capacity due to the high concentration of hexanal.

Research has shown that *Ocimum basilicum* oil has a DPPH radical scavenging action with an IC_{50} of 11.23 μg/mL, which can be attributed to the high phenolic content in its composition. Furthermore, *Kickxia aegyptiaca* oil exhibited significant antioxidant activity with IC_{50} of 30.48 mg/L (DPPH) and 35.01 mg/L (ABTS) (Hou et al. 2022).

Moreover, Jerônimo et al. (2021) evaluated the essential oils from the Brazilian Amazon, such as *Psidium guineense*, *Psidium guajava*, *Myrcia sylvatica*, *Myrcia splendens*, *Eugenia stipitata,* and *Eugenia patrisii*, and reported that these samples exhibited weak-to-moderate antioxidant activity, with rates of 11% and 5% to 38.6% scavenging of DPPH radicals at a concentration of 10 mg/mL.

3.5 Anticancer

Cancer is a major health challenge worldwide, the second disease that kills the most, with approximately 17% of deaths. Recently, synthetic or semisynthetic anticancer drugs have promoted numerous side effects, such as reduced white blood cell count, impaired immunity, bone marrow depression, alopecia, and weakness. Therefore, the anticancer activity of essential oils has been investigated to avoid drug resistance and adverse effects caused by antitumor drugs (Bayala et al. 2014; Nguyen et al. 2022; Alipanah et al. 2022).

Initial assays determined that the rate of mutation within cells could be driven by oxidative stress acting in a damaging manner to DNA. In this perspective, reactive

oxygen species contribute to tumor development by activating signaling pathways that promote stages of carcinogenesis, such as cell transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis. In this way, the components of essential oils can be efficient against cancer cells. Effects on oral, bone, breast, cervical, colon, kidney, liver, lung, ovarian, pancreas, prostate, and uterine cancers have been investigated. In addition, properties against glioblastoma, melanoma, and leukemia have also been reported (Bayala et al. 2014).

In this perspective, the oils of *Eugenia* spp. exhibited activity against colorectal, gastric, and melanoma cancer cell lines (da Costa et al. 2020c). Investigations on the essential oil of *Cymbopogon flexuosus* indicated the ability to imbalance of cell death (apoptosis) in human leukemia cells. Furthermore, the oil extracted from the leaves of *Malus domestica*, at 1000 μg/mL, caused an inhibition of the human acute monocytic leukemia cell. Moreover, *Patrinia scabra* root oil showed inhibitory activity on human ovarian tumor cells (Bayala et al. 2014).

Cymbopogon flexuosus oil and isointermedeol sesquiterpene promoted the inhibition of human leukemia cell proliferation with IC_{50} values equal to 30 and 20 μg/mL, respectively. Thus, the anticancer effect of *Cymbopogon citratus* oil and citral (neral + geranial) for cervical cancer cell lines was evaluated, citral oil and emulsion caused apoptosis by decreasing cell proliferation (Angelini et al. 2018). Furthermore, *Eugenia uniflora* oil and curzerene sesquiterpene displayed cytotoxic activity against lung, colon, stomach, and melanoma cancer cell lines. Thus, curzerene induced apoptosis at 5.0 μM and 10.0 μM compared to Doxorubicin, exhibiting a decrease in cell migration at 5.0 μM and 10.0 μM after 30 hours of treatment (Figueiredo et al. 2019).

In vivo studies, *Cymbopogon citratus* oil exhibited cytotoxicity against Chinese hamster ovarian cells (Bayala et al. 2014). Another assay evaluated the antitumor activity through the subcutaneous implantation of *Curcuma zedoaria* essential oil at doses of 2, 4, 12, 60, and 240 mg/kg in lung carcinoma cells of mice and it was concluded that the administration of the oil for 3 weeks caused a dose-dependent inhibition of tumor volume and a reduction in tumor weight (Chen et al. 2013).

To investigate the cytotoxic property against lung cancer of the essential oil of *Origanum majorana*, a therapy was performed in mice to evaluate lymph node metastasis using the xenograft model of cancer cells. The treatment dose of 400 mg/kg/day was observed for 24 days, and the oil reduced the growth and occurrence of metastasis; after 24 days, 71% of mice were free of lymph node metastases (Arafat et al. 2022).

4 Toxicity

The drug development is a slow and costly process, and few products reach the market due to the high incidence of failure in the clinical trial phase due to low efficacy and high toxicity. In this perspective, the pharmacological and toxicological properties of the constituents need to be analyzed carefully (Radulović et al.

2013; Tomiotto-Pellissier et al. 2022). In this way, essential oils stand out for quickly crossing biological barriers to finally reach the circulatory system (Nair et al. 2022).

Due to the high absorption rate of essential oils and their lipophilic properties, the organism's toxicity risk must be evaluated (Nair et al. 2022). The active constituents interfere the toxicity of essential oils according to their chemical structure and concentration. In this way, research on toxicity enables an answer about its potentiating effects and its safe use (Herman and Herman 2015).

Systemic toxicity of essential oils can be considered low or moderate. Clinical studies in humans based on the topical use of *Pinus sylvestris* and *Eucalyptus globulus* oils reported good tolerance when used by inhalation and topically (Reichling et al. 2009). An acute toxicity test in mice classified the association of essential oils from *Croton argyrophyllus* and *C. tetradenius* (1:1) as medium toxicity when administered intraperitoneally, so the combination from this route of administration should be careful. On the other hand, when used orally, it did not manifest toxicity, being safe in mammals (da Cruz et al. 2020).

Studies generally recognize that the oils of the *Curcuma* genus are safe due to their low irritability in mice. In rats, when administered orally, the average lethal dose (LD_{50}) was greater than 5 g/kg. However, it is reported that despite not presenting acute toxicity or adverse reactions, *Curcuma zedoaria* oil should not be recommended during pregnancy and breastfeeding, as it presented embryotoxicity in mice when administered intraperitoneally at 300 mg/kg and by intravaginally at doses of 60 or 400 mg/kg/day in rabbits (Dosoky and Setzer 2018).

5 Incorporation of Essential Oils in Pharmaceutical Formulations

Nanotechnology is expanding, which can be defined as the use of matter in atomic area, supramolecular and molecular scale, providing viable forms of inclusion for compounds of natural origin with a lipid character in cosmetics and pharmaceutical products (Nahar et al. 2021).

Essential oils have volatile and fragile constituents, which limits their use due to enzymatic reactions and phenomena that interfere with their activities, which may increase toxicity. In this way, nanotechnology improves the distribution system of essential oils in the body, increasing their bioavailability, potency and enabling a good pharmacokinetic profile (Cimino et al. 2021).

According to the lipid formulations, nanoparticles, nanoencapsulation, and nanoemulsions stand out, with differences indicated in Fig. 3, as they present pharmaceutical benefits such as ease of production, good solubility, and drug safety (Masiero et al. 2021).

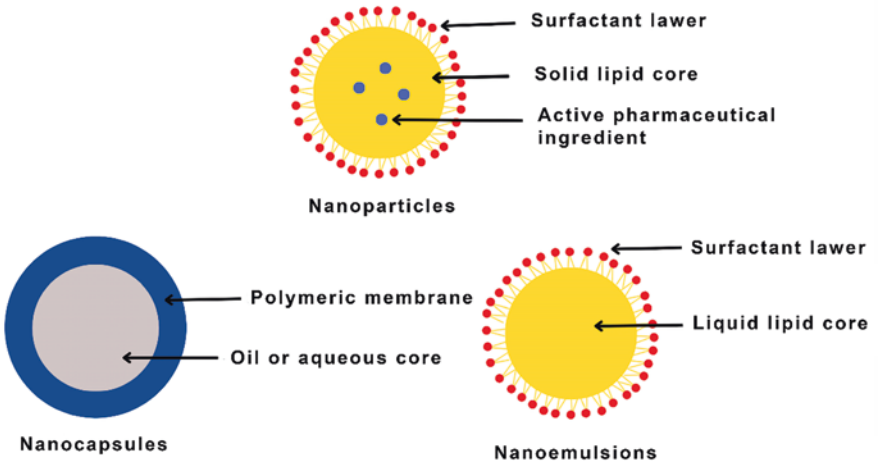

Fig. 3 Lipid dosage forms used with essential oils

5.1 Nanoparticles

Nanoparticles have 1 to 100 nanometers, which makes it possible to develop new treatment and diagnostic strategies, considered an innovation for pharmaceutical sciences, thus improving biological mechanisms (Nair et al. 2022). In this sense, nanoparticles have different forms that depend on the specific condition and are of great importance in combating many pathologies, playing a key role in biomedical applications (Rai et al. 2017). In this way, nanoparticles have the potential to protect essential oils from degradation, heat, ensuring greater stability, increasing the therapeutic effects and shelf life of the product (Nair et al. 2022).

Drug release mechanisms consist of three stages. The first stage involves the release of the active compound on the nanoparticle‘s surface. The second involves kinetic release through factors such as the dissolution medium, concentration gradient, and diffusion medium. The final stage involves degradation of nanoparticle components. In this perspective, nanoparticles are a revolutionary advance capable of expanding the stability of the drug, increasing the transport capacity, facilitating the different routes of administration, incorporating hydrophobic and hydrophilic compounds and the controlled release of the drug, bringing benefits to the bioavailability and improving the nonadherence problem (Masiero et al. 2021).

Chitosan nanoparticles containing the essential oil of *Elettaria cardamomum*, proved to be effective and safe, with positive results for the absence of necrosis and hemolysis in mammalian cells. According to the authors, the oil-laden nanoparticles provided high solubility, chemical stability, decreased volatility, increased shelf life, and good antimicrobial action. In addition, studies on the essential oil of *Mentha spicata* have shown that its adsorption on the surface of hydroxyapatite nanoparticles can improve the production of implants that reduce postoperative infections (Nair et al. 2022).

5.2 Nanocapsules

Nanocapsules are encapsulation systems composed of an oil and aqueous phase core of approximately 115 nm, which protect the active compounds against photodegradation through a natural or synthetic polymeric membrane (Bilia et al. 2017).

Presenting an oily phase, the nanocapsule promotes the ideal transport for lipophilic molecules, such as essential oils, while enabling a controlled release of constituents, with a regulated penetrability rate, from polymers and surfactants during formulation. In addition, it also reduces irritability and harmful effects due to biocompatibility with tissues and cells, being a system commonly applied topically and associated with semisolid systems, such as creams. Currently, research has successfully developed responsive nanocapsules capable of transporting essential oils (da Costa et al. 2020b; Deng et al. 2020; Oliveira et al. 2022; Gupta et al. 2022).

Kalita et al. (2017) evaluated the antibacterial activity of *Cymbopogon flexuous* essential oil nanoencapsulation with chloramphenicol and reported significant improvement in its action. The adequate particle size, low cytotoxicity, and sustained release of the active compounds contributed to the bioavailability and therapeutic efficacy against bacterial resistance.

Furthermore, the literature reports that the nanoencapsulation with *Origanum glandulosum* oil showed a greater inhibitory capacity against human liver cancer cell line than the non-nanoencapsulated oil (Ali et al. 2020). Meanwhile, another study shows that different methods, such as nanoprecipitation, emulsion-coacervation, and polymer-coating can improve the pharmacokinetics of nanocapsules, allowing a better controlled release and contributing to the anticancer activity of the essential oil (Rahman et al. 2020).

5.3 Nanoemulsion

Nanoemulsions are colloidal dispersions with an average droplet size of less than 200 nm, composed of a liquid phase and an oil phase, with a structure represented by an oil core surrounded by mono- or multilayers of a surfactant with the nonpolar tails oriented toward the hydrophobic core. The polar ends toward the hydrophilic medium allow the combination of two immiscible liquids, thereby reducing interfacial tension (Pavoni et al. 2020).

Nanoemulsions are excellent carriers of lipophilic drugs as they increase drug absorption due to the high surface area and low energy demand to prepare them, in addition to providing controlled drug release (Barradas and de Holanda e Silva 2021; Masiero et al. 2021).

The most used essential oil-based lipid nanocarriers are nanoemulsions due to their physicochemical stability, ability to reduce the hydrophobicity of oils, serve as protection against environmental degradation and early evaporation, and good

performance in in vitro and in vivo assays (Barradas and de Holanda e Silva 2021; Masiero et al. 2021).

Nanoemulsions containing essential oils from *Foeniculum vulgare* or *Syzygium aromaticum* could modulate the transdermal release of oils and skin retention. Specifically, the oil of *Syzygium aromaticum* associated with the surfactant Pluronic F68 caused an increase in retention and low skin permeation. While *F. vulgare* oil is associated with the emulsifying agent, Cremophor RH40 contributed to greater penetration and retention in the skin (Barradas and de Holanda e Silva 2021).

Nanoemulsions with the antitumor agent mitomycin C, associated with essential oils of *Zingiber officinale* and *Boswellia carteri*, resulted in greater nuclear apoptotic activity, relative to free mitomycin C, in cervical and breast cancer cells. Furthermore, it was reported that the *Cymbopogon flexuosus* oil nanoemulsion demonstrated high antimicrobial activity against *Candida albicans*, *Cryptococcus grubii*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus* when compared to the free oil (Masiero et al. 2021). Furthermore, (de Moraes et al. 2018) evaluated the leishmanicidal activity of nanoemulsions of *Copaifera* sp. and *Carapa guianensis* in mice with promastigotes of *Leishmania infantum* and *Leishmania amazonensis* and concluded that treatment with nanoemulsions reduced the size of lesions caused by these pathogens with a decrease in parasites in the liver and spleen.

6 Standardization of Essential Oils: Influences of Biotic and Abiotical Factors

The composition and content of essential oils can be affected by biotic, abiotic, environmental, and genetic aspects (Kumar et al. 2021). Moreover, other factors can also influence constituents, such as adulteration and mislabeling. Therefore, the authenticity of essential oils must be ensured through standardized methods for their application in pharmaceutical industries (Ordoudi et al. 2022).

The oils can be influenced by environmental and genetic factors (da Costa et al. 2020a). The collection period of *Chamomilla recutita* with and without weed was evaluated at the following times: 6 am, 12 am, and 6 pm. The results showed that the essential oil content was higher in chamomile flowers without grass compared to with grass. The highest oil content of *C. recutita* without grass was detected between 12 and 18 hours. This increase may be related to the temperature and light intensity increase, which improves metabolic reactions by increasing the synthesis of secondary plant metabolites (Kumar et al. 2021).

The production of plant secondary metabolites can be altered by seasonality since the seasonality can modify the metabolic route leading to the synthesis of different compounds (de Castro et al. 2022). *Psidium acutangulum* oil was evaluated for the influence of climatic factors (humidity, sunlight, and precipitation) on its yield and composition, showing seasonal variability. Thus, a prior investigation must be carried out before its application (Santos et al. 2022). On the other hand, the

essential oil of *Lippia alba* showed geraniol as the predominant constituent with few quantitative variations during the seasonal study. However, environmental factors had little effect on chemical composition, suggesting that seasonal changes do not alter herbal biological activities (de Sousa Peixoto Barros et al. 2022).

As an adaptation mechanism, Eucalyptus species may change due to growth, development, morphology, and physiological functions due to environmental variations directly affecting the oil quantitatively and qualitatively. In addition, plant age also contributes to changes in chemical composition. *Eucalyptus tereticornis* sampled at different ages reported that the 1,8-cineole content of the 25-year-old sample was higher than the 28-year-old sample (de Castro et al. 2022).

Thus, analysis of the chemical composition of essential oils is critical to ensure their safety (Pandey et al. 2020; Kumar et al. 2021). Moreover, it is essential to carry out phytochemical studies to control the quality of the oil, in addition to using the data obtained from the pharmacopeia and other studies as a reference (de Castro et al. 2022).

Therefore, essential oils have pharmacological properties with the potential for therapeutic use and the development of new drugs. In addition, they can be a useful tool for safe and effective therapy against various pathologies. However, further studies are needed to deepen the pharmacokinetic mechanisms and verify toxicity profiles and quality control of essential oils.

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Essential Oil-Derived Monoterpenes in Drug Discovery and Development

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Abstract Essential oils are complex mixtures of plant secondary metabolites composed mostly of terpenoids, aliphatic and aromatic hydrocarbons, and their derivatives such as aldehydes, ketones, alcohols, and esters. They play important roles as defense compounds against microbes, herbivores, and other ecological stress factors according to their structural designs. Among the secondary metabolites, monoterpenes (C_{10}) form the major group. Several reports have shown that both natural monoterpenes and their synthetic derivatives exhibit a wide range of biological activities. In this chapter, a review of the anti-inflammatory, analgesic, antitumor, anticonvulsant, cardioprotective, gastroprotective, wound-healing, antifungal, antibacterial, and antiviral properties of different classes of monoterpenes is discussed.

Keywords Plant secondary metabolites · Essential oils · MEP pathway · MVA pathway · Monoterpenoids · Acyclic monoterpenes

1 Introduction

Essential oils are highly concentrated hydrophobic volatile oily liquids (secondary metabolites) responsible for the characteristic odor in aromatic plants. They can be synthesized by all plant organs, i.e., buds, flowers, leaves, stems, twigs, seeds, fruits,

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roots, wood, or bark, and are stored in secretory cells, cavities, canals, epidermic cells, or glandular trichomes (Baby and George 2009; Chouhan et al. 2017). The chemical fingerprint of the essential oil varied in quality, quantity, and composition according to agroclimatic (climatic, seasonal, and geographical) conditions, stage of maturity, and the adaptive metabolism of plants (Angioni et al. 2006; Ahmed et al. 2019). In plants, they function as important chemical mediators of antagonistic and mutualistic ecological interactions (Nunes et al. 2016) and are also involved in plant growth promotion, light harvesting, and photoprotection (Bhatla 2018). Apiaceae, Asteraceae, Clusiaceae, Cupressaceae, Fabaceae, Geraniaceae, Hypericaceae, Lamiaceae, Lauraceae, Liliaceae, Malvaceae, Myristicaceae, Myrtaceae, Oleaceae, Pinaceae, Poaceae, Rosaceae, Rutaceae, and Zingiberaceae are examples of essential oil-bearing plant families (Pragadheesh et al. 2020).

Terpenes or terpenoids or isoprenoids are structurally diverse and the most abundant secondary metabolites (Pragadheesh et al. 2020). It comprises over 80,000 compounds produced by plants, fungi, insects, marine organisms, and animals (Christianson 2017). Essential oils are complex mixtures of terpenes (terpenoids), aliphatic and aromatic hydrocarbons, and their derivatives such as aldehydes, ketones, alcohols, and esters. Terpenoids are classified according to the number of incorporated five-carbon isoprene molecules and are distinguished into hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}), and tertraterpenes (C_{40}) (Baby and George 2009). Among these, monoterpenes are the major component of essential oils, followed by sesquiterpenes (Falleh et al. 2020). Diterpenes, triterpenes, and tetraterpenes with their oxygenated derivatives are also detected in small amounts (Stephane and Jules 2020; Masyita et al. 2022). In certain plant genera, phenylpropanoids are also found in the essential oil (Sadgrove et al. 2022), sometimes as the main component (e.g., cinnamaldehyde in *Cinnamomum zeylanicum* and eugenol in *Syzygium aromaticum*).

2 Biosynthesis of Monoterpenes

Monoterpenes are biosynthetically derived from two 5-carbon-base isoprene precursors, namely isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are synthesized via either the methylerythritol 4-phosphate (MEP) or the mevalonate-dependent (MVA) pathways (Fig. 1). The MVA pathway operates in the cytosol and starts with the condensation of two molecules of acetyl-coenzyme A (CoA) to yield acetoacetyl-CoA, which undergoes another condensation to form 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA). With the help of an enzyme HMG-CoA reductase, HMG-CoA is reduced to (R)-mevalonate which is phosphorylated to get mevalonate-5-phosphate by mevalonate-5-kinase. The mevalonate-5-phosphate is again phosphorylated to yield mevalonate pyrophosphate by phosphomevalonate kinase. The resulting mevalonate pyrophosphate undergoes decarboxylation to get IPP by an enzyme called mevalonate pyrophosphate decarboxylate. The conversion of IPP to DMAPP is enabled by isopentyl pyrophosphate

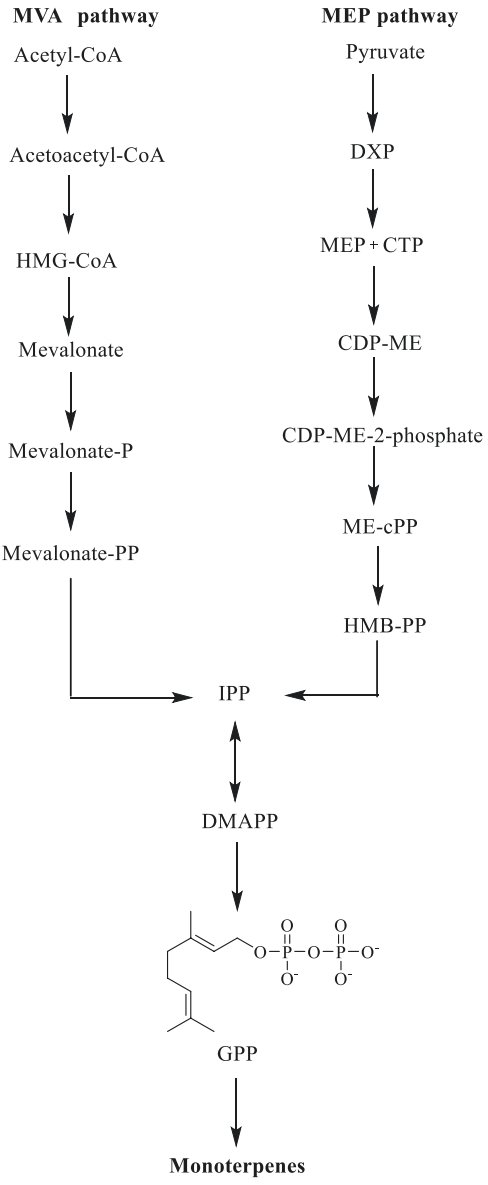


Fig. 1 Monoterpene biosynthetic pathway

isomerase (Baser and Demirci 2007; Lange and Ahkami 2013; Zuzarte and Salgueiro 2015; Haymond et al. 2014; Zebec et al. 2016).

The 2C-methyl-D-erythritol-4-phosphate (MEP) or non-mevalonate or deoxyxylulose phosphate pathway starts off with condensation of D-glyceraldehyde-3-phosphate and pyruvate affording 1-deoxy-D-xylulose-5-phosphate (DXP). The next step is reductive isomerization of DXP to MEP by DXP reductoisomerase.

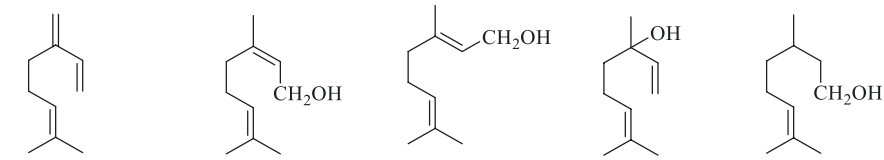
Then, coupling between MEP and cytidine triphosphate (CTP) is catalyzed by CDP-ME synthetase which produces methylerythritol cytidyl diphosphate (CDP-ME), which is phosphorylated to 4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate (CDP-MEP-2-phosphate). The CDP-MEP is cyclized by 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (IspF) to 2-C-methyl-D-erythritol2,4-cyclodiphosphate (MEc-PP), which further undergoes ring opening of the cyclic pyrophosphate followed by the C3-reductive dehydration to produce 4-hydroxy-3-methyl-butenyl-1-diphosphate (HMBPP). Finally, the enzyme 4-hydroxy-3-methylbut-2-enyl diphosphate reductase or isoprenoid synthesis H (IspH) converts (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) to both IPP and DMAPP. A head-to-tail condensation of IPP with DMAPP in the presence of prenyltransferase forms geranyl diphosphate or geranyl pyrophosphate (GPP, C_{10}), the common precursor for monoterpenes (Baser and Demirci 2007; Lange and Ahkami 2013; Zuzarte and Salgueiro 2015; Haymond et al. 2014; Zebec et al. 2016).

3 Classification of Monoterpenes

Monoterpenes represent a major group of secondary metabolites. Monoterpenes comprises of two isoprene units ($C_{10}H_{16}$) and are found in plants, fungi, bacteria, etc. (Ninkuu et al. 2021). They are odoriferous and one of the major components in the essential oils. Monoterpene motif also containing heteroatoms such as oxygen or nitrogen are recognized as monoterpenoids (Volcho and Anikeev 2014). They occur as acyclic (e.g., geraniol, citral) and cyclic compounds, the cyclic compounds occurring as monocyclic (e.g., carvone, thymol), and bicyclic (e.g., camphor, thujone). The bicyclic monoterpenes may be further divided into another three classes according to the size of the second ring. The first ring being cyclohexane in each class and the second ring may be three, four, or five membered (Waser and Rinner 2016; Ninkuu et al. 2021).

3.1 Acyclic Monoterpenes

Acyclic monoterpenes are relatively few, unstable, highly volatile, and their oxygenated derivatives are widely distributed in nature. These are biogenetically derived from common precursor, geranyl pyrophosphate. For example, elimination of the pyrophosphate moiety from geranyl pyrophosphate leads to the formation of *β*-myrcene and ocimene and the hydrolysis of the phosphate groups give geraniol (*trans*-3,7-dimethyl-2,6-oktadien-1-ol). Moreover, oxidation, reduction, and rearrangement provide various alicyclic monoterpenes such as citral, citronellal, citronellol, linalool, and many others (Fig. 2) (Wise and Croteau 1999; Bicas et al. 2009).



β-Myrcene (Z)–β-Ocimene Geraniol Linalool Citronellol

Fig. 2 Some acyclic monoterpenes

3.2 Cyclic Monoterpenes

Cyclic monoterpenes are highly volatile secondary metabolites commonly used in pharmaceutical and cosmetic industry. They include limonene, menthol, camphor, and carvone. These are the active constituent of lemon oil, mint oil, camphor oil, and caraway oil. They are further subdivided into monocyclic such as menthane and bicyclic such as pinane, camphane, isocamphane, fenchane, carane, and thujane type. Monoterpenoids are biosynthetically derived from menthyl or α-terpinyl cation which undergo series of cyclizations, hydride shifts or Wagner–Meerwein rearrangements, deprotonation, and addition of a nucleophile enable the synthesis of a broad variety of mono- and bicyclic monoterpenes (Fig. 3) (Loza-Tavera 1999; de Carvalho and da Fonseca 2006; Degenhardt et al. 2009; Zebec et al. 2016).

3.2.1 Monocyclic Monoterpenes

Monocyclic monoterpenes are structural homologs of cyclohexane ring and are derived from dehydrogenation of methyl-isopropyl cyclohexane. Menthane is fundamental unit of monocyclic monoterpenes and exist in three isomeric forms: ortho-, meta-, and para-menthanes. Carvone from the leaves of *Salvia karelinii* (syn. *Perovskia angustifolia*), menthol from *Mentha arvensis*, thymol from *Thymus pubescens,* γ-terpinene from *Cinnamomum longepaniculatum,* and limonene from *Citrus* × *limon* (syn. *Citrus* × *bergamia*) are some of the examples of monocyclic monoterpenes (Fig. 4) (Loza-Tavera 1999; de Carvalho and da Fonseca 2006; Degenhardt et al. 2009; Zebec et al. 2016).

3.2.2 Bicyclic Monoterpenes

Bicyclic monoterpenes are more complex than monocyclic monoterpenes and comprises of two cyclic rings that are condensed together. Depending on the size of the second ring, bicyclic monoterpenes are further divided into three classes, the first being a six-membered cyclohexane moiety in each class while the second can be either a three (6 + 3; e.g., thujone, δ-3-carene), four (6 + 4; e.g., α-pinene, β-pinene), or five (6 + 5; e.g., borneol, camphor) membered rings. Thujane-type monoterpenes

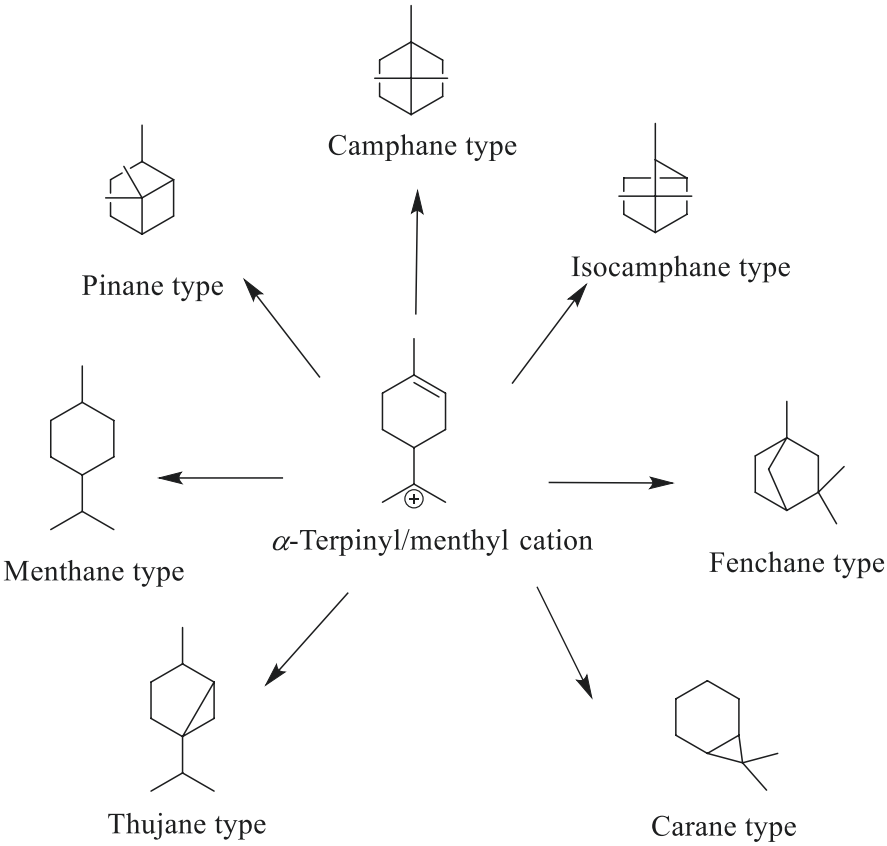


Fig. 3 Different types of cyclic monoterpenes

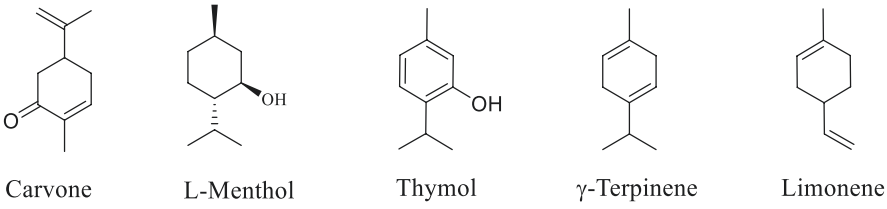


Fig. 4 Some monocyclic monoterpenes

are unusual monoterpenes with a cyclopropane ring in a bicyclo[3.1.0] skeleton, which is formed from terpinen-4-yl cation. Carane-type also contain cyclopropane ring in a bicyclo [4.1.0] skeleton. Pinane monoterpenes are bicyclic [3.1.1] skeleton resulting from intramolecular rearrangement of the α-terpinyl cation. The bornane-, camphane-, and fenchane-type monoterpenes have [2.1.1] bicyclic skeleton formed

Fig. 5 Some bicyclic monoterpenes

from different cyclisation of the terpinyl cation (Fig. 5) (Loza-Tavera 1999; de Carvalho and da Fonseca 2006; Degenhardt et al. 2009; Zebec et al. 2016).

4 Bioactivity of Monoterpenes

Studies around the globe have shown that monoterpenes have diverse biological properties. In this chapter, we have discussed the anti-inflammatory (de Cássia da Silveira e Sá et al. 2013; Quintans et al. 2019), analgesic (Guimarães et al. 2013), antitumor (Sobral et al. 2014; Machado et al. 2022), anticonvulsant (Zhu et al. 2014; da Fonsêca et al. 2019), cardioprotective (Santos et al. 2011; de Andrade et al. 2017; Silva et al. 2021), gastroprotective (Périco et al. 2020), wound-healing (Barreto et al. 2014), and antimicrobial (Baby and George 2009; Marchese et al. 2017; Mahizan et al. 2019) properties of various monoterpenes isolated from plants (Kozioł et al. 2014; Salakhutdinov et al. 2017; Zielińska-Błajet and Feder-Kubis 2020; Yang et al. 2020).

4.1 Anti-inflammatory Activity

In an electrophysiological study of HEK293T epithelial cells, Ye et al. (2019) found that geraniol reversibly blocked Kv1.3 (voltage-gated potassium channel) currents in a voltage-dependent manner (half maximal inhibitory concentration [IC_{50}] of 490.50 1.04 M at +40 mV). Geraniol also inhibited the secretion of cytokines such as interleukin (IL)-2, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ by activated human T cells. In an imiquimod-induced psoriasis-like animal model, geraniol significantly reduced psoriasis area and severity index scores. Su et al. (2010) found that geraniol and citronellol inhibited nitric oxide (NO) and prostaglandin E_2 (PGE_2) production in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages. Katsukawa et al. (2011) found that geraniol and citronellol reduced LPS-induced cyclooxygenase (COX)-2 protein and messenger ribonucleic acid (mRNA) expression and activated peroxisome proliferator-activated receptor (PPAR)-α and PPARγ.

Limonene effectively inhibited LPS-induced NO, PGE_2, and proinflammatory cytokine production in RAW 264.7 macrophages (Yoon et al. 2010).

Paeoniflorin isolated from the dried rhizome of *Paeonia lactiflora* and its derivatives such as 4-O-methyl paeoniflorin, 4-O-methylbenzoyl paeoniflorin inhibited the production of NO, IL-6, and TNF-α induced by LPS (Bi et al. 2017). 1,8-Cineole showed anti-inflammatory activity in various animal models and in in vitro studies (Juergens et al. 1998a, 1998b, 2003, 2004; Santos and Rao 2000; Santos et al. 2004; Bastos et al. 2011). A double-blind, placebo-controlled clinical trial on the effect of 1,8-cineole evidenced a mucolytic and steroid-saving effect in bronchial asthma patients (Juergens et al. 2003). Juergens et al. (1998) found that l-menthol significantly reduced the production of leukotriene B_4 (LTB_4), PGE_2, and IL-1β in LPS-stimulated monocytes. El Mezayen et al. (2006) found that administration of thymoquinone effectively reduced COX-2 protein expression in sensitized mouse lungs.

El Gazzar et al. (2006) found that thymoquinone treatment markedly decreased lung eosinophilia and the production of T-helper 2 (Th2) cytokines following stimulation of lung cells with ovalbumin. Liu et al. (2011) found that borneol ameliorated oxygen glucose deprivation and reperfusion (OGD/R)-induced neuronal injury, nuclear condensation, intracellular free radical generation, and mitochondrial membrane potential dissipation. Borneol inhibited the nuclear factor kappa B (NF-κB) p65 nuclear translocation induced by OGD/R, thereby reduced the release of proinflammatory cytokines. He et al. (2006) found that experimental rats given borneol had fewer intercellular adhesion molecule (ICAM)-1-positive vessels, IL-1β-positive cells, TNF-α-positive cells, and neutrophils, indicating anti-inflammatory potential. Bornyl acetate showed anti-inflammatory activities in various experimental models (Wu et al. 2004). Sousa et al. (2020) found that (S)-(+)-carvone (100μg/ml) significantly decreased the expression of nitric oxide synthase (Nos)2 and IL-1β in murine macrophages and in a primary human chondrocyte model of osteoarthritis.

Riella et al. (2012) discovered that thymol (10, 30, and 100 mg/kg, i.p.) significantly reduced edema, inhibited myeloperoxidase (MPO) activity, and decreased leukocyte influx in carrageenan-induced paw edema in rats. Sosa et al. (2005) found that carvacrol (0.1, 1, 10, 50, and 100μM) significantly inhibited the production of PGE_2 catalyzed by COX-2 in in vitro. Carvacrol inhibited LPS-induced COX-2 mRNA in human macrophage-like U937 cells (Hotta et al. 2010). Silva et al. (2012) found that carvacrol significantly reduced paw edema induced by histamine, dextran, and substance P by 46%, 35% (50 mg/kg), and 46% (100 mg/kg), respectively. Batista et al. (2010) found that (−)-linalool significantly reduced complete Freund's adjuvant (CFA)-induced mechanical hypersensitivity and produced an effective reduction in CFA-induced paw edema. Peana et al. (2002) found that (−)-linalool, its racemate form, i.e., (±)-linalool, and linalyl acetate showed anti-inflammatory activity in a carrageenan-induced edema rat model. Gomes et al. (2017) found that myrtenol (12.5, 25, and 50 mg/kg, p.o.) reduced oxidative stress and neutrophil migration in a CFA-induced arthritis model and carrageenan-induced peritonitis in rats.

Ramos et al. (2020) found the anti-edematogenic and anti-inflammatory potential of isopulegol in rodent models. Isopulegol significantly reduced inflammatory activity by decreasing albumin extravasation, leukocyte migration, and MPO concentration, as well as IL-1β and TNF-α exudate levels. Siqueira et al. (2016) discovered that α-phellandrene (50, 100, and 200 mg/kg, p.o.) inhibited neutrophil migration, proinflammatory cytokine production (TNF-α and IL-6), and mast cell degranulation. Rufino et al. (2015) found that myrcene (IC_{50} = 37.3μg/ml) had significant anti-inflammatory activity in human chondrocytes by inhibiting IL-1β-induced NF-κB, *c*-Jun *N*-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK) activation, and the expression of induced nitric oxide synthase.

D-Limonene increased the survival of lymphoma-bearing BALB/c mice and delayed the hypersensitivity reaction to 2,4-dinitrofluorobenzene (Del Toro-Arreola et al. 2005). Hydroxydihydrocarvone obtained by hydration of the natural compound (R)-(−)-carvone exhibits anti-inflammatory activity in a carrageenan-induced paw edema rodent model (de Sousa et al. 2010a). Ramalho et al. (2015) found that γ-terpinene significantly alleviated inflammatory parameters such as edema and proinflammatory cytokine production, as well as cell migration in different Swiss mouse models of inflammation. Ozbek (2007) found that fenchone at a dose of 0.20 ml/kg exerted significant anti-inflammatory activity (70.6%) in a carrageenan-induced right hind-paw edema rat model. (S)-cis-Verbenol reduced the cerebral ischemic injury caused by a 1.5-hour middle cerebral artery occlusion followed by 24-hour reperfusion (Choi et al. 2010). The structures of the abovementioned monoterpenes with anti-inflammatory activity are given in Fig. 6.

4.2 Analgesic Activity

Pereira et al. (2022) found that limonene significantly reduced acute and chronic corneal nociception and formalin-induced temporomandibular joint nociception. Limonene decreased the TNF-α levels, downregulated the NF-κB and p38 MAPK signaling pathways and reduced protein kinase C (PKC) substrate phosphorylation and cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) immunocontent. Kaimoto et al. (2016) found that limonene significantly reduced the hydrogen peroxide-induced nociceptive behaviors through transient receptor potential ankyrin (TRPA)-1 activation tested in a mouse model. de Santana et al. 2015 discovered that p-cymene (25, 50, and 100 mg/kg) significantly reduced the hyperalgesia induced by carrageenan, TNF-α, dopamine, and PGE_2, implying a possible opioid system involvement and modulation of some proinflammatory cytokines. p-Cymene at a dose range of 25 to 100 mg/kg showed significant antinociceptive activity in male Swiss mice demonstrated in the tail flick test and showed an increase in dose-dependent reaction time (de Santana et al. 2015). Quintans-Júnior et al. (2013) analyzed the comparative antinociceptive activity of p-cymene, (+)-camphene, and geranyl acetate (50, 100, and 200 mg/kg, i.p.) in male Swiss mice tested in the acetic acid-induced writhing and formalin models. They

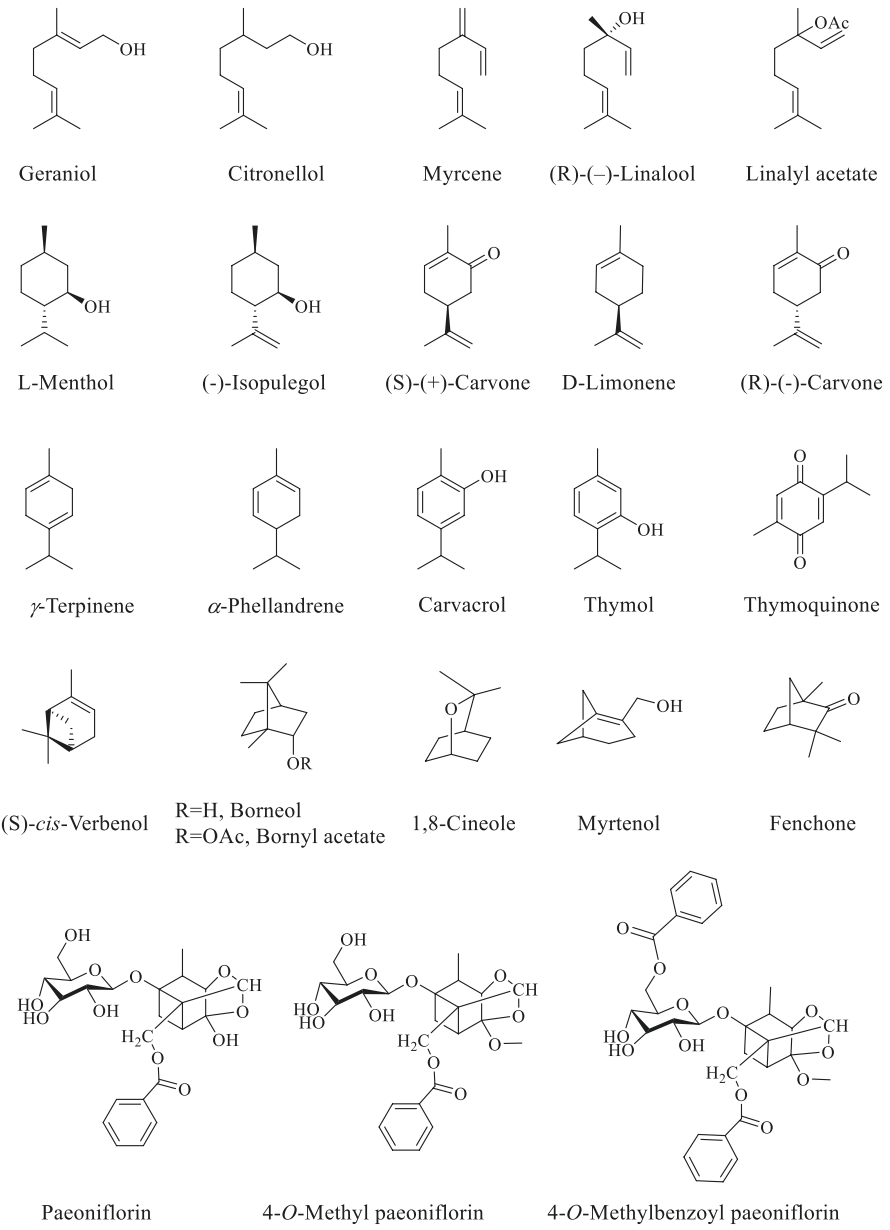


Fig. 6 Some monoterpenes with anti-inflammatory activity

discovered that p-cymene had a strong antinociceptive effect at all doses, while (+)-camphene and geranylacetate (200 mg/kg) had a moderate effect. They also found that p-cymene at doses of 25, 50, and 100 mg/kg, i.p., demonstrated orofacial antinociceptive activity in Swiss mice. Bonjardim et al. (2012) reported that p-cymene at doses of 50 and 100 mg/kg, i.p., significantly decreased the number of

writhes in the acetic acid-induced writhing model and licking time in the first and second phases of the formalin test.

Intraplantar injection of (±)-linalool (5 and 10μg/paw) in male ddY-strain mice effectively and dose-dependently suppressed behavioral responses to paclitaxel-induced mechanical allodynia and hyperalgesia (Katsuyama et al. 2012). Tashiro et al. (2016) found that linalool exhibited antinociceptive activity in vapor exposure mediated by hypothalamic orexin neurons. Dal Bó et al. (2013) discovered that eugenol (3–300 mg/kg, p.o.) had antinociceptive action via opioid receptor involvement, glutamatergic receptor modulation, and TNF-α inhibition. Ferland et al. (2012) found that eugenol (40 mg/kg, p.o.) showed antinociceptive activity in monoiodoacetate-induced osteoarthritis in Sprague-Dawley rats. Wang et al. (2015) found that methyl eugenol dose- and voltage-dependently inhibited the peripheral nerve Nav1.7 (voltage-gated sodium channel) currents in the whole-cell patch-clamp method (IC_{50} of 295μmol/l at a − 100 mV holding potential). Pan et al. (2012) found that menthol (50 and 100 mg/kg, i.p.) suppressed ipsilateral and contralateral pain hypersensitivity induced by complete Freund's adjuvant reduction and reduced nociceptive activity up on formalin injection in both phases tested in cluster of differentiation (CD)-1 male mice.

Citronellal at doses of 50, 100, and 200 mg/kg administered intravenously resulted in dose-dependent significant reduction in nociception in various models (Quintans-Júnior et al. 2011a). Citronellal reduced nociception induced by TNF-α and carrageenan at doses of 25, 50, and 100 mg/kg, i.p. (de Santana et al. 2013). Citronellol at doses of 25, 50, and 100 mg/kg i.p. showed a significant reduction in nociception in acetic acid-induced writhing, formalin-induced pain, the hot plate test, and orofacial nociception models. Citronellol also reduced neutrophil infiltration and TNF-α levels in carrageenan-induced pleurisy exudates (Brito et al. 2012, 2013, 2015). α-Phellandrene at doses ranging from 3.125 to 50 mg/kg p.o., as well as citronellyl acetate at 100 and 200 mg/kg, significantly reduced nociception in chemically induced acute pain models in mice (Lima et al. 2012; Rios et al. 2013). Quintans-Júnior et al. (2011b) discovered that α-terpineol (25, 50, and 100 mg/kg, i.p.) reduced nociception significantly in all doses tested in chemically induced mouse models. Safaripour et al. (2018) discovered that α-terpineol (40 and 80 mg/kg) exerted antinociceptive activity in mice via the L-arginine/ *S*-nitroso-*N*-acetylpenicillamine (SNAP)/NO/cyclic guanosine monophosphate (cGMP)/adenosine triphosphatase-sensitive potassium (KATP) channel pathway. Bilbrey et al. (2022) found the analgesic potential of α-terpineol, β-caryophyllene, and γ-terpinene in the mouse chronic constriction injury model of neuropathic pain. Hernandez-Leon et al. (2020) discovered that β-caryophyllene (3.16 to 10 mg/kg) exhibited significant analgesic properties in various rodent models via receptors such as opioids, benzodiazepines, and serotonin 1A receptor, as well as nitric oxide.

Gonçalves et al. (2008, 2013) found the antinociceptive action of carvone in both the central and peripheral nervous systems. They reported that carvone increased cytosolic calcium levels in dorsal root ganglion (DRG) neurons by activating transient receptor potential vanilloid (TRPV)1 channels. Xu et al. (2015) found that thymol increased the frequency of spontaneous excitatory postsynaptic current

through activation of TRPV1 channels and produced membrane hyperpolarization without TRP activation in substantia gelatinosa neurons of adult rat spinal cord slices. Parvardeh et al. (2018) reported the central and peripheral antinociceptive activity of thymoquinone through the L-arginine/NO/cGMP/KATP channel pathway. de Sousa et al. (2011a) found that (R)-(+)-pulegone (31.3, 62.5, and 125 mg/kg, i.p.) showed potent antinociceptive activity in formalin and hot plate test mouse models. Guimarães et al. (2012a, b) found that carvacrol, at a dose range of 25 to 100 mg/kg i.p., markedly reduced the nociception in a male Swiss mouse model induced with carrageenan, formalin, capsaicin, and glutamate. Luo et al. (2014) reported that carvacrol treatment significantly increased the secretion of L-glutamate from nerve terminals by activating TRPA1 and produced membrane hyperpolarization in adult rat spinal cord slices.

Citral (25, 100, and 300 mg/kg, p.o.) showed potent antinociceptive activity against acute and chronic nociceptive mouse models and found that citral has the potential for the treatment of inflammatory and neuropathic pain (Nishijima et al. 2014). Almeida et al. (2013) found that borneol (5, 25, and 50 mg/kg, i.p.) exhibits significant central and peripheral antinociceptive and anti-inflammatory properties. In another study conducted by Jiang et al. (2015), it was found that (+)-borneol (125, 250, and 500 mg/kg, p.o. or i.t.) showed antihyperalgesic activity on neuropathic and inflammatory pain in different animal models. Borneol dose-dependently decreased mechanical hypersensitivity in both segmental spinal nerve ligation-induced neuropathic pain and complete Freund's adjuvant-induced chronic inflammatory pain models. Silva et al. (2014) reported that myrtenol (25–75 mg/kg, i.p.) showed antinociceptive and anti-inflammatory activities in mouse models. Myrtenol inhibited the cell migration and signaling pathways of receptors involved in the transmission of pain. The structures of the abovementioned monoterpenes with analgesic activity are given in Fig. 7.

4.3 Antitumor Activity

Geraniol is a well-known acyclic monoterpene alcohol found in many essential oil-bearing plants. Studies have shown that geraniol has both therapeutic and prophylactic effects on different types of cancer, such as lung cancer (Galle et al. 2014), colon cancer (Carnesecchi et al. 2001), prostate cancer (Kim et al. 2011), pancreatic cancer (Burke et al. 1997), and liver cancer (Ong et al. 2006). In the breast cancer cell line MCF-7, geraniol inhibits tumor cell growth by blocking the G1 phase of the cell cycle (Duncan et al. 2004). Geraniol reduced tumor weight and volume in mice bearing tumors that formed from human pulmonary adenocarcinoma A549 cells (Galle et al. 2014). In Caco-2 cells (a human colon cancer cell line, geraniol at a dose of 400μM caused a 70% inhibition of cell growth (Carnesecchi et al. 2001).

Perillyl alcohol showed significant broad-spectrum antitumor activity in various cancer cell lines (Chen et al. 2015; Yang et al. 2020). Treatment with perillyl alcohol at a dose of 1 to 2 g/kg in rats significantly reduced the incidence and multiplicity

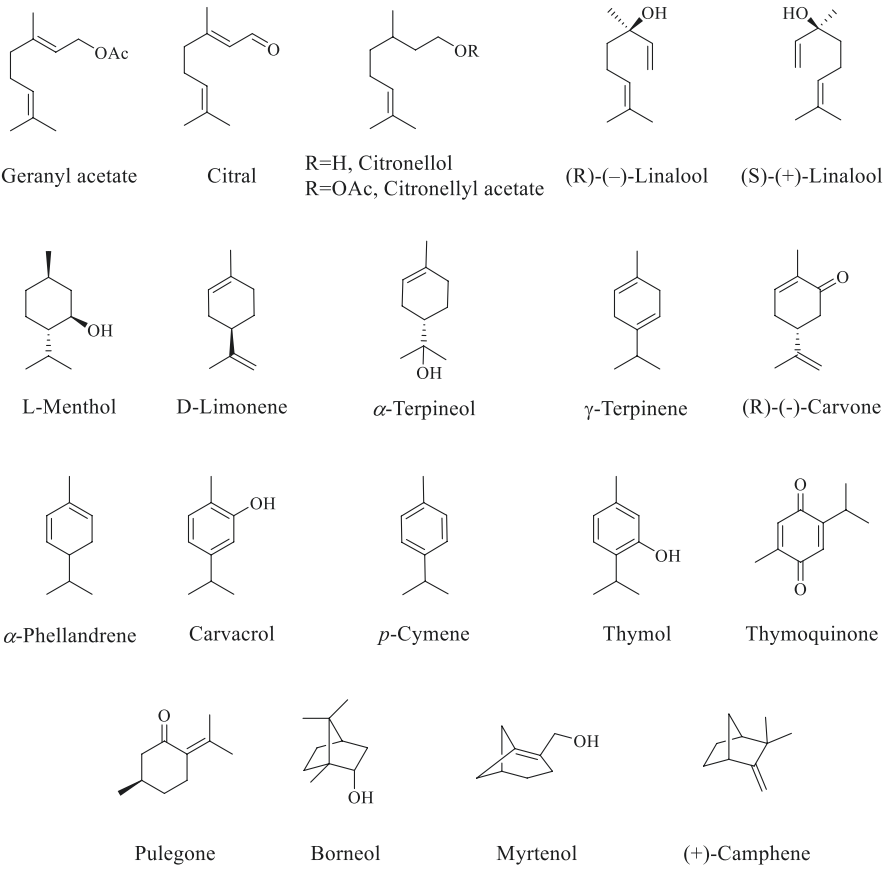


Fig. 7 Some monoterpenes with analgesic activity

of colonic invasive adenocarcinoma induced by azomethane injection (Chen et al. 2015). 4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone-induced lung cancer in mice was significantly inhibited by perillyl alcohol at a dose of 75 mg/kg (i.p.), three times per week (Lantry et al. 1997; Chen et al. 2015). Perillyl alcohol showed cytotoxic effects in OVCAR-8 (human ovarian epithelial cancer cell line), HCT-116 (human colon cancer cell line), and SF-295 (human glioblastoma cell line) cell lines, with a 90.92%–95.82% range (Andrade et al. 2015). Andrade et al. (2016) found that perillyl alcohol showed 35.3% and 45.4% inhibition of tumor growth in mice with a dose of 100 and 200 mg/kg, respectively. Oturanel et al. (2017) reported that perillyl alcohol showed cytotoxicity against human liver cancer HepG2 cells with an IC_{50} of 409.2μg/ml.

Hou et al. (2022) found that linalool inhibits colorectal cancer progression by modulating the protein kinase B (Akt)/mammalian target of rapamycin (mTOR) and Janus kinase (JAK)2/signal transducer and activator of transcription (STAT)3 pathways. Linalool (2 mM) and 1,8-cineole (8 mM) inhibited cell proliferation by

inducing G0/G1 and/or G2/M cell cycle arrest in lung adenocarcinoma A549 cells without affecting the viability of normal lung WI-38 cells (Rodenak-Kladniew et al. 2020). Rodenak-Kladniew et al. (2020) found that 1,8-cineole inhibited cell proliferation by promoting G0/G1 arrest in HepG2 cells through oxidative stress and MAPK, adenosine monophosphate-activated protein kinase (AMPK), and Akt/mTOR pathways. Linalyl acetate, α-terpineol, and camphor inhibited the growth of human colon cancer cell lines (HCT-116 p53+/+ and p53−/−) while being nontoxic to normal human intestinal FHs 74 Int cells (Itani et al. 2008). Hassan et al. (2010) reported that α-terpineol inhibits tumor cell growth by acting on the NF-κB. Allo-ocimene showed a significant cytotoxic effect in mouse P388 leukemia cells (Okamura et al. 1993). Menthol showed a cytotoxic effect in human gastric cancer cells (SNU-5) by inhibiting the expression of topoisomerases I, IIα, and IIβ and promoting the expression of NF-κB (Lin et al. 2005). Wang et al. (2012) found that menthol inhibited the proliferation and motility of prostate cancer DU145 cells. Li et al. (2009) and Okamoto et al. (2012) found that menthol induced cell death via the transient receptor potential melastatin subtype (TRPM)8 channel in human bladder cancer and oral squamous carcinoma cells.

Jo et al. (2021) found that α-pinene treatment caused cytotoxicity in natural killer cells (NK-92MI) cells via the extracellular signal-regulated kinase (ERK)/Akt pathway. Furthermore, in CT-26 colon cancer cells allografted into Bagg albino (BALB/c) mice, α-pinene inhibited tumor growth. Matsumura et al. (2001) reported that 0.32 μm/ml γ-thujaplicin inhibited human gastric cancer KATO-III and mice Ehrlich's ascites adenocarcinoma cell lines by 85% and 91%, respectively. Su et al. (2013) found that borneol potentiates selenocysteine-induced apoptosis in human hepatocellular carcinoma cells by enhancement of cellular uptake and activation of reactive oxygen species (ROS)-mediated DNA damage. Ascaridole showed antitumor activity in a Swiss mouse tumor model with sarcoma 180 cells with an inhibition percentage of 33.9% at a dose of 10 mg/kg (Bezerra et al. 2009). Horváthová et al. (2006, 2007) demonstrated that carvacrol showed cytotoxicity against K562, HepG2, and Caco2 cells and significantly reduced the hydrogen peroxide-induced DNA damage. Slamenová et al. (2007) also found the cytotoxic and DNA-protective effects of carvacrol in mammalian cells. Jaafari et al. (2007) found that carvacrol dose-dependently inhibited P815 mastocytoma cell growth. Arunasree (2010) found that carvacrol dose-dependently inhibited the growth of MDA-MB-231 human metastatic breast cancer cells. Jaafari et al. (2009) demonstrated that carvacrol induced apoptosis in the P815 tumor cell line via cell cycle arrest at the S phase. Carvacrol showed inhibition in myoblast cells even after activation of a mutated N-ras oncogene (Zeytinoglu et al. 2003).

Paramasivam et al. (2012) reported that thymol exhibited cytotoxicity in mouse neuroblastoma (Neuro-2a) cells with an IC_{50} value of 88.5 μg/ml. Yin et al. (2012) found that thymol induced cell cycle arrest at the G0/G1 phase in the human hepatocellular carcinoma cell line HepG2. Deb et al. (2011) found that thymol exhibited an apoptotic effect in HL-60 cells via caspase-dependent and caspase-independent pathways. Thymohydroquinone exhibited antitumor activity in murine tumor models with an inhibition rate of 52% (Ivankovic et al. 2006). Johnson et al. (2006)

studied the comparative cytotoxic effect of thymoquinone and thymohydroquinone in human prostate cancer PC-3 cells and found that there is a 1.7-fold decrease in the cytotoxicity of thymohydroquinone when compared to thymoquinone. Cecarini et al. (2010) found that thymoquinone induced time-dependent selective proteasome inhibition in glioblastoma cells, thereby inducing apoptosis in cancer cells. When treated against nonsmall cell lung cancer (NSCLC) and small cell lung cancer (SCLC) cell lines, Jafri et al. (2010) found that thymoquinone alone and in combination with cisplatin inhibited cell proliferation (90%) and induced apoptosis. Gali-Muhtasib et al. (2008) found that thymoquinone induced the inactivation of the stress response pathway sensor checkpoint kinase (CHEK)1 and contributed to apoptosis in colorectal cancer cells. Roepke et al. (2007) found that thymoquinone showed p53-independent apoptosis in human osteosarcoma cells. Peng et al. (2013) found that thymoquinone exhibited an antitumor and antiangiogenesis effect on osteosarcoma through the NF-κB pathway. Yazan et al. (2009) discovered that thymoquinone was toxic to HeLa cells in a dose- and time-dependent manner, inducing apoptosis through a p53-dependent pathway. Thymoquinone induced apoptosis in Hep-2 human laryngeal carcinoma cells by depleting glutathione (GSH) and activating caspase 3 (Rooney and Ryan 2005). Thymoquinone activated caspase-3, causing apoptosis in p53-null HL-60 cancer cells (El-Mahdy et al. 2005). Rajput et al. (2013) found that thymoquinone promoted G1 arrest through the inhibition of cyclin D1 and induced apoptosis in breast cancer cells.

Bai and Tang (2020) found that myrcene (0.25, 0.50, and 1.0μg/ml) mediates the anticancer activity of A549 lung adenocarcinoma cells through the activation of the apoptosis mechanism via mitochondria-mediated cell death signaling and induction of oxidative stress. Myrcene showed a cytotoxic effect against HeLa (cervical cancer cells), A-549 (lung carcinoma epithelial cells), HT-29 (colorectal adenocarcinoma cells), and Vero (cells derived from the kidney of an African green monkey) cell lines (Silva et al. 2007). Sobrerol exhibited anticarcinogenic activity during the initiation phase of 7, 12-dimethylbenz[a]anthracene (DMBA)-induced carcinogenesis (Elegbede et al. 1993). Kawamori et al. (1996) found the apoptotic effect of D-limonene in human leukemia HL-60 cells is via the activation of caspase-8. Chen et al. (1998) found that limonene showed anticancer activity via the inhibition of the membrane association of p21ras protein and increased gap junction intercellular communication. Haag et al. (1992) and Chander et al. (1994) found that limonene induced regression of mammary carcinomas. Limonene in combination with 4-hydroxyandrostenedione showed greater regression of the rat mammary tumor (83.3%). Elegbede and Gould (2002) found that limonene significantly inhibited aflatoxin-DNA adduct formation in hepatocytes. Kawamori et al. (1996) found that D-limonene inhibited the development of colonic aberrant crypt foci induced by azoxymethane in F344 rats. Zheng et al. (1994) found that p-mentha-2,8-dien-1-ol and p-mentha-8(9)-en-1,2-diol inhibited benzo[α]pyrene-induced carcinogenesis in mice. The structures of the abovementioned monoterpenes with antitumor activity are given in Fig. 8.

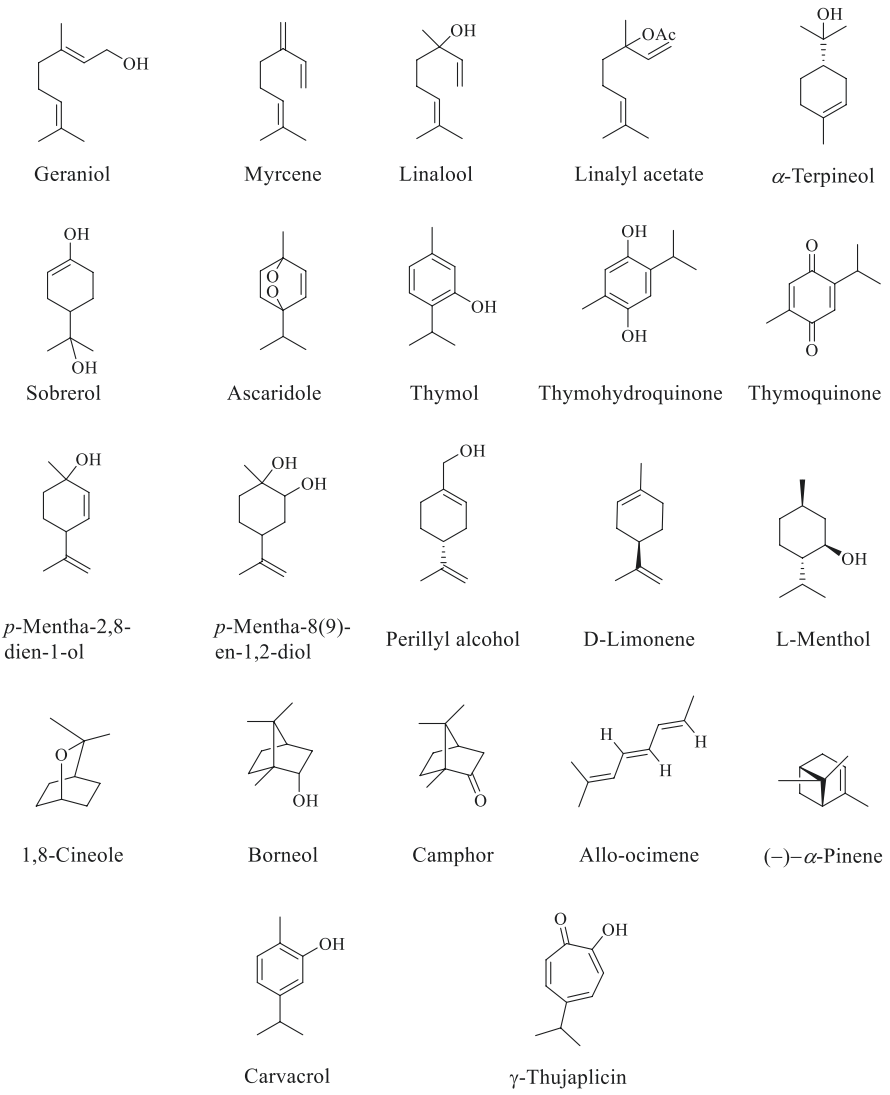


Fig. 8 Some monoterpenes with antitumor activity

4.4 Anticonvulsant Activity

Quintans-Júnior et al. (2010a) and Viana et al. (2000) reported that treatment with 400 mg/kg citral orally increases the latency and inhibits convulsions by 40% induced by pentylenetetrazol (PTZ) and maximal electroshock (MES). They have established the agonist effect of citral on the gamma-aminobutyric acid type A (GABAA) receptor. Citronellal at doses of 100, 200, and 400 mg/kg increased the

latency time, inhibited convulsions, and decreased mortality in mouse seizure models induced by injecting PTZ and picrotoxin (PIC) (Melo et al. 2011a). The anticonvulsive effect of citronellal is due to its GABAA agonist action (Melo et al. 2011b), glutamatergic receptor-modulating effect (Santos et al. 2016), blockage of voltage-gated sodium ion (Na^+) channels (Quintans-Júnior et al. 2010b), activation of the potassium ion (K^+) channel (de Santana et al. 2013), and attenuation of inflammation and oxidative stress (Melo et al. 2011b). The anticonvulsant effect of (+)-citronellol at different doses (100, 200, and 400 mg/kg, i.p.) was evaluated by de Sousa et al. (2006) in convulsion models, and it was found that the time of latency increased. Aoshima and Hamamoto (1999) and Kessler et al. (2014) showed the effect of citronellol on the GABAA receptor. Ziemba et al. (2015) found that citronellol acts as a blocker of the 5-HT3A receptor.

Geraniol at a dose of 200 mg/kg (i.p.) decreases seizure signs by 50% and mortality by 100% in the PTZ-induced seizure model (Lins et al. 2014). Ziemba et al. (2015) and Medeiros et al. (2018) reported that geraniol has the ability to block the 5-hydroxytryptamine receptor 3A (5-HT3A) receptor. (S)-(+)-Linalool and (R)-(−)-linalool enantiomers, as well as the racemic mixture (±)-linalool at doses of 200 and 300 mg/kg i.p., showed anticonvulsive effects in different seizure models (de Sousa et al. 2010a, b). Elisabetsky et al. (1999) found that linalool reduces convulsions induced by *N*-methyl-*D*-aspartate (NMDA) and quinolinic acid via competitive antagonism of the L-[3H] glutamate receptor. Leal-Cardoso et al. (2010) and Venâncio et al. (2011) found that linalool also modulated neural excitability through the blockade of voltage-dependent Na^+ channels. Sabogal-Guáqueta et al. (2018) found that linalool exhibited neuroprotective effects in hippocampal and motor cortex regions through the reduction of astrogliosis and microgliosis. da Guedes et al. (2022) found that trans-anethole at a dose of 400 mg/kg attenuated seizures by increasing the time for the onset of spasms and convulsions and reducing the duration of seizures. The electroencephalographic profile substantiates the above results and showed a reduction in the amplitude of waves compared to the PTZ-induced group.

In an acute PTZ-induced model, β-myrcene reduced convulsions (Viana et al. 2000). Carvacrol at a dose of 100 mg/kg, i.p., showed a protective effect on a 6 Hz psychomotor convulsion model (Mishra and Baker 2014). Administration of carvacrol three times a day (75 mg/kg, i.p.) prevents the reoccurrence of early status epilepticus, reduces early seizure frequency without altering chronic epilepsy (Khalil et al. 2017). Pires et al. (2015a) found that carvacryl acetate increases seizure latency and decreases the seizure rates and mortality of animals induced with pilocarpine (PILO), PTZ, and PIC. Furthermore, carvacryl acetate increased the activities of Na^+, K^+-adenosine triphosphatase (ATPase) and δ-aminolevulinic acid dehydratase.

(−)-Carvone and (+)-carvone modulate the seizure signal by voltage-gated sodium channel blockade (Gonçalves et al. 2010). However, (−)-carvone and (+)-carvone inhibit GABAA receptors (Sánchez-Borzone et al. 2014). p-Cymene reduces cholinergic signs, tremors, and the induction of seizures by PILO (de Oliveira et al. 2014). γ-Decalactone showed anticonvulsant activity in different

models (de Oliveira et al. 2008; Pfluger et al. 2018a, b). Isopulegol at doses of 100 and 200 mg/kg in mice increased seizure latency similar to diazepam (Silva et al. 2009). Viana et al. (2000) found that limonene (200 and 400 mg/kg i.p.) showed anticonvulsant effects against PTZ-induced seizures. Limonene decreased glutamate levels while increasing GABA levels in the brain (Zhou et al. 2009). Souto-Maior et al. (2016) found that linalool oxide at doses of 50, 100, and 150 mg/kg, i.p., showed anticonvulsant activity. Zhang et al. (2008) found that menthol promotes anticonvulsive effects in models induced by PTZ. (R)-(+)-Pulegone was found to increase the seizure latency time in a PTZ-induced acute model (de Sousa et al. (2011a).

Apart from the monoterpenes mentioned above, safranal (Hosseinzadeh and Sadeghnia 2007), (±)-α-terpineol (de Sousa et al. 2007), terpinen-4-ol (de Sousa et al. 2009; Nóbrega et al. 2014), thymol (Mishra and Baker 2014), thymoquinone (Hosseinzadeh and Parvardeh 2004; Hosseinzadeh et al. 2005; de Sousa et al. 2011b; Velagapudi et al. 2017; Zeinvand-Lorestani et al. 2018; Alkharfy et al. 2018; Arjumand et al. 2019), (−)-borneol (Granger et al. 2005; Quintans-Júnior et al. 2010a; Jiang et al. (2015); Tambe et al. 2016; Madhuri and Naik 2017; Skalicka-Woźniak et al. 2018), and 1,8-cineole (de Figuêiredo et al. 2019), (1S)-(−)-verbenone (de Melo et al. 2017) also demonstrated anticonvulsant properties, owing primarily to the GABAA agonist effect, as well as antioxidant and anti-inflammatory activity by modulating opioid and 5-HT receptors. α-Pinene at doses of 0.2 and 0.4 mg/kg, i.p., and β-pinene at a dose of 400 mg/kg, orally demonstrated anticonvulsant activity (Yang et al. 2016; Zamyad et al. 2019; Felipe et al. 2019; Ueno et al. 2020). The structures of the abovementioned monoterpenes with anticonvulsant activity are given in Fig. 9.

4.5 Cardiovascular Protective Activity

Magyar et al. (2002) reported that thymol induced cardiac arrhythmias by inhibiting K^+ and calcium ion (Ca^{2+}) currents in ventricular myocytes isolated from dogs. Magyar et al. (2004) showed that thymol inhibits L-type Ca^{2+} currents in human and canine ventricular cardiomyocytes. Aydin et al. (2007) found that carvacrol at a dose of 100 mg/kg, i.p., reduced blood pressure and heart rate and inhibited the hypertension induced by N^G-nitro-L-arginine-methyl ester (L-NAME) in normotensive rats. Peixoto-Neves et al. (2010) proved that carvacrol induced an endothelium-independent relaxation, possibly involving inhibition of Ca^{2+} influx through the membrane. Pires et al. (2015a, b) showed that carvacrol promoted an increased influx of calcium by activating the TRPV3 channel. Carvacrol showed a hypotensive effect that was probably due to bradycardia and peripheral vasodilatation (Dantas et al. 2015). Đukanović et al. (2022) reported that carvacrol (1 mmol/l) showed vasorelaxation through the blockage of L-type Ca^{2+} channels on smooth muscle cells (human umbilical arteries).

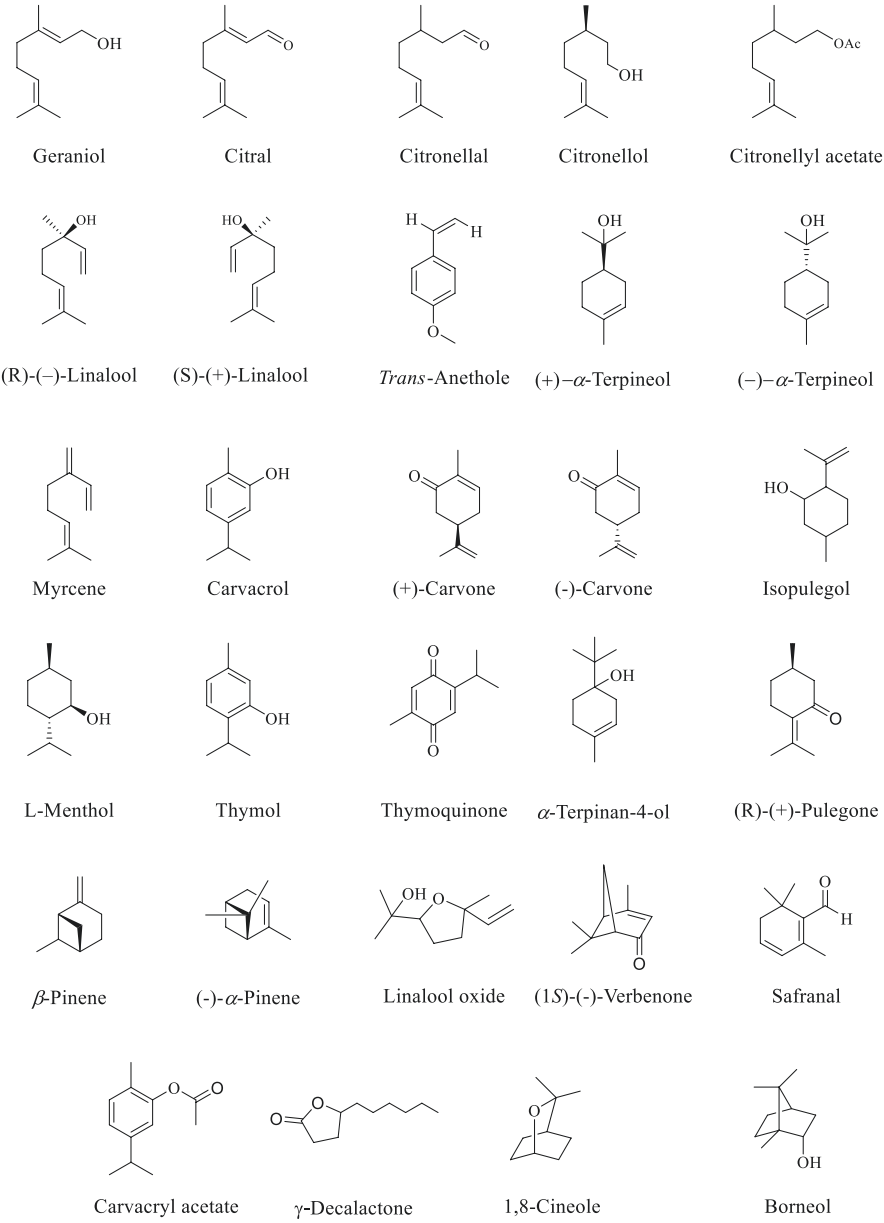


Fig. 9 Some monoterpenes with anticonvulsant activity

Lahlou et al. (2002a) found that intravenous administration of eucalyptol significantly reduced the blood pressure of both conscious and anaesthetized rats and showed vasorelaxant activity. Pinto et al. (2009) reported that the vasorelaxation property of eucalyptol depended on the integrity of the vascular endothelium and

nitric oxide release. Soares et al. (2005) found that eucalyptol effects papillary muscle relaxation in the preparations from the rat ventricle. Johnson et al. (2009) revealed that menthol causes dilatation in human forearm cutaneous vessels via activation of muscarinic receptors and/or production of nitric oxide. Rotundifolone is the major component (63%) of the essential oil of *Mentha* x *villosa* (Guedes et al. 2002). Guedes et al. (2004) reported that intravenous administration of rotundifolone in rats significantly reduced blood pressure and heart rate via induction of negative inotropic and chronotropic effects in the atrium. Guedes et al. (2002) also found that the vasorelaxation was due to the inhibition of Ca^{2+} influx through the membrane and the release of Ca^{2+} from intracellular stores.

Saito et al. (1996) showed that α-terpineol at a dose of 5 mg/kg administered intravenously had a hypotensive effect in rats. α-Terpineol induced vasorelaxation in the perfused rat mesenteric vascular bed, which was terminated in the presence of L-NAME, indicating the involvement of NO (Magalhães et al. 2008). According to Ribeiro et al. (2010), the hypotensive and vasorelaxant properties of α-terpineol are primarily due to NO release and activation of the NO-cGMP pathway. Intravenous administration of α-terpinen-4-ol resulted in an immediate reduction in blood pressure in both normotensive (Lahlou et al. 2002b) and hypertensive (Lahlou et al. 2003) rats. Lahlou et al. (2003) revealed that a depolarizing solution of K^+ up on α-terpinen-4-ol treatment precontracted rat aorta preparation. Höferl et al. (2006) found that the optical isomers (+) and (−)-linalool showed opposite effects on blood pressure and heart rate, administered by inhalation. The (+)-linalool showed a stimulating effect on the cardiovascular system, while the (−)-linalool found to have a depressing effect. Menezes et al. (2010) found that (±)-linalool in nonanesthetized normotensive rats induced hypotension associated with tachycardia.

Demirel (2022) discovered that geraniol (0.4 to 3.2μg/ml) and β-citronellol (1.6, 3.2, and 6.4μg/ml) dilate the rat thoracic aorta. Citronellol caused vasorelaxation in isolated rings of the superior mesenteric artery of rats by inhibiting Ca^{2+} influx through the membrane and releasing Ca^{2+} from intracellular stores (Bastos et al. 2010). In rats, oral administration of limonene and sobrerol at a dose of 400 mg/rat significantly decreased the changes in pulmonary hypertension and right ventricular hypertrophy induced by monocrotaline (Touvay et al. 1995). Both limonene and sobrerol also reduced the increase in medial thickness of the pulmonary artery. El Tahir et al. (2003) found that intravenous administration of α-pinene and p-cymene caused hypotension and bradycardia in urethane-anesthetized rats. Menezes et al. (2010) demonstrated that intravenous administration of (+)-α-pinene and (−)-β-pinene showed a hypotensive effect associated with tachycardia in nonanesthetized normotensive rats. Saito et al. (1996) found that intravenous administration of myrtenal, myrtenol, and perillyl alcohol at doses of 1 and 5 mg/kg showed hypotensive activity in rats.

Ghayur et al. (2012) found that thymoquinone exerted relaxant activity in the rat aorta by blocking voltage-operated Ca^{2+} channels. Silva-Filho et al. (2012) demonstrated the relaxation effect of borneol in phenylephrine or potassium chloride (KCl) contracted aortic rings. Kundu et al. (2014) found that carvone showed a vasorelaxant effect on aortic rings and guinea pig tracheas through its action on calcium

voltage-dependent channels. de Sousa et al. (2015) reported that there is no difference in the pharmacological action of (+)- and (−)-enantiomers of carvone. They also found the relaxant effects of (+)-limonene and (−)-limonene enantiomers on the tracheas and aortic rings independent of the endothelium. Cheang et al. (2013) demonstrated that menthol suppressed the $CaCl_2$-induced contraction in rat aortae, mesenteric, and coronary arteries by inhibiting calcium influx. da Silva et al. (2020) revealed that (−)-carveol possesses a vasorelaxant effect in human umbilical arteries (HUAs) through the opening of calcium and potassium channels. Cardoso-Teixeira et al. (2018) found that limonene, carveol, and perillyl alcohol showed a relaxant effect on the aorta smooth muscle of rats by the mechanism of inhibition of protein kinase C and inositol trisphosphate pathways. The structures of the above-mentioned monoterpenes with cardiovascular protective activity are given in Fig. 10.

4.6 Gastroprotective Activity

Several monoterpenes isolated from various plant species showed significant gastroprotective effects against nonsteroidal anti-inflammatory drugs (NSAIDs) in experimental animals. The gastrointestinal complications caused by NSAIDs are mainly due to the inhibition of COX, a key enzyme in the production of prostaglandins (Laine et al. 2008; Sostres et al. 2010). Koc et al. (2020) reported that thymol at low doses (50, 100, and 200 mg/kg) significantly improved the gastroprotection in an indomethacin-induced gastric ulcer model. Various studies have shown that thymol (Ribeiro et al. 2016), ascaridole (Zhu et al. 2012), citral (Nishijima et al. 2014), eucalyptol (Rocha Caldas et al. 2015), epoxy-carvone (Siqueira et al. 2012), menthol (Rozza et al. 2013, 2014), α-terpineol (Souza et al. 2011), thymoquinone (Zeren et al. 2016), carvacrol (Oliveira et al. 2012), limonene (Rozza et al. 2011), and β-myrcene (Bonamin et al. 2014) possesses gastroprotective property against NSAIDs. Limonene ameliorated 99% of indomethacin-induced gastric ulcers (Rozza et al. 2011). At a dose of 25 mg/kg, citral prevented 76% of gastric ulcers (Nishijima et al. 2014). Thymol, menthol, limonene, and carvacrol showed gastroprotective effects via stimulating mucus secretion and/or PGE_2 production, thereby increasing gastric mucosal integrity. Thymoquinone showed gastroprotective effects by increasing the antioxidant defense mechanisms of the cells. Serafim et al. (2021) revealed that (−)-carveol showed gastroprotective effects in ethanol, stress, and NSAID-induced animal models. (−)-Carveol (25, 50, 100, and 200 mg/kg, p.o.) significantly reduced the ulcerative lesion. Gastroprotective activity of (−)-carveol is related to antisecretory, antioxidant, and immunomodulatory mechanisms.

Monoterpenes showed significant gastroprotective effects against experimental models induced with ethanol, 70% ethanol, or alcoholic hydrochloric acid (HCl). Carvacrol (Oliveira et al. 2012), geraniol (de Carvalho et al. 2014), epoxy-carvone (Siqueira et al. 2012), α-pinene (Pinheiro Mde et al. 2015), myrtenol (Viana et al. 2016), α-terpineol (Souza et al. 2011), linalyl acetate (Barocelli et al. 2004),

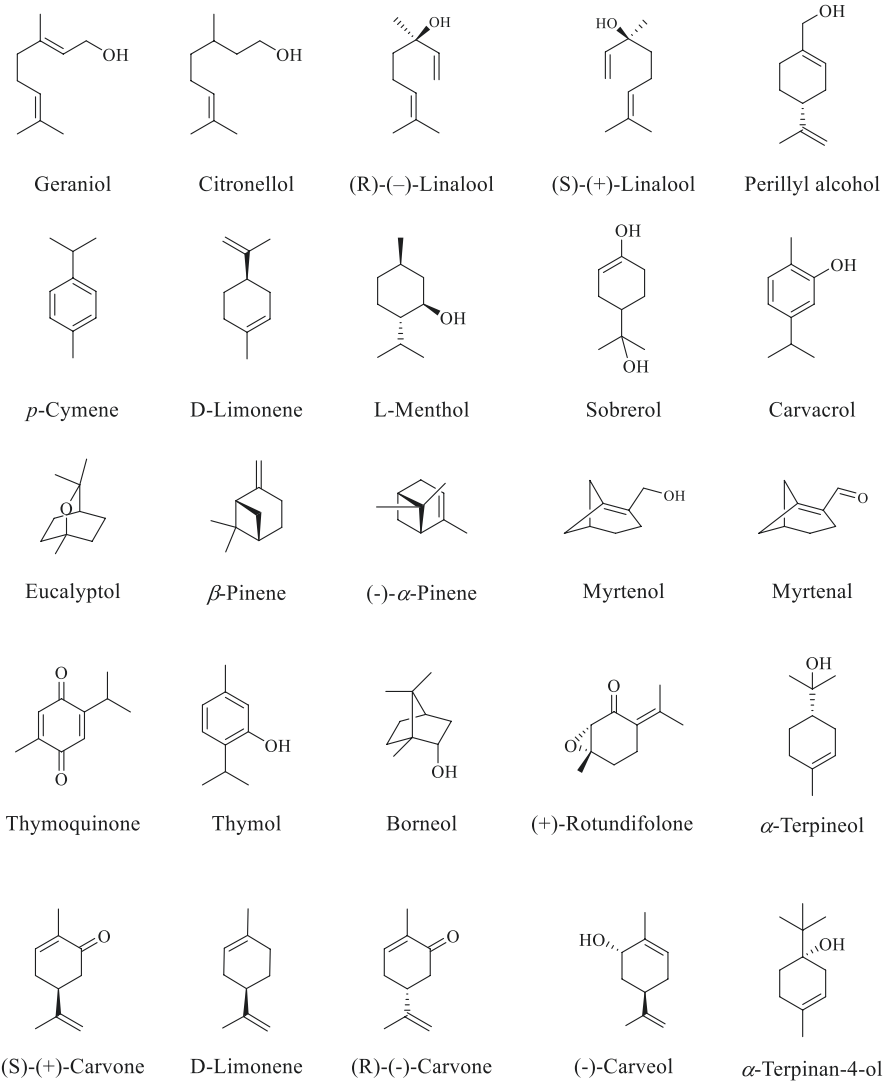


Fig. 10 Some monoterpenes with cardiovascular protective activity

menthol (Rozza et al. 2013, 2014), nerol (González-Ramírez et al. 2016), eucalyptol (Rocha Caldas et al. 2015), limonene (Rozza et al. 2011; de Souza et al. 2019), thymol (Ribeiro et al. 2016), and β-myrcene (Bonamin et al. 2014) reduced the gastric lesions induced by ethanol up to 100% via increased production of mucus, PGE_2, NO, and sulfhydryl compounds. According to Vespermann et al. (2017), the isomeric forms α-pinene and β-pinene have completely different activities. In an ethanol-induced gastric lesion, α-pinene (30 mg/kg) showed up to 44% protection, and β-pinene (33 mg/kg) did not show any protective effect. In an I/R model,

carvacrol and β-myrcene significantly reduced gastric lesions by 38% and 86%, respectively (Oliveira et al. 2012; Bonamin et al. 2014). β-Myrcene improved the antioxidant status of the mucosal tissue of the stomach.

The acetic acid-induced gastric lesions resemble human ulcers in terms of pathological and healing mechanisms (Tarnawski 2005; Silva and de Sous 2011). Bhattamisra et al. (2018) found that geraniol showed antiulcer activity against acetic acid and anti-*Helicobacter pylori* activity in rats. Geraniol at a dose of 30 mg/kg significantly increased the gastric pH along with a reduction in total acidity and gastric juice volume. Geraniol also enhanced the antioxidant status of the gastric mucosa. A rapid urea test revealed that geraniol cured 33% of *H. pylori* infections. Carvacrol (Silva et al. 2012), linalool (da Silva et al. 2016), eucalyptol (Rocha Caldas et al. 2015), thymol (Ribeiro et al. 2016), ascaridole (Zhu et al. 2012), and geraniol (Venzon et al. 2022) significantly protected from acetic acid induced gastric lesions in different experimental models. Ascaridole induces antisecretory effects that inhibit acid secretion and accelerate ulcer healing (Zhu et al. 2012). Carvacrol inhibits the release of inflammatory mediators and induces PGE_2 production (Silva et al. 2012). Geraniol also enhanced oxidative status and prevented the production of IL-6 and TNF-α (Venzon et al. 2022). Eucalyptol (Rocha Caldas et al. 2015) and linalool (da Silva et al. 2016) showed significant ROS scavenging and gastric cell regeneration properties.

One of the main therapeutic modalities for treating gastric ulcers is the elimination of *Helicobacter pylori* infection. Some monoterpenes with gastroprotective activity also had anti-*H. pylori* activity (Zielińska-Błajet and Feder-Kubis 2020). Both carvacrol and geraniol inhibited 92% of the *H. pylori* growth with minimal inhibitory concentrations (MICs) of 40 mg/l and 2 mg/l, respectively (Boyanova and Neshev 1999; Bergonzelli et al. 2003). The MICs for limonene and β-myrcene were 75μg/ml and 500μg/ml, respectively (Rozza et al. 2011; Bonamin et al. 2014). De Monte et al. (2015) reported that safranal showed a 32μg/ml MIC. The structures of the abovementioned monoterpenes with gastroprotective activity are given in Fig. 11.

4.7 Wound-Healing Activity

Pivetta et al. (2018) found that carbopol gel containing nanoencapsulated thymol (50% w/w) at a concentration of 12.5μM appeared to stimulate the growth of keratinocytes and promote cell viability. Thymol showed wound-healing activity by increasing the production of macrophage migration inhibitory factor and enhancing fibroblast growth (Riella et al. 2012). They further found that the anti-inflammatory effect of thymol is due to the inhibition of MPO activity and decreased leukocyte influx. In human neutrophils stimulated with N-formyl-methionyl-leucyl-phenylalanine, thymol delayed the elastase activity (Braga et al. 2006). Thymol

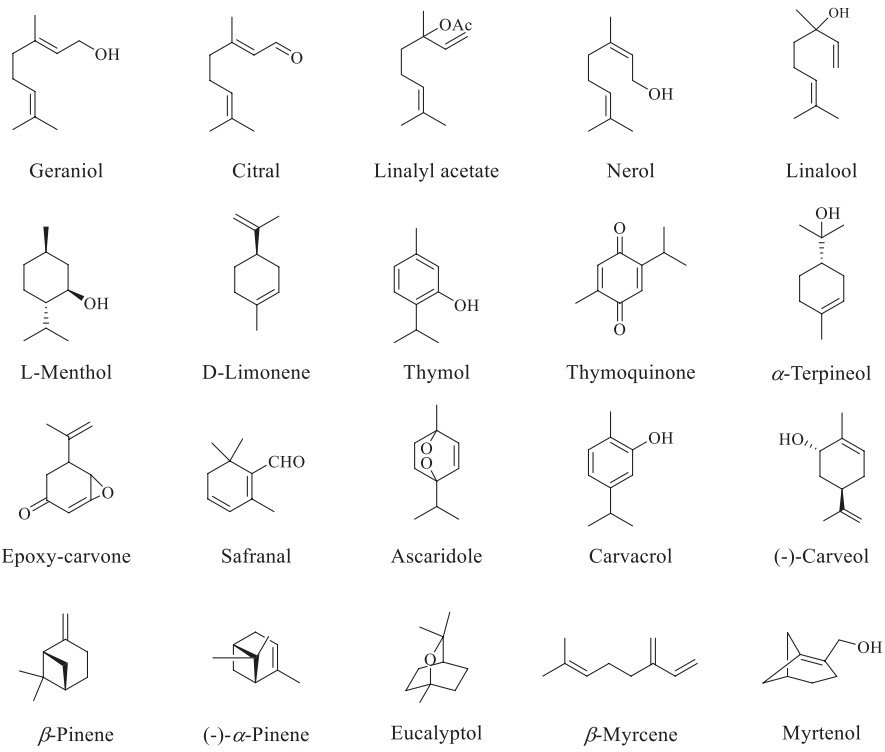


Fig. 11 Some monoterpenes with gastroprotective activity

significantly inhibited COX-1 (Marsik et al. 2005) and inducible lymphocyte proliferation (Amirghofran et al. 2011).

Gunal et al. (2014) reported that carvacrol (12.5%) diluted in sunflower oil (2%) significantly reduced the surface of the lesion and promoted changes in granulation tissue thickness and lesion depth. Carvacrol also influenced the release of TNF-α, transforming growth factor (TGF)-α, and IL-1 during tissue repair. Carvacrol (0.5% or 1%) incorporated into chitosan films reduced wound areas and tissue edema, induced earlier granulation tissue formation, increased cell proliferation, increased epithelialization rates, and improved collagenization on excision wounds in rats (Barreto et al. 2015). Using an in vitro scratch assay, de Christo Scherer et al. (2019) found that terpinolene and α-phellandrene stimulate fibroblast proliferation and migration. Salas-Oropeza et al. (2021) found that α-pinene (9%) and α-phellandrene (1%) produce stress-resistant scars and accelerate wound contraction through collagen deposition in the early stages of the wound.

Borneol showed wound-healing properties (Mai et al. 2003) through antimicrobial (Unlü et al. 2002) and anti-inflammatory (Almeida et al. 2013) activities. Borneol inhibited leukocyte migration (Almeida et al. 2013), fibroblast growth, and matrix metalloproteinase (MMP)-2 activity, and it further inhibited collagen and

TIMP-1 production (Dai et al. 2009). In another study, borneol suppressed the production of the proinflammatory cytokines IL-1β and IL-6 (Park et al. 2003). Villegas et al. (2001) reported the wound-healing potential of (+)-epi-α-bisabol and α-terpineol isolated from *Peperomia galioides*. α-Terpineol inhibits neutrophil influx (Oliveira et al. 2012) and selectively inhibits ovine COX-2 (Kawata et al. 2008). The structures of the abovementioned monoterpenes with wound-healing potential are given in Fig. 12.

4.8 Antifungal Activity

Raut et al. (2013) found that menthol showed significant inhibitory activity on *Candida albicans*. Menthol tested against *Fusarium verticillioides* showed a 75% reduction in growth (Dambolena et al. 2008). 1,8-Cineole showed good activity against *Aspergillus carbonarius* (Dammak et al. 2019). Venkatesh et al. (2017) found that *Boswellia serrata* essential oil containing monoterpenes 3-carene and β-ocimene showed inhibitory activity against *Alternaria brassicicola*, *A. geophila*, and *Curvularia tetramera*. Geraniol showed better efficacy against *Trichophyton rubrum*, *T. mentagrophytes*, and *Microsporum canis* when compared with terbinafine and miconazole (Miron et al. 2014). The trans isomers showed higher antifungal activity than cis, signifying the importance of configurational isomerism in bioactivity (Miron et al. 2014). Singh et al. (2016) found that geraniol may inhibit the calcineurin pathway, damage to the plasma membrane and the cell wall of *Candida albicans*. Thymol and carvacrol showed potent antifungal activity against *Cryptococcus neoformans* and *C. laurentii* (Kumari et al. 2017). Ahmad et al. (2013) found that eugenol and thymol showed antifungal activity in *Candida albicans* through the inhibition of hydrogen ion (H^+)-ATPase activity. Ahmad et al. (2011) also found the synergistic antifungal activity of thymol and carvacrol against *Candida albicans* through the inhibition of efflux-pump genes (CDR1 and MDR1) overexpression.

Nikitina et al. (2021) found that (−)-myrtenol exhibits good activity against both yeast (*Candida albicans*) and mycelial (*Rhizopus nigricans*, *Aspergillus fumigatus*, and *Fusarium solani*) fungi species. Scariot et al. (2021) found that citral, geraniol, citronellol, and citronellal showed antifungal activity against *Saccharomyces cerevisiae* through cell membrane damage with minimum inhibitory concentration and minimum fungicidal concentration values between 0.64 and 3.68 mM, and 1.56 and 6.25 mM, respectively. Kaur et al. (2019) found that citral at a concentration of 0.2μl/ml exhibited strong fungicidal effect against *Fusarium oxysporum* and *Sclerotium rolfsii* while geraniol showed better activity against *S. rolfsii* at a concentration range of 0.2 to 2μl/ml. de Oliveira Lima et al. (2017) found that linalool (minimum inhibitory concentration 256μg/ml) caused leakage of intracellular material in clinical isolates of fluconazole resistant *Trichophyton rubrum*. Pereira Fde et al. (2015) found that geraniol at a minimum inhibitory concentration range of 16–256 μg/ml and citronellol at a minimum inhibitory concentration range of 8 to

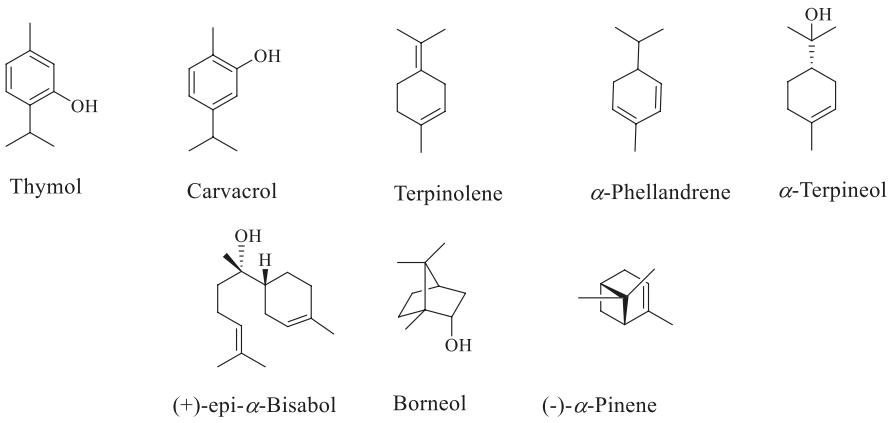


Fig. 12 Some monoterpenes with wound-healing activity

1024μg/ml inhibited *Trichophyton rubrum* mycelial growth and conidia germination. Chaillot et al. (2015) found that carvacrol exhibits antifungal activity against *Candida albicans* by altering endoplasmic reticulum integrity. The structures of the abovementioned monoterpenes with antifungal activity are given in Fig. 13.

4.9 Antibacterial Activity

Contreras Martínez et al. (2022) found that isoespintanol isolated from *Oxandra xylopioides* showed biofilm eradication potential against the clinical isolate *Pseudomonas aeruginosa* after 1 hour of exposure. Menthol exhibited broad spectrum antibacterial activity (Pattnaik et al. 1997; Osawa et al. 1999; Inouye et al. 2001; Trombetta et al. 2005). Trombetta et al. (2005) reported the antibacterial effect of (+)-menthol, thymol, and linalyl acetate against *Staphylococcus aureus* and *Escherichia coli*. Thymol was found to be effective against *Salmonella typhimurium* and *Escherichia coli* with minimum inhibitory concentration values of 1.0 and 1.2 mmol/l, respectively (Olasupo et al. 2003). Nostro et al. (2004) found that all the *Staphylococcus aureus* and *Staphylococcus epidermidis* clinical isolates tested were susceptible to thymol, with minimum inhibitory concentration values ranging from 0.03 to 0.06% v/v. There is no difference in methicillin-resistant and methicillin-sensitive *Staphylococci*. Xu et al. (2008) found that thymol at a concentration of 200 mg/ml inhibited the growth of *Escherichia coli*.

Carson and Riley (1995) found that terpinen-4-ol, (+)-α-terpineol, and ρ-cymene inhibited the growth of *Acinetobacter baumannii*, *Aeromonas veronii* biogroup *sobria*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica* subsp. *enterica* serovar *Typhimurium*, *Serratia marcescens*, and *Staphylococcus aureus*. Kotan et al. (2007) found that oxygenated monoterpenes such as nerol, linalool, α-terpineol, fenchol, and terpinen-4-ol showed broad

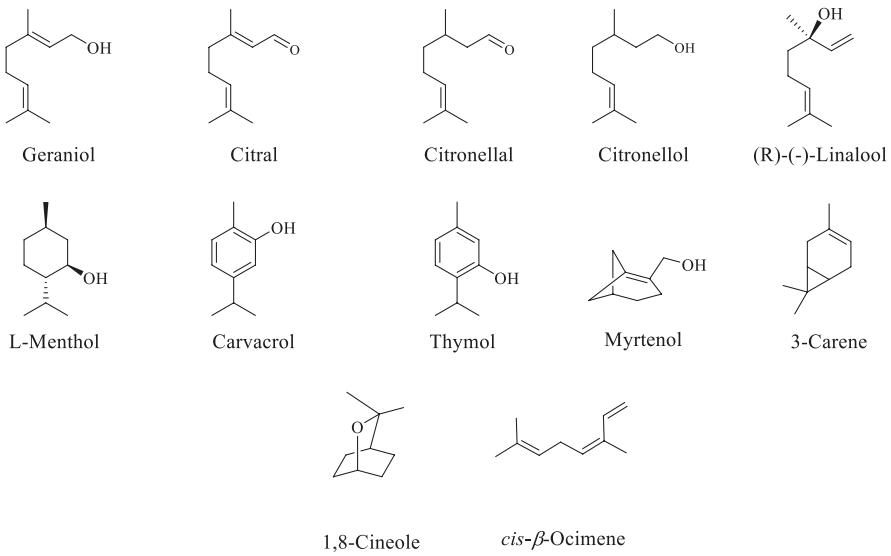


Fig. 13 Some monoterpenes with antifungal activity

spectrum antibacterial activity. Muilu-Mäkelä et al. (2022) found that α-pinene, β-pinene, R-limonene, S-limonene, and 3-carene are effective against *Escherichia coli*. Coêlho et al. (2016) found that nerol showed antibacterial activity against *Escherichia coli*. In addition, nerol synergistically enhanced the activity of norfloxacin against *Staphylococcus aureus*.

The optical isomers of carvone, (4R)-(−)-carvone, and (4S)-(+)-carvone are effective against *Campylobacter jejuni* and *Listeria monocytogenes*, respectively. Carvone also showed good inhibition against *Enterococcus faecium* and *Escherichia coli* (Friedman et al. 2002). Rivas da Silva et al. (2012) found that a mixture of (+)-α-pinene and (+)-β-pinene showed potent activity against *Staphylococcus aureus*. Ellouze et al. (2012) found that (−)-linalool showed potent antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Porphylomonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*. Park et al. (2012) found that (−)-linolool showed antibacterial activity against *Staphylococcus aureus* resistant to vancomycin and *Pseudomonas aeruginosa*. Liu et al. (2020) found the minimum inhibitory concentration (431μg/ml) and minimum bactericidal concentration (862μg/ml) of linalool against *Pseudomonas aeruginosa*. The structures of the abovementioned monoterpenes with antibacterial activity are given in Fig. 14.

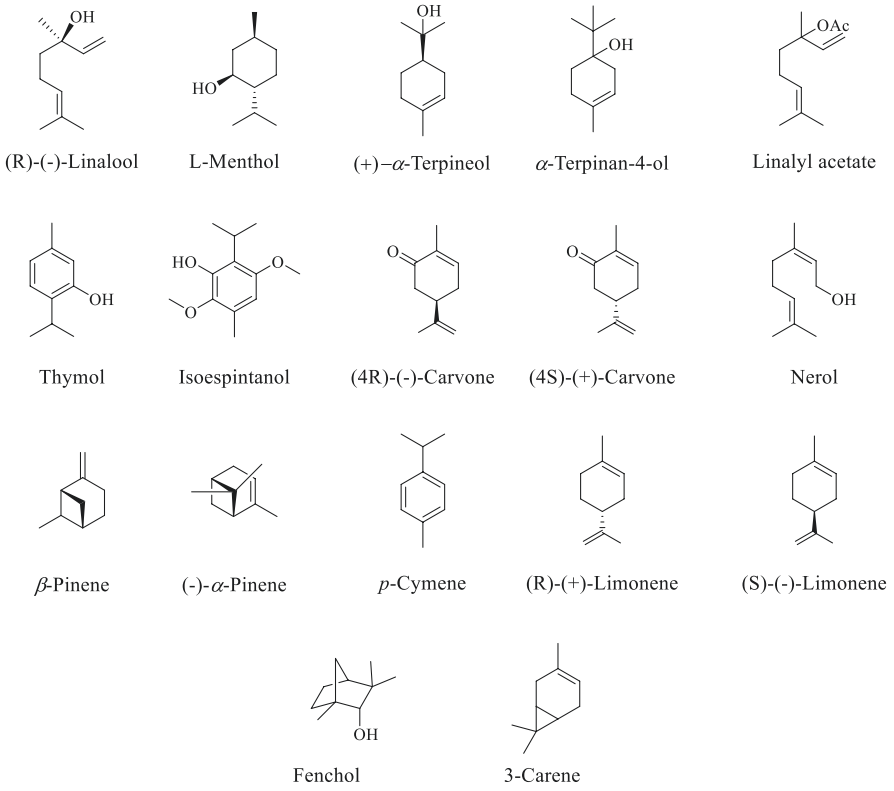


Fig. 14 Some monoterpenes with antibacterial activity

4.10 Antiviral Activity

Borneol and isoborneol showed activity against herpes simplex virus-1 (HSV-1). Isoborneol showed total inhibition of HSV-1 replication at a concentration of 0.06% (Armaka et al. 1999). Astani et al. (2010) found that thymol, α-terpinene, γ-terpinene, 1,8-cineole, α-terpineol, and citral isolated from tea tree, thyme, and eucalyptus exhibited significant activity (>80%) against HSV-1. Mundinger and Efferth (2008) found the anti-HSV-1 activity of 1,8-cineole. Orhan et al. (2012) found the anti-HSV-1 activity of citral. (−)-α-Pinene and (−)-β-pinene at a concentration of 1 mM exhibited anti-IBV (infectious bronchitis virus) activity (Yang et al. 2011). Garozzo et al. (2011) and Orhan et al. (2012) found that citral, citronellal, and citronellol showed anti-herpes simplex virus-1 activity. Zamora et al. (2016) found that terpinen-4-ol present in the tea tree oil (*Melaleuca alternifolia*) showed significant activity against the influenza A H1N1/Puerto Rico/8/34 and West Nile viruses. The structures of the abovementioned monoterpenes with antiviral activity are given in Fig. 15.

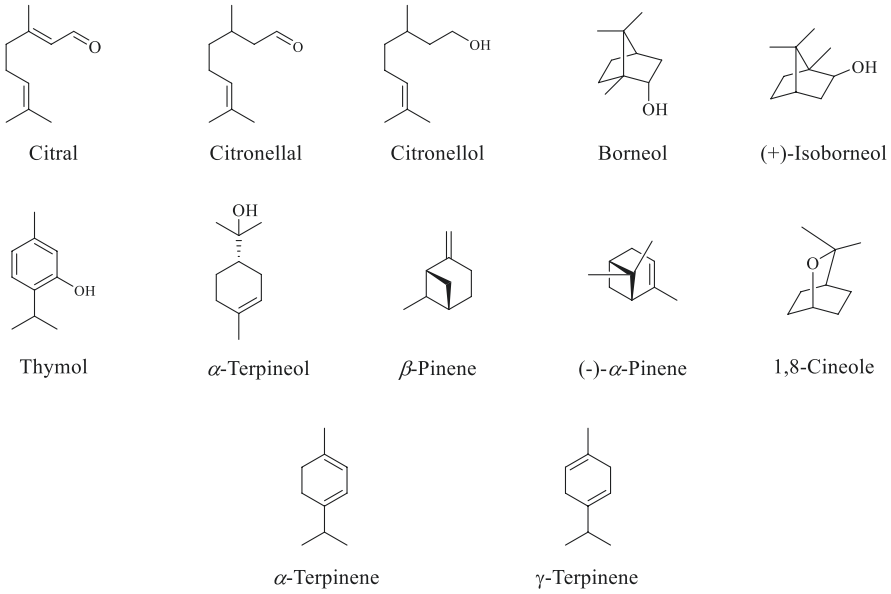


Fig. 15 Some monoterpenes with antiviral activity

5 Conclusion and Future Prospects

Emerging and re-emerging pathogens, lifestyle-related metabolic disorders, and chronic diseases continue to pose a major threat to global public health. During the past 3 years, mankind has faced a hitherto unknown global outbreak of the SARS-Cov-2 virus, which caused COVID-19 disease and associated complications. Similarly, we are facing regional epidemics caused by several new and emerging pathogens such as H1N1 Swine Flu, Nipah, Zika, Ebola, Dengue, Chikungunya, MERS, and SARS. Malaria is another epidemic that is reappearing in spite of its eradication toward the end of the twentieth century. This emergence of new pathogens, their variants, and drug resistance has thrown a challenge to the medicinal chemists. Although there are several drugs in clinical trials, no one knows at the moment how effective they will be or whether they will arrive in time to make any difference to the present challenge.

As detailed in this chapter, bioactive monoterpenes have a significant role in future drug development processes, which requires further in-depth research in a combination of recent "omics" biology and network pharmacology approaches. In addition to this, the use of bioactive monoterpenes as chemical scaffolds for novel compounds with improved drug-likeness, pharmacokinetic, and pharmacodynamic properties is another promising strategy in the drug discovery process. Recent advancements in synthetic biology and metabolic engineering may further accelerate the drug development process by enabling sustainable and economically viable large-scale production processes of desired bioactive molecules.

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Part II
Initial Processes for Identifying and Extracting Compounds: Isolation and Identifying These Structures

Chromatographic Methods for Separation and Identification of Bioactive Compounds

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Abstract Chromatographic methods are widely used in the separation and identification of bioactive compounds in complex samples. Chromatography is a technique that is based on the separation of the components in a mixture into a stationary and mobile phase. There are several types of chromatography, including liquid chromatography (HPLC), gas chromatography (GC), thin-layer chromatography (TLC), and ion-exchange chromatography. Each type of chromatography has its advantages and disadvantages and is more suitable for different types of samples and compounds. For example, HPLC is often used to separate high-polarity compounds, while GC is best suited for volatile compounds. Chromatography can be combined with other analytical methods, such as mass spectrometry, to help identify specific compounds in a sample. Together, these chromatographic and spectrometric methods have been widely used in the identification and characterization of bioactive compounds in plants, microorganisms, and other natural matrices.

Keywords Chromatography · Spectroscopy · NMR · Mass spectrometry · FTIR · GC · LC/MS · HPLC · Natural product · Separation

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1 Introduction

The separation of complex mixtures from natural matrices still represents a challenge for chemists, even with substantial advances in separation techniques (Bucar et al. 2013). Bioactive compounds are often in low concentrations, so the development of a selective and effective separation method is essential for obtaining higher yields of these substances (Zhang et al. 2018; Abdelmohsen et al. 2022).

Since the 1990s, interest in natural product research has increased considerably. The emergence of new chromatographic techniques and the improvement of existing ones, as well as spectroscopic techniques and sensitive bioassays, allowed the isolation and structural elucidation of new chemical entities (Sticher 2008). Even the use of more-primitive chromatographic techniques, such as thin-layer chromatography (CCD), still find applications in the purification of natural molecules (Wilson and Poole 2023).

Currently, the use of hyphenated techniques such as gas chromatography-mass spectrometry (GC/MS), liquid chromatography-mass spectrometry (LC/MS), capillary electrophoresis-mass spectrometry (CE/MS), and nuclear magnetic resonance-liquid chromatography (NMR/LC), among others, takes the leading role in the search for natural products with pharmacological potential (Sarker and Nahar 2012).

In the following sections of this chapter, we will discuss the main chromatographic techniques currently employed in the isolation of bioactive compounds from natural matrices, as well as the main spectroscopic techniques used for the structural elucidation of these compounds, in order to show the principles of these techniques and their improvements.

2 Gas Chromatography

Evaluating the chemical constituents of plant material is a challenging task, as the investigated matrices are generally complex. For the most part, compounds with higher biological potential are present in very small amounts and often have similar structures (Ganzera and Murauer 2017). Thus, the separation of the chemical components of complex matrices via chromatographic methods is a major concern for researchers in the area of natural products and also for those who evaluate chemical compounds of natural origin in biological and active activity tests (Friesen et al. 2015). Thus, it is necessary to use techniques that meet strict requirements for selectivity, versatility, and sensitivity.

Large parts from volatile compounds are directly subjected to analysis by using the gas chromatography (GC) technique, which has a unique separation capacity. This technique can offer high sensitivity and selectivity when combined with identification methods, such as mass spectrometry (Stalikas 2007).

Commonly, GC is versatile for analyzing nonpolar and semipolar chemicals and volatile and semivolatile chemicals. Without chemical derivatization, such a technique is often used for the analysis of oils, short-chain fatty acids, sterols, aromas, and constrictors that provide flavors to food (Lehotay and Hajšlová 2002). In addition, the different methods of gas chromatography are also essential for the elucidation of different bioactive compounds, including pesticide agents. An example of its convenience is the use of the gas chromatography (GC) technique in conjunction with high-efficiency liquid chromatography (HPLC) in combination with prior sample separation through modern microextraction systems, which have proven to be valuable in rapidly controlling food quality, providing assurance and safety in its application for such purposes (Parys et al. 2021).

In the contemporary period, the market has benefited from the wide application of volatile oils, which has generated the need to ensure that the content of the complexes sold remains consistent. However, the characterization of volatile oils, such as essential oils, is an extremely laborious and complex task because these oils are chemically diverse mixtures. Therefore, GC has been an indispensable technique for identifying the unknown constituents of volatile oils. Methods, based on various retention rates that can be calculated from patterns, have been proposed to chemically characterize unknown substances in volatile oils. More often, the identification and confirmation of the components of these matrices is completed by analyzing gas chromatography coupled with a mass spectrometer (GC-MS) or by comparing them with authentic patterns (He and Beesley 2005).

Recent research seeks to evaluate the presence of secondary metabolites via exclusive multidimensional GC methods, seeking to innovate techniques in order to observe the complexities in structural analyses of volatile compounds. Multidimensional GC has been a successful technique for the isolation of sesquiterpenes in essential oils, and GC-MS is the most important technique for the characterization of components in the analysis of this type of sample. The quality of the data obtained in these analyses proportionally increases and becomes very useful after the elution time of the constituent has been determined and combines with the mass spectrum. In addition, it has been a common practice to use infrared spectroscopy with mass spectrometry (IR-MS) and combined with GC-MS as a practical solution for the separation and detection of volatile compounds (Waseem and Low 2015).

The separation time of gas chromatography can be decreased in different ways, such as by increasing the flow of drag gas, heating the column faster, increasing the column diameter, shortening the column, reducing the thickness of the column film, or reducing the viscosity of the drag gas. However, compensating for a higher speed causes the sample capacity to be reduced, generating higher detection limits and/or worse separation efficiency. In practice, the shortest GC analysis time should be projected according to the necessary selectivity (i.e., separation of the analytes and matrices). Thus, one possibility would be the use of selective detectors to improve the selectivity of the analyte matrix. Mass spectrometry detection generally improves selectivity, which reduces dependence on GC separation and can lead to faster analysis times for a given list of analytes and matrices (Lehotay and Hajšlová 2002).

Although the CG technique is widely disseminated and used, it is important to highlight that there is a need to optimize the speed of analysis.

3 High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is one of the most widely used analytical methods, by different research laboratories, for the separation of complex mixtures of substances with satisfactory accuracy and commercially available equipment. Having started in the mid 1900s, it was formerly known as classical liquid chromatography or liquid column chromatography. The whole process was developed manually and by using glass columns filled with solid particles adsorbed in solvents, called the stationary phase (Snyder 2000).

In general, after the preparation of the stationary phase, the solubilized sample is applied to the top of the column, and a solvent, mobile phase is poured. The solvent descends the column thanks to gravity and interaction with particles, and thus, analytical separation occurs by obtaining analyses of interest at different velocities. Although it is a method still widely used, with the advancement of science, its theories, and the need for types of solvents that were faster, more efficient, and applicable to different types of samples, the use of equipment with even-smaller particles and that supported high solvent pressures in the columns was emerging (Meyer 2010).

Because of this, sometimes this technique was also called high-pressure liquid chromatography (HPLC). In this system (Fig. 1), the equipment is designed commonly containing the following modules: pump, injector, column oven, detector, and a data-retrieval system. In its operation, the moving phase leaves the reservoir to the pump, which controls the solvent flow and the pressure required for this solvent to pass through the column. The sample is injected into the column with the aid of an automatic sampler or injector, and separation occurs inside the column, which can have its temperature controlled in the oven. Depending on the concentration of the analyses, the response occurs in the detector, and the data system monitors and processes the data obtained (Dong 2019).

Each of the modules can be changed according to the characteristic of the sample to be analyzed, thus leading to the different types of liquid chromatography existing today, defined by the stationary phase and the separation modes. Among them, we can mention chromatography via ion exchange, adsorption, exclusion, affinity, and chiral. The performance of each equipment intended for these analyses depends on the accuracy of the modules and, more, the sensitivity of the detector (Dong 2013).

The great advantage of this system goes beyond its use in different types of analytes, such as small organic molecules, large biomolecules, and even polymers (Dong 2013). Hyphenation with mass spectrometry improved the technique, which combined the great separation capacity of analytes in chromatography with the specificity and sensitivity of the mass spectrometer, aiming to overcome the possible limitations that would be found in the HPLC, thus expanding its use to several new areas and applications (Ganzera and Sturm 2018).

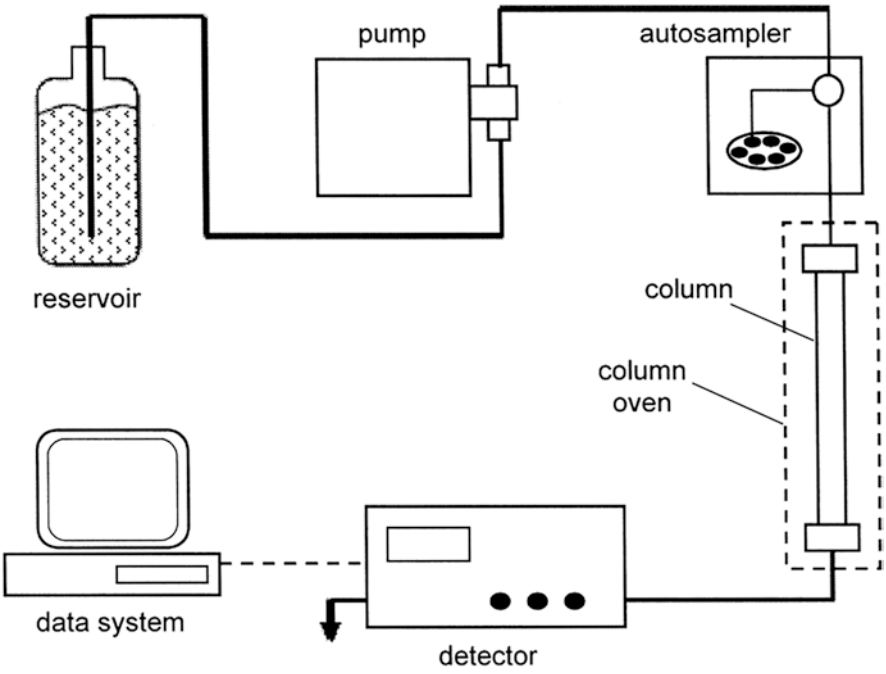

Fig. 1 System of an HPLC with the essential components (Snyder 2000)

One of the most used techniques, reverse-phase liquid chromatography (RP-HPLC), has a separation system that is based on the hydrophobicity of solute molecules in the stationary phase (Pratab et al. 2013). In it, the form of elution can be a gradient of solvents, where the concentration of the solvent gradually increases over time, or an isocratic condition, with a maintained concentration (Aguilar 2004). In the study by Mohamed et al. (2022), the research strategies of some organic acids in vinegar were used, using RP-HPLC to detect and identify possible adulterations. The technique proved to be accurate and had a high recovery rate, and it is recommended for different real samples.

In the case of complex samples, combined separation techniques, such as two-dimensional chromatography, have become options. In this method, two modes of applications of the technique can be performed. In heart cutting or LC-LC, only the fractions selected in a first column, called the first dimension, are directed to the second column, called the second dimension. In the comprehensive LC or LC × LC, the entire sample can be separated by using both columns—i.e., in both dimensions (Ganzera and Sturm 2018).

Varfaj et al. (2023), using the two-dimensional chromatographic technique to analyze branched-chain amino acids (BCAAs) in dietary supplements, applied the achiral–chiral heart-cutting method (mLC-LC) in an enantioselective analysis. This method proved advantageous not only for dealing with the limited chemoselectivity of some techniques but also for obtaining more information with only one analysis and for green analytical chemistry.

In the recent literature, we can find its use in the analysis and identification of pigments in cultural heritage objects (Careaga et al. 2023), separation and detection of organic substances in extracts of natural products and supplements (Shafaei et al. 2022; Pereira et al. 2023), and quantification of compounds (Gao et al. 2018), in addition to analyses that combine different techniques (Proch and Niedzielski 2021; Baj et al. 2022; Da Silva et al. 2023). This shows that for a successful execution of the technique, the analysis will require more than the simple push of a button. It is essential to plan it by incorporating data from advanced studies, gaining assistance from a qualified professional, and using equipment with accessories designed to obtain the best chromatographic conditions for the analysis of the sample of interest.

4 Countercurrent Chromatography (CCC) and Centrifugal Partition Chromatography (CPC)

Countercurrent chromatography (CCC) is based on liquid–liquid partition systems, without the use of any solid adsorbent, where two immiscible liquid phases are used, in which one is in the stationary phase and the other is in the mobile phase (Conway 1995). The stationary phase is maintained within the column via a centrifugal field or a gravitational field. The first type of equipment was based on the gravitational field, such as the countercurrent distribution created by Lyman Craig in 1940 (Craig 1950). Thanks to technological advances, the new separation equipment in countercurrent chromatography is based on the centrifugal field, and its development is directly associated with the name Yoichiro Ito (Pauli et al. 2008).

Centrifugal field techniques are divided into two groups: centrifugal partition chromatography (CPC) and high-speed countercurrent chromatography (HSCCC). In CPC, there is a hydrostatic balance between the two liquid phases that is caused by the rotation of the column on the centrifuge axis (Berthod et al. 2009b; Spînu et al. 2020), and in this technique, it is possible to operate the equipment in ascending mode and descending mode. In ascending mode, the mobile phase (FM) is the least dense of the liquid–liquid partition, which is pumped by the bottom of the column containing the stationary phase (FE); it rises to the top of the column, separating the analytes according to their affinity with the phases (Fig. 2a). In descending mode, the denser phase is used as FM, which is pumped by the top of the column (Fig. 2b).

At HSCCC, the two phases are in hydrodynamic equilibrium thanks to the movement of the two axes of rotation (Ito 2005a; Berthod et al. 2009b); the spiral column rotates on its own axis and the centrifuge axis, similar to a planetary system. The synchronous combination of these rotation and revolution movements maintains the less-dense phase at one end of the column, which Ito called the head, and the denser phase at the other end, called the tail. Therefore, in the HSCCC the equipment can be operated in two ways: (1) in normal mode, the lower phase (denser) is used as FE to fill the column, and the upper phase (less dense) is pumped by the tail of the column as FM, and (2) in reverse mode, the less-dense phase is used as FE, and the

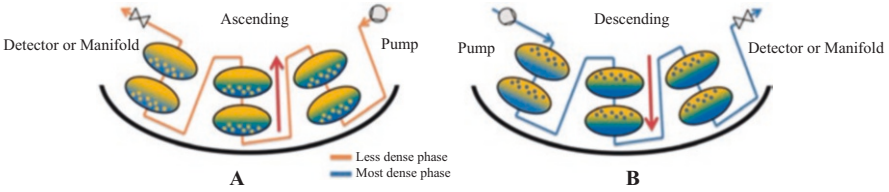

Fig. 2 Modes of operation for the CPC: (**a**) represents the ascending mode and (**b**) the descending mode. (Adapted from Örkényi et al. (2017))

densest phase is FM, which is pumped by the head of the spine. For a thorough investigation of the entire development of CCC-based techniques, see *Countercurrent Chromatography: Apparatus, Theory and Applications*, by Von W. D. Conway (1990).

The choice of solvent composition for FM and EF is of fundamental importance because its composition demands 90% of the time required for developing the chromatographic method in HSCCC and CPC (Ito 2005b). For a complex sample such as plant extracts, and the literature on established systems, such as Ito (n-hexane, AcOEt, MeOH, n-BuOH, and H_2O) (Ito 2005b; Liu et al. 2018), ARIZONA (n-heptane, AcOEt, MeOH, H_2O) (Liu et al. 2018), HEMWat (n-hexane, AcOEt, MeOH, H_2O) (Friesen and Pauli 2015; Liu et al. 2018), and HBAW (n-hexane, ACN, n-BuOH, H_2O) (Costa and Leitão 2010; Liu et al. 2018), needs to be reviewed. In these solvent-proportioned systems, it is possible to work with low-, medium-, and high-polarity molecules, from aqueous and nonaqueous systems, and they may be mixtures of two, three, or more solvents. They must not form emulsion, or this emulsion mist disappear in less than 30 seconds after agitating the phases in a test tube (Costa and Leitão 2010). The sample needs to be soluble in order to prevent any obstructions from entering the column.

The choice of the composition of FM and EF is directly related to the partition coefficient (K), calculated by the solute concentration in EF by the concentration of solute in FM (Ito 2005b). If the generated value is below 0.5, it has greater affinity with FM, and values above 2.0 indicate affinity with E, causing long runtimes for chromatographic runs. The ideal range of K values is from 0.5 to 2.12 for extracts of natural products; the calculation of K can be impeded, so thin-layer chromatography (CCD) is used. The analyst must use a capillary to solubilize the sample in the chosen solvent system and shake the vial to separate the phases. They analyst must make two application points in the CCD, namely the upper and lower phases, after eluting the CCD in an appropriate solvent. The visual information on the chromatographic plate is sufficient to choose the modes of operation for the equipment, where metabolites should be present in both the upper and lower phases to properly following the chromatographic method.

For isolating flavonoids, the HEMWat system is the most cited method in the literature, and to this class of metabolites is attributed numerous biological activities, such as antioxidant, anticancer, anti-inflammatory, antimicrobial, and antiviral activities (Dias et al. 2021). Costa and Leitão (2010) used the HEMWat

(Hex-EtOAc-MeOH-H_2O) solvent system to isolate free, prenylated and diprenylated flavonoids. Boonloed et al. (2016) and Giesbers et al. (2019) used CPC with the ARIZONA solvent system for xylidine isolation from the fungus *Chlorociboria aeruginosa*, a molecule that could be used as a material for sustainable semiconductors. Huang et al. (2022) used the HSCCC to isolate and purify naphthoquinones in fractions obtained from the hexagonal extract of *Arnebia euchroma*, which previously worked in silica gel and liquid chromatography in reverse mode. Li et al. (2015) developed a method in HSCCC to isolate six compounds from a *Panax japonicus* plant extract, and it inhibited xanthine oxidase, an enzyme that produces uric acid and is associated with gout.

Different elution modes can be used in CCC: pH gradients, FM composition, flow, extrusion elution, dual-mode elution, etc. (Huang et al. 2016), as seen in recent progress made in different modes of elution. The advantages of HSCCC and CPC are as follows: They use only liquid phases, such as FM and FE; samples can be fully recovered, eliminating irreversible adsorption problems; each proportion of the FE solvent can be considered a new column; and the analyses are in contact with the entire volume of FE (Berthod et al. 2009a). This versatility assists in the isolation and purification of synthetic molecules and natural products.

5 Capillary Electrophoresis (CE)

Capillary electrophoresis (CE) was introduced in the mid 1980s and since then has been improved. It is a technique that presents more-attractive characteristics compared to other separation techniques such as high simplicity in configuration and miniaturization, rapid separation with high resolution and efficiency, and low sample and solvent consumption. Thus, EC is constantly applied in the biomedical, forensic, environmental, and food areas, for qualitative and quantitative analysis of analytes ranging from small ions and proteins to the metabolites of plants and microorganisms (Unger 2009; Gao and Zhong 2022).

In capillary electrophoresis, the dissections are based on the migration of the analytes in an electric field that runs through a narrow capillary filled with a background electrolyte. In this method, the migration occurs through two distinct mechanisms: electro-osmotic flow (EOF) and electrophoretic mobility (EM) (Suntornsuk 2010). The electro-osmotic flow consists of the mass flow of the background electrolyte. Thanks to the electric field in a fused silica capillary, EOF moves from the anode toward the delicacy because of the negative surface load of the capillary and the presence of the electric field. In electrophoretic mobility, the size of the molecule determines the speed of transport because the analyte is attracted by the anode or cameo. These two modes of transport usually occur simultaneously in most CE sections. During a CE separation with the anode at the injection site in a bare molten silica capillary, when EOF is present, the analyte migrates in the following order: positively charged molecules, positively charged

larger molecules, comigration-neutral molecules, negatively charged large molecules, smaller molecules, and negatively charged molecules (Lu et al. 2018).

As shown in Fig. 3, the separation via CE is performed through the passage of an electric current between two reservoirs containing buffer solutions that are joined by a filled and fused silica capillary. This results in the generation of electro-osmotic flow, which allows target molecules to be charged from one electrode to the other. The capillaries are 30–50 cm long with 50–75 μm i.d. (thin) walls, allowing the quick and efficient change of Joule heating that results from the high voltages required for electrophoretic partitioning. The outside of the fused silica capillary is coated with a layer of polyamide that confers excellent tensile strength to the fragile capillary. The polyamide coating is carefully burned from a small portion of the capillary to expose a section of silica. This transparent section of the capillary is inserted into the light path of a UV detector and becomes the flow cell. As the protein and peptide molecules are swept through the capillary by the EOF, they pass through the path of the detector light and are recorded on the UV monitor. In fact, the capillary becomes a very low-volume flow cell (Burgi and Smith 2001).

The capillary electrophoresis technique has become an important ally in the separation and resolution of a complex mixture of natural products thanks to the versatility among injection modes, its high separation efficiency, and its low consumption of samples and reagents (Tubaon et al. 2014). These attributes determined the phytochemical profile of *Rourea minor*, in which several phenolic compounds were identified, derived mainly from bergenins, catechins, and lignans, in addition to fatty acids (Ngoc et al. 2019). Moreover, some adaptations, such as capillary zone electrophoresis, allowed the technique to perform the separation of phenolic compounds, alkaloids, and phenolic acids (Gotti 2011).

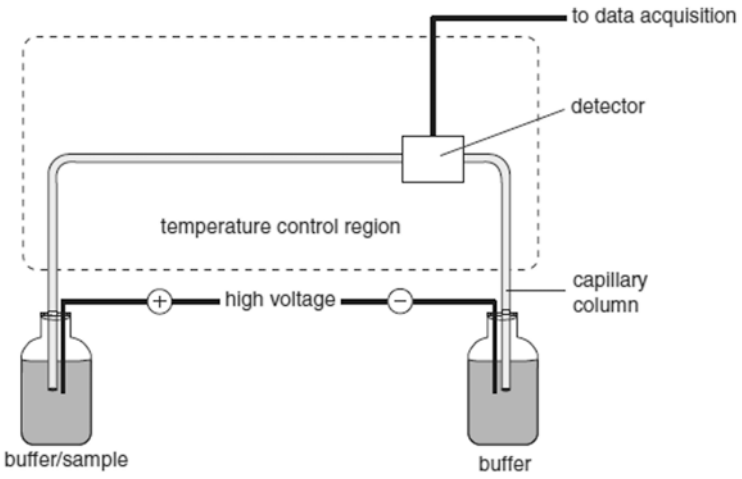

Fig. 3 Capillary electrophoresis equipment scheme (Burgi and Smith 2001)

6 Spectroscopic Methods for Structural Elucidation of Natural Products

6.1 Infrared (IR) Spectrometry

Infrared (IR) spectrometry is an analytical technique that involves the interaction of molecules with electromagnetic energy (Pavia et al. 2009; Mcmurry 2011; Silverstein et al. 2014). This analytical tool is essential for analyzing chemical structures (Xia et al. 2022) and performs fast, accurate, and nondestructive analyses (Ng and Simmons 1999; Arrigone and Hilton 2005). IR spectroscopy has great potential in quality assessments of a variety of products, including agricultural, food, pharmaceutical, soil, and petrochemical products (Ackerman and Hurtubise 2002) and in the evaluation of different materials (Kim et al. 2013). In addition, more-recent advances in IR development have increased the ability of this technique to analyze various types of biological specimens (Su and Lee 2020).

The vast majority of compounds that have covalent bonds, whether organic or inorganic, absorb various frequencies of electromagnetic radiation in the infrared region. This region involves wavelengths greater than those associated with visible light, ranging from approximately 400 to 800 nm (Pavia et al. 2009; Silverstein et al. 2014). However, most chemists refer to radiation in the vibrational infrared region of the electromagnetic spectrum by using units of wave number (n) data in (cm^{-1}) instead of wavelength (μ or μm). The wave number is preferred as a unit because it is directly proportional to the energy. Therefore, in terms of wave number, vibrational infrared ranges from 4000 to 400 cm^{-1}. This range corresponds to wavelengths of 2.5 to 25 μm (Pavia et al. 2009). In organic chemistry, there has been huge interest in the regions of near infrared, from 14,290 to 4000 cm^{-1}, as well as far infrared, from 700 to 200 cm^{-1} (Silverstein et al. 2014).

Infrared absorption comprises energy changes of the order of 8–40 kJ/mol. The radiation in this range encompasses vibrational frequencies from the stretching and folding of bonds in most covalent molecules. In this process, the level of radiation that is absorbed is equivalent to the natural vibrational frequencies of the molecule, and the absorbed energy serves to increase the amplitude of the vibrational movements of the bonds. However, not all bonds in a molecule are capable of absorbing energy in infrared, even if the frequency of radiation is the same as that of vibrational motion. Only connections that have a dipole moment that changes as a function of time are able to absorb radiation in infrared. Symmetric bonds, such as hydrogen gas (H_2) and chlorine gas (Cl_2), do not absorb radiation in infrared (Pavia et al. 2009).

The mechanism that obtains the infrared absorption spectrum of a compound is called an infrared spectrometer or spectrophotometer. In chemistry laboratories, dispersive spectrophotometers and Fourier transform (FT) spectrophotometers are widely used. These instruments provide composite spectra in the range of 4000 to 400 cm^{-1}. The two devices produce almost identical spectra, but the FT

spectrophotometers generate spectra with higher speeds than those of the dispersive spectrophotometers (Su and Lee 2020).

Fourier transform infrared (FTIR) spectrophotometers have become dominant equipment for measuring infrared spectra (Su and Lee 2020). In FTIR spectroscopy, schematized in Fig. 4, an external light-beam infrared radiation source accompanies the beam separator. A part of it is reflected in a mirror fixed at a 90° angle, while another part is transmitted to a moving mirror. After being reflected in the respective mirrors, the beams make their way back to the beam divider. When the two beams meet in the beam divider, they recombine. The difference in the optical path can be controlled by moving the moving mirror. Owing to interference, the intensity of each beam that passes to the detector and returns to the source depends on the difference in the path of the beams in the two arms of the interferometer. The combined beam containing these interference patterns produces the interferogram (Su and Lee 2020).

The beams produced by the divider then pass through the sample, and it simultaneously absorbs all the frequencies found in its infrared spectrum. The

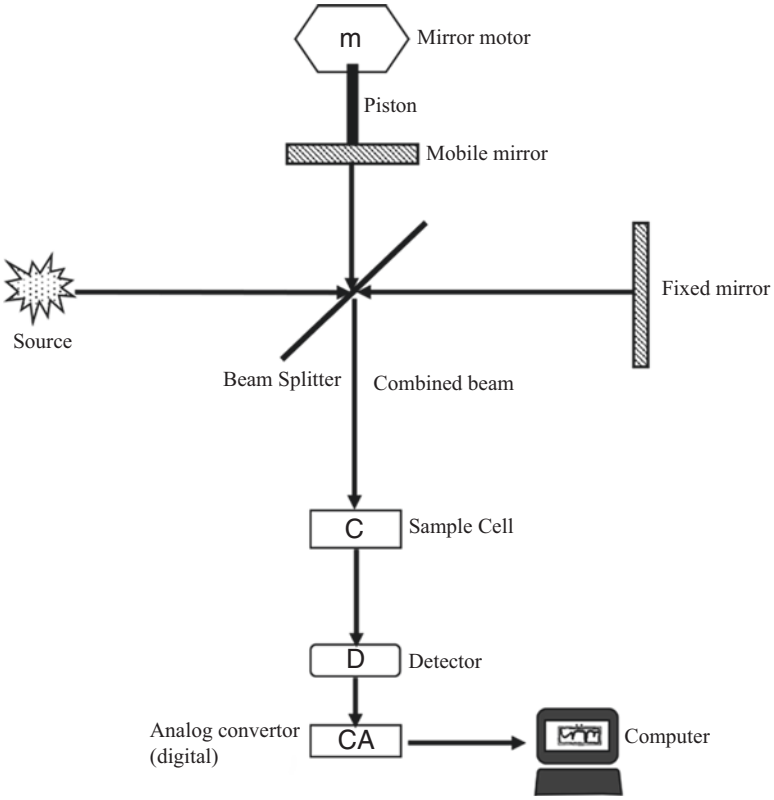

Fig. 4 Scheme of an FTIR spectrophotometer (Pavia et al. 2010)

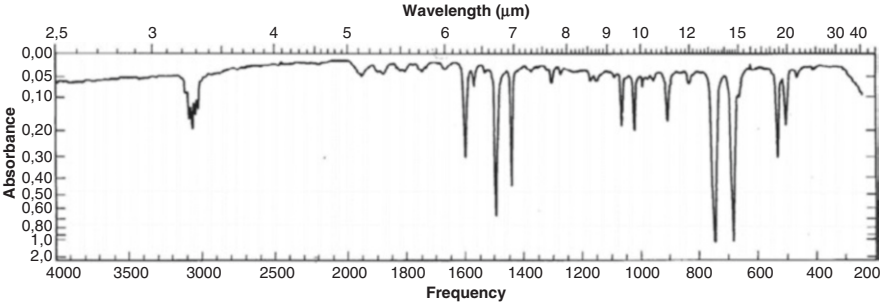

Fig. 5 IR spectrum (Pavia et al. 2010)

interferogram signal then reaches the detector that contains information about the amount of energy absorbed in each frequency. Thus, using the final interferogram, the computer performs a mathematical operation called the Fourier transform, producing an infrared spectrum (Fig. 5) (Pavia et al. 2009).

6.2 Mass Spectrometry

Mass spectrometry (MS) is currently one of the main analytical techniques used. It can analyze a large number of small molecules in complex samples; more specifically, it can identify and quantify metabolites. MS is a widely used technique thanks to its high sensitivity, high yield, and ability to detect a significant number of molecules in samples (Zhang et al. 2020).

In MS, molecules are first ionized by colliding them into a high-energy electron beam. The shock between the electrons and the analyte molecules provides enough energy to leave them in excited states (Skoog et al. 2007). Fragmented ions are magnetically arranged according to their mass/load (m/z) ratios. In this process, the precursor ion (molecular ion, referring to the loss of electrons in the molecule) undergoes fragmentation, generating other ions (Pavia et al. 2009; Mcmurry 2011; Silverstein et al. 2014). This is exemplified in the case of ethylbenzene (Fig. 6), in which the main product is the molecule $C_6H_5CH_2^+$, which arises from the loss of a methyl group (CH_3); in this case, other small fragments are also formed (Skoog et al. 2007). In turn, these ions make it possible to obtain information on the nature and structure of its precursor molecule (Mcmurry 2011).

Generally, the mass spectrometer equipment consists of five main components (Fig. 7). The first is the sample input unit, where the sample can be a gas, a liquid, or a solid. The sample is then converted into steam to obtain a flow of molecules, which is directed to the ionization source, where the molecules are transformed into gas-phase ions; these ions are accelerated by an electromagnetic field, and next, the mass analyzer strains the ions from the sample according to their m/z ratios. The ions then reach the detector and are counted, and the signal is processed and recorded by the data system, usually on a computer. The result of the data is a mass spectrum

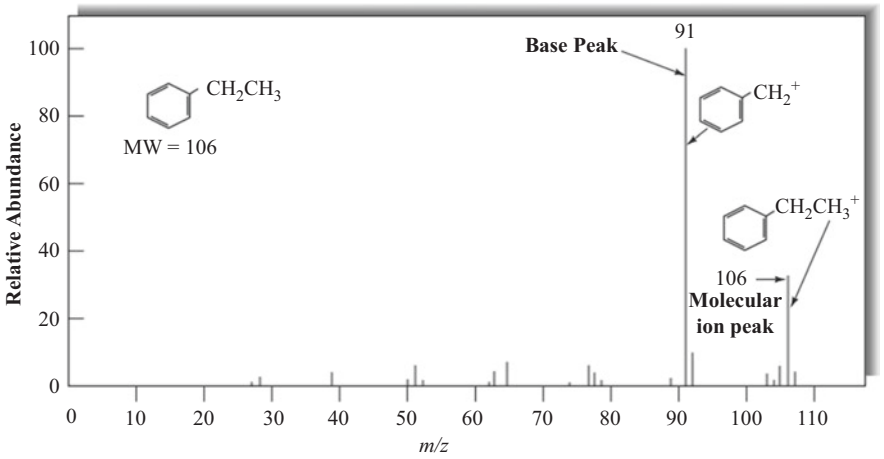

Fig. 6 Ethylbenzene mass spectra (Skoog et al. 2007)

Fig. 7 Process phases in mass spectrometry equipment (Pavia et al. 2009)

graph that represents the number of ions that were detected to have an m/z ratio function, as shown earlier (Pavia et al. 2009; Silverstein et al. 2014).

In metabolomic analysis, there are three more common MS techniques, including gas chromatography-mass spectrometry (GC/MS), liquid chromatography-mass spectrometry (LC/MS), and image mass spectrometry (IMS) (Zhang et al. 2020).

Currently mass spectrometry is used in a multitude of activities. The biotechnology industry uses MS to examine and sequence proteins, oligonucleotides, and polysaccharides (Pavia et al. 2009), and it is also used in the determination of polypeptide structures and other high-molecular-mass biopolymers (Skoog et al. 2007). MS is essential in the pharmaceutical industry because it is used in all phases of the drug-development process, from the identification of compounds to structural analyses and the production of synthetic products (Pavia et al. 2009). In the area of health, this technique is widely used in clinical research, blood tests, urine tests, and drug tests and in the identification of compounds designated as pathological markers (Zhang et al. 2020).

6.3 Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance spectroscopy is the ideal technique in numerous applications because experiments that use it are not destructive. Analyses using NMR spectroscopy are quantitative (with proper experimental assemblies), which allows them to determine the concentrations of analytes (Ben-Tal et al. 2022). It is applicable in a wide spectrum of the common nuclei of organic and organometallic substances and in molecular interaction studies. It is reasonably sensitive and provides detailed structures. Spectroscopy also provides data on the spatial arrangement of molecules; for this, both one-dimensional and two-dimensional experiments are used. Thus, experiments should be designed to determine the number of chiral and/or enantiomeric molecules.

According to Ben-Tal et al. (2022), through NMR, it is possible to monitor the reaction mechanisms of organic biomolecules in order to verify the analysis approach, reaction conditions, temperature, speed, symmetry, resolution, and complexity of kinetics, among other parameters. It also has applications in analyses using synthesis and isotopic markers.

The identification of components in complex mixtures has led to the development of a new trend in the chemistry of natural products. One method of such identification call metabolomics. The goal of metabolomics is to detect and identify all the metabolites involved in specific processes, an objective that cannot be realistically achieved by any of the existing analytical methods (Ge et al. 2018). Therefore, the development of new analytical platforms is an important issue in the field of metabolomics. These combined techniques are often called the hyphenated method, characterized by a combination of chromatography techniques and/or extraction-based techniques with spectroscopic analytical instruments. Among them are platforms that combine spectroscopy and spectrometry with LC (Wu et al. 2008; Porzel et al. 2014; Mung and Li 2018).

As an emerging field, metabolomics requires a new test approach, different from the NMR technique, to enable a simple and rapid screening of constituents in mixtures (Castejón et al. 2014; Pinheiro et al. 2022)—such as determining the chemical profile of fatty acid contents to classify the quality of edible oils (Jabeur et al. 2014). This approach has been made possible thanks to technical advances in the field of automatic sample exchangers, improvements in the quality of spectrometers, and the development of new software for processing spectral data. It also involves a fully automated system comprising the preparation, acquisition, processing, analysis, and interpretation of spectral data (Spraul et al. 2009; Monakhova et al. 2014; Castejón et al. 2016).

Thus, technique spectrometry is associated with a range of applications, including the identification and quantification of isolated structures, the monitoring of chemical reactions, and the screening of samples in mixtures. In this way, its use in the chemistry of organic molecules is essential.

6.4 X-Ray Diffraction

After X-rays were discovered in 1895 by Conrad Wilhelm Röntgen, in 1912, Max Von Lou and coworkers observed that X-rays interact with crystalline substances, producing interference patterns, which were interpreted as being the result of a regular three-dimensional arrangement (Coppens and Penner-Hahn 2001; Wagner and Kratky 2015).

X-rays are generated from a tube that is composed of a metallic filament that acts as the cathode, which is the opposite of the anode (larger than the filament) of the tube (Haschke 2014; Skoog 2017). After applying a high voltage to the filament, high-energy electrons are produced and travel toward the anode, and after this collision, X-rays are generated (Haschke 2014; Skoog 2017). Cu ($K\alpha^1 = 0.154056$ nm) and Mo ($K\alpha^1 = 0.070930$ nm) are the elements most used as sources of radiation (Přichystal et al. 2016). The adjusted level of the applied voltage indicates the energy of the electrons (Haschke 2014).

Next, when the target material interacts with electromagnetic radiation, the elastic scattering (diffraction) of a small fraction of the incoming light occurs, producing X-ray spectra characteristic of the sample (Bunaciu et al. 2015). When it is not possible to obtain single crystals of the substance, the technique can be applied to the analysis of powder samples (Jegorov and Hušák 2014). Hence, X-ray diffraction (also called crystallography) is one of the most powerful techniques for determining the details of the three-dimensional structures of molecules.

X-ray diffraction can help explain these compounds' biological activities by confirming their structure, stereochemistry, and relative or absolute configuration. For example, a pair of enantiomeric alkaloid dimers, (+)- and (−)-pestaloxazine A (Fig. 1), isolated from a *Pestalotiopsis* sp. fungus, shows anti-enterovirus activity (IC_{50} 14.2 and 69.1 μM, respectively), which indicates that the stereochemistry of the spiro center might contribute to this antiviral activity (Jia et al. 2015).

The (S)- enantiomer of 8-formyl-5,7-dihydroxyflavanone (Fig. 1), a formylated flavonoid, had higher antifungal activity against *Cryptococcus neoformans* (IC_{50} 70.4 μM) compared to the (R)- enantiomer (no obvious activity), which indicates that the configuration of the C2 atom is associated with this data (Zaki et al. 2016).

In one study, the difference in the immunosuppressive activity of the murine splenocytes of fischeramides A (IC_{50} 7.08 μM) and B (no obvious activity), geometric isomers, and alkaloids suggested that the C10–C11 double bond (highlighted in red in Fig. 1) in *E*-geometry (fischeramide A) was more beneficial than that in *Z*-geometry (fischeramide B) at improving inhibitory potency (Lin et al. 2020).

Two lignan isomers, (cis)-pensione and (trans)-penchinone, exhibited different protective activities against acetaminophen (AP)-induced damage to hepatocytes (trans showed effective protection, while cis showed weak activity), demonstrating that the configuration of the propenyl unit was related to this result (He et al. 2015).

Dong et al. (2020) identified a new bilobalide isomer (Fig. 1), a terpene from an extract of *Ginkgo biloba*, and after confirming the structure and absolute

configuration, this terpene showed no obvious antiplatelet aggregation activity, which was a divergence from the activity obtained from this same test with another bilobalide isomer (IC_{50} 37.34 μg/mL), identified by (Zheng et al. 2019).

The anti-inflammatory effects from (±)-homocrepidine A (Fig. 1), isolated from *Dendrobium crepidatum*, on the production of nitric oxide indicated that racemic alkaloids have different biological activities and that (+)-homocrepidine A (IC_{50} 3.6 μM) (Fig. 1) had much stronger anti-inflammatory activity than (−)-homocrepidine A (IC_{50} 22.8 μM) (Hu et al. 2016).

After confirming the absolute configuration of the triterpene dichapegenins A (Fig. 1), via X-ray crystallography, triterpene dichapegenins A showed better cytotoxic activity against human tumor cells (human Burkitt's lymphoma IC_{50} 5 μM; human alveolar basal epithelial IC_{50} 6.9 μM; and human liver hepatocellular carcinoma cell line IC_{50} 6 μM) compared to isomer dichapegenins B (no activity against tumor cells) (Zhou et al. 2021).

Wang et al. (2021) reported that the synthesis of 32 stereochemically diverse isomers of spirooliganin and their structures were determined via spectroscopic techniques and also supported by the X-ray of crystal products. Only one isomer had a similar level of activity to that of spirooliganin against coxsackievirus B3 (spirooliganin = IC_{50} 2.1 μM; isomer spirooliganin = IC_{50} 3.7 μM) (Fig. 8). X-ray diffraction is as important a tool as NMR spectrometry in characterizing natural compounds, although one of the advantages of X-rays is the exact reflection of the structure of the substance independent of the influences of neighboring atoms, whereas the NMR technique is affected by such influences. In this way, crystallography is a powerful ally in the discovery of new bioactive compounds with therapeutic properties.

6.5 Ultraviolet-Visible Spectroscopy (UV-Vis Spectroscopy)

UV-vis spectrophotometry is a simple and versatile analytical technique that has been widely used for the quantification and characterization of the structure of various compounds. This method is based on the absorption of light by a sample, and the quantification of this measurement provides key information for the analyst. In different regions of the electromagnetic spectrum, atomic and molecular transitions can be generated when the molecule interacts with radiation, which provides important information about it in that each substance can absorb at specific frequencies of electromagnetic radiation. In the case of UV-vis spectrophotometry, the wavelength range for the UV region is between 180 and 380 nm. The Beer–Lambert law relates to the process of absorption of radiation by the analyte and its concentration: when light passes through the absorbing sample, the intensity decreases thanks to the excitation of the analyte. In this way, it is possible to measure the amount of analyte by developing a calibration curve that compares the absorbance or transmittance of light with the concentration of the sample, showing a directly proportional relationship: The higher the concentration, the higher the absorption or

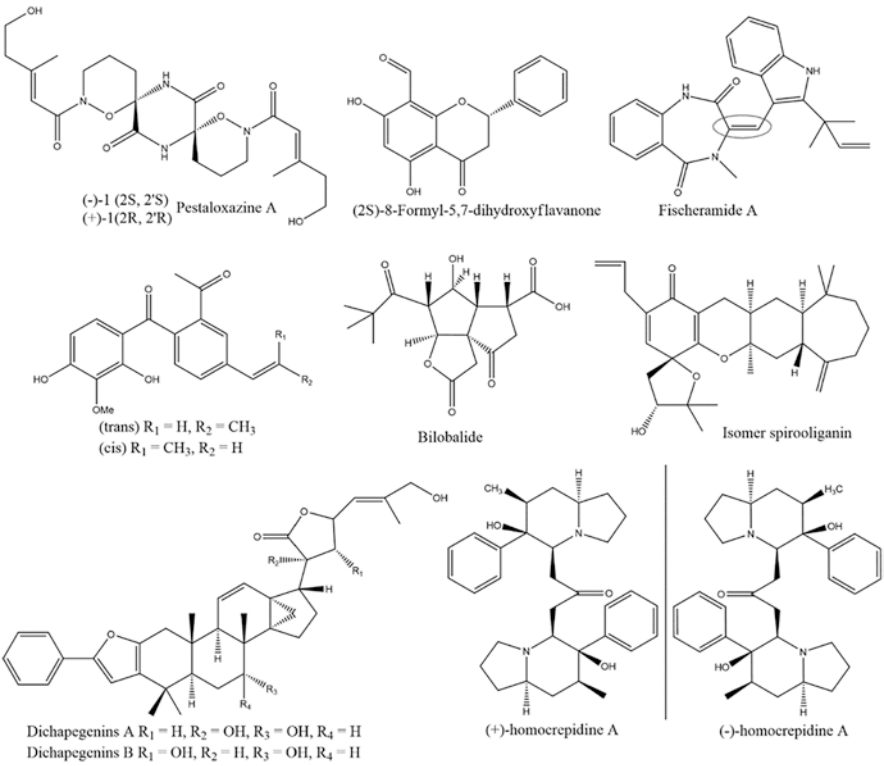

Fig. 8 Natural compounds with their structures and configurations confirmed by X-ray diffraction (Wang et al. 2021)

absorbance. An absorption spectrum is a graph of the absorbance of the sample versus the wavelength. Molar absorptivity as a function of wavelength is independent of concentration and is characteristic of each molecule, allowing these graphs to be used for the identification or confirmation of a compound (Skoog et al. 2007).

Spectroscopy equipment is composed mainly of the following components: (1) a radiation source, (2) a wavelength selector, (3) one or more containers for the sample, (4) a radiation detector, and (5) a data system that processes the signals and readings. In the case of UV spectrophotometry, the lamp most used to emit radiation is deuterium, or hydrogen, which provides a useful continuous spectrum, in the region of 160–375 nm. Many equipments use a monochromator or filter to select the desired wavelength so that only a band of interest is detected and measured, which is achieved by the diffraction grating that these devices have, and that allows the light to be scattered in its wavelengths. The detector converts the radiation into an electrical signal that can then be amplified and converted into numbers proportional to the magnitude of the original quantity. The most commonly used detectors for UV are phototubes and photomultiplier tubes that work within a wavelength range from 150 to 1000 nm. The spectroscopic instrument used to measure sample

absorption is called a spectrometer, and it uses a monochromator or polychrome in combination with a detector to convert radiation intensity into electrical signals. A spectrophotometer is a type of equipment that can measure the relationship between the radiation between two rays, which is necessary to measure absorbance. This comes with the notable advantage of continuous variation in wavelengths, which allows absorption spectra to be recorded (Skoog et al. 2007).

Organic molecules can absorb radiation at wavelengths between 180 and 780 nm thanks to the interactions of photons and electrons involved in bond formation or around nitrogen, oxygen, sulfur, and halogen atoms. Organic functional groups that absorb radiation in UV-vis are known as chromophores (Maleš et al. 2022), including alkenes, alkynes, carbonyls, carboxyls, amides, azo groups, nitrous groups, nitrous groups, nitrates, and some aromatics. Therefore, ultraviolet spectrophotometry may be useful for the detection of chromophore groups and aromatic rings in various samples thanks to the electron transitions of π bonds, σ bonds, and lone-electron pairs (Patle et al. 2020). Among these are phenolic compounds and flavonoids, which are biomolecules in natural products and provide health benefits for humans (Lin et al. 2016). Phenols are molecules whose structures contain phenolic rings, carboxylic acids, and hydroxyl groups such as gallic acid, ferulic acid, coumaric acid, and caffeic acid, among others. Flavonoids are polyphenols that have at least two phenolic rings, and about 4000 compounds of this type have been identified in different plant species (Marinova et al. 2005; Aziz et al. 2022). Flavonoids and polyphenols show antimicrobial, anticancer, antioxidant, and antidiabetic properties (Lin et al. 2016).

Patle et al. (2020) used UV-vis spectrophotometry to screen phytochemicals in plant samples, where through absorption spectra, they showed the presence of flavonoids, phenolic acids, and tannins by using as standards gallic acid, quercetin, rutin, and tannic acid, which exhibit absorption levels between 250 and 370 nm. Scano (2021) carried out the characterization of polyphenols in red and white wines by integrating the spectral data of the techniques of FTIR (Fourier transform infrared spectroscopy) and UV-vis spectrophotometry, which allowed them to identify composites such as flavonoids and flavonols in the ultraviolet region in such a way that the complementary use of these two instrumental techniques was confirmed to provide valuable information on the fingerprint of the fractions of polyphenolic compounds in red and white wines. On the other hand, some researchers applied chemometrics to data obtained from NMR (Nuclear Magnetic Resonance Spectroscopy) and UV-vis spectroscopy as a way to evaluate artichoke extracts and concluded that they are efficient techniques for the structural characterization of cynaropicrin, a bioactive lactone that was obtained in high proportions from this plant product (Boffo et al. 2022). Giglio et al. (2023) also used chemometry from data obtained by UV-vis spectroscopy and high-performance liquid chromatography (HPLC) for the quantification of various phenols in New Zealand pinot noir wines. They concluded that UV-vis spectroscopy can be used to perform calibrations for a wide variety of phenolic compounds in commercial wines. In addition, they obtained accurate models for higher-concentration molecules such as malvidin-3-glucoside

and caftaric acid, which may be useful for small wine companies that aim to quantify these types of molecules at a low cost and with a high degree of accuracy.

Song et al. (2020) used data from the UV-vis and UHPLC/Q-TOF-MS spectrophotometry techniques to predict the antioxidant capacity of and total phenol content in Gayuba leaves. In the experimental part, they observed two peaks in the UV spectrum, at 280 and 358 nm, which corresponded to electronic transitions n-π^* that were caused by aromatic compounds and some chromophores, which were assigned to phenolic acids and flavonols (Aleixandre-Tudo and du Toit 2018). In addition, the samples that had high antioxidant capacities provided higher absorbances at these two wavelengths, which may be markers that predict antioxidant capacities. The results of the study indicate that the data obtained by UV-vis spectrophotometry are suitable for the prediction of this capacity and are comparable with techniques such as FTIR or NIR in other studies (Silva et al. 2014). On the other hand, research has also been carried out on the rapid quantification of total phenols and total ferulic acids in whole wheat by using the data provided by this analytical technique and developing partial least squares models, which provided very accurate predictive models, especially for the quantification of the total content of phenols (Tian et al. 2021). In conclusion, UV-vis spectrophotometry is an efficient technique for the determination of the chromophore groups present in the bioactive compounds of plants.

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Supercritical Fluid for Extraction and Isolation of Natural Compounds

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Abstract Supercritical fluid (SCF) extraction has emerged as an effective and efficient method for separating important phytoconstituents. The extraction process is simple and environmentally friendly, generating minimal to no waste. This procedure offers various advantages over traditional extraction techniques. This chapter discusses the procedure, advantages, and different types of phytoconstituents isolated using supercritical fluids, with a preference for natural products.

Keywords Supercritical · Extraction · Phytoconstituents · Solvent · CO_2 · Dissolve · Oil · Alkaloids · Flavonoids · Terpenes

1 Introduction

Supercritical fluid (SCF) extraction is an analytical method to separate the analyte from the sample matrix using supercritical fluids as solvents (Hedrick et al. 1992). This technique is rapid, inexpensive, sustainable, and simple to execute, compared to the traditional Soxhlet extraction, where solvent costs are usually high, requiring several hours accompanied by an additional concentration step that aids pollution (Sapkale et al. 2010).

SCF extraction was initiated along with supercritical fluid chromatography in the late twentieth century for isolating forensically relevant compounds (Khaw et al. 2017). It later gained popularity when supercritical toluene was used mainly in the petroleum industry with many commercial interests.

Over the last few years, SCF extraction has gained recognition for its many established advantages, particularly supercritical carbon dioxide, because of its easy-to-use properties (2017). CO_2 has a near ambient critical temperature of 31 °C,

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allowing many biological materials and natural products to be processed around 35 °C without denaturation. It is being extensively used in decaffeination and power generation processes and is widely used to extract natural products, leaving no toxic residues behind (Khaw et al. 2017).

The advantage of supercritical CO_2 ($ScCO_2$) is that its extraction properties can be precisely varied with just minute changes in temperature and pressure. The properties can also be modified using solvents like ethanol (Camel 2001). Other than CO_2, various solvents are used to extract bioactive components from plants, namely, propane, DME, SF2, and ethanol (Bizaj et al. 2021).

2 Methodology/Mechanism

A supercritical fluid is a substance whose thermodynamic properties are higher than the critical temperature and pressure of the source compound. The maximum temperature, beyond which the gaseous state of a substance cannot be liquified, irrespective of the amount of pressure applied, is called the critical temperature of the substance. Critical pressure is the minimum pressure required to condense a gaseous substance to a liquid at its critical temperature (Alekseev et al. 2020). For carbon dioxide, the critical temperature is 304.2 K and 73.0 atm.

In the supercritical region, a homogenous fluid materializes, which has unique physiochemical properties. In this region, the surface tension of the supercritical fluid is equal to zero, the dissolving and swelling capacity increases, and the viscosity decreases (Alekseev et al. 2020). The physiochemical properties can be modulated by changing the parameters of the supercritical state. The density of supercritical fluids changes with variations in pressure and temperature; a slight increase in pressure can cause a drastic increase in the density of the supercritical fluid, which in turn causes an increase in the solubility of the supercritical solvent. Once extraction is complete, solvent recovery is relatively simple due to the volatility of the supercritical fluid leaving behind the extracted analyte (Pourmortazavi et al. 2014). Such manipulations of the physiochemical properties make SCFs an excellent solvent for extraction due to their high selectivity, solubility, and extraction efficiency (Yousefi et al. 2019).

The setup (Fig. 1) for supercritical fluid extraction involves a pump, a pressurized compartment, and a collecting vessel. The solvent is commonly stored in a tank connected to a pressurized pump. The commonly used solvent is CO_2, pumped into the system as a liquid below 5 °C and at around 50 bars of pressure. The fluid is cooled to remain a liquid but heated to critical condition after pressurization. The pressure must be maintained in the extraction cell, and heating should be provided to counteract the cooling caused by the adiabatic expansion of the CO_2. Raw material from which the natural product is extracted is placed in the extraction cell, where pressure and temperature are controlled. The raw material is also pre-treated to modulate the moisture content and particle size for optimal extraction. The supercritical fluid is allowed to enter the pressurized extraction cell, where the natural

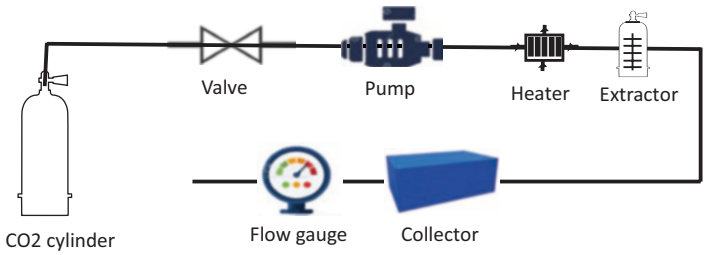

Fig. 1 Setup of $ScCO_2$ extraction

products to be extracted dissolve in the supercritical fluid based on its solubility, which in turn is dictated by its density and pressure. Once the extraction is completed, the fluid with the dissolved natural product is passed through a chamber with lesser pressure, reducing the fluid's dissolving power, and the natural product gets precipitated out. The depressurization of the supercritical CO_2 causes the fluid to become a gas and can be collected separately for further use (Sapkale et al. 2010).

Table 1 lists the chemicals/phytochemicals extracted from the species. Some of the chemicals listed in this table are generic in name, as the literature does not specify the individual compound. The table is arranged in alphabetical order of the species name.

Table 1 List of chemicals isolated from different species using $ScCO_2$

Sl no	Species name	Chemicals/phytochemicals	Ref
1	*Abelmoschus manihot*	Rutin, hyperin, isoquercetin, hibifolin, myricetin, quercetin-3′-O-glucoside, quercetin	Li et al. (2016a)
2	*Acacia dealbata*	Oxygenated triterpenes	Casas et al. (2021)
3	*Acacia dealbata*	Lupenyl acetate, lupenone, tetracosanoic acid, hexacosan-1-ol	Rodrigues et al. (2021)
4	*Acanthophoenix rubra*	Vitamin E	2018)
5	*Acanthus ilicifolius*	2-benzoxazolinone	Arumugam and Thiruganasambantham (2018)
6	*Acer nikoense*	Diarylheptanoids	Alberti et al. (2018)
7	*Alnus glutinosa*	β-Sitosterol, betulin, betulinic acid, lupeol	Felföldi-Gáva et al. (2012)
8	*Alnus hirsuta*	Diarylheptanoids	Alberti et al. (2018)
9	*Aloysia citrodora*	Phenylpropanoids, Flavonoids	Leyva-Jiménez et al. (2020)
10	*Alpinia blepharocalyx*	Diarylheptanoids	Alberti et al. (2018)
11	*Alpinia officinarum*	Diarylheptanoids	Alberti et al. (2018)

(continued)

Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
12	*Amaranthus cruentus*	Linoleic acid, decadieneal, linoleic acid propyl ester, 2.5-pentadecadiene-l-ol, 9-oxononanoic acid	Velikorodov et al. (2018)
13	*Ananas comosus*	Esters, ketones, alcohols, aldehydes, acids	Mohamad et al. (2019)
14	*Andrographis paniculata*	Rosmarinic acid, eurycomanone, andrographolide	Abd Aziz et al. (2021)
15	*Andrographis paniculata*	Andrographolide	Kumoro et al. (2019)
16	*Andrographis paniculata*	Andrographolides	Kumar et al. (2014)
17	*Annona muricata*	Flavonoids, Tannins, Phenolics, Phytate	Mesquita et al. (2021)
18	*Aquilaria malaccensis*	n-Hexadecanoic,1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	Eissa et al. (2018)
19	*Artemisia annua*	Artemisinin	Baldino and Reverchon (2018)
20	*Ascophyllum nodosum,*	Alginate, agar, carrageenan	Abdul Khalil et al. (2018)
21	*Azadirachta indica*	Terpinen-4-ol, 1,2,4-Trithiolane, 3,5-diethyl, allyl isopropyl sulphide, Cycloisolongifolene, á-Bisabolene, (−)-α-Panasinsen, Isocaryophyllene, trans-Sesquisabinene hydrate, 1-Naphthalenol	Swapna Sonale et al. (2018)
22	*Baccharis uncinella*	α-Pinene, β-pinene, limonene, (E)-caryophyllene, germacrene D, bicyclogermacrene, spathulenol, caryophyllene oxide	Minteguiaga et al. (2021)
23	*Betula platyphylla*	Diarylheptanoids	Alberti et al. (2018)
24	*Boswellia serrata*	α-Thujene, camphene, β-pinene, myrcene, limonene, m-cymene, cis-verbenol	Ayub et al. (2018)
25	*Brassica campestris*	Linolenic acid amide, linolenic acid glyceride, linolenic acid, palmitic acid	Li et al. (2016c)
26	*Brassica napus*	Phytosterols	Jafarian Asl et al. (2020)
27	*Bryonopsis laciniosa*	Linoleic acid, linolenic acid, β-sitosterol stigmasterol	Balkrishna et al. (2022)
28	*Calendula officinalis*	Bioactive pentacyclic triterpenes	Villanueva-Bermejo et al. (2019)
29	*Calluna vulgaris*	Bioactive pentacyclic triterpenes	Villanueva-Bermejo et al. (2019)

(continued)

Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
30	*Camellia oleifera*	Palmitic acid, stearic acid, oleic acid, linoleic acid, α-tocopherol, β-carotene, squalene phytosterol. 3-hydroxytyrosol, benzoic acid, catechins, 4-hydroxybenzoic acid, chlorogenic acid	Fang et al. (2015)
31	*Cananga latifolia*	Phenolic acids, flavonoids, tannins, alkaloids	Chhouk et al. (2018)
32	*Cannabis sativa*	Tetrahydrocannabinol	Gallo-Molina et al. (2019)
33	*Cannabis sativa*	ω-6 linoleic acid, ω-3 α-linolenic acid	Devi and Khanam (2019b)
34	*Cannabis sativa*	Cannabidiol	Marzorati et al. (2020)
35	*Capsicum annuum*	γ-Tocopherol	Cvetković et al. (2020)
36	*Capsicum chinense*	Rutin, vicenin-2	de Aguiar et al. (2019)
37	*Capsicum frutescens*	Capsaicinoids	de Aguiar et al. (2018)
38	*Carica papaya*	Oleic acid	Devi and Khanam (2019a)
39	*Catharanthus roseus*	Vincristine	Karimi and Raofie (2019)
40	*Chaenomeles japonica*	α-Tocopherol, β-tocopherol, γ-tocopherol	Górnaś et al. (2019)
41	*Chenopodium quinoa*	Tocopherol	Benito-Román et al. (2018)
42	*Cinnamomum cambodianum*	Phenolic acids, flavonoids, tannins, alkaloids	Chhouk et al. (2018)
43	*Cinnamomum verum*	Cinnamaldehyde, eugenol	Masghati and Ghoreishi (2018)
44	*Cinnamomum verum*	Eugenol, eugenol acetate	Khalil et al. (2017)
45	*Citrus grandis*	7-Methoxy-8-(2-oxo-3-methylbutyl) coumarin, (6E,8E,10E)-2,6,11,15-tetramethyl-2,6,8,10,14-hexadecapentaene, γ-sitosterol, hexadecanoic acid, (E,E)-2,4-decadienal, pentacosane	Gyawali et al. (2012a)
46	*Citrus grandis*	(Z)-9-Octadecenoic acid, limonene, α-Terpineol, (E,E)-2,4-decadienal, hexadecanoic acid, pentacosane, stigmasterol, γ-sitosterol	Gyawali et al. (2012b)
47	*Citrus hassaku*	(Z)-9-Octadecenoic acid, limonene, α-Terpineol, (E,E)-2,4-decadienal, hexadecanoic acid, pentacosane, stigmasterol, γ-sitosterol	Gyawali et al. (2012b)
48	*Citrus Iyo*	(Z)-9-Octadecenoic acid, limonene, α-Terpineol, (E,E)-2,4-decadienal, hexadecanoic acid, pentacosane, stigmasterol, γ-sitosterol	Gyawali et al. (2012b)

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Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
49	*Citrus maxima*	Terpenes, terpenoids, aldehydes, alcohols, esters	Chen et al. (2018a)
50	*Citrus reticulata*	Nobiletin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, tangeretin	Long et al. (2019)
51	*Citrus sinensis*	α-Terpineol, D-Limonene, hesperidin	Barrales et al. (2018)
52	*Citrus sinensis*	Limonene, Hesperidin	Jokić et al. (2020)
53	*Colchicum speciosum*	Colchicine	Bayrak et al. (2019)
54	*Corallina officinalis*	Acyclic alkanes, branched alkanes, alkenes, organobromine compounds, organosulfur compounds, aromatic compounds, monoterpenes, sesquiterpenes, diterpenes, triterpene	Djapic (2018)
55	*Coriandrum sativum*	Linalool, camphor, linalool oxide, p-cymene, α-pinene, limonene, geranyl acetate	Choi and Lee (2018)
56	*Corylus avellana*	Diarylheptanoids	Alberti et al. (2018)
57	*Crocus sativus*	Crocetin sugar esters, picrocrocin, safranin	Kyriakoudi and Z. Tsimidou (2018)
58	*Crocus sativus*	Apocarotenoids, anthocyanins, flavonoids, anthocyanidins, phenolic compounds	Bakshi et al. (2022)
59	*Croton Polycarpus*	Flavanols, sesquiterpenoids	Aponte-Buitrago et al. (2017)
60	*Cucumis melo*	Linoleic acid, oleic acid, palmitic acid, stearic acid	Bouazzaoui et al. (2018)
61	*Cucurbita maxima*	Tocopherols	Rohman and Irnawati (2020)
62	*Cucurbita pepo*	Desmosterol, campesterol, stigmasterol, β-sitosterol, spinasterol, Δ7,22,25-stigmastatrienol, Δ7-stigmastenol, Δ7,25-stigmastadienol, Δ7-avenasterol	Hrabovski et al. (2012)
63	*Cuminum cyminum*	Cumin aldehyde, γ-terpinene, β-pinene, β-Cumic aldehyde, α-phellandrene	Fang et al. (2018)
64	*Curcuma caesia*	Beta-elemene, curzerenone, boldenone, 2-cyclohexen-1-one, 4-ethynyl-4-hydroxy-3, 5, 5-trimethyl.	Chaturvedi et al. (2020)
65	*Curcuma longa*	Tumerone, ar-turmerone, curlone	Haiyee et al. (2016)
66	*Curcuma longa*	Turmeric oil	Priyanka and Khanam (2018)
67	*Cymbopogon citronella*	Essential oil	Wu et al. (2019)
68	*Cymbopogon winterianus*	Citronella oil	Salea et al. (2018)

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Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
69	*Cynomorium coccineum*	Glucose, Fructose, Sucrose, Alanine, Asparagine, Glutamine Proline, Valine, Acetate, Citrate, Formate, Fumarate, Malate, Malonate, Succinate, Betaine, Choline	Attia et al. (2018)
70	*Cyphomandra Betacea*	Linoleic acid, oleic acid, palmitic acid, stearic acid, linolenic acid, palmitoleic acid, squalene, β-sitosterol, cycloartenol, dihydrolanosterol, sterols, γ-tocopherol	Dorado Achicanoy et al. (2018)
71	*Dalbergia ecastophyllum*	Artepillin C, p-coumaric acid	Machado et al. (2016)
72	*Daucus carota*	Carotenoids	Miękus et al. (2019)
73	*Derris elliptica*	Rotenoids	Baldino et al. (2018b)
74	*Descurainia sophia*	Sinapic acid	Hadinezhad et al. (2015)
75	*Dialium cochinchinense*	Phenolic acids, flavonoids, tannins, alkaloids	Chhouk et al. (2018)
76	*Dipteryx odorata*	Alcohols, carbonyl compounds, acids, esters, terpenes, terpenoids, lactones, aliphatic aromatic hydrocarbons	Bajer et al. (2018)
77	*Duguetia furfuracea*	Alloaromadendrene oxide-1, β-caryophyllene oxide,(+)-Spathulenol, Spathulenol,(−)(−) Caryophyllene oxide, Methyl eladiate, Aromadendrene oxide-2,Alloaromadendrene oxide-2,(−)-Spathulenol,Isoaromadendrene epoxide, 2-methylenecholestran-3-ol, α-tocoferol,Palmitic acid,3-Deoxyestradiol,2 Methyhexadecan-1-ol	Favareto et al. (2019)
78	*Echinacea purpurea*	Caftaric acid, cichoric acid, chlorogenic acid, cynarin, echinacoside	Konar et al. (2014)
79	*Eichhornia crassipes*	Stigmasterol, cholesterol, β-sitosterol	Martins et al. 2016)
80	*Elaeagnus angustifoli*	Linoleic acid, decadieneal, linoleic acid propyl ester, 2.5-pentadecadiene-l-ol, 9-oxononanoic acid	Velikorodov et al. (2018)
81	*Elaeagnus mollis*	Linoleic acid, oleic acid, palmitic acid	Mu et al. (2021)
82	*Elaeis guineensis*	Phenolics, flavonoids, carotenoids	Bezerra et al. (2018)
83	*Elaeis guineensis*	Phenolic compounds	Chan et al. (2018)
84	*Elaeis guineensis*	Vitamin E	Damrongwattanakool and Raviyan (2018)
85	*Elaeis guineensis*	Hexadecanoic acid, octadecanoic acid	Jaafar et al. (2011)

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Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
86	*Elaeis guineensis*	α-Carotene, β-carotene	Carmona et al. (2018)
87	*Elettaria cardamomum*	1,8-cineol	Ghosh et al. (2015)
88	*Eremanthus erythropappus*	α-Bisabolol	Náthia-Neves et al. (2020)
89	*Eucalyptus globulus*	Quinolizidine alkaloids, ß- Carotenes, Saponins, tannins, steroids, flavonoids	Abd Hamid et al. (2018)
90	*Eucommia ulmoides*	Linolenic acid	Zhang et al. (2018)
91	*Eugenia involucrata*	α-Tocopherol	Barzotto et al. 2019)
92	*Eurycoma longifolia*	Rosmarinic acid, eurycomanone, andrographolide	Abd Aziz et al. (2021)
93	*Ficus hirta*	Elemicin, Psoralen, Palmitic acid, Bergapten, Linolenic acid, Medicarpin, Retinoic Acid, Maackiain, Squalene	Deng et al. (2018)
94	*Foeniculum vulgare*	Sterols	Bettaieb Rebey et al. (2019)
95	*Furcraea selloa*	Saponins	Ramli et al. (2019)
96	*Ganoderma lucidum*	Oleic acid, palmitic acid, linoleic acid, Ergosta-7, 22-dien-3β-ol, ergosterol	Li et al. (2016b)
97	*Garcinia mangostana*	Squalene, α-Cubebene	Hamid et al. (2013)
98	*Garcinia Mangostana*	α-Mangostin	Hamid et al. (2018)
99	*Gardenia angkorensis*	Phenolic acids, flavonoids, tannins, alkaloids	Chhouk et al. (2018)
100	*Glycine max*	Phytosterol, tocopherol	Han et al. (2016)
101	*Glycine max*	Polyene phosphatidyl choline	Jiang et al. (2016)
102	*Glycyrrhiza uralensis*	1-Methoxyerythrabyssin II, 6,8-diprenylgenistein, gancaonin G, isoglycyrol, licorisoflavan C, licoricidin, licorisoflavan D, licorisoflavan E	Villinski et al. (2014)
103	*Haematococcus pluvialis*	Astaxanthin	Cheng et al. (2018)
104	*Haematococcus pluvialis*	Phorbol 12-myristate 13-acetate, doxycycline	Chou et al. (2016)
105	*Haematococcus pluvialis*	Astaxanthin, lutein, fatty acids	Di Sanzo et al. (2018)
106	*Hancornia speciosa*	Amyrin, lupeol, α-amyrin, β-carotene	Maia et al. (2018)
107	*Helianthus annuus*	Chlorogenic acid	Daraee et al. (2019)

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Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
108	*Hippophae rhamnoides*	β-Sitosterol, α-tocopherol	Dienaitė et al. (2021)
109	*Hippophae rhamnoides*	Zeaxanthin, β-carotene, lycopene, α-tocopherol, β-tocopherol, δ-tocopherol, β-sitosterol	Mihalcea et al. (2021)
110	*Humulus lupulus*	Xanthohumol, desmethylxanthohumol, bitter acids, phenolic compounds	Bizaj et al. (2021)
111	*Humulus lupulus*	Phenolic acids, ferulic acid, flavonoids, resveratrol, xanthohumol	Veiga et al. (2021)
112	*Hylocereus polyrhizus*	Linoleic acid	Abdullah et al. (2018)
113	*Ilex guayusa*	Caffeine, squalene, α-amyrin.	Cadena-Carrera et al. (2019)
114	*Inula racemose*	Alantolactone, isoalantolactone	Chi et al. (2016)
115	*Iris lactea*	Linoleic acid, oleic acid, docosahexaenoic acid.	Luan et al. (2020)
116	*Isatis tinctoria*	Isatin, tryptanthrin, deoxyvasicinone, isaindigotone, isaindigotidione, quinazolines, indolinone, benzodiazepine, glucoraphanin progoitrine, glucobrassicine, aromatic, aliphatic carboxylic acids	Hamburger (2002)
117	*Juniperus communis*	Sesquiterpene, diterpene alcohols, terpene oxides, ketones	Bogolitsyn et al. (2019)
118	*Laminaria digitata*	Alginate, agar, carrageenan	Abdul Khalil et al. (2018)
119	*Laminaria hyperborean*	Alginate, agar, carrageenan	Abdul Khalil et al. (2018)
120	*Larix sibirica*	Dehydroquercetin	Averyanova et al. (2018)
121	*Lavandula angustifolia*	Linalyl acetate	Győri et al. (2019)
122	*Lavandula angustifolia*	Tannins, flavonols, anthocyanins	Tyskiewicz et al. (2019)
123	*Leucas cephalotes*	Oleanolic acid	Kaushik et al. (2021)
124	*Linum usitatissimum*	α-Linolenic acid, lignans, proteins, dietary fibers	Tang et al. (2021)
125	*Lippia graveolens*	Flavonoids	Arias et al. (2020)
126	*Lippia origanoides*	Flavonoids	Arias et al. (2020)
127	*Lupinus luteus*	Apigenin, fisetin	Buszewski et al. (2019)
128	*Lycopodium clavatum*	Quinolizidine alkaloids, ß-Carotenes, Saponins, tannins, steroids, flavonoids	Abd Hamid et al. (2018)
129	*Macrocystis pyrifera*	Alginate, agar, carrageenan	Abdul Khalil et al. (2018)

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Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
130	*Mangifera indica*	Pectin, phenolic compounds, carotenoids (mainly all-trans-ß-carotene), various vitamins	Sánchez-Camargo et al. (2019)
131	*Mangifera indica*	Mangiferin, isomangiferin, quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-xyloside, quercetin 3-O-arabinoside, quercetin, kaempferol	Meneses et al. (2015)
132	*Marrubium vulgare*	Marrubiin	Gavarić et al. (2021)
133	*Matricaria chamomilla*	Cycloalkane polyols	Al-Suod et al. (2019)
134	*Melaleuca cajuputi*	Caryophyllene, humulene	Kueh et al. (2018)
135	*Melissa officinalis*	Eugenol, geraniol, D-limonene, ortho-cresol	Zaid et al. (2020)
136	*Mitragyna speciosa*	Quinolizidine alkaloids, ß-Carotenes, Saponins, tannins, steroids, flavonoids	Abd Hamid et al. (2018)
137	*Momordica cochinchinensis*	β-Carotene, lycopene	Kha et al. (2014)
138	*Moringa oleifera*	Quinolizidine alkaloids, ß-Carotenes, Saponins, tannins, steroids, flavonoids	Abd Hamid et al. (2018)
139	*Moringa oleifera*	Gallic acid, vanillic acid, p-coumaric acid, catechin, 1-triacontanol, nonacosane, heptacosane, phytol, γ-tocopherol, α-tocopherol	da Silva et al. (2022)
140	*Morus nigra*	Phenolic acids, flavonoids	Nastić et al. (2018)
141	*Muricauda lutaonensis*	Zeaxanthin	Hameed et al. (2011)
142	*Musa paradisiaca*	Lupenone, methyl 2-hydroxy-2-(3-nitrophenyl)-2-(4-nitrophenyl)-acetate, pentacosane, 3,6,9-nonacosatriene, 10-hentriacontene, 7,23-dimethyltritriacontane	Correa et al. (2016)
143	*Myrcia blanchetiana*	Myrciaine	de Cerqueira et al. (2013)
144	*Myrica rubra*	Diarylheptanoids	Alberti et al. (2018)
145	*Myrmecodia pendans*	Gallic acid, catechin, ferulic acid, caffeic acid, p-coumaric acid, quercetin, luteolin, kaempferol	Sanjaya et al. (2014)
146	*Myrtus communis*	Quinolizidine alkaloids, ß- carotenes, Saponins, tannins, steroids, flavonoids	Abd Hamid et al. (2018)
147	*Narcissus poeticus*	Benzyl benzoate, benzyl linoleate, benzyl alcohol α-Terpineol, Limonene, (3E)-hexenol, heneicosanol, dihydroactinidiolide, 4,8,12,16-tetramethyl heptadecan-4-olide, heptanal, nonanal, (2E,4E)-decadienal, octadecanal	Baranauskienė and Venskutonis (2022)

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Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
148	*Nelumbo nucifera*	Linoleic acid, decadieneal, linoleic acid propyl ester, 2.5-pentadecadiene-l-ol, 9-oxononanoic acid	Velikorodov et al. (2018)
149	*Nicotiana tabacum*	Nicotine, neophytadiene, 4,8,13-duvatriene-1,3-diol. Palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid	Banožić et al. (2021)
150	*Nigella sativa*	Thymoquinone, thymol, p-cymene, chlorquinaldol, amylmetacresol, 2,4-dichlorobenzyl alcohol	Gawron et al. (2019)
151	*Ocimum basilicum*	1,8-cineole, linalool, eugenol, germacrene D, T-cadinol	Occhipinti et al. (2013)
152	*Ocimum basilicum*	Linalool, estragol	Győri et al. (2019)
153	*Ocimum sanctum*	Eugenol	Ghosh et al. (2013)
154	*Ocimum tenuiflorum*	Eugenol, eugenol acetate	Khalil et al. (2017)
155	*Odontonema strictum*	Flavonoids	Ouédraogo et al. (2018)
156	*Oenocarpus distichus*	Oleic acid, palmitic acid, linoleic acid	Cunha et al. (2019)
157	*Olea europaea*	Polyphenols	Trucillo et al. (2018)
158	*Olea europaea*	Oleuropein (OLE)	Baldino et al. (2018a)
159	*Olea europaea*	Oleuropein, luteolin-7-glucoside were the main phenolic antioxidants	Cejudo Bastante et al. (2018)
160	*Olea europaea*	Oleuropein	Uzel (2018)
161	*Olea europaea*	β-Cyclodextrin	Jaski et al. (2019)
162	*Opuntia ficus-indica*	Isorhamnetin-3-O-glucosyl-rhamnosyl--rhamnoside, isorhamnetin-3-O-glucosyl-rhamnosyl-pentoside, isorhamnetin-3-O-glucosyl-rhmanoside	Antunes-Ricardo et al. (2018)
163	*Orbignya phalerata*	Lauric acid, oleic acid, lauric acid	de Oliveira et al. (2019)
164	*Origanum majorana*	Cis-sabinene hydrate	Busatta et al. (2017)
165	*Origanum vulgare*	Cis-sabinene hydrate	Busatta et al. (2017)
166	*Origanum vulgare*	α-Linolenic acid, palmitic acid, oleic acid, linoleic acid, carvacrol, heneicosane, nonacosane, docosane, borneol, thymol	García-Pérez et al. (2019)
167	*Oroxylum indicum*	Phenolic acids, flavonoids, tannins, alkaloids	Chhouk et al. (2018)
168	*Orthosiphon aristatus*	Rosmarinic acid, eurycomanone, andrographolide	Abd Aziz et al. (2021)

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Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
169	*Orthosiphon stamineus*	Sinensetin	Aziz et al. (2018)
170	*Orthosiphon stamineus*	Sinensetin, Isosinensetin, Rosmarinic Acid	Abdul Aziz et al. (2020)
171	*Parthenium argentatum*	Terpenoids, phenolics, alkaloids, sterols, fatty acids/triglycerides	Dehghanizadeh and Brewer (2020)
172	*Passiflora mucronate*	β-amyrin, β-sitosterol, stigmasterol, oleanolic acid	da Silva et al. (2020)
173	*Petroselinum crispum*	Apigenin	Saotome and Imai (2018)
174	*Physalis angulate*	Trinitrobenzenesulphonic acid	Almeida Jr. et al. (2017)
175	*Picea Abies*	Methyl dehydroabiatate	Burčová et al. (2018)
176	*Picea abies*	Catechin, dihydroquercetin, astringin, isorhapontin	Ferrentino et al. (2021)
177	*Pimpinella anisum*	Sterols	Bettaieb Rebey et al. (2019)
178	*Piper amalago*	Pyrrolidine Alkaloid	Carrara et al. (2017)
179	*Piper betle*	Phenolic compounds	Pise et al. (2022)
180	*Piper betle*	Tannins, quercetin, eugenol, hydroxychavicol, chavibetol	Azahar et al. (2020)
181	*Piper hispidum*	Cinnamoyl pyrrolidine amides	Lima et al. (2020)
182	*Piper klotzschianum*	Germacrene D, pipercallosidine, 14-oxy-α-muuroleno, bicyclogermacrene, (E)-caryophyllene	Lima et al. (2019)
183	*Piper nigrum*	Eugenol, eugenol acetate	Khalil et al. (2017)
184	*Piper nigrum*	Piperine, piperlonguminine, piperanine, pipercallosine, dehydropipernonaline, pipernonatine, retrofractamide B, pellitorine, guineensine	Yu et al. (2022)
185	*Pistacia lentiscus*	α-Pinene, terpinene-4-ol	Aydi et al. (2020)
186	*Pistacia vera*	α-Pinene, β-myrcene, limonene-D, α-terpinolene	Demirkoz et al. (2018)
187	*Pleurotus ostreatus*	Heteropolysaccharides, β-glucans, α-glucans, oligosaccharides	Barbosa et al. (2020)
188	*Pongamia pinnata*	Oleic acid, arachidic acid, cis-10-pentadecenoic acid, stearic acid, cis-8,11,14-Eicosatrienoic acid, linolenic acid, gamma(γ)-linolenic acid, cis-11-Eicosenoic acid	Suryawanshi and Mohanty (2018)
189	*Populus balsamifera*	Pinostrobin, tectochrysine, pinocembrin, chrysin	Adekenov et al. (2020)

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Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
190	*Prunus armeniaca*	Tocopherols, Amygdalin, Fatty Acids	Pavlović et al. (2018)
191	*Punica granatum*	Punicic acid, tocopherols, phytosterols, triterpenes, phospholipidsquercetin, epicatechin, catechins, delphinidin, pelargonidin, cyanidin, punicalagin, punicalin, gallic acids, caffeic acids, chlorogenic acids	El-Shamy and Farag (2021)
192	*Punica granatum*	Punicic acid, linoleic acid, oleic acids	Khoddami et al. (2014)
193	*Putranjiva roxburghii*	β-Sitosterol, oleic acid, linoleic acid	Balkrishna et al. (2021)
194	*Rhodiola rosea*	Salidroside, rhodioloside B, rhodioloside C, rhodiosin, luteolin, catechin, quercetin, quercitrin, herbacetin, sacranoside A, vimalin, dihydroquercetin, acacetin, mearnsetin, taxifolin-O-pentoside, tricetin trimethyl ether 7-O-hexosyl-hexoside, tricin 7-O-glucoronyl-O-hexoside, tricin O-pentoside, tricin-O-dihexoside, eriodictyol-7-O-glucoside; flavan-3-ols: gallocatechin, hydroxycinnamic acid caffeoylmalic acid, di-O-caffeoylquinic acid, esculetin, esculin, fraxin, lignans: hinokinin, pinoresinol, L-ascorbic acid, glucaric acid, palmitic acid, linolenic acid	Zakharenko et al. (2021)
195	*Rhus punjabensis*	Dihydrofisetin	Dong et al. (2020)
196	*Rosa canina*	Linoleic acid, linolenic acid, palmitic acid, stearic acid	Jahongir et al. (2019)
197	*Rosa damascene*	Citronellol, geraniol, nerol, nonadecane, nonadecene, heneicosane, heptadecane	Antonova et al. (2021)
198	*Roselle calyces*	Anthocyanins	Idham et al. (2021)
199	*Rosmarinus eriocalyx*	β-Amyrin, camphor, tetradecenoic acid, linolenic acid	Bendif et al. (2018c)
200	*Rosmarinus officinalis*	Carnosic acid, carnosol, rosmanol, genkwanin, cirsimaritin, homoplantaginin, ursolic acid	Sharifi-Rad et al. (2020)
201	*Rosmarinus officinalis*	Carnosic acid, carnosol, methyl carnosate, rosmanol, rosmarinic acid. Moreover, carnosic acid, carnosol	Fornari et al. (2014)
202	*Rosmarinus officinalis*	Verbenone, cirsimaritin, salvigenin, carnosol, carnosic acid	Kuo et al. (2011)
203	*Rosmarinus officinalis*	Essential oils, phenolic compounds	Ali et al. (2019)
204	*Rosmarinus officinalis*	Palmitic acid, α-linolenic acid, linoleic acid, oleic acid, stearic acid, d-camphor, eicosane, 1,8-cineole, tetracosane, borneol, β-caryophyllene	García-Pérez et al. (2020)

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Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
205	*Rubia tinctorum*	Alizarin, lucidin, rubiadin	Yekefallah and Raofie (2022)
206	*Rubus idaeus*	Fatty acids, tocopherols	Marić et al. (2020)
207	*Ruellia angustiflora*	Fatty acids, triterpenes, tetraterpenes, tocopherols, phytosterols	Pires et al. (2021)
208	*Saccharum officinarum*	Alcohols, esters, hydrocarbons, ketones, aldehydes	Ahmed Baloch et al. (2018)
209	*Saccharum officinarum*	Long-chain fatty alcohols, phytosterols	Albarelli et al. (2018)
210	*Salvia hispanica*	Linoleic acid, a-linolenic acid, tocopherols, polyphenols	Ixtaina et al. (2014)
211	*Salvia hispanica*	Squalene, sterols, tocopherols, polyphenols, carotenoids	Dąbrowski et al. (2018)
212	*Salvia officinalis*	1,8-cineole, α-/β-thujone, camphor, α-humulene, viridiflorol, manool	Jokić et al. (2018)
213	*Salvia officinalis*	Carnosic acid, carnosol	Pavić et al. (2019)
214	*Salvia Rosmarinus*	α-Pinene	Allawzi et al. (2019)
215	*Salvia Rosmarinus*	Carnosic acid, rosmarinic acid, carotenoids, chlorophyll	Lefebvre et al. (2021)
216	*Salvia viridis*	Vanillin, Ethyl syringate, Syringaldehyde (3,5-Dimethoxy-4-hydroxybenzaldehyde), Antiarol (3,4,5-Trimethoxyphenol), Indole-4-carbaldehyde, Coumarin, Coniferyl aldehyde (4-Hydroxy-3-methoxycinnamaldehyde), N-(2-Phenylethyl)acetamide, Sinapyl aldehyde (3,5-Dimethoxy-4-hydroxycinnamaldehyde), Dimethoxy-trihydroxy(iso)flavone isomer 1, Dihydroxy-dimethoxy(iso)flavone, Dimethoxy-trihydroxy(iso)flavone isomer 2, Genkwanin, Dihydroxy-trimethoxy(iso)flavone, Hydroxy-trimethoxy(iso)flavone, Hydroxy-tetramethoxy(iso)flavone, 1-Oxomicrostegiol, Viroxocin, Apigenin-4′,7-dimethyl ether (4′,7-Dimethoxy-5-hydroxyflavone), 3-Oxomicrostegiol, Hexadecanedioic acid, Viridoquinone	Zengin et al. (2019)
217	*Sambucus nigra*	Quercetin, kaempferol, rutin	Anusha Siddiqui et al. (2022)
218	*Satureja montana*	Thymol, carvacrol, γ-terpinene, p-cymene	Damjanović-Vratnica et al. (2016)

(continued)

Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
219	*Saururus chinensis*	Aurantiamide acetate, echinuline, (−) -(7R, 8R) -7-O-acetylpolysphorin, elemicin, isoelemicin, 1, 4-bis (3, 4-dimethyoxyphenyl) 2, 3-dimethyl-1, 4-butanedione, saucerneol D, (2R) -3-(3′, 4′, 5′-trimethoxyphenyl) -1, 2-propanediol, grandisin, rel-(7R, 8R, 7′R, 8′R) 3′, 4′- methylenedioxy-3, 4, 5, 5′-tetramethoxy-7, 7 -epoxylignan, zanthopyranone, (±) -eritro-1-(3, 4, 5-trimethoxy) -1, 2 -propanodiol, threo-3, 4, 5-trimethoxy-7-hydroxy-1′-allyl-3′, 5′-dimethoxy-8. O. 4′-neolignan, (+) -(8R) -(2, 6 -dimethoxy-4-propenylphenoxy) -1-(3, 4, 5-trimethoxyphenyl) propan-1-one, meso-dihydroguaiaretic acid, (−) -galbacin, (−) - (7R, 8R) -7-O-acetylraphidecursinol B	Chen et al. (2018b)
220	*Scenedesmus almeriensis*	Lutein	Mehariya et al. (2019)
221	*Schinus terebinthifolia*	Germacrene D, sabinene, β phellandrene, α- phellandrene	Andrade et al. (2017)
222	*Schinziophyton rautanenii*	Campesterol, stigmasterol, β-sitosterol, Δ5- avenasterol, 22-dihydrospinasterol, Δ7-avenasterol, lanosterol, Δ5,23-stigmastadienol, Δ7-campesterol, clerosterol, obtusifoliol, Δ 5,24(25)-stigmastadienol, α-amyrin, gramisterol, cycloeucalenol, cycloartenol, stigmasta-8,24-dienol-3-β-ol, 28-methylobtusifoliol, 24-methylenecycloartenol, citrostadienol, β-sitosterol, Δ5-avenasterol, campesterol.	Gwatidzo et al. (2014)
223	*Serenoa repens*	Fatty acids, beta-sitosterol, fatty alcohols	Bartolomé Ortega et al. (2017)
224	*Sesamum indicum*	Sesamin, sesaminol, sesamolinol	Hu et al. (2004)
225	*Sesamum indicum*	γ-Tocopherol, lignan	Shi et al. (2018)
226	*Sesamum indicum*	Sesamin, sesamolin, tocopherols, linoleic acid, oleic acid	Buranachokpaisan et al. (2021)
227	*Sida rhombifolia*	Isoquercitin	Ferro et al. (2019)

(continued)

Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
228	*Sideritis sipylea*	ß-Caryophyllene, α -Humulene, 9-epi-(E)-Caryophyllene, Germacrene D, Bicyclogermacrene, cis-Sesquisabinene hydrate, Spathulenol, Caryophyllene oxide, Humulene epoxide II, (E)-Sesquilavandulyl acetate, Cyclopentadecanolide, Hexahydrofarnesyl acetone, (Z)-Lanceol acetate, Isopimara-9(11),15-diene, Totarene, Beyerene, Geranyl-α-terpinene, Geranyl-p-cymene, (Z,Z)-Geranyl linalool, Dolabradiene, Sclarene, (E,Z)-Geranyl linalool, (Z,E)-Geranyl linalool, 13-epi-Dolabradiene, 13-epi-Manool oxide, (E,E)-Geranyl linalool, Manool, 13-epi-Manool, Phytol, Abienol, Abieta-8(14),13(15)-diene, Sandaracopimarinal, Sclareol, 7-α-hydroxy-Manool, 3-α-hydroxy-Manool, Isopimarol, Sideridiol, n-Hexacosane, 7-Epicandicandiol, Siderol, n-Heptacosane, n-Octacosane, n-Nonacosane, Sidol, Sesquiterpene hydrocarbons, Oxygenated sesquiterpenes, Diterpene hydrocarbons, Oxygenated diterpenes, lkanes	Axiotis et al. (2020)
229	*Solanum lycopersicum*	Polyphenols, flavonoids, lycopenes, carotenoids	Haddadin and Haddadin (2015)
230	*Solanum lycopersicum*	Lycopene	Inakuma (2015)
231	*Solanum lycopersicum*	Lycopene, β-carotene	Cante et al. (2022)
232	*Solanum lycopersicum*	Lycopene	Reverchon et al. (2022)
233	*Solanum lycopersicum*	α-Tocopherol, γ-tocopherol, lycopene, β-carotene	Romano et al. (2020)
234	*Solanum viarum*	1,2-Benzenedicarboxylic acid, quinic acid, octadecenoic acid, solasodine	Confortin et al. (2019)
235	*Sophora flavescens*	Genistein	Han and Kang (2015)
236	*Sorbus aucuparia*	Linoleic acid, oleic acid, palmitic acid	Bobinaitė et al. (2020)
237	*Sorghum bicolor*	Linoleic acid, decadieneal, linoleic acid propyl ester, 2.5-pentadecadiene-l-ol, 9-oxononanoic acid	Velikorodov et al. (2018)
238	*Spina gleditsiae*	Saponins	Liu (2018)
239	*Spinacia oleracea*	Lutein, chlorophyll	Derrien et al. (2018)
240	*Spinacia oleraecea*	Phenolics	Lee et al. (2018)

(continued)

Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
241	*Stellera chamaejasme*	Hexanedioic acid, bis(2-ethylhexyl) ester, πsitosterol, 7-methyl-Z-tetradecen-1-ol acetate, 9-hexadecenoic acid-hexadecyl ester (Z), 1,2- benzenedicarboxylic acid-diisooctyl ester, (3π24Z) stigmasta-5,24(28)-dien-3-ol, stigmastan-3,5-diene, squalene	Bai et al. (2012)
242	*Stevia rebaudiana*	Polyphenols, chlorophylls, carotenoids	Bursać Kovačević et al. (2018)
243	*Sucupira branca*	Alpha-humulene, beta-caryophyllene, alpha-copaene, (−)-beta-elemene, (E)-germacrene D(−)-gamma-elemene, spathulenol	Chañi-Paucar et al. (2022)
244	*Swietenia mahagoni*	Linoleic acid	Hartati et al. (2018)
245	*Syzygium aromaticum*	Eugenol, eugenol acetate	Idowu et al. (2021)
246	*Syzygium aromaticum*	Eugenol, chavicol, n-pentacosane, hexacosanal, vitamin E	Frohlich et al. (2019)
247	*Syzygium aromaticum*	Eugenol, caryophyllene, eugenol acetate	Győri et al. (2019)
248	*Syzygium aromaticum*	Eugenyl acetate, β-caryophyllene, α-humulene	Haro-González et al. (2021)
249	*Syzygium campanulatum*	Flavanones, chalcone, triterpenoids	Memon et al. (2016)
250	*Tagetes erecta*	Lutein	Pal and Bhattacharjee (2018)
251	*Tanacetum parthenium*	Parthenolide, sudachitin, aceronin, nevadensin	Végh et al. (2018)
252	*Teucrium polium*	Germacrene D, β-eudesmol, shyobunol, δ-cadinene	Bendif et al. (2018b)
253	*Theobroma cacao*	Polyphenols, mainly procyanidins, flavan-3-ols	Hernández et al. (2019)
254	*Thymus mastichina*	Thymol, α-terpinene, p-cymene	Kessler et al. (2022)
255	*Thymus munbyanus*	Tocopherol	Bendif et al. (2018a)
256	*Thymus vulgaris*	Chlorophyll b, chlorophyll a	Hamdan and Daood (2011)
257	*Trachyspermum ammi*	Thymol, o-Cymene, γ-Terpinene, 2-methyl-5-(1-methylethyl)-phenol	Bhatt et al. (2018)
258	*Trifolium pratense*	Isoflavonoids (3-phenyl chromones), flavonoids (2-phenyl chromones)	Klejdus et al. (2005)
259	*Triticum Vulgare*	Tocopherol	Özcan and Ören (2019)

(continued)

Table 1 (continued)

Sl no	Species name	Chemicals/phytochemicals	Ref
260	*Vaccinium Meridionale*	Anthocyanins (ACNs)	Colorado et al. (2020)
261	*Vaccinium myrtillus*	Anthocyanins, flavonols, tocopherols. Polyunsaturated fatty acids, vitamin E.	Gustinelli et al. (2018)
262	*Viburnum opulus*	β-Sitosterol, α-tocopherol	Dienaitė et al. (2021)
263	*Viburnum opulus*	Phenolic acids, iridoids, quercetin, (epi) catechina, flavalignans, procyanidins, anthocyanins	Dienaite et al. 2020)
264	*Virola surinamensis*	Steroids, terpenes, coumarins, phenolics	Cordeiro et al. (2019)
265	*Vitis vinifera*	1-Hexacosanol, 1-octacosanol,1-triacontanol, α-tocopherol, β-sitosterol, β-amyrin	de Melo et al. (2020)
266	*Xanthium strumarium*	Linoleic acid, decadieneal, linoleic acid propyl ester, 2.5-pentadecadiene-l-ol, 9-oxononanoic acid	Velikorodov et al. (2018)
267	*Xinjiang jujube*	Quercetin-3-O-robinobioside, Rutin (Quercetin-3-O-rutinoside), Hyperoside (Quercetin-3-O-β-d-galactoside), Quercetin-3-O-β-d-glucoside, Kaempferol-3-O-robinobioside, Kaempferol-3-O-glucoside, Quercetin-3-O-β-l-arabinosyl-(1 → 2)-α-l-rhamnoside, Quercetin-3-O-β-d-xylosyl-(1 → 2)-α-l-rhamnoside.	Song et al. (2019)
268	*Zingiber officinale*	α-Zingiberene	de Souza Junior et al. (2020)
269	*Zingiber officinale*	6-Gingerol	Gan et al. (2016)

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Microwave-Assisted Extraction of Phytochemicals

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Abstract Microwave-assisted extraction (MAE) has emerged as a promising technique for the extraction of phytochemicals and has received substantial scientific attention in recent years. MAE involves the utilization of microwaves to heat the sample, which facilitates the release of bioactive compounds from the plant matrix. MAE offers several advantages over traditional extraction methods, including faster extraction times, higher extraction yields, and reduced solvent consumption. To improve the efficiency of the extraction process, research has concentrated on optimizing various parameters, including the extraction temperature, extraction time, and solvent type. Additional studies have investigated the effect of MAE on the chemistry and bioactivity of the extracted phytochemicals. Several classes of phytochemicals, including phenolic compounds, flavonoids, and alkaloids, have been successfully extracted using MAE. These compounds possess various biological activities, such as antioxidant, antimicrobial, and anticancer properties. Essential oils from aromatic plants have also been extracted using MAE, which is widely employed in the food, cosmetic, and pharmaceutical industries. Despite its many advantages, the major challenge in the application of MAE is the potential degradation of the extracted compounds due to the high-temperature and high-pressure conditions during extraction. Additionally, the cost of microwave equipment and the need for specialized expertise may stunt its widespread adoption. In diverse omics disciplines, MAE shows promise, notably for the development of analytical platforms for research in genomics, proteomics, metabolomics, and related subdisciplines. Nonetheless, more investigation is required to optimize the extraction conditions and guarantee that the chemical makeup and biological activity of the isolated phytochemicals are preserved.

Keywords Microwaves · Phytochemicals · Natural pigments · Omics · Pharmaceuticals

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1 Introduction

For the separation, identification, and usage of valuable chemicals from various plants, extraction is a crucial step. Depending on the characteristics of the desired chemical, a suitable technique must be chosen in order to achieve the highest yield and purity. Compounds from plants are extracted using a wide range of chemical and mechanical procedures, including solvent extraction and steam distillation. The extraction of essential oils, fats, and oils is now done using Soxhlet, hydrodistillation, and alcohol-maceration methods. Reproducibility is a significant problem because these techniques involve manual operations. Low extraction yields are the result of the heating process degrading thermally sensitive components. The mentioned restrictions, along with the sharp rise in demand for bioactive components, essential oils, fat, and oils, have motivated the need for appropriate, selective, economical, and environmentally friendly extraction technologies that are quick and yield more (Stévigny et al. 2007).

Many efforts have been made to enhance the Soxhlet extraction in order to shorten the extraction time, use less solvent, and do away with the requirement for concentration and evaporation at the end of the extraction. Randall presented a three-step extraction process that includes boiling, rinsing, and solvent removal as a significant enhancement to the Soxhlet extraction equipment (Randall 1974). It is well known that microwave energy, with a frequency of 2.45 GHz, significantly affects the speed of numerous processes in the chemical and food industries. The use of microwave dielectric heating in analytical chemistry has garnered a lot of attention due to the shorter analysis times, easier manipulation, and improved purity of the end result. All of the applications that have been documented have demonstrated that microwave-assisted solvent extraction (MAE) is a practical substitute for traditional methods for such matrixes. The key advantages are the decreases in solvent, energy, and extraction time (Virot et al. 2008).

2 Fundamentals of Microwave Extraction (Microwave Theory)

Gedye, Giguere, and Ganzler were the first to discuss the use of microwave energy in chemical laboratories for organic synthesis and the extraction of biological matrices for the creation of analytical samples, respectively, in 1986 (Ganzler et al. 1990). Ultrasound-assisted extraction (UAE) is a technique that is around 35 years older than MAE. The huge potential of this non-conventional energy source for synthetic, analytical, and processing applications has nevertheless been investigated in great detail by numerous laboratories. Dielectric heating has been used in synthesis and extraction thus far, and over 7000 and 2000 articles, respectively, have documented this utilization. Early investigations, which did not measure the temperature or power, described the microwave-assisted extraction in a

screw-capped vial with a volume of only 3 mL using conventional microwave ovens. Techniques for extracting bioactive chemicals from plants were developed. Microwaves (MW) are electromagnetic radiation with frequencies ranging from 300 MHz (radiofrequency radiation) to 300 GHz. In chemistry research, 2.45 GHz and 915 MHz are employed as frequencies for lab equipment and industrial equipment, respectively.

The following categories can be used to group microwave interactions with materials: (Gupta and Wai Leong Wong 2007)

Opaque Materials Microwave applicators are made of opaque materials, which are often conducting materials with free electrons, such as metals, that reflect electromagnetic waves but do not allow them to pass through.

Transparent Materials Transparent materials, such as low-loss dielectric materials or insulating materials like glass, ceramics, and air, allow microwaves to travel through easily with minimum attenuation. These materials are used to make reactors that are put within microwave applicators.

Absorbing Materials Materials that absorb energy include those with conductivity and insulating characteristics. These materials, which are the focus of microwave extraction, are commonly referred to as high-loss dielectrics or high dielectric loss materials because they absorb electromagnetic energy and convert it to heat.

Since it is lower than the typical ionization energies of chemical bonds (3–8 eV) or even hydrogen bonds, the MW photon energy corresponding to the frequency used in microwave heating systems, ranging from 3.78×10^{-6} to 1.01×10^{-5} eV, acts as a non-ionizing radiation that has no effect on the molecular structure (0.04–0.44 eV). The interaction with materials happens by heating them since microwave radiation is non-ionizing. Only substances that can take in microwave energy can be heated. Heating can come from dielectric and magnetic losses caused by interactions between the microwave's electric and magnetic field components and the materials. For non-metal materials, the importance of the dielectric losses is greater. Ionic conduction and dipole rotation serve as their foundations. Ionic conduction, the first of these, is the term used to describe the induced electrophoretic migration of charge carriers (such as ions and electrons) when the electric field of the microwaves is present. The migrating ions and the medium experience "friction" as a result of the migration, and this can result in heating. When dipolar molecules strive to align themselves with the alternating electric field in a medium created by microwaves, the second principle, known as dipole rotation, takes place (Zhang et al. 2011). These dipolar species' oscillation causes them to collide with nearby molecules, which generates heat. Temperature is a major factor in determining the relative importance of the energy conversion mechanisms, dipole rotation, and ionic conduction. Ionic conduction rises with rising sample temperature, whereas dipole rotation decreases for small molecules like water and some other solvents. This means that when microwave energy is used to heat a sample that contains both ionic and polar chemicals, the heating is first dominated by the contribution of dipole

rotation and, as the temperature rises, it becomes dominated by ionic conduction. The mobility, concentration, and sample relaxation time of the ions all affect the relative contribution of these two heating mechanisms. This information is crucial for the MAE (Lee et al. 2016). The extraction yield drastically alters when the plant material's microwave absorption capacity exceeds that of the solvent. Having an estimate of the medium's reaction to microwave radiation is crucial for achieving the best possible microwave applicator design (permittivity). This information will enable the process to be characterized (including its heating rate, penetration depth, and temperature distribution, among other things), and it will aid in understanding its behavior (Vinatoru et al. 2017).

MAE extraction technology has been used for both large-scale and laboratory applications. The MAE method has been applied in recent years to isolate essential oils, fats, and oils. In terms of speed, safety, and cost, microwave technology has been found to be an effective extraction method (Bélanger et al. 1997). The closed-vessel MAE system and the open-vessel system comprise the two types of MAE systems. Closed containers are used for extraction. Whereas open-vessel systems are for extractions of target chemicals at high-temperature and high-pressure conditions performed under circumstances of atmospheric pressure.

The selection of the proper solvent is a key element that influences extraction. The choice of solvent is mostly determined by the desired analyte's solubility, the solvent‘s capacity to interact with the matrix, and its microwave absorption. The chosen solvent should be compatible with additional chromatographic analysis steps and should have a high selectivity of the target analyte over matrix components. Transparent solvents are not heated in the microwave, and those that have a high capacity for absorption are heated more quickly to speed up the extraction. Hexane is regarded as a top-notch solvent for absorbing microwave energy (Virot et al. 2008). For the best extraction yields, researchers have combined solvents with high and low microwave absorbabilities.

3 Instrumentation of the Microwave Extraction

Instrumentation systems for microwave-assisted extraction and its laboratory application are available in two varieties (Pastor et al. 1997; Luque-García and Luque de Castro 2004), namely:

(a) Closed extraction vessels/multimode microwave ovens.
(b) Focused microwave ovens.

Controlled pressure and temperature drive extraction in a closed extraction vessel/multimode microwave oven. In contrast, in focused microwave-assisted Soxhlet or solvent extraction (FMASE), as the name implies, only the portion of the extraction vessel containing the sample is targeted for microwave irradiation. Closed-vessel and focused vessel systems are both commercially available as multimode and

single-mode or focused systems (Luque-García 2003). A multimode system provides for the random distribution of microwave radiation throughout the microwave cavity, ensuring that every zone in the cavity and the sample is evenly irradiated. Focused MAE systems allow subjecting the sample to a much restricted and focused delivery of microwave radiations under a strong electric field. Domestic microwave oven behaves as a modified multimode open-vessel extraction system (Moen et al. 2012).

The sample and solvent are also situated within the sealed vessel, which is commonly made of microwave-transparent materials such as polyether imide or trifluoromethoxy polymers in the pressurized MAE system (Fig. 1). The following are the general operating conditions for MAE:

- Pressure: 200 psi
- Temperature: Between 110 °C and 145 °C
- Power Setting: 100% at 900 W

3.1 Fundamental Components in an MAE Device

The microwave extraction assembly is composed of four major parts:

1. Magnetron/ Microwave Generator: Used to generate microwaves.
2. Wave Guide: Used to direct the microwaves from the source to the microwave cavity.
3. Applicator: Contains the sample holder which houses the sample.
4. Circulator: Used to regulate microwave propagation only in the forward direction.

In the case of multimode systems, the applicator is a closed cavity within which a random dispersion of microwaves occurs (Kristenson et al. 2006). Beam reflectors or turntables aid in the consistent distribution of microwave energy inside the cavity, regardless of sample placement position. The microwave waveguide serves as the applicator in focused microwave systems, and the extraction vessel is placed immediately in the cavity. Only a few inches of the vessel's bottom are exposed to microwaves, and because glass is microwave-transparent, the upper area of the vessel remains cool. As a result of the microwave's integrated architecture, an effective condensing process occurs (Figs. 2 and 3).

3.2 Advantages of Closed-Vessel Systems

Higher temperatures can be obtained in a closed-vessel system due to greater pressure inside the vessel, which raises the boiling point of the solvents utilized. In a closed vessel system, there is virtually no loss of volatile compounds and just a little

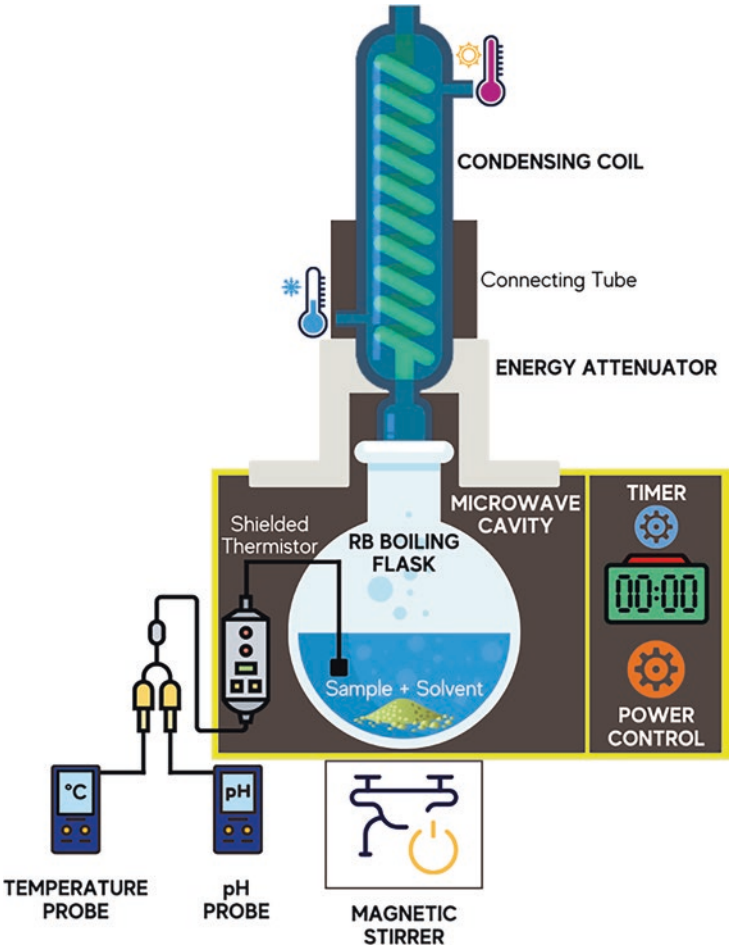

Fig. 1 Scheme of a modified multimode domestic microwave oven for MAE (open-vessel extraction)

amount of solvent is required. Because there is no need to add solvent/s frequently, the risk of air-borne contamination is reduced. The vessel is capable of containing all the potentially hazardous fumes produced during an acid microwave extraction and doesn't require any additional provision for the same (Tatke and Jaiswal 2011).

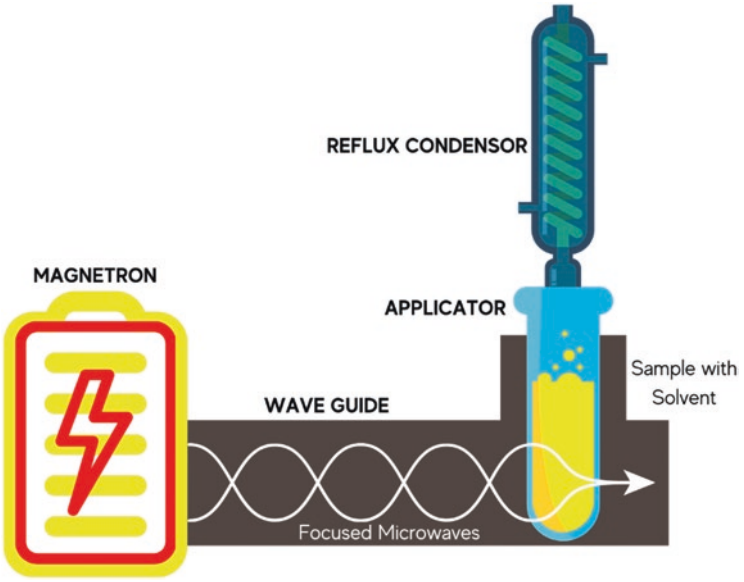

Fig. 2 Schematic view of focused microwave oven

3.3 Limitations of Closed-Vessel Systems

A closed-vessel system's shortcomings include the risk of using high pressures and the limited volume of samples that can be processed. The material utilized for vessel construction, such as PTFE (polytetrafluoroethylene), does not allow for high temperatures, and when utilizing volatile compounds, the vessel must be opened only after a cooling process to avoid the loss of extracted volatile elements. The high pressures used in closed-vessel systems pose a safety risk as they are prone to explosions. The single-step procedure also excludes the addition of reagents or solvents during the operation of the system (Tatke and Jaiswal 2011).

3.4 Atmospheric Pressure or Open MAE System

Atmospheric pressure or open-vessel systems offer much more effective microwave sample preparation than closed-vessel systems. The usage of atmospheric pressure systems has several substantial advantages over pressurized-vessel systems (Tatke and Jaiswal 2011), including:

(a) Open vessels have increased safety as they can be operated at atmospheric pressure and the reagents can be added at any time during the treatment.
(b) The oven containers can be made of PTFE, glass, or quartz, and surplus solvents can be easily removed.

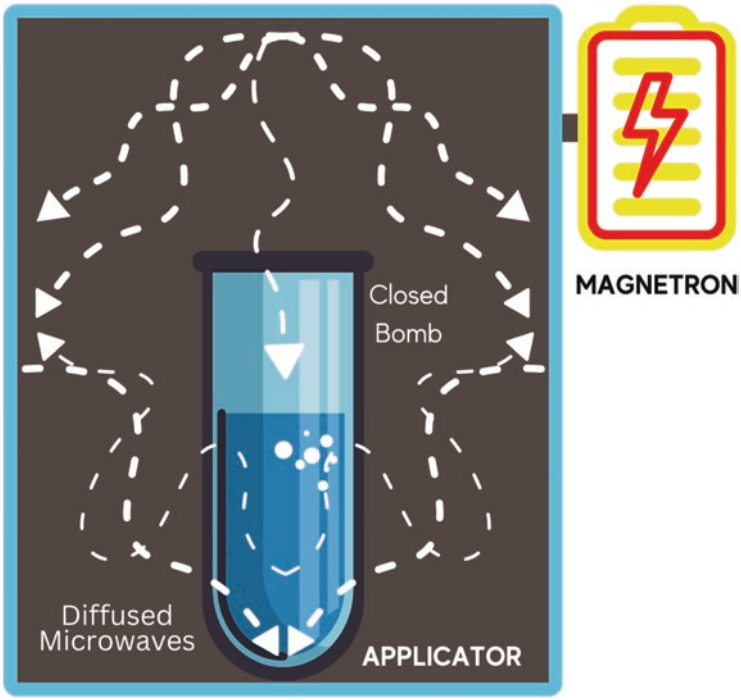

Fig. 3 Schematic view of multimode microwave oven

(c) The instrument's main advantage is its capacity to process huge samples without the need for a cooling process.
(d) The equipment is inexpensive, and complete automation with open-vessel operation is possible.
(e) It has the ability to go through leaching cycles until quantitative removal of the target species is achieved.
(f) The atmospheric system is best suited for thermolabile species as it utilizes lower temperatures compared to closed-vessel systems.

Notwithstanding their many benefits, open-vessel systems have significant shortcomings (Tatke and Jaiswal 2011), which are as follows:

(a) Methods employed in open-vessel systems are typically less precise than those used in closed-vessel systems.
(b) The open-vessel system cannot process many samples at the same time, but closed-vessel systems can handle 10 to 14 samples at a time.
(c) Open-vessel systems require longer extraction durations to achieve extraction efficiencies comparable to those of closed-vessel systems.

4 Scaleup of Microwave-Assisted Extraction

Although MAE has been successfully employed for several years and laboratory studies have shown promising industrial potential, the commercialization of this technology seems very stale. Environment Technology Centre (ETC) and Environment Canada made the first step towards the scale-up of MAE technology. The instrumentation is represented in Fig. 4. As depicted, the system is a continuous process where materials and solvents are pumped into the TEFLON tube located in a microwave cavity. Within the cavity, microwave-assisted extraction occurs. This flowing continuous process allows this technique to be scaled up to 0.5 tonnes/hr. with a microwave power of 6 kW. System analyses have shown that the continuous-flow pipe system used for this technique can only be applied when the temperature is below the boiling point of the solvent (preferably nonpolar) used for the extraction with a mechanism given by Paré and Bélanger in 1997 (Bélanger et al. 1997). Notwithstanding, in most cases, the extraction is performed in reflux conditions for a short duration (a few minutes to hours) where the equipment cannot be employed. In such cases, a batch-fed MAE system equipped with a condenser is much more economical (Dai 2006).

5 Factors Influencing Microwave-Assisted Extraction

When we speak in terms of extraction procedures, there are various methods that can be followed. One of the most commonly followed extraction method is microwave-assisted extraction. In this method, there are various factors which will

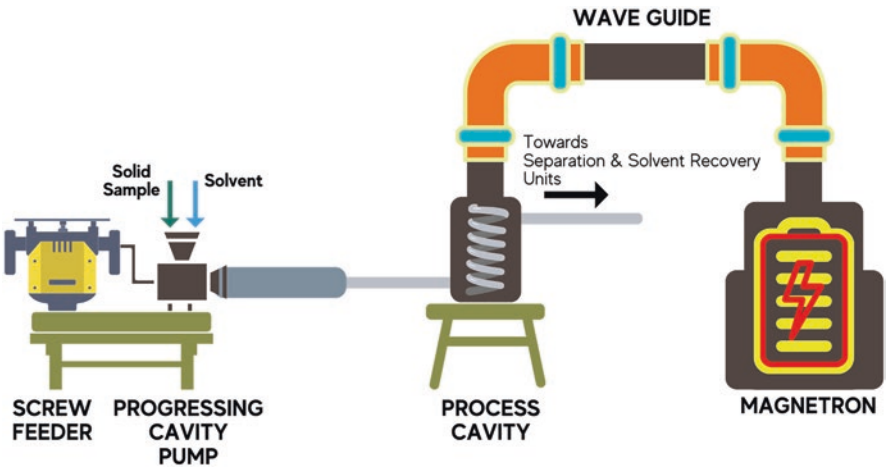

Fig. 4 Schematic diagram of a scaled-up MAE equipment

influence the extraction procedure of which some will accelerate the process and others will hinder the process of extraction (Bagade and Patil 2019).

Here are some key factors which will influence the process of extraction:

- A blend of microwave-assisted solvents should be used for the isolation of specific active compounds from the plant sample. This helps to obtain a good yield (Bagade and Patil 2019).
- Time of heating (temperature) during extraction is a very important factor because if more heat is provided then the main elements to be extracted may also get degraded. The temperature should be optimized and generally set in a range of 60 °C to 120 °C (Llompart et al. 2019).
- The microwave power should be appropriate or else will lead to the loss of plant constituents.
- Extraction efficiency of microwave-assisted extraction is affected by the particle size of the matrix (Bagade and Patil 2019) (Fig. 5).

6 Microwave-Assisted Extraction of Fats and Oils

A group of lipids known as acylglycerides, or esters in which two or three fatty acids are linked to a glycerol molecule to create monoglycerides, diglycerides, or triglycerides, respectively, are collectively referred to as fats. Triglycerides, which are triesters of glycerol and fatty acids and can either be solid or liquid at room temperature depending on their specific structure and composition, are the most

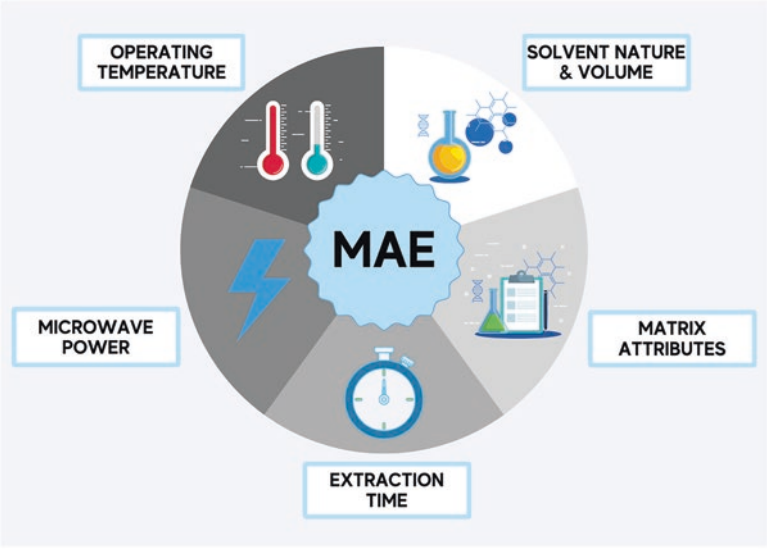

Fig. 5 Factors affecting microwave-assisted extraction of herbal matrices

prevalent types of fats. The terms "oils," "fats," and "lipids" are frequently used to refer to fats, but they typically refer to lipids that are liquid or solid at room temperature, respectively. The main sources of raw materials with extractable fats and oils are either plants or animals. A unique process is used for each raw material to produce ambient-temperature solid fats made from plant sources (such as cocoa butter, coconut fat, and palm butter). These fats are commonly utilized as fat filling in the food industry (e.g., cocoa butter in the chocolate industry) (de Castro et al. 2012).

Olives are an example of a fruit from which vegetable oils are derived (e.g., sun flower, soybean, corn, cotton). Fruit or seed oils are used for frying in both domestic and commercial settings (de Castro et al. 2012). Seed oils can be used to make margarine by hydrogenating them to raise their melting point. Additionally, albeit less frequently, edible oils can be made from grape seeds and dried fruits like pecans, hazelnuts, or almonds (a by-product of wine production). The cosmetics sector also makes use of several vegetable fats and oils. Linseed oil is also utilized in varnishes and paints because of its high polyunsaturated fatty acid content and semidrying qualities.

7 Types of MAE Extractants

Depending on the individual extractant, there are a variety of methods by which extraction under heating might occur. The sample can therefore be submerged in a single solvent or a mixture that can effectively absorb large amounts of microwave energy (mechanism I). As an alternative, the sample can be extracted into a mixture of solvents with a changeable ratio of both high and low dielectric losses (mechanism II). A sample with a significant dielectric loss can also be extracted using a solvent that is transparent to microwaves (mechanism III). Using specialized bars made of a chemically inert fluoropolymer can achieve heating if the sample and extractant are both transparent to microwaves (mechanism IV). Usually, one of these mechanisms—or a combination of them—is used for solute extraction and partitioning.

In order to assure the quantitative extraction of both neutral lipids and membrane-associated polar lipids, the analytical extraction of lipids from tissues necessitates the use of an extractant or extractant mixture that is sufficiently polar. The solvents that are most typically employed for MAE of lipids are mixtures of n-hexane and acetone (Lopez-Avila 1999). However, combinations of ethyl acetate and cyclohexane have also proven successful for tissue extraction. The ethyl acetate/cyclohexane azeotrope has a composition that is approximately equal in volume (54:46) and a boiling point that is 23 °C higher than the n-hexane/acetone mixture's boiling point at 72.8 °C (Shackelford and Alexander 2000). Additionally, it doesn't require a microwave transformer to be heated directly because it has a high enough dielectric constant. It also performs a dual function since it functions as a nonpolar system but accepts some water. Water in the sample evaporates, breaking down the

cell's structure and enabling the separation of lipids from their interaction with lipoproteins and cell membranes. From this perspective, having water in the sample is beneficial for speeding up extraction (particularly under microwave irradiation). Water, on the other hand, makes the extraction solvent more polar, which reduces its effectiveness and, potentially, lipid yields. Aqueous tissue samples are often dried before extraction because of this. The debate over whether to use less toxic or nontoxic solvents in place of toxic ones like n-hexane has been reignited by the current trend toward green processes in extraction (Virot et al. 2008). One example is the discovery that limonene, a significant component of the citrus fruit industry's by-products, is more effective than n-hexane for the MAE of oil from olive drupes (de Castro et al. 2012).

8 Microwave-Assisted Extraction of Antioxidants

Without endangering essential molecules, antioxidants interact with free radicals to stop chain events, such as those that lead to lipid peroxidation. Balance reactive oxygen species (ROS) by eating foods high in antioxidants to reduce such health hazards.

In order to extract high-quality antioxidant extracts from a range of plant matrices, microwave-assisted extraction (MAE) is a very helpful technique. Its benefits come from microwave (MW) heating, which is quick and efficient since heat is supplied directly to the material. As a result, phytochemical substances are released from plant cell compartments more rapidly and easily (Camel 2000). Additionally, MAE operations take much less time and use less solvent than Traditional Solvent Extraction. The most efficient parameters in the microwave-assisted extraction process were determined to be temperature, microwave power, solvent concentration, solid-to-solvent ratio, and extraction time (Hayat et al. 2009). A closed-vessel microwave technology is generally utilized, which automatically adjusts power to balance temperature variations, and the temperature is optimized rather than the power. According to temperature variations, the system-applied microwave power is in the range of 0 to 1500 watts (Şen et al. 2019).

Response surface methods is used to optimize the key parameters, including extraction temperature, extraction time, solvent concentration (ethanol in water), and solid-to-solvent ratio. The extraction efficiency for antioxidants rose as the ethanol content of the water decreased from 80% to 58%, whereas there was little change at lower concentrations. It is believed that ethanol breaks the link between antioxidant chemicals and plant matrix while water acts as a plant-blowing agent. The concentration of 58% ethanol in water was found to be the ideal solvent composition, and the mixture of water and ethanol was found to be the most effective solvent for the extraction of antioxidants. For a high extraction yield, the ethanol concentration in the water should have a limiting value to speed up the damage to the plant cell membranes. However, once the critical ethanol concentration is reached, protein coagulation and impurity extraction may take over as the main

factors that negatively affect solvent penetration (González and González 2010). The solvent ratio of ethanol to water is found to have a statistically significant impact on extraction yield, reducing power, and total phenolic content for lemon grass, galangal, holy basil, and rosemary. Solvent concentration is the parameter that has the greatest impact on MAE antioxidant yield.

Because the analytes are more soluble in the matrix's active sites at higher temperatures, extraction efficiencies rise as a result. Additionally, the analytes can be dissolved by the solvents at higher temperatures, and as the temperature rises, the surface tension and viscosity of the solvents both decrease, improving sample wetting and matrix penetration, respectively. The extraction of antioxidants is said to increase with temperature in the literature, while structural disintegration begins at high temperatures. High temperatures should also not be used for safety and energy efficiency reasons. Therefore, the ideal working temperature may be 78 °C (Şen et al. 2019). The MAE system offers advantages over traditional extraction methods due to features like multiple sample extraction, the capacity to work in closed vessels beyond the boiling point, speed (minutes), simplicity of use, automatic temperature control using power at various voltages, and high extraction efficiency. The MAE approach is therefore probably a significant alternative method for the easy, affordable, quick, and highly successful extraction of antioxidants from plants (Dorta et al. 2013).

9 Extraction of Natural Pigments by Microwave-Assisted Technology

Nearly all plant parts, including leaves, flowers, fruits, seeds, and roots, can be used to make natural colorants and dyes. Because chlorophyll can transform sunlight into chemical energy through photosynthesis, green is thought to be the hue that is most frequently found in plant leaves. In addition to shielding plants from natural predators, other colors in plants can also draw in insects or other creatures that can act as intermediaries in pollination and ultimately reproduction (Dangles 2012).

In order to make our living world vibrant and colorful, it is important to note that all of these natural hues can be utilized to add color to an infinite range of products (textiles, food, varnishes, cosmetics, etc.). The majority of the color compounds that make up natural pigments are from one or more of the following groups: carotenoids, anthocyanins, betanin, chlorophyll, curcumin, and flavonoids (Velíšek et al. 2008). Additionally, chromophores and auxochromes are the two main chemical groups that make up a pigment molecule, according to chemical studies of pigment. The chromophore is typically compared to an aromatic ring with unsaturated bonds because of its coloring property, and the number of unsaturated bonds determines the color intensity. In order to impart color, the auxochrome can aid in combining the pigment molecule with the substrate (Siva 2007). In order to improve the aesthetic value of foods, colorants have been widely utilized in a variety of food

products. The use of natural colorants and dyes has been rapidly declining as a result of rising market demands, which have led to an increase in the usage of artificial colorants and dyes made from petrochemical sources. However, due to its benefits to human health, safety, and the environment, people choose to utilize natural colors (Velíšek et al. 2008). Natural colors haven't, however, been a commercial success due to several technical issues (lack of extraction knowledge, challenging plant gathering, etc.). The greatest class of naturally occurring colorants are carotenoids. In fact, carotenoids, which are orange-red pigments found in many plant species including tomato, orange, and carrot as well as in some animals, make up a large portion of the natural food colors (Dangles 2012).

A chain of isoprene units makes up the structure of carotenoids. The number of distinct carotenoid molecules has increased to above 500. With at least 40 carbon atoms and a long chain of carbon–carbon conjugated double bonds, carotenoids are hydrophobic compounds. Because carotenes only contain carbon and hydrogen and xanthophylls also contain oxygen atoms, they can be distinguished from one another in the carotenoids class (Gedye et al. 1986).

9.1 Recovery of Natural Pigments by Microwave Assistance

In order to improve the aesthetic appeal of foods, colorants have been utilized for a very long time in a variety of food products. The use of natural colorants and dyes has rapidly decreased as a result of rising market demand, which has led to an increase in the usage of artificial colorants and dyes made from petrochemical sources. Although several technical issues (lack of extraction knowledge, difficulty in plant gathering, etc.) have stopped natural colors from being successful commercially, people are eager to utilize them because of their health, safety, and environmental benefits. One of the cutting-edge extraction methods, microwave-assisted extraction, has been used to get beyond natural color extraction.

Red raspberries were processed by Sun et al. using the MAE technique to extract anthocyanins (Acys) (Sun et al. 2007). Twelve different types of Acys were successfully extracted without causing any damage to the chemical structure, and the compositions of the extracted Acys were comparable to those obtained using traditional solvent extraction (Liazid et al. 2011). Additionally, Liazid et al. created a new technique for analyzing anthocyanins in grapes and discovered that the solvent employed in MAE is the key factor in determining the optimal Acys extraction yield (Chen et al. 2006). Additionally, the MAE technique has been used to study other natural colors like curcumin and carotenoids, with results that are comparable (Lianfu and Zelong 2008; Mandal et al. 2008). Safflower yellow and flavonoids were extracted using dynamic microwave-assisted extraction as opposed to traditional techniques (Gao et al. 2006). By using this derivative technique, the extraction process may be conveniently tracked and continuously measured. Vacuum microwave-assisted extraction (VMAE), a derivative MAE technique, was put up against MAE in a comparison of the extraction of microalgal pigments

(Dabiri et al. 2005). They found that the mechanical barrier—that pigment must be extracted from microalgae with a robust frustule—can be significantly reduced by the use of microwaves. In order to extract safflomin A from Chinese herbs, Wang et al. also used this method; nevertheless, they concluded that VMAE was preferable for extracting thermosensitive chemicals (Sun et al. 2007). An intriguing study on coupling techniques for lycopene extraction found that ultrasound and microwave-assisted extraction (UMAE) produced a higher yield of lycopene (97.4%) while using less solvent and less time (367 s) than ultrasound and microwave-assisted extraction (UAE), which produced a yield of (89.4%) in 29.1 minutes.

With the presence of water in the extract, the combined UAME approach may prevent the generation of hydroxyl radicals by the ultrasonic cavitation effect, which could break down lycopene. Green extraction has emerged as the future trend, as we covered in the antioxidant part. Zill-e-Huma et al. (2011) explored a solvent-free microwave hydrodiffusion and gravity extraction of flavonol from onions. The advantages of the prior microwave-assisted approach were preserved by this new, original methodology, but they were also enhanced in terms of extraction time, solvent, efficiency, etc. It is noteworthy to note that microscopic examinations of the extracted tissues revealed that microwave irradiation might cause a significant disturbance in the structure of plant tissue (cell walls, vacuoles, etc.), allowing for the effective extraction of target substances (Hemwimon et al. 2007).

10 Extraction of Personal Care Products

Pharmaceuticals and personal care products (PPCPs) have been reported in a variety of natural matrices from numerous locations (Golet et al. 2002; Ternes et al. 2004). PPCPs include medications ranging from analgesics and antibiotics to contraceptives and lipid regulators, in addition to the active ingredients in soaps, detergents, perfumes, and skin, hair, and dental care products (Peck and Hornbuckle 2003; Ferrer et al. 2004). Continuous introduction of PPCPs into the environment, multiple dispersal mechanisms, and their pharmacological activities may result in detrimental impacts on wildlife and humans (Kolpin et al. 2002; Ternes et al. 2004).

The extent of exposure from contaminated matrices remains largely unknown; however, studies have reported the bioaccumulation of some PPCPs in lobster, clams, and human breast milk (DiFrancesco et al. 2003; Ohoro and Okoh 2019). Increased hermaphroditism in organisms exposed to female reproductive hormones has also been observed (Kuster et al. 2004). In addition, increased bacterial resistance among colonies subjected to widely used antibacterial agents has been documented (Prat et al. 2006; Lima et al. 2022). The discovery of multiple classes of PPCPs coincident in environmental samples further necessitates the consideration of potential interactive effects (Kolpin et al. 2002). For instance, a mixture of 13 pharmaceuticals resulted in a 10–30% reduction in growth of human embryonic kidney cells after 2 days of exposure in vitro, while no effects were observed when one of the chemicals was presented individually (Prat et al. 2006). Wastewater

treatment plant (WWTP) effluents are considered a primary source for PPCP introduction to the environment. Pharmaceuticals may enter wastewater via excretion or disposal of unused medications (Cunningham et al. 2006).

Personal care products are incorporated through washing and bathing practices. During wastewater treatment, some removal of PPCPs occurs through sorption to sludge (Ternes et al. 2004). Over one-half of the sewage sludge generated annually in the United States is further stabilized, then referred to as biosolids, and applied to agricultural fields, golf courses, and residential lawns as fertilizer/soil conditioner (Renner 2000; la Guardia et al. 2003). The remainder is placed in landfills or incinerated (Kuster et al. 2004). Following biosolid application onto land, incorporated contaminants may enter soil or be translocated via leaching, volatilization, or transport on eroded particles (DiFrancesco et al. 2003; la Guardia et al. 2003). Some contaminants in biosolids are likely to be available for uptake by plants, microorganisms and animals which inhabit or feed on soil and sediment (Renner 2000; DiFrancesco et al. 2003; Kuster et al. 2004). Biosolids have high nutrient and organic carbon content; however, the identities and levels of organic contaminants therein are largely unknown and unregulated. Diverse chemicals constitute the compound class known as PPCPs and, hence, such chemicals will exhibit a variety of fates in WWTPs and the natural environment. The incorporation of polar functional groups in many suggests considerable water solubility, while others are more hydrophobic or possess positively charged moieties which may lead to significant interaction with solids. Many PPCPs contain combinations of these structural properties, complicating the prediction of their behavior and necessitating their quantification in multiple matrices (Ternes et al. 2004). The synthetic steroid Ethinyl-estradiol has been detected in sewage effluent, surface waters, activated and digested sludge, and river sediment (Gomes et al. 2004), while the over-the-counter anti-histamine diphenhydramine was found in sediment at concentrations that are believed to exceed those in aqueous matrices by three orders of magnitude (Ferrer et al. 2004) (Table 1).

11 Extraction of Pharmaceuticals

More than 3000 distinct compounds, such as antibiotics, antidiabetics, beta-blockers, contraceptives, lipid regulators, antidepressants, or nonsteroidal anti-inflammatory medications, are utilized as pharmaceutical components to treat illnesses in humans or animals (NSAIDs) (Guedes-Alonso et al. 2016; Muzammil et al. 2023). Human-use pharmaceuticals and their metabolites are partially eliminated in urban Wastewater Treatment Plants (WWTPs) from domestic and medical effluent. As a result, several environmental components (such as water, sediments, but also biota) play a role in their ultimate fate (Kumirska et al. 2015).

The majority of the samples of sewage sludge from wastewater treatment plants have been subjected to analytical procedures based on MAE to identify pharmaceuticals (WWTP). Numerous classes of pharmaceutical substances have

Table 1 Target compounds selected for development of mixed PPCP method

Target compound	Function	References
Caffeine	Stimulant	Kuster et al. (2004)
Diphenhydramine hydrochloride	Anti-histamine	Ferrer et al. (2004)
Epicoprostanol	Molecular marker for fecal waste (steroid)	Kuster et al. (2004)
17-Estradiol	Female reproductive hormone	Gomes et al. (2004)
Ibuprofen	Anti-inflammatory; analgesic	Ferrer et al. (2004)
Ketoprofen	Anti-inflammatory; analgesic	Ferrer et al. (2004)
Musk ketone	Synthetic fragrance	Peck and Hornbuckle (2003)
Naproxen	Anti-inflammatory; analgesic	Gomes et al. (2004)
Triclosan	Anti-bacterial agent	Ferrer et al. (2004)

been examined in this matrix, including steroid hormones (Snow et al. 2012), nonsteroidal anti-inflammatory medicines (NSAIDs) (Petrie et al. 2016), antiepileptic drugs (Mohapatra et al. 2012), and antibiotics (Montesdeoca-Esponda et al. 2011; Dorival-García et al. 2013). Additionally, MAE has been effectively used to identify pharmaceuticals in a variety of solid matrices, including compost (Speltini et al. 2015), sediments (Tong et al. 2016), biota (Kazakova et al. 2018), and air samples (Jiao et al. 2014). The most significant class of steroid hormones are the estrogens, and their release into the environment, particularly into aquatic environments, can have detrimental impacts on aquatic animals.

The major extraction solvents used for the MAE optimization of steroid hormones in sewage sludge and sediments were MeOH (Snow et al. 2012), water (Azzouz and Ballesteros 2015), and a combination of MeOH/water (3:2, v/v) (Kumirska et al. 2015). Following MAE, extract evaporation and reconstitution in MeOH was used as a concentration step before the LC-MS analysis of 20 synthetic and natural steroids and their associated metabolites in sediments (Snow et al. 2012) and 15 sex hormones and corticosteroids in sludge (Guedes-Alonso et al. 2016). Prior to analysis, SPE was also used as an extra clean-up step. In every instance, LODs and good recoveries between 71% and 102% were realized at the low ng/g^{-1} (Vega-Morales et al. 2013).

Antibiotics are used to treat bacterial infections. Due to their wide range of activity and effective oral absorption, fluoroquinolones are arguably the most significant class of synthetic antibiotics. They have been found in wastewater effluents, and because they are lipophilic, they can gather in sediments or sludge (Dorival-García et al. 2013; Alves et al. 2023). Fluoroquinolones have been successfully extracted by MAE from compost and sewage and wastewater sludge (Montesdeoca-Esponda et al. 2011).

In order to extract 54 multiclass pharmaceuticals (NSAIDs, sedatives, sulfonamides, quinolones, and other popular medications) and PCPs from fish samples, innovative techniques including MAE combined with hollow fiber-liquid/solid phase microextraction (HF-L/SME) have recently been developed

(Zhang et al. 2017). In this instance, microwave energy was used during the HF-L/SME operation on a synthesized SPME fiber since it demonstrated high capacity, concentration rate, and efficiency. Combining these two options provides for a faster rate of target compound diffusion, which cuts down on extraction time (12 min). For all substances, LODs between 0.01 and 0.50 ng/g^{-1} were attained (Huang et al. 2016) (Table 2).

12 The Role of Microwaves in Omics Disciplines

12.1 Omics

Genomics is the exploration of all genes and their interrelationships in order to determine their collective influence on an organism's growth and development. Proteomics, on the other hand, is concerned with the study of the expression, localizations, functions, and interactions of all proteins expressed by an organism's genetic code. Finally, metabolomics is concerned with quantifying all low-molecular-weight metabolites (sugars, amino acids, organic acids, fatty acids, and others) in an organism's cells at a certain time under precise environmental/biological conditions. Omics is currently a magical suffix from the Latin "ome," which means mass or gigantic, and alludes to the massive amount of analytical data generated and required to gain the information sought by the so-called omics disciplines. To gather the data required for an omics investigation, an analytical procedure must be performed. Microwave (MW) energy can be used to expedite, improve, or allow the gathering of target analytical data during one or more steps of the analytical process (Delgado-Povedano and Castro 2017) (Fig. 6).

12.2 Microwave Equipment for Assisting Omics

Microwaves have been employed to varying degrees to facilitate research on analytical platforms in various omics disciplines. Metabolomics has reaped maximum advantage of the great number of methodologies created under the umbrella of reductionist theory in molecular biology over several decades by employing microwaves to optimize sample preparation stages. Other omics, on the other hand, have used MWs primarily to speed up sluggish procedures like sample preparation and/or detection, which are substantially slower when done without the aid of microwaves. In the three major omics and their subdisciplines, a number of microwave devices have been employed to perform analytical MW-assisted tasks (especially sample preparation). For this reason, both monomode and multimode MW generation have been utilized. In addition, to speed up standard omics procedures, commercially available devices, laboratory-made designs, and household ovens have

Table 2 List of pharmaceuticals extracted from MAE

Analyte	Sample	Sample pretreatment	MAE condition	Extraction treatment	Determination	LOD	*References*
Sex Hormones and Corticosteroids	Sludge	Freeze-dried	10 mL MeOH MAE (500 W, 65 °C, 4 min)	Filtration, Evaporation and Reconstitution	LC-MS/MS	1.1 to 7.9 ng g^{-1}	Snow et al. (2012)
19-Norethindrone, DES, Norgestrel	Sewage Sludge	Homogenization and Air-Dried	5 mL MeOH MAE (200 W, 6 min)	Filtration, Dilution and SPE	LC-MS/MS	0.1–0.7 ng g^{-1}	Kolpin et al. (2002)
Fluoroquinolones	Compost	Homogenization and Air-Dried	10 mL mg (NO3)2 6 H_2O and NH3 aqueous solution MAE (200 W, 135 °C, 20 min)	Dilution and SPE	LC-MS/MS	2.2–3 ng g^{-1}	DiFrancesco et al. (2003)
Multiclass Antibiotics	Aquifer Sediment	Air- Dried and Sieved	MeOH MAE 5 min ramp up to 60 °C (held at 25 °C),100 W	Centrifugation and SPE	LC-Q-Orbitrap MS	0.1–3.8 ng g^{-1}	Kumirska et al. (2015)
Sulphonamides, Tetracyclines, Fluoroquinolones, Amphenicols, and NSAIDs	Fish	Lyophilization	50 mL Proteinase-K solution+5 mL formic acid +5 mL ACN/H_2O (1:1, v/v) MAE (5 min)	Evaporation and reconstitution	LC-MS/MS	0.6–12 ng g^{-1}	Kuster et al. (2004)

(continued)

Table 2 (continued)

Analyte	Sample	Sample pretreatment	MAE condition	Extraction treatment	Determination	LOD	*References*
Tetracyclines, Oxytetracyclines, Chlortetracyclines, Deoxytetracyclines	Soil, Sludge, Atmospheric Particulate Matter	Lyophilization and Homogenization	5 mL MeOH + mSPE device MAE (400 W, 60 °C, 20 min)	Sonication with MeOH	LC-UV	0.1–6.3 ng g^{-1}	Morales-Toledo et al. (2016)
Carbamazepine	Waste water sludge	Freeze-Dried and Homogenization	20 mL MeOH MAE (1200 W, 10 min ramp up to 110 °C, Held for 10 min)	Concentration and Redissolution (Water)	LDTD-APCI-MS/MS	12 ng g^{-1} (Waste water) 3.4 ng g^{-1} (Sludge)	Huang et al. (2016)

Abbreviations: *LC* Liquid Chromatography; *LDTD-APCI* Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization; *LOD* Limit of Detection; *MAE* Microwave-Assisted Extraction; *MeOH* Methanol; *MS* Mass Spectrometry; *MS/MS* Tandem Mass Spectrometry; *NSAIDs* Nonsteroidal Anti-inflammatory Drugs; *DES* Diethylstilbestrol

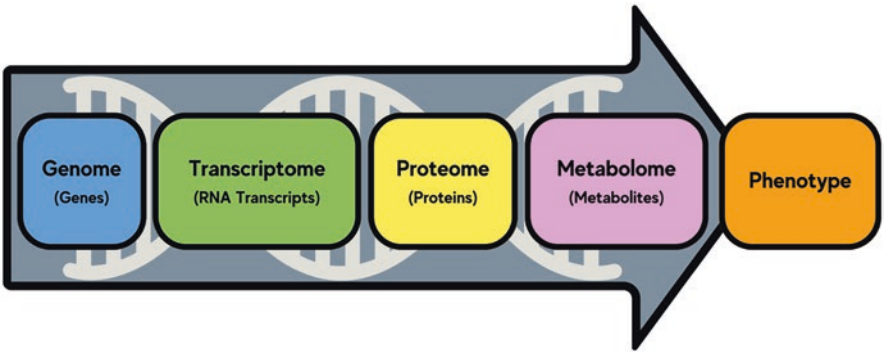

Fig. 6 General scheme of omics disciplines

been used. Two factors characterize the use of MW in omics disciplines: (1) The device used to apply this sort of energy, which entails decisions on two aspects: MW mode and continuous or batch performance of the MW-subjected target step; (2) The medium or solvent with which the sample (or, after separation, the portion of it to be analyzed) comes into contact when MW is applied (de Castro et al. 2012).

A number of MW systems have been commercialized for specialized biological and biochemical applications. CEM, for example, sells a piece of specialized equipment for high-throughput digestion. The setup consists of a Discover system with a screw-top container that can store numerous microvials or Eppendorf tubes, as well as an insert for a fiber-optic temperature probe. The fiber-optic probe is designed to aid in temperature stabilization by monitoring magnetron power while simultaneously cooling to allow energy input while keeping the comparatively cool temperature required for the desired reaction. This equipment is appropriate for metabolite digestion and/or extraction (particularly from solid matrices). CEM offers a 45 mL vapor-phase hydrolysis jar for use with the Discover MW device, which can handle up to ten 300 mL samples in concurrently. A valve panel is included in the system to simplify connecting the hydrolysis vessel to the vacuum and nitrogen sources. To promote hydrolysis under inert, anaerobic conditions and hence prevent oxidative deterioration of the sample components, the sealed sample vessel is alternatively vacuum evacuated and purged with nitrogen (Delgado-Povedano and Castro 2017; Almeida et al. 2022).

Furthermore, numerous businesses have marketed systems designed with omics operations in mind. The CEM MARS 5, a microwave system with PTFE tanks for MW-assisted digestion, is one example. The MW-accelerated reaction system was created for digesting, dissolving, and/or hydrolyzing a wide range of materials in the laboratory. It rapidly heats samples in polar or ionic liquids at high pressures using MW energy. Its primary application is to prepare samples for atomic absorption (AA), inductively coupled plasma emission spectroscopy (ICP), or gas or liquid chromatography. A number of laboratories employ the vacuum-assisted automatic MW histoprocessor MFX-800-3, which has an in-built vacuum system and temperature stabilizer to allow for quick tissue processing without compromising

the original structure. This is an environmentally friendly, quick, cost-effective, fully automatic microprocessor-controlled histoprocessor that can also be used manually and is ideal for a wide range of applications (Delgado-Povedano and Castro 2017).

12.3 Microwave-Assisted Steps in Various Omics

Microwave irradiation can help to speed up some procedures in genomics, proteomics, metabolomics, and related subdisciplines. The specific omics procedures that can be accelerated by employing microwaves (MWs) vary, as do the MW devices used to accomplish this, which include focused or multimode MWs, single continuous or high-throughput formats, and laboratory-adapted, commercial, or dedicated equipment. Cell fixation, DNA extraction, deparaffinization, digestion, PCR hybridization, rolling circle amplification, and metal-enhanced fluorescence are the specific activities most efficiently aided by MW in genomic applications. Proteomics can advantage from MW effects for operations such as enzyme quenching and proteolysis (enzymatic or chemical), identification and characterization of posttranslational modifications or metal-catalyzed reaction sites on proteins and lipase selectivities, dissociation of protein complexes and protein quantitation using commercially available processes such as ICATR® and iTRAQR®, or traditional procedures based on sensitive phenomena such as fluorescence. In any event, metabolomics has benefited the most from MW assistance, particularly for drying, digesting, solid–liquid extraction (or, more precisely, "leaching"), steam distillation, liquid–liquid extraction, and derivatization of a wide range of metabolites from diverse matrices (Delgado-Povedano and Castro 2017).

12.4 Solvents Used in MW-Assisted Steps in Various Omics Disciplines

Because MW heating can induce a sudden increase in the internal temperature of a solution, which can result in an explosion, solvent characteristics must be thoroughly tested ahead of time. In order to accelerate proteolytic digestion, it has become more popular to incorporate a tiny quantity of organic solvent in digestion buffers to partially denature the substrate protein, allowing better access to the proteolytic enzyme. Furthermore, the presence and composition of surfactants in the working medium can have an impact on the MW-assisted sample preparation stage (Lin et al. 2005). Enzymes that can catalyze in non-aqueous environments, on the other hand, are frequently highly compatible with MW assistance, in which they tend to be exceedingly thermally stable without notable inactivation. Because of the large

range of metabolite polarity, choosing an adequate solvent in metabolomics is more complex (Sandoval et al. 2007). There is no such thing as an ideal solvent when it comes to extraction. When designing the overall analytical process, consider the option of directly introducing the digested extract, eluate, and so on (i.e., the analytical sample) into the analytical equipment. This entails choosing a solvent that satisfies the unique needs of the analytical equipment being employed.

12.5 Microwave Assistance Trends in the Omics Approach

Despite its rapid expansion, MW's analytical help in omics is still in its infancy. Unanswered questions include the precise mechanisms of action of MW in comparison to conventional heating, as well as the actual usage and potential of this area. So far, the kinetics and specificity of MW-assisted incubations and reactions in genomics, transcriptomics, and proteomics have only been investigated in a few regions and on a few systems. MW-assisted processes involving metabolites, on the other hand, have been developed almost from the advent of MW devices in the analytical laboratory.

Past research and current demands indicate the following predictable trends in the use of MW to help omics:

(a) Magnetite beads are used to speed up MW-assisted enzymatic digestion and other SP procedures (Chen and Chen 2007).
(b) Quantum dots, which are widely utilized as fluorescent reporters in biomedical research and are now being used in the omics arena (Dua et al. 2010), will almost certainly necessitate technological improvements based on MW support.
(c) Nanostructured materials, which have been widely used in the therapeutic field (Phan et al. 2009; Feliu and Fadeel 2010), would benefit from MWs to improve target processes, notably in integrated omics investigations (Gibb et al. 2011).
(d) Microfluidic technologies, which are becoming more prevalent in omics (Brouzes et al. 2009), nanomedicine in general (Sakamoto et al. 2010), and nanoscale platforms (Soundararajan et al. 2010), can be predicted to gain from MW support, increasing and accelerating their performance.
(e) Bioinformatic approaches (Cho et al. 2007), such as nanoparticle ontology (Thomas et al. 2011) and nanoinformatics (Maojo et al. 2011), could make it easier to interpret interactions of micro- and nano-omics systems with MW.
(f) New commercially available miniaturized MW devices can solve the question of what type of MW device to utilize for MW-assisted omic processes at the micro- and nanoscale (Aydoğan et al. 2020).

13 Conclusion

As a result of groundbreaking research, microwaves are being used to extract phytoconstituents from diverse herbal specimens. Conventional extraction procedures are time-consuming, need more solvents, and are no longer appropriate for thermally sensitive plant components. The extraction stage must be more yielding; rapid, specific, and solvent-free, while also preserving the stability of thermolabile components, and microwave extraction meet these demands. Heat is generated while employing microwave energy in this unconventional method. The significant characteristics that determine extraction efficiency are solvent attributes, volume, duration of exposure, microwave control, system attributes, temperature, and application. Microwave-assisted extraction is a green technology when compared to other extraction techniques. Thus, MAE is an effective green technology that has become one of the key strategies for extracting bioactive components from environmental, biological, and geological matrices.

MAE approach was developed in the mid-1980s to isolate bioactive chemicals from plants. From an existing perspective of green chemistry, this is an environmentally benign and human-friendly technique. It is currently widely accepted as a method for extracting bioactive natural chemicals from plant sources. In today's herbal isolation context, extraction techniques for plant matrix are key tasks that must be completed in order to meet quality control attributes. In this case, microwave-aided extraction can be used as a guide for the extraction of new and selective bioactive compounds from the plant sample matrix. This method outperforms conventional methods in terms of selectivity, specificity, and extraction efficiency. Furthermore, several research studies reveal that microwave-assisted extraction offers substantial advantages over conventional approaches, such as shorter extraction times, higher extraction yield, and lower solvent usage.

Furthermore, heat- or oxygen-sensitive chemicals should be extracted under controlled circumstances (such as a vacuum or inert atmosphere) to avoid oxidative and thermal destruction. Solvent-free microwave extraction yields a more valuable product with higher levels of oxygenated chemicals. Some highly polar chemicals can be employed in suitable proportions in a solvent blend under increased heat and pressure. As a result, microwave-aided extraction can produce higher quality samples (in particular chemical classes of substance) than other conventional approaches. It also allows full control over extraction factors like as duration, energy, and temperature, which improves reproducibility. Several aspects, including solvent composition, solvent quantity, and plant material loading, influence microwave-aided extraction. To efficiently recover the chemicals of interest, thorough process optimization with parameter regulation and control is required. New batch microwave assemblies are more intelligent than prior designs. For sample loading and recognition, these systems employ a sample set time program. At a single moment in time, microwave operators can now choose the temperature for each sample as well as the sample type for different extraction systems.

Unfortunately, relatively few studies have been published on microwave-assisted extraction of bioactive components from plants on a significant scale. This could be attributed to crucial control points in industrial microwave design, such as the processing facility, processing conditions, safety and operation-related hazards, and product parameters. As a result, specific efforts should be undertaken to address technical challenges connected to the design of microwave extractors and their suitability for isolating bioactive components from plant matrices in order to encourage the use of microwave-aided extraction in the food and pharmaceutical industries. To facilitate the application of microwave-assisted extraction to the food and drug industries, special efforts should be made to solve conceptual and practical problems such as the unveiling of the extraction mechanism, the development of microwave-based extractors, and the diagnostic testing of plant materials.

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Part III
Screening of Compounds Using Molecular Modeling Approaches: Optimization of Natural Compounds Using *In Silico* Methods

Software for Drug Discovery and Protein Engineering: A Comparison Between the Alternatives and Recent Advancements in Computational Biology

Tathagata Adhikary and Piyali Basak

Abstract "Omic" technologies (such as genomics, transcriptomics, proteomics, and metabolomics) generate huge databases that demand computational approaches to state novel conclusions. With the advent of machine learning and artificial intelligence algorithms, the analysis of biological data and protein engineering has taken a step forward. Different virtual screening servers and standalone software paved their importance in the initial phase of drug discovery, aiding in drug repurposing and high-throughput screening. Besides, interaction networks, often encountered in polypharmacology and network pharmacology, guide a researcher in target fishing and developing drug combinations. Visualization and prediction of molecular structures, modeling antibodies, and peptides including homology modeling are crucial to bioinformaticians and clinical biologists. Biological network analysis, pharmacophore modeling, molecular docking, and dynamics simulation are broadly exploited in the domain of computational biology and elucidate the mechanisms underlying biomolecular interactions, consequently revealing the orchestra of biological pathways. Considering the intended purposes, advantages, and limitations of the existing software, this chapter highlights only a fraction of popular platforms and encourages the readers to explore other alternatives in various domains of drug discovery and protein engineering.

Keywords Big data · Virtual screening · Biological networks · Pharmacophore · Homology modeling

1 Introduction: The Need for Computational Biology

Discovering novel drug molecules strictly demands huge investments in terms of time, infrastructure, and labor to identify, optimize, and validate the drug-likeliness of such molecules by conducting in vitro, in vivo, and preclinical experiments

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(Lei et al. 2016; Rifaioglu et al. 2019). To ease the process, a shift toward the application of computational tools is witnessed in the early stages of identifying drug-like molecules. Constant advancements in software and its algorithms aid to bridge the "innovation gap" that exists due to higher investments and lower approval rates. The process of drug discovery sequentially includes the identification and validation of disease targets, lead compound identification and its optimization, and finally success in clinical trials. Accordingly, establishing a drug can take around 10 to 13 years with huge capital expenditure (Malathi et al. 2018). Challenges arising due to the pleiotropic nature of biomolecules and the interaction of chemical compounds with multiple pharmacological targets (often encountered in combinatorial/multi-targeted approaches) can be addressed by chemo- and bioinformatics tools that make use of databases on physicochemical characteristics and therapeutic use of compounds (Lagunin et al.). The fact that the primary healthcare of 80% of the population in developing countries counts on the conventional herbal remedies and the steep rise of 380% in plant-based supplements' sales in the United States from 1990 to 2000 encouraged the development of numerous databases on ethnomedicine (Dunkel et al. 2006; Mosihuzzaman and Choudhary 2008). This expands the prospects of utilizing traditional knowledge on medicinal plants in modern-day drug discovery and drug repurposing.

In 1971, the database Protein Data Bank (PDB) came into existence, being the first open-access digital repository in the field of biology. It is a collection of 3D structures (resolved by laboratory experimentations namely X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy) of biological macromolecules and receptor–ligand complexes (https://www.rcsb.org/) (Burley et al. 2017). ChemCom (Chemical Comparator) is an application based on Java Web Start (JavaWS) technology and includes UnionBit Tree Algorithm to search and compare large chemical libraries (Saeedipour et al. 2015). The list of such repositories can be long enough (Lagunin et al.). A few open-source databases on medicinal plants, phytochemicals and other chemical compounds can be listed as follows: Plants For A Future (PFAF), Indian Medicinal Plants Phytochemistry And Therapeutics 2.0 (IMPPAT 2.0), Native American Ethnobotany database, SuperNatural 3.0, The Natural Compound (NC) collection, NCBI PubChem, ChEMBL, Collection of open natural products (COCONUT), Traditional Chinese Medicine Information Database (TCMID), Dr. Duke's Phytochemical and Ethnobotanical Databases, Aromatic and Medicinal Plants Index (by Purdue University), Agricultural Science and Technology (or AGRIS supported by the Food and Agriculture Organization (FAO) of the United Nations), Compendium of Ayurveda Medicinal Plants of Sri Lanka, Botanical.com, Chinese Herbal Medicine Dictionary (by Complementary and Alternative Healing University), Clinicaltrials.gov database, Medicinal Plant Database (by Botanical Survey of India), EcoPort, TIPdb (a database of indigenous and endemic plant species in Taiwan), Traded Medicinal Plants Database, Herbal Medicines Compendium Medicinal Herbs and Plant Database, Drugs Herbs and Supplements by MedlinePlus, ZINC database, Marowina database medicinal support, Natural Medicines, Herbs at a Glance, Prelude Medicinal Plants Database, Raintree Tropical Plant Database, The World Flora Online, and TRAMIL database

(Xie et al.; Duke 2020). Commercial databases with paid access include Chemical Abstracts Service (CAS), HerbalThink-TCM, Dictionary of Natural Products (DNP), and HerbMed.

The increasing data relating to the bioactivities of a chemical compound, the composition of phytoconstituents in an extract, and the target receptors responsible for specific bioactivity need to be stored and be able to be retrieved systematically. "Omic" technologies have led to the development of diverse databases, and interpreting/interconnecting them or data mining from them is a major challenge to human capabilities. Hence, computational approaches including artificial intelligence and machine learning algorithms (e.g., artificial neural networks (ANN), Naive Bayes, K-Means, support vector machine (SVM), random decision forest, etc.) are widely adopted to provide solutions to complex biological questions (Gupta et al. 2021; Muzammil et al. 2023). Continuous development of in silico tools for chemoinformatics and bioinformatics provides insight to the vast multiomics data and adds different perspectives to the scientists in the domain of drug discovery. Chemoinformatics particularly aims to model a statistical correlation between the observed bioactivity and structural parameters. These approaches relating to computer-aided drug design have gained noteworthy momentum in the drug discovery process. Genome-wide functional genetic screening (e.g., using deep learning algorithms) is a cutting-edge technique that has led to the discovery of genotype–phenotype interconnections and established new phenotypes (Zhang et al. 2011). Genomics and proteomics analyses in high-throughput screening have shown promising results to rationalize the drug discovery process; however, the cost inflation incurred due to these technologies does not meet the expected growth of the drug's approval rate. Freely available software that are frequently employed in machine learning and statistical analysis of data are R, PSPP by the GNU Project, and WEKA while commercial ones include MATLAB, SAS/STAT, SIMCA, SPSS Statistics by IBM, and TIBCO Data Science/Statistica (Dzemyda et al. 2019).

The first thing that needs to be checked while selecting a software for computer-aided drug designing is its vendor and license—whether it is under academia, commercial, open-source, or in-house software. Open-source software are popular among academic personnel as, unlike commercial software, no license fee is required, their source code is made available freely and can be modified by a user. Based on the intended use, license fee, and characteristic features of the software/platforms, attempts are made to categorize and list the in silico tools employed in the various domains of drug discovery (Singh et al. 2021). Molecular docking, pharmacophore modeling, methods relating (Q)SAR, molecular dynamics simulation, network pharmacology and machine learning algorithms accelerate the drug discovery process and complement the traditional bioactivity-guided fractionation, high-throughput screening and systems biology approaches. In this chapter, the tables summarizing the in silico tools only provide a fraction of popular platforms and encourage the readers to explore other alternatives in various domains of drug discovery and protein engineering.

2 Visualization of Molecular Structures

Molecular graphics enhances the experience of representing, modeling and analyzing multifaceted biochemical systems. Besides modeling the 3D architecture of molecular structures, 2D illustrations of molecules have gained interest among chemical scientists and biologists in the field of theoretical chemistry and discovery because of their clear representation of structural characteristics and interactions between atoms (Zhou and Shang 2009). Visualizing molecular structures in any virtual reality environment demands rapid high-quality rendering of geometries to build molecular models with intuitive and informative interactions. Different visualization techniques (such as the space-filling model, the ball-and-stick model, and the reconstruction of the surface of the secondary structures alpha helixes and beta sheets) are used while representing a molecular model, and some available platforms to design, analyze and visualize molecular structures are listed in Table 1.

3 Prediction of Pharmacokinetic/Pharmacodynamic Profile

Evaluating the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties of a molecule is a major step in discovering novel drug compounds. In general, compounds having natural origin tend to have desirable ADMET properties compared to synthetic compounds. Early prediction of ADMET properties of a chemical compound can be of utmost importance since most drug failures occur in the later phases due to undesirable pharmacokinetics and toxicological characteristics. Lipinski's rule of five is often checked to predict the drug-likeliness (in humans) of an oral-administered compound (Lipinski 2004; Rego et al. 2022). According to it, a drug molecule can have at most one violation among these five rules: (a) ligand's molecular weight should be less than or equal to 500 Daltons, (b) the number of H-bond donors should be less than 5, (c) the number of H-bond acceptor should be less than 10, (d) value of octanol partition coefficient (miLogP) should be less than 5 and (e) the number of rotatable bonds should be less than 10.

Most of the software packages that predict the ADMET of compounds (e.g., their affinity toward transporter proteins, blood proteins and drug-metabolizing enzymes P450 cytochromes isoforms, etc.) consider their structural/physicochemical characteristics to develop (Q)SAR models. Derek Nexus (Lhasa Ltd.), TOPKAT (Accelrys), OSIRIS Property Explorer, MCASE (Multicase) and PASS can be opted to predict various toxicities and report the teratogenic, mutagenic, cardiotoxic, hepatotoxic, carcinogenic, and renal-toxic nature of the compounds (Kar et al. 2018). The online server of GUSAR (www.way2drug.com) predicts the LD_{50} values of query compounds on rodents when administered via four different routes. Some other software/web-servers to study the ADMET properties are listed in Table 2.

Table 1 Software/servers for designing and visualizing molecular structures

Software/platform	Description	URL
3DNA	It is used to analyze, rebuild and visualize 3D structural architecture of nucleic acids and ligand/protein-nucleic acid complexes. The parameters relating to the spatial relationship of the base pairs present in the nucleic acids are evaluated by using a simple matrix-based scheme (Lu and Olson 2003)	https://x3dna.org/
iMol	A free tool exclusively designed for the Mac OS X operating system to visualize molecular structures and molecular dynamics trajectories	https://www.pirx.com/iMol/index.shtml
VMD (Visual Molecular Dynamics)	It is an open-source software that utilizes graphics processing units (GPUs) to model, visualize and analyze complex biomolecular systems comprising proteins, DNAs, RNAs, etc. Animation and analysis of molecular dynamics simulation trajectories can also be performed using VMD, thus acting as a graphical interface for an external simulation program. It can read Protein Data Bank (PDB) file format (Humphrey et al.)	https://www.ks.uiuc.edu/Research/vmd/
Swiss-PdbViewer or DeepView	It allows simultaneous analysis of several proteins and their superimposition that helps compare the active/binding sites, amino acid mutations, H-bonds, atomic angles, and distances. It is a 32 bits application and is not supported by OSX Catalina or newer versions of OSX (Johansson et al. 2012)	https://spdbv.unil.ch/
RasTop	Adapted from RasMol, it is an open-source platform under the General Public License (GPL) for molecular visualization (specifically for academic purposes). It is supported in Windows and Linux operating systems	https://www.geneinfinity.org/rastop/
The Ramachandran Plot Explorer	It analyses the conformation of a polypeptide along with the effect of conformational changes in the energy parameters of the system (Hayward et al. 2014)	https://boscoh.com/ramaplot/
QuteMol	It exploits the GPU of a computer system (through OpenGL shaders) for high-quality molecular visualization (in 3D) of biological systems (Tarini and Cignoni 2006)	https://qutemol.sourceforge.net/
PyMOL	It is a user-sponsored commercial molecular visualization product and is compatible with Windows, Linux and macOS (Yuan et al. 2017)	https://pymol.org/2/

(continued)

Table 1 (continued)

Software/platform	Description	URL
ProteinShader	It is a free, open-source (under GNU GPL) molecular visualization program. It requires Java 1.5 (or newer versions) and a graphics card that supports at least OpenGL 2.0. It allows cartoon-type (artistic) illustrations of the protein structures (Weber 2009)	https://proteinshader.sourceforge.net/
MOLMOL	It is a molecular graphics project to display, analyze, and manipulate the 3D structure of biological macromolecules and study their structures obtained from NMR spectroscopy	https://sourceforge.net/projects/molmol/
Jmol/JSmol	It is a molecular viewer for 3D structures. It can take several many file types as its input. This includes PDB, CIF, SDF, MOL, PyMOL PSE files, and Spartan files, as well as output from Gaussian, GAMESS, MOPAC, VASP, CRYSTAL, CASTEP, QuantumEspresso, VMD, and other quantum chemistry programs	https://sourceforge.net/projects/jmol/
ICM-Browser	It is compatible with Windows, Mac and Linux platforms. It can build fully annotated and interactive 3D molecular structure files. ActiveICM plugin allows those files to display directly in PowerPoint and on the web. It can load and display sequence file types and generates publication-quality images. It allows the superimposition of protein structures and calculates atomic distances and angles (both planar and torsion angles) (Magnotti et al. 2019)	https://www.molsoft.com/icm_browser.html#ibFeatures
The BIOVIA Discovery Studio Visualizer	It is a free molecular visualization software for molecular modeling and analyzing the structures (of both proteins and small molecules) with advanced features such as depth cueing, blur and shading	https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/visualization/
NOC	It is a free molecular structure visualizer. Its source code is open for research purposes and allows nonprofit commercial redistribution	https://noch.sourceforge.net/#Downloading
Cn3D	It can simultaneously display structure, sequence, and alignment with advanced options for annotation and alignment editing (Stasinakis et al. 2017)	https://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml

SmilesDrawer	It is used to parse and draw SMILES strings using client-side JavaScript (Probst and Reymond 2018)	https://github.com/reymond-group/smilesDrawer
PlexView	It generates the 2D image of protein-ligand interactions that includes hydrogen bonding and pi-pi stacking. If the protein is not protonated, PlexView will protonate it at pH 7; however, the ligand needs to be protonated previously to correctly highlight the H-bonds	https://playmolecule.com/PlexView/
Ketcher	It is developed using JavaScript and serves as an open-source web-based editor to modify/draw structures of chemical compounds and reactions	https://lifescience.opensource.epam.com/ketcher/
NCIPLOT	It provides a visualization index for noncovalent interactions. It plots reduced density gradient (RDG) as a function of the density across a molecule (Laplaza et al. 2021)	https://www.lct.jussieu.fr/pagesperso/contrera/index-nci.html
PoseView	It is used in the 2D illustrations of protein-ligand interactions. It identifies hydrogen bonds, hydrophobic contacts and the interacting amino acids residues are selected using the FlexX-library (Stierand and Rarey 2010)	https://www.zbh.uni-hamburg.de/en/forschung/amd/server/poseview.html
bioRENDER	It helps to create professional scientific images using premade icons and templates. Its basic version is free for educational purposes but other features require a paid subscription	https://biorender.com/
Servier Medical Art	It allows the free use of over 3000 medical images to illustrate in publications/presentations	https://smart.servier.com/
LeView (Ligand Environment Viewer)	It is a java program (requiring Java Runtime Environment (JRE) to be preinstalled and enabled) that generates 2D representations of protein-ligand interaction from their complex (in PDB file)	http://www.pegase-biosciences.com/leview-ligand-environment-viewer/
Smi2Depict	It is used in the generation of 2D figures of molecular structure from SMILES	http://cdb.ics.uci.edu/cgibin/Smi2DepictWeb.py
IBS 2.0	It is a web-based open resource used for visualizing biological sequences (including both proteins and nucleotides) and highlights the functional elements to assist experimentalists in generating publication-quality images (Xie et al. 2022)	https://ibs.renlab.org/#/home

(continued)

Table 1 (continued)

Software/platform	Description	URL
CIME (ChemInformatics Model Explorer)	It is an interactive web-based tool for inspecting data sets of chemical compounds and their subgroups and visualizing models (Humer et al. 2022)	https://github.com/jku-vds-lab/cime
Img2Mol	It aims to automatically recognize the molecular content and predict the SMILES strings from molecular graphics (Clevert et al. 2021)	https://chemrxiv.org/engage/chemrxiv/article-details/60c756c6f96a00bff5288b68
DECIMER (Deep lEarning for Chemical ImagE Recognition)	It is an Optical Chemical Structure Recognition (OCSR) tool used to generate the SMILES strings by taking the input of the chemical structures as image files or PDF documents (Rajan et al. 2021)	https://decimer.ai/
ChemPix	It uses deep learning algorithms and neural networks to map and recognize hand-drawn hydrocarbon molecules and present the corresponding machine-readable SMILES (Weir et al. 2021)	https://github.com/mtzgroup/ChemPixCH
Chemtool	It is a LINUX-based program to draw chemical structures	http://ruby.chemie.uni-freiburg.de/~martin/chemtool/
Molsketch	It is an editor for 2D molecular structures that generates high-quality vector images	https://sourceforge.net/projects/molsketch/
JChemPaint (JCP)	It is a java application that can edit and view 2D molecular structures	http://jchempaint.github.io/
SketchEl	It is an interactive sketching tool for chemical structures and exports molecule diagrams as Scalable Vector Graphics (SVG)	https://sketchel.sourceforge.net/
BKChem	It is a python based free software to draw chemical structures	https://bkchem.zirael.org/
DataWarrior	It is an open-source platform with embedded chemical intelligence to visualize and analyze data using scatter plots, box plots, bar charts and pie charts. Considering various chemical descriptors, it can predict physicochemical properties and structure–activity relationships and display activity cliffs (Sander et al. 2015)	https://openmolecules.org/datawarrior/index.html

Skylign	It creates logos to represent sequence alignments and profile hidden Markov model	http://skylign.org/
TMRPres2D (TransMembrane protein Re-Presentation in 2 Dimensions)	It is a tool that provides a graphical user interface to automatically create uniform 2D graphical models of alpha-helical or beta-barrel transmembrane proteins from the corresponding sequences (Spyropoulos et al. 2004)	http://bioinformatics.biol.uoa.gr/TMRPres2D/
ChemTreeMap	It is an open-source interactive tool used in the visualization of chemical similarity/diversity in a library of chemical compounds (Lu et al.)	http://ajing.github.io/ChemTreeMap/
LigPlot+	It automatically generates a high-quality 2D image of ligand-protein interaction	https://www.ebi.ac.uk/thornton-srv/software/LigPlus/
Protein Contacts Atlas	It provides insight into the protein structures by visualizing and analyzing noncovalent contacts	http://pca.mbgroup.bio/

Table 2 In silico tools used in the prediction of ADMET properties of small molecules

Software/platform	Description	URL
DMFGAM	It uses a deep learning algorithm on molecular fingerprints to predict hERG channel blockers and cardiotoxicity (Wang et al.)	https://github.com/zhaoqi106/DMFGAM
TOXRIC	It is an online platform/database consisting of toxicity-related data of 113,372 compounds. It uses molecular fingerprints, transcriptome profiles, metabolic reactions and other chemical descriptors to predict 13 toxicity categories (such as acute toxicity, cardiotoxicity, endocrine disruption, etc.) with 1474 toxicity endpoints (Wu et al.)	https://toxric.bioinforai.tech/home
DIADpredictor	Using machine learning it employs support vector machine (SVM) and uses SMILES strings to predict drug-induced autoimmune diseases	http://diad.sapredictor.cn/
DDInter	It is an open-access database to find drug-drug interactions and performs risk assessment in medications for improved clinical decisions	http://ddinter.scbdd.com/
cardioToxCSM	It is a web-server that takes SMILES strings of small molecules (a maximum of 1000 molecules) as the input for predicting six types of cardiotoxicities (namely arrhythmia, cardiac failure, heart block, hERG toxicity, hypertension, and myocardial infarction)	https://biosig.lab.uq.edu.au/cardiotoxcsm/
ToxSTAR	It combines in vitro, in vivo, and in silico data to build the prediction models that include the prediction of drug-induced cholestasis, cirrhosis, hepatitis, steatosis, and drug-induced liver injury (DILI)	https://toxstar.kitox.re.kr/about_toxstar
Deep-B^3	It is a deep learning-based model that uses molecular descriptors and fingerprints, images, and SMILES strings to predict the blood-brain barrier permeability of molecules (Tang et al.)	http://cbcb.cdutcm.edu.cn/deepb3/
toxCSM	It is used in the prediction of toxicity profiles of chemical compounds including environmental toxicity	https://biosig.lab.uq.edu.au/toxcsm/
NR-Profiler	It is used in the prediction of nuclear receptor profiling and identifies the potential nuclear receptor modulators	https://github.com/jywangECUST/NR-Profiler
NURA (NUclear receptor activity dataset)	It integrates several toxicological and pharmacological databases and provides information on the bioactivity of 15,206 molecules and 11 nuclear receptors (Valsecchi et al.)	https://michem.unimib.it/download/data/nura/

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Super-PRED	It is based on the Anatomical Therapeutic Chemical (ATC) classification system published by the World Health Organization (WHO) that is used for the target prediction of small molecules using Pubchem name or SMILES string	https://prediction.charite.de/index.php
PredMS	One can use the SMILES string or draw the ligand structure in a molecule editor for the prediction of its metabolic stability in human liver microsomes	https://predms.netlify.app/
ICDrug	Apart from ADMET prediction, it can be used as a ligand designer, protein designer, and to analyze protein-protein or protein-ligand interactions	http://www.icdrug.com/
PK-DB	It is a pharmacokinetics open database built using the results of numerous clinical and preclinical research (Grzegorzewski et al. 2021)	https://pk-db.com/
ToxicoDB	It is a curated database (four datasets consisting of 234 compounds and 32,000 genes) of toxicogenomics data and allows mining multiple databases	https://www.toxicodb.ca/
vNN-ADMET	Based on variable nearest neighbor (vNN) methodology, it is a publicly available web server that uses 15 prediction models to predict the ADMET properties (Schyman et al. 2017)	https://vnnadmet.bhsai.org/vnnadmet/login.xhtml
SwissADME	It inputs multiple SMILES strings to study the pharmacokinetics and drug-likeness of chemical compounds (Daina et al.)	http://www.swissadme.ch/
admetSAR	It is a free tool that can predict multiple (24) endpoints such as human intestinal absorption (HIA), renal clearance, organ/genomic/environmental toxicity, etc.	http://lmmd.ecust.edu.cn/admetsar2/
OCHEM	It is a platform for ADMET prediction and can generate quantitative structure–activity and structure–activity models	http://www.eadmet.com/en/ochem.php
SApredictor	It identifies and collects structural alerts (i.e., structural fragments) that have the potential to influence chemical toxicity	http://www.sapredictor.cn/
VenomPred	It uses various machine learning algorithms to predict the potential mutagenicity, hepatotoxicity, carcinogenicity and estrogenic effect of chemical compounds using their SMILES strings (Galati et al. 2022)	http://www.mmvsl.it/wp/venompred/

4 Prediction of Structures Including Homology Modeling

Molecular modeling based on structure-based drug designing requires 3D structures of the receptor and ligand molecules (experimentally determined by X-ray crystallography and NMR spectroscopy). In cases where experimental data are unavailable, the existing data and sequences can be used to predict the structures by homology-based modeling, sometimes referred to as comparative modeling of protein. The amino acid sequence of a protein (acquired from NCBI or UniPort) is used to generate the structure using computational tools. Evolutionarily related proteins share a similarity in sequences and homologous proteins exhibit similarity in their protein structure (substitution matrices such as Blosum 60 describe such homology). The 3-D protein structure is found to be evolutionarily more conserved compared to the sequence conservation alone (Kaczanowski and Zielenkiewicz 2010). Homology modeling starts with recognizing a template that shows similarity in sequence (searching is accomplished by employing BLAST (Basic Local Alignment Search Tool) or PSI-BLAST (Position-Specific Iterated BLAST) or fold recognition methods) and subsequent alignment of the known structures (resolved by experiments) in the database. A similarity of less than 30% is generally not preferred in homology modeling. BLAST compares a query sequence with the existing database and identifies the most suitable sequence with significant similarity, i.e., it identifies the homologous sequences. Alignments with an expectation value (E-value) closer to zero indicate a higher similarity index. A higher E-value makes the alignment of two sequences strenuous, thus considering sequences from other homologous proteins can help in this scenario (Pearson 2013; Alves et al. 2023). Multiple Sequence Alignment programs, e.g., CLUSTALW, can align sequences by performing insertions and deletions. Alignment correction, if done not properly, will generate defective structures. Some of the methods that are used to build models are spatial restraint, rigid-body assembly, segment matching and artificial evolution. Modeling tools, namely Modeller or CASP, can be used to build the backbone from the aligned sequences. Most often, aligning the model sequence with the template sequence creates gaps that can be resolved by considering conformational changes, insertions/deletions/substitutions of amino acid residues. Thus, refining the model includes loop modeling and side-chain modeling following the principles of molecular dynamics, Monte Carlo, and genetic algorithms. After modeling, structures are energetically minimized by employing force fields (for instance OPLS, AMBER, MM3, and CHARMM22 force fields) (Lewis-Atwell et al.). Loop modeling can be knowledge-based or energy-based. Knowledge-based loop modeling, sometimes referred to as template-based or homology-based, searches existing databases to identify known loop conformations that match the input sequence and geometric descriptors about the anchoring points (Karami et al.). It does not require complex simulations and high computation power; however, it relies on the availability of appropriate loop conformations present in the existing repositories of protein structures to consider the entire conformational space. Energy-based loop modeling corresponds to nontemplate-based or de novo methods that use an energy function and

minimizes it by Monte Carlo methods or molecular dynamics to optimize the loop conformation. Proteins that share structural similarity also exhibit similarity in torsion angle about Ca-Cb bond (psi angle) when side-chain conformations are considered. The entire conserved residues can be taken from the template and copied to the model to yield highly accurate results when compared to the methods that copy the backbone or predict the side chains. Modeling of side chains includes knowledge-based methods to extract a library of rotamers from known crystallographic structures and substitutes the side chains on the backbone structure. After side chain modeling, the analysis is done by using their root mean square deviation (RMSD) values. The errors found in the final model are dependent on the extent of similarity between the template and the target. If it is >90%, then the crystallographic structure is fairly predicted, whereas for a value <90%, the r.m.s.d errors will be significant. The estimation of errors can be done by using a force field to calculate the model's energy and checking if the bond lengths and angles are exhibiting a value in the normal range (Dolan et al. 2012; Wink et al. 2019; Lima et al. 2022). However, this method does not evaluate the folding nature of the model and the misfolding in proteins is taken care of by 3D distribution functions. Model validation is necessary to establish the prediction accuracy.

The stereochemical aspects of the protein can be explored by WHATCHECK, WHAT IF, VADAR, and PROCHECK. Ramachandran plot, obtained by plotting the torsional angles of amino acids φ (phi) and ψ (psi) in a protein sequence two-dimensionally, is used to analyze the stereochemical and geometrical nature of the structure and verifies the presence of geometries in the electrostatically unfavored regions of the plot. A higher proportion of residues in the favored region indicates the structural feasibility of the model (Agnihotry et al. 2022). Popularly used tools for homology-based modeling are MODELLER, SWISS PDB VIEWER, SWISS MODEL and COMPOSER (Malathi et al. 2018). MODELLER is also used for sequence searching, comparing and clustering protein structures or sequences. In brief, steps in homology modeling take into account template identification, sequence alignment, structural modification, energy minimization and model validation to predict the 3D structure.

5 Interaction Networks

Hopkins in 2007 brought the concept of network pharmacology that makes use of network analysis algorithms (on the existing knowledge of biological networks consisting of structural/physicochemical properties of proteins/ligands, the interaction of a protein/gene with another protein/gene/ligand, signaling and metabolic pathways) to predict the therapeutic action of small molecules, elucidate their mechanism of action, and understand the drug-disease relationships at the system-level (Csermely et al. 2013). Visualization of biological networks (such as pie-nodes and edge-pie matrix visualization) and network comparison (by employing network alignment and computing pair-wise similarity between selected networks) is

essential for network analysis, identification of key components/nodes/interactions in a concerned biological system, and highlighting the union/intersection/complement regions in a set of biological networks. Networks have the capability to highlight the interacting elements within a complex biochemical system, thus aiding in the visualization and exploration of big data. However, the challenges relating the large size and high complexity of biological networks generate the so-called "hairballs" in the networks. Hence, there is a need for an efficient and interactive graphical user interface for network comparison and visualization (Pirch et al. 2021; Almeida et al. 2022). One needs to consider several types of relationships (namely "target–effect," "target–pathway," "pathway–effect," and "target–pathway–effect" relationships) to investigate the pleiotropic and synergistic effects of a drug compound or a combination of drug compounds. The benefit of conceptualizing such "cause–effect" relationships unfold gradually—if the bioactivity of a drug relates to certain molecular targets and their corresponding pathways are established, then other mode-of-actions of influencing those pathways can yield similar effects.

Analysis of biological pathways (such as signaling pathways, regulatory pathways, metabolic pathways, signal transduction pathways, etc.) makes use of various pathway databases (Lagunin et al.). To name a few, WikiPathways (https://www.wikipathways.org), HumanCyc (https://humancyc.org/), NetPath (http://www.netpath.org/), Reactome (https://reactome.org/), KEGG (https://www.genome.jp/kegg/pathway.html), SignaLink (http://signalink.org/), and Small Molecule Pathway Database (https://www.smpdb.ca/). QIAGEN Ingenuity Pathway Analysis (IPA) is an online platform that is used to analyze, integrate, model and interpret the nexus of data from "omic" technologies including RNAseq experiments and Single-Nucleotide Polymorphism (SNP) microarrays. It aids in the identification of genes and pathways that functionally interact with the drug molecules and compares the gene regulatory circuits involved in the phenotypic responses. Connectivity Map (CMap) connects the genes and the drugs (currently in use) underlying various diseases and enables us to perform data-driven analysis of repurposing/reprofiling/repositioning of drugs (it does so by analyzing the disease-specific and drug-specific gene signatures). A user provides the "gene hit lists" (aka "signatures") to the CMap for its comparison with a gene differential expression (DE) database (obtained by perturbation of cell lines with numerous drug-like molecules) to output a rank of compounds that exhibit similarity in expression patterns considering the query hit list. The CMap resource hosts over 1.5 million gene expression profiles from around 5000 chemical compounds and 3000 genetic reagents that are tested in various cell lines (Lim and Pavlidis 2021). The similarity in the gene expression profiles based on drug–drug, drug–disease, and disease–disease relationships is used to create the disease–drug networks for studying the potential side effects, targets and pathways associated with the drug compound. Aside CMap, Gene Expression Omnibus (GEO), and the Comparative Toxicogenomics Database (CTD) can be opted to create such disease-specific gene expression signatures. DIGEP-Pred is a free web-based platform that considers the structural characteristics of compounds to predict drug-induced variations in gene expression profiles (Lagunin et al. 2013). Natural Product-based Drug Combination and Its Disease-specific Molecular Regulation

(NPCDR) is an interactive database that shares knowledge on drug combinations (of natural products) with clinical or experimental validations. It also provides information on disease-specific molecular recognition and pathways and allows integration of available databases, easing the research on network pharmacology and medicinal chemistry (Sun et al. 2022).

The platforms that are free for academic use in bioinformatics and systems biology researches to analyze complex data from "omic" technologies are OmicsNet (https://www.omicsnet.ca/) (Zhou and Xia 2018), Cell Illustrator (http://www.cellillustrator.com/home), Cytoscape (https://cytoscape.org/), ConsensusPathDB (http://cpdb.molgen.mpg.de/), Gene Set Enrichment Analysis or GSEA (https://www.gsea-msigdb.org/gsea/index.jsp), The Database for Annotation, Visualization and Integrated Discovery or DAVID (https://david.ncifcrf.gov/), VANESA (https://cbrinkrolf.github.io/VANESA/). Other software with paid licenses include the geneXplain platform (https://genexplain.com/), QIAGEN Ingenuity Pathway Analysis (https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa/), and Elsevier's Pathway Studio (https://www.elsevier.com/en-in/solutions/pathway-studio-biological-research). Other alternatives that can be explored in this domain of research are presented in Table 3.

6 Pharmacophore Modeling and Molecular Docking

From a large library of chemical compounds, virtual screening identifies the lead compounds having a specific bioactivity. There exists structure-based and ligand-based virtual screening. The former approach utilizes the 3D structure of the target protein and performs molecular docking to report the potential active compounds that exhibit good binding affinity/score with the target receptor structure. Molecular docking is a structure-based approach and is used in the prediction of the 3D orientation of the ligand molecule with respect to a particular conformation of the receptor molecule when both are interacting and forming a stable complex (Sahoo et al.). It is one of the first-line tools used in discovering/designing novel drug molecules that predict the binding affinity of a chemical compound with the target receptor and ranks the ligands based on their respective docking scores. Molecular docking predicts the atomistic model of the receptor–ligand interactions and their binding orientations. In site-specific or targeted docking, the active sites of the target protein are reviewed or predicted by using programs such as CASTp, Q-SiteFinder, LigA Site, and MetaPocket, while blind docking considers the entire protein structure as the probable region of ligand interaction (Wong and Kwan 2015). Searching algorithms that fish out favorable conformations from infinite possibilities include matching algorithms, incremental construction methods, multiple copy simultaneous searching, Monte Carlo and genetic algorithms. Scoring functions (either empirical, force field, or knowledge-based) of a docking software estimate the binding affinity of the ligand with the target receptor and rank the ligands based on docking scores.

Table 3 Some software/servers to generate, visualize, and analyze biological networks

Software/platform	Description	URL
Cytoscape	It is an open-source platform used in the integration and graphical interpretation of complex networks and analysis of human-curated pathway datasets such as WikiPathways, Reactome, and KEGG (Shannon et al.)	https://cytoscape.org/
PathVisio	Developed in Java, it is a free open-source software for pathway analysis that allows a user to draw, edit, and analyze biological pathways. It is the pathway editor for WikiPathways	https://pathvisio.org/
ToxPi (Toxicological Prioritization Index)	It is a free interface distributed under the GNU GPL. It allows the integration of multiple data sources to generate visual profiles and clustering of data (Marvel et al. 2018)	https://toxpi.org/
ReactomeFIViz	It is used to build subnetworks and search pathways and network patterns related to several diseases including cancer. It has access to the Reactome Functional Interaction (FI) network. It needs Cytoscape to be preinstalled where it can be found as "ReactomeFIPlugIn"	https://reactome.org/tools/reactome-fiviz#Overview
PyPathway	It is a python package for analyzing and visualizing biological networks	https://pypi.org/project/pypathway/
Conan	It is a C++/Python library developed for generating, inferencing and analyzing complex networks (Honorato-Zimmer et al. 2010)	https://github.com/rhz/conan/
CyTargetLinker	This tool allows the extension of biological networks and comes with Cytoscape automation feature	https://apps.cytoscape.org/apps/cytargetlinker
multiSLIDE (Multi-omics Systems-Level Interactive Data Exploration)	It is a web server to analyze multiomics data and explore the interconnection of components of biological pathways (the connected molecular features are visualized in heatmaps) (Ghosh et al. 2021)	https://github.com/soumitag/multiSLIDE
VRNetzer	It allows efficient visualization, interactive exploration, customization, integration of external databases and extension of highly complex networks (Pirch et al. 2021)	https://menchelab.com/vrnetzer
MONGKIE (Modular Network Generation and Visualization Platform with Knowledge Integration Environments)	It is a single platform that analyzes (such as performing network clustering and over-representation analysis) and visualizes integrated networks generated from multiomics data	http://yjjang.github.io/mongkie/
CellDesigner	This modeling tool is a kind of editor that provides an intuitive GUI to draw gene-regulatory and biological networks (Funahashi et al.)	https://www.celldesigner.org/index.html

(continued)

Table 3 (continued)

Software/platform	Description	URL
RING (Residue Interaction Network Generator)	It is used in the identification of noncovalent interactions in a given protein structure. It can create probabilistic networks and conformational-dependent contact maps	https://ring.biocomputingup.it/submit
MinePath	It analyses gene expression data and identifies differentially expressed functional paths or subpaths within a gene regulatory network (Koumakis et al. 2016)	http://www.minepath.org/
Pathview	It is used for integrating and mapping diverse biological data on pathways and produces top-notch hyperlinked graphs (Luo et al. 2017)	https://pathview.uncc.edu/
NAPS (Network Analysis of Protein Structures)	It is a web-based platform that models proteins as a network of noncovalent interactions between amino acid residues (rather than the traditional way of analyzing the secondary structure and fold arrangement) to describe the topological characteristics and structure–function relationship (Chakrabarty and Parekh 2016)	https://bioinf.iiit.ac.in/NAPS/
ProSNEx (Protein Structure Network Explorer)	The web service is used to construct Protein Structure Networks (PSNs) and allows sequence conservation, annotation, and analysis of amino acid flexibility (Aydlnkal et al. 2019)	http://prosnex-tool.com/demos/technical/

Qualitative "structure–activity relationships" (i.e., SAR) and quantitative structure–activity relationships (i.e., QSAR) are used in virtual screening (and target fishing) if the structures of the chemical compounds are available or predicted/designed. These approaches assume the bioactivity of a ligand as a function of its structural or physicochemical characteristics. Analysis and comparison of the structures are achieved with the help of some descriptors (such as structural fragments, fingerprints, constitutional, topological, electro-topological, quantum-chemical and physicochemical descriptors) (Lagunin et al.). Pharmacophore modeling considers a group of atoms in the structure whose presence directs the pharmacological effect of the ligand. Ligand-based virtual screening employs QSAR approaches that aim to develop mathematical models to study the correlation between the observed bioactivities and structural/physicochemical characteristics. Software such as Sybyl-X 2.0 and E-Dragon perform QSAR studies (Browne et al.; Fedyushkina et al. 1990).

Two techniques, namely comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA), are encountered in 3D QSAR for ligand-based drug designing (Chavda and Bhatt 2019). In CoMFA, a library of ligands comprising their physicochemical characteristics and biological activities is created. These bioactive compounds vary between themselves by some substitutions. Seventy percent of data in the database is fed as the input to the training set (regression models are generated using it following the Partial Least Squares (PLS) regression and correlating the models with the pIC50 value), whereas the rest

of the 30% data is kept as the test set (used to establish the prediction accuracy of the QSAR regression models). Finally, the models undergo leave-one-out (LOO) cross-validation. The descriptors in CoMFA are determined by the sp^3 probe. Columbic potential energy calculates the electrostatic field and Lennard Jones potential energy describes the bond energy curves for Van der Waals bonding. The 3D steric and electrostatic contour plots depict the variation in bioactivity with the alteration of molecular fields. The SEAL similarity method in CoMSIA takes into account the electrostatic, steric, hydrogen bonding and hydrophobic descriptors to predict the similarity between molecules using Gaussian functions. The contour plots produced by the CoMSIA portray the favorable and unfavorable regions for the interaction of ligands (Bordás et al. 2003).

Approaches to build pharmacophore-based models identify the molecular characteristics that direct the macromolecular recognition of ligands, thus triggering the biological response. The aromaticity, hydrophobicity, presence of hydrogen bond acceptors/donors and anion/cation residues are considered to model pharmacophores that act as a query to search the potential bioactives from a database of compounds in virtual screening. Developing pharmacophore models can follow either structure-based or ligand-based approaches. The former approach relies on the availability of X-ray crystallographic or NMR spectroscopic 3D structure of the receptor molecule/target protein. The active sites and the spatial interactions are described by certain physicochemical properties that complement the interacting ligands and selectively identify the compounds with high binding affinity. A good model must incorporate protein flexibility to consider the structural changes that occur during the formation of the receptor–ligand complex. Ligand-based modeling is useful in cases where the 3D molecular structure of the receptor molecule is not available and the pharmacophores are generated by studying the common features (e.g., hydrophobic and electrostatic interaction, hydrogen bonding, etc.) that exist at the same position in the ligand structures. In ligand-based pharmacophore modeling, chemical compounds in the training set create a conformational space that takes care of ligand flexibility (Braga et al.). HipHop, DISCO, HypoGen, and PHASE are some software for generating pharmacophore models.

The structural data generated by NMR, X-ray crystallography, and homology modeling are static in nature that fails to describe the dynamic nature of the biorecognition process during receptor–ligand binding. These experimental data highlight the binding sites for some endogenous agonists; however, other active sites (including the allosteric and cryptic binding sites) are often not identified. Neither the receptor nor the ligand is a frozen/rigid entity; instead, the structures are interacting under constant motion in a solution (any biological fluid). Moreover, an approaching ligand can cause a series of conformational changes in the receptor structure to improve its binding affinity. In order to consider the flexibility of the macromolecular structures, the relaxed complex scheme (RCS) has been developed that extracts several conformations of the receptor sampled using simulation and then performs molecular docking of the ligand with each of the conformations. The scoring functions often consider conformational entropy and solvation energy as

negligible parameters while calculating the binding affinity to make the process computationally less expensive (but compromising with the model's accuracy).

Often researchers employ both QSAR modeling and molecular docking to predict the bioactivity and investigate the mechanism of action of compounds. In a study, the immunomodulatory effect of the ligands is evaluated by employing forward stepwise multiple linear regression to develop a QSAR model with 52 physical-chemical descriptors (important ones are namely dipole moment, steric energy, amide group count, λmax (UV-visible) and molar refractivity) using the SCIGRESS platform. Finally, molecular docking is performed to predict their binding affinity with immunomodulatory targets namely TLR-4, iNOS, COX-2, CD14, IKK b, CD86, and COX-1 (Yadav et al. 2010). A similar QSAR model with 50 descriptors from SYBYL-X 1.3 is used to study the cytotoxicity of ursolic acid analogs against human glioblastoma and lung cancer cell lines. The model exhibited a good regression coefficient (r^2) and the cross-validation regression coefficient (r_{cv}^2) (values ranging from 0.8 to 0.96). The relevant parameters for cytotoxicity are found to be LUMO energy, ring count, dipole vector and solvent-accessible surface area (Kalani et al. 2012).

Some freely available software and webservers to generate descriptors (that include arithmetical, topological, constitutional, geometrical, electrostatic, thermodynamic, quantum-chemical descriptors and other molecular fingerprints) are AFGen (http://glaros.dtc.umn.edu/gkhome/afgen/overview), ISIDA-fragmentor (https://complex-matter.unistra.fr/equipes-de-recherche/laboratoire-de-chemoinformatique/software-development/#c89382), E-DRAGON (http://www.vcclab.org/lab/edragon/), Open3DQSAR (https://open3dqsar.sourceforge.net/), ToMoCoMD-CARDD (http://tomocomd.com/), MOLGEN (http://molgen.de/?src=documents/molgenqspr.html), Mold2 (https://www.fda.gov/science-research/bioinformatics-tools/mold2), Toxicity Estimation Software Tool or TEST by United States Environmental Protection Agency (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test) and Open Babel (http://openbabel.org/wiki/Main_Page) while commercial alternatives are The CODESSA PRO project (http://www.codessa-pro.com/). Along with the model's high internal accuracy (i.e., $R^2 > 0.9$ and $Rcv^2 > 0.8$ calculated using the training set only), external validation of the (Q)SAR model with experimental data is desirable as per the OECD guidelines (www.oecd.org/env/ehs/risk-assessment/37849783.pdf). In order to better correlate the structural characteristics with the bioactivities, one must use molar units (such as mol/kg, or mmol/kg) instead of mass units (i.e., mg/kg) in the models (Dearden et al. 2009). In inverse docking or target fishing, identification of the possible targets/receptors for the query ligand is performed by software such as GOLD, FlexX, TarFisDock, TarSearch-X, and TarSearch-M.

The evaluation of the bioactivities of a novel compound (i.e., the potential drug targets) can be accomplished by using pair similarity with known compounds (e.g., ChEMBL database calculates the Tanimoto coefficient based on fingerprints), molecular docking, pharmacophore modeling, Bayesian statistics and designing substructural descriptors or fingerprints. However, one must take to avoid the

"activity-cliff" problem in the model that arises when the compounds share analogous structural characteristics but exhibit dissimilar bioactivity spectra. Despite being a rapid and efficient technique in virtual screening, pharmacophore modeling essentially relies on the knowledge of reported active ligands, necessitates sampling conformers using a search algorithm, and is based on a rigid framework for searching hit compounds from the database (Horvath 2010; Kaushik et al. 2018; Lans et al. 2020).

Some platforms to perform protein-protein or protein-DNA docking include SPServer (http://aleph.upf.edu/spserver/), pyDockDNA (https://model3dbio.csic.es/pydockdna), CoDockPP (http://codockpp.schanglab.org.cn/), DOCKSCORE (http://caps.ncbs.res.in/dockscore/), PIIMS Server (http://chemyang.ccnu.edu.cn/ccb/server/PIIMS/index.php), GalaxyDomDock (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=DOMDOCK_INTRO), P3DOCK server, HDOCK server, and GRAMM (Global RAnge Molecular Matching) (https://gramm.compbio.ku.edu/). ezCADD is a fast 2D/3D molecular visualization software that allows small-molecule docking, protein-protein docking, prediction of binding sites, identification of drug targets, homology modeling and structure quality assessment (Tao et al. 2019). FragVLib is an open-source software (distributed under the GNU General Public License) that generates a virtual library of ligand fragments (used for structure-based drug designing) by searching the binding pocket similarity considering a database of ligand-receptor complexes (Khashan 2012). eMolFrag is used for the virtual fragmentation of molecules and extracts the molecular fragments to build a library for virtual screening (Liu et al. 2017). Other software/servers used in virtual screening (structure-based and/or ligand-based) can be listed as follows, although other popular platforms do exist: DENVIS (https://github.com/deeplab-ai/denvis), ReMODE (Receptor-based MOlecular Design for de novo drug designing available at http://cadd.zju.edu.cn/relation/remode/), Pocket2Drug (https://github.com/shiwentao00/Pocket2Drug), DrugRep (http://cao.labshare.cn/drugrep/), DockingPie (a docking plugin for PyMOL), CB-Dock2 (https://cadd.labshare.cn/cb-dock2/php/index.php), PharmRF (https://github.com/Prasanth-Kumar87/PharmRF), DeepDock (https://github.com/OptiMaL-PSE-Lab/DeepDock), Knime workflow (https://hub.knime.com/), RNALigands (http://rnaligands.ccbr.utoronto.ca/php/downloads.php), AutoDock Vina (https://vina.scripps.edu/), eSPC (https://spc.embl-hamburg.de/), RASPDplus (https://github.com/HITS-MCM/RASPDplus), LigRMSD (https://ligrmsd.appsbio.utalca.cl/), LeDock (http://www.lephar.com/index.htm), VSpipe (https://github.com/sabifo4/VSpipe), PyRx (https://pyrx.sourceforge.io/), LiSiCA ((Ligand Similarity using Clique Algorithm available at http://insilab.org/lisica/), ALIDE (http://chemyang.ccnu.edu.cn/ccb/server/AILDE/), Open3DALIGN (https://open3dalign.sourceforge.net/), PrepFlow (https://ifm.chimie.unistra.fr/prep-flow), QSAR-Co-X (https://github.com/ncordeirfcup/QSAR-Co-X), PyRMD (https://github.com/cosconatilab/PyRMD), SwissSimilarity (http://www.swisssimilarity.ch/), PharmMapper (http://lilab-ecust.cn/pharmmapper/), and ZINCPharmer (http://zinc-pharmer.csb.pitt.edu/).

7 Molecular Dynamics Simulation

The deterministic approach of the quantum-mechanical model of motion in the macroscopic world contrasts the use of probability functions that describe the motion in the microscopic world. This is because the electron clouds (that interact while bonding) exhibit wave-particle duality and not simple mechanical bonding. Simulating the system of proteins and other receptor molecules interacting with ligands at the atomistic level has paved its importance to the drug discovery process. The breakthroughs in hardware-based computational power and the development of new algorithms ease the calculation of molecular forces that exist in the system. The limitations of the conventional "lock and key" model of receptor–ligand interaction (where the receptor is held rigid and conformational sampling of the ligand is done, restricting the atomistic motions to keep the model simple) are overcome by such simulations. This considers the dynamic nature of the proteins, thus sampling numerous conformational states and selectively stabilizing them when an agonist or antagonist interacts. Any simulation starts with the modeling of the receptor–ligand system (using the data obtained from NMR, crystallography, or homology modeling), subsequently, the forces experienced by each atom (present in the system) are estimated and the positional changes of atoms are done following Newton's laws of motion. These forces are the results of bonded interactions (i.e., charged/electrostatic interactions that use Coulomb's law to generate the model) and nonbonded interactions (i.e., van der Waals interactions that use the Lennard-Jones 6–12 potential for modeling). Virtual springs and sinusoidal functions are used in the estimation of the difference in potential energy between eclipsed and staggered conformations. The parameters used in the functions identify the stiffness and lengths of the springs, estimate the atomic angles (and dihedral angles), calculate the partial atomic charges (responsible for electrostatic interactions), and predict the van der Waals atomic radii. These parameterizations form the basis of a "force-field" that depicts the nature of molecular dynamics under the influence of several atomic forces. Finally, the simulation time is advanced (by 1–2 femtoseconds, i.e., 10^{-15} s), and the process is iterated (in the order to 10^6) (Durrant and McCammon 2011). Different force fields exist depending on how they are parameterized, although they mostly generate similar outputs. AMBER, CHARMM, and GROMOS force fields are generally encountered in simulation modeling. Molecular dynamics simulation demands performing a huge number of calculations; hence, computer clusters or supercomputers with numerous processors need to operate parallelly. Message Passing Interface (MPI) compatible simulation software like NAMD, CHARMM, and AMBER help in connecting multiple processors so that they can be simultaneously used to execute a complex assignment. Such simulations can estimate the values of NMR-related parameters (e.g., spin relaxation), thus allowing comparison between the theoretical prediction and experimental value.

Simulating molecular systems follows Newton's laws of motion. Such simulations output trajectory graphs for evaluating the stability of the target protein or its docked complexes. In order to perform molecular dynamics simulation, the protein

topology is generated by applying force fields such as Amber and Gromos (using GROMACS or LEaP program), while the PRODRG server can be used for obtaining ligand topology (Strasser and Wittmann 2013). The structures are placed inside a cube and solvation is done using the flexible simple point-charge (SPC) water model. Followed by system neutralization, the steepest descent algorithm minimizes the energy of the system. At a particular temperature (let's say 300 K), position-restraining simulations are performed for a certain period of time under constant volume and temperature dynamics (NVT) and pressure and temperature dynamics (NPT). LINear Constraint Solver (LINCS) algorithm is frequently reviewed for molecular simulations with bond constraints (Hess et al. 1997). The Particle Mesh Ewald algorithm estimates the electrostatic energy (Madelung energy) of the complex/crystal. After performing the molecular dynamics simulation, the trajectories (w.r.t. time) are generated by the XMGrace tool and the parameters namely the root mean square deviation (RMSD), root mean square fluctuation (RMSF), the radius of gyration (Rg), and intermolecular hydrogen bond formations are considered to analyze the stability of the protein-ligand complex (Van Der Spoel et al. 2005). The advantages of molecular dynamics simulation come with a cost—the process becomes computationally expensive. Lower simulation time will reflect the inadequacy (of the model) in the conformation sampling step. Force fields are used in the approximation of the quantum-mechanical model of motion at the atomistic level; hence, molecular dynamics simulations fail largely for the systems having dominant quantum effects such as bonds involving transition metal atoms (Durrant and McCammon 2011). The tools/platforms that can be employed to perform molecular dynamics simulations and analyze the output files post simulation are reviewed in Table 4.

8 Conclusion

To ease the process of drug discovery, a shift toward the application of computational tools is witnessed in the current era of research. Challenges arising due to the pleiotropic nature of biomolecules and the interaction of chemical compounds with multiple pharmacological targets (often encountered in combinatorial/multitargeted approaches) can be addressed by chemo- and bioinformatics tools that make use of databases on physicochemical characteristics and therapeutic use of compounds. Early prediction of ADMET properties of a chemical compound can be of utmost importance since most drug failures occur in the later phases due to undesirable pharmacokinetics and toxicological characteristics. Simulating the system of proteins and other receptor molecules interacting with ligands at the atomistic level has paved its importance to identifying novel drug-like compounds. This considers the dynamic nature of the proteins, thus sampling numerous conformational states and selectively stabilizing them when an agonist or antagonist interacts. The breakthroughs in hardware-based computational power and the development of new algorithms ease the calculation of molecular forces that exist in the system. Biological

Table 4 Platforms to perform molecular dynamics simulations and analysis of output files

Software/platform	Description	URL
YASARA (Yet Another Scientific Artificial Reality Application)	It is compatible with Windows, Linux, macOS and Android and requires a license fee to design photorealistic molecular graphics, and models and perform simulations	http://www.yasara.org/
Abalone	It is a package of molecular graphics used for molecular modeling, geometry optimization, and simulations of proteins, nucleic acids, and ligands. It includes features such as semiautomated parameterization of force fields, GPU acceleration, interfacing with quantum chemical programs, and scripting	http://www.biomolecular-modeling.com/Abalone/index.html
NAMD	It is based on Charm++ parallel objects and exhibits high-performance molecular dynamics simulation of complex systems (having a large number of atoms). It uses VMD (a tool for molecular graphics) for analyzing trajectories	https://www.ks.uiuc.edu/Research/namd/
LARMD Server (Ligand and Receptor Molecular Dynamics)	It is used to study the molecular dynamics of protein-ligand interactions. It helps in structure preparation, building force field libraries for molecules, energy calculation (MM/PBSA), and analysis of hydrogen bonds, trajectories, Root-Mean-Square Deviation (RMSD), Radius of Gyration (Rg), Fraction of Native Contacts (Q), Root Mean Square Fluctuation (RMSF), B-factor, Principal Component Analysis (PCA) being some features among others	http://chemyang.ccnu.edu.cn/ccb/server/LARMD/
GROMACS	A free and open-source platform for performing molecular dynamics simulations and their output analysis	https://www.gromacs.org/
GROMITA	Written in Perl language, it is a GUI (providing both a window-based environment and a terminal mode) front-end for GROMACS (Sellis et al. 2009)	http://gromita.bio.demokritos.gr/
YAMACS	It is a collection of plugins for performing GROMACS simulations through the YASARA platform	https://github.com/YAMACS-SML/YAMACS
3dRS (3-dimensional structure Representation Sharing)	It is used to visualize 3D biological structures and molecular dynamics trajectories (Bayarri et al. 2021)	https://mmb.irbbarcelona.org/3dRS/
LAMMPS	It stands for Large-scale Atomic/Molecular Massively Parallel Simulator. It runs on a single processor and also supports Message Passing Interface for parallel operation	https://www.lammps.org/
BitClust	It is based on Python and follows Daura's algorithm and is used for efficient clustering of relatively long molecular dynamics trajectories	https://pypi.org/project/bitclust/
MDAnalysis	It is a Python library exclusively used in the analysis of molecular dynamics trajectories	https://www.mdanalysis.org/

(continued)

Table 4 (continued)

Software/platform	Description	URL
Tinker-HP	It utilizes multiple CPUs and GPUs and supports MPI to ease long molecular dynamics simulations	https://tinker-hp.org/?Download-instructions
QwikMD	It provides an easy connection between VMD and NAMD for performing hassle-free simulations following a few steps	http://www.ks.uiuc.edu/Research/qwikmd/
PREFMD	It stands for Protein structure REFinement via Molecular Dynamics. The input file must be in PDB format	http://feig.bch.msu.edu/prefmd
Amber	It is a collection of molecular dynamics simulation programs (Salomon-Ferrer et al. 2012)	http://ambermd.org/
DynOmics ENM server	It is used to study the dynamics of biological systems. It integrates two elastic network models (ENMs)—the Gaussian Network Model (GNM) and the Anisotropic Network Model (ANM) (Li et al.)	https://dyn.life.nthu.edu.tw/oENM/

networks have the capability to highlight the interacting elements within a complex biochemical system, thus aiding in the visualization and exploration of big data. In brief, molecular docking, pharmacophore modeling, methods relating (Q)SAR, molecular dynamics simulation, network pharmacology, and machine learning algorithms accelerate the drug discovery process and complement the traditional bioactivity-guided fractionation, high-throughput screening, and systems biology approaches. The examples that are listed/tabularized in this chapter highlight only a fraction of popular software/platforms and encourage the readers to explore other alternatives in various domains of drug discovery and protein engineering.

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Part IV
Synthesis and Encapsulation of Compounds of Natural Origin

Multicomponent Reactions for the Synthesis of Natural Products and Natural Product-Like Libraries

Miriam Ruiz-Serrano and J. Carlos Menéndez

Abstract Multicomponent reactions involve at least three starting materials that are combined in a single operation and are convergent, atom-economic, and step-efficient. They provide a highly efficient alternative to sequential multistep procedures and are therefore ideally suited for simplifying target-oriented synthetic efforts. In this context, this chapter reviews the use of multicomponent reactions as key step for natural product synthesis.

Keywords Strecker reaction · Mannich reaction · Petasis reaction · Povarov reaction · Passerini reaction · Ugi reaction · [C + NC + CC] coupling · Anion relay chemistry · Catellani reaction

1 Introduction

Transformations that generate several bonds, thus minimizing the number of synthetic operations, are very attractive when planning routes to complex targets such as natural products. Among the various types of multiple bond-generating transformations, multicomponent reactions stand out as particularly interesting due to their flexibility. For the purposes of this chapter, we will define multicomponent reactions as "one-pot processes that combine three or more substrates either simultaneously or through a sequential-addition procedure that does not involve any change of solvent" (Touré and Hall 2009), in such a way that the reaction products contain significant fragments of each individual component.

Although some of the classical multicomponent reactions were discovered during the very early development of organic chemistry, their systematic use in synthesis has taken a long time to take off, until the advent of combinatorial chemistry led

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to the identification of multicomponent reactions as an ideal technology to assemble compound libraries, especially in medicinal chemistry contexts.

There is a great deal of literature on the application of multicomponent chemistry to natural product synthesis, as testified by the abundance of review articles on the various aspects this topic (Touré and Hall 2009; Perreault and Rovis 2009; Dömling et al. 2012; Smietana et al. 2018; Ziarani et al. 2019). We have striven to produce a balanced, reasonably concise summary of the current status of the field.

2 Imine-Initiated Multicomponent Reactions

2.1 Strecker Reaction

The preparation of α-aminonitriles from amines, aldehydes or ketones and a cyanide salt was first reported in 1850 by the German chemist Adolph Strecker (Strecker 1850; Kouznetsov and Galvis 2018). This classical reaction holds the distinction of being the first multicomponent reaction described in the literature and it also enabled the first synthesis of an amino acid, even before their isolation from natural sources. Some methodologies that have been shown to improve the Strecker reaction include ultrasound irradiation (Menéndez et al. 1986), flow chemistry (Wiles and Watts 2008) and mechanochemical activation (Hernández et al. 2016). The Strecker reaction has become an important tool in the synthesis of complex heterocyclic systems, including natural products (Grundke et al. 2020).

The first step of the mechanism of the Strecker reaction (Li 2007) is the acid-promoted condensation of the amine and carbonyl components, affording the α-aminoalcohol intermediate **1** (Singh et al. 2022) and then imine **2**. The cyanide anion attacks the iminium intermediate arising from imine protonation to give the corresponding α-amino nitrile **3**. The initial step competes with cyanohydrin formation, but this reaction is reversible under the reaction conditions and the equilibria are displaced toward α-aminonitrile formation (Fig. 1).

The synthesis of reserpine by the Stork group (Stork et al. 2005) provides an example of the use of the Strecker reaction as a key step in alkaloid synthesis. Reserpine was first isolated from the dried root of *Rauvolfia serpentina*, used in traditional Indian medicine under the name *sarpagandha* as a tranquilizer and for other applications. This compound was introduced in clinical practice in the 1950s as an antihypertensive agent and is still employed, in combination with diuretics, when the more usual treatments fail. The Stork total synthesis of reserpine has as the key step the reaction of 6-methoxytryptamine, aldehyde **4** and potassium cyanide, which led to the formation of the 2-cyanodecahydroisoquinoline **6**, presumably through the intermediacy of the initial Strecker product **5**. Compound **6** was transformed into reserpine by a Pictet–Spengler cyclization in acidic conditions, with concomitant cyanide elimination, followed by acylation of the secondary hydroxyl with 3,4,5-trimethoxybenzoyl chloride (Fig. 2).

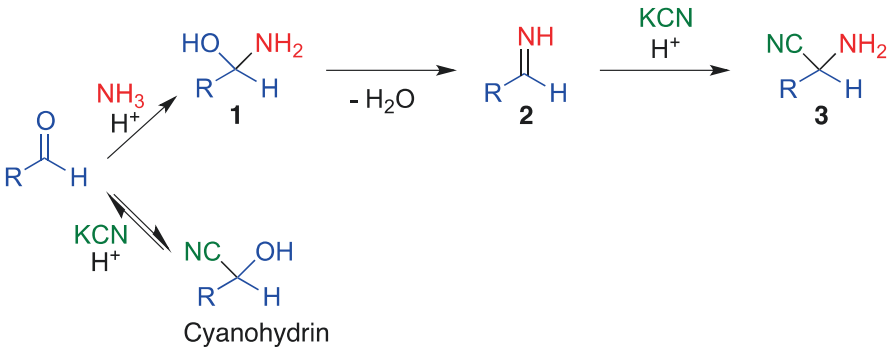

Fig. 1 Mechanism of the Strecker reaction

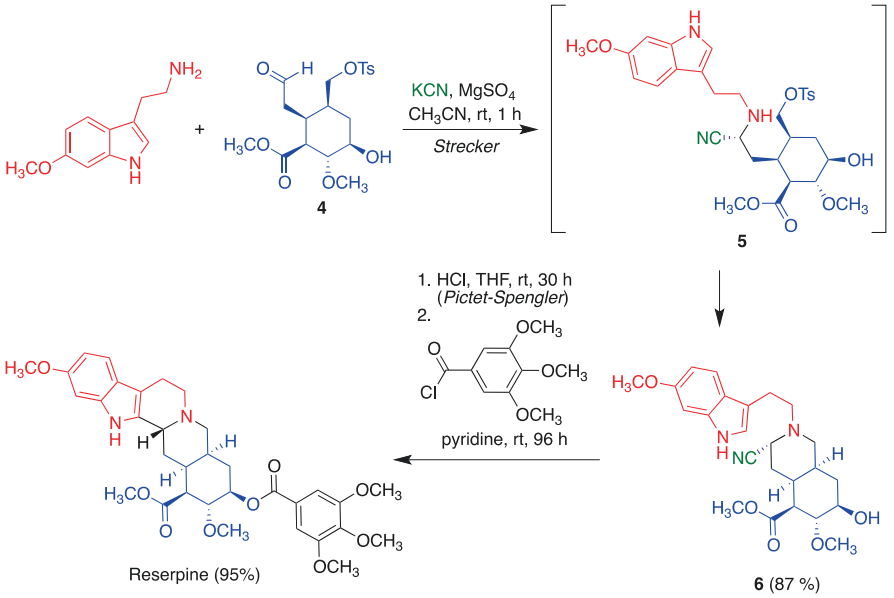

Fig. 2 Stork's total synthesis of reserpine

Another example of a diastereoselective Strecker reaction from chiral starting materials is found in the work by the Corey group on the total synthesis of the anticancer natural product trabectedin (ecteinascidin-743), commercialized under the brand name Yondelis® for the treatment of advanced soft-tissue sarcoma and ovarian cancer. This marine alkaloid is highly complex, containing the pentacyclic framework characteristic of the tetrahydroisoquinoline family of natural products plus an additional tetrahydroisoquinoline moiety attached to the B-ring by a chain that forms a 10-membered lactone. One of the key steps of the Corey synthesis of this alkaloid is the Strecker reaction of aldehyde **7**, tetrahydroisoquinoline derivative **8**

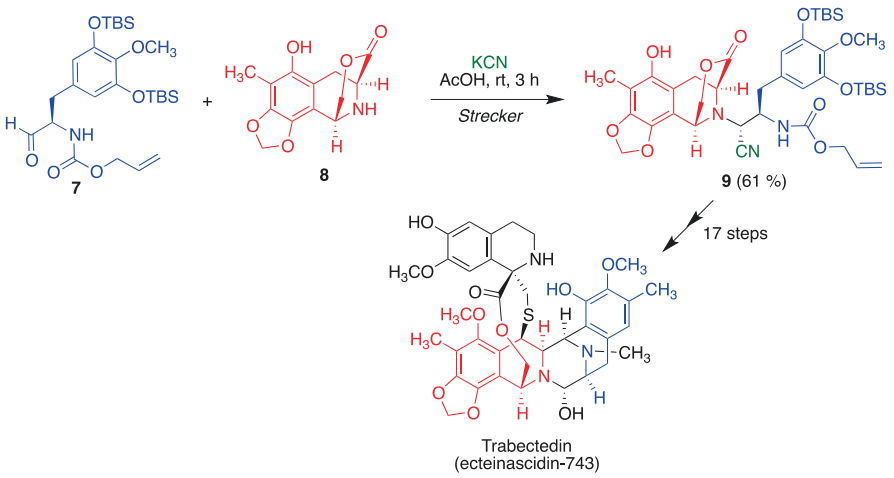

Fig. 3 A Strecker reaction as part of Corey's total synthesis of trabectedin

and potassium cyanide in acetic acid that furnished α-aminonitrile **9**, from which the polycyclic scaffold of trabectedin was built in 17 additional steps (Fig. 3) (Corey et al. 1996).

2.2 Mannich Reaction

The Mannich reaction, or Mannich aminomethylation, is a three-component reaction that combines ammonia or a primary or secondary amine, an aldehyde (often formaldehyde) and a compound having at least one active hydrogen atom to furnish aminomethyl derivatives of the latter component (Allochio Filho et al. 2017). The Mannich reaction is normally performed under acid catalysis, and starts with the reaction of the amine with the nonenolizable aldehyde to give a hemiaminal whose dehydration leads to an iminium cation **10**. This intermediate reacts with the enolizable carbonyl compound through an aldol-type reaction resulting in the formation of the final product (Fig. 4).

Nakadomarin A is a cytotoxic marine alkaloid of the manzamine family that was isolated from the sponge *Amphimedon* sp. and contains a very unusual 8/5/5/5/15/6 ring system. The Dixon group has reported a synthesis of this alkaloid that has a nitro-Mannich reaction as one of its key steps (Faisca Phillips et al. 2020). Thus, the reaction between compound **11**, bearing a nitroalkane unit, 5-hexenamine and formaldehyde afforded compound **12** via a nitro-Mannich/lactamization domino sequence, and this intermediate was then transformed into the natural product in four additional steps (Fig. 5) (Jakubec et al. 2009). A similar strategy is being studied for the synthesis of additional members of this alkaloid family, such as keramaphidin (Jakubec et al. 2016).

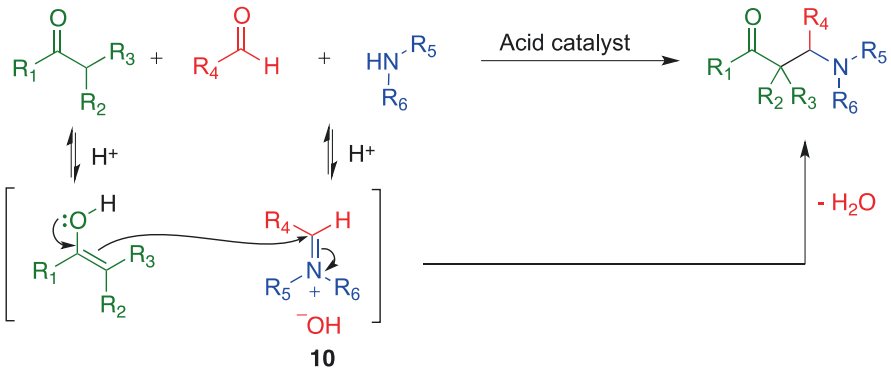

Fig. 4 Mechanism of the acid-promoted Mannich reaction

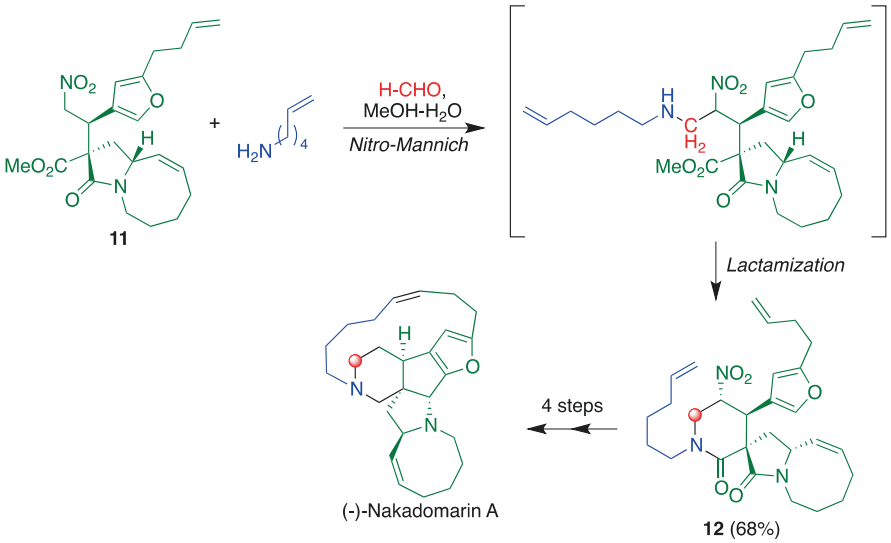

Fig. 5 Synthesis of nakadomarin A by Dixon, featuring a nitro-Mannich reaction

Although not strictly multicomponent, we will also discuss intramolecular Mannich reactions due to their importance in natural product synthesis (Shi et al. 2018).

Morphine, a *Papaver somniferum* alkaloid, is one of the most widely employed narcotic analgesics, and a popular target for total synthesis. In 2006, the Fukuyama group reported a total synthesis of morphine, in racemic form, featuring construction of one ring via an intramolecular Mannich reaction (Uchida et al. 2006). This double cyclization was induced by refluxing compound **13** in methanolic HCl, which presumably induced first the deprotection of the acetal moiety in the starting material, followed by intramolecular cyclocondensation with the carbamate group nitrogen to generate an eight-membered ring bearing an iminium function

(intermediate **14**). An intramolecular Mannich reaction would then complete the formation of the pentacyclic ring system of the alkaloid and gave compound **15**, which was transformed into morphine in nine additional steps (Fig. 6).

Plants of the *Lycopodium* genus have been used in traditional Chinese medicine and some of their alkaloids have shown interesting pharmacological properties. Within this group, lycojaponicumin D is a challenging synthetic target due to its particularly unusual architecture. Fan and coworkers (Zhao et al. 2017) have reported the first total synthesis of (±)-lycojaponicumin D starting from its putative biosynthetic precursor lycodoline, as summarized in Fig. 7. α-Hydroxylation of this starting material gave (±)-lycoposerramine G, another *Lycopodium* alkaloid, whose oxidation with hydrogen peroxide afforded (±)-lycoposerramine F. Treatment of the latter with triphosgene afforded lycojaponicumin D via a domino ring opening-ring closure sequence, where the latter process involved an intramolecular Mannich reaction.

Robinson's pioneering synthesis of tropinone (Robinson 1917; Medley and Movassaghi 2013), the first example of a biomimetic synthesis, is a landmark of natural product total synthesis and involved the construction of a bicyclic nitrogen heterocycle via a multicomponent domino process comprising inter- and intramolecular Mannich steps (Fig. 8). The reduction of tropinone to tropine followed by esterification with tropic acid affords atropine, an antimuscarinic alkaloid present in *Atropa belladonna* and other plants of the *Solanaceae* family that is employed to treat bradycardia and poisoning by organophosphate pesticides. A related flow protocol for atropine synthesis has also been developed (Dai et al. 2015).

Vinylogous Mannich reactions have also found widespread application in natural product synthesis (Sánchez-Roselló et al. 2016). One example is summarized in Fig. 9, representing a unified organocatalytic synthesis of indolizidine alkaloids

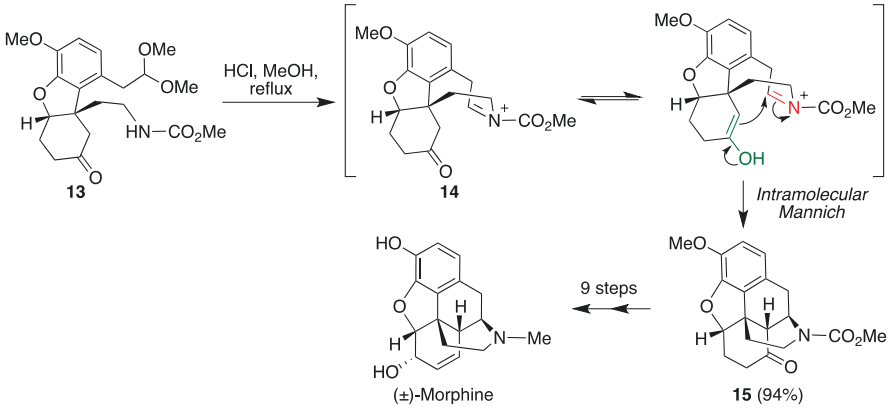

Fig. 6 Generation of the morphinan B-ring using an intramolecular Mannich reaction in the Fukuyama synthesis of (±)-morphine

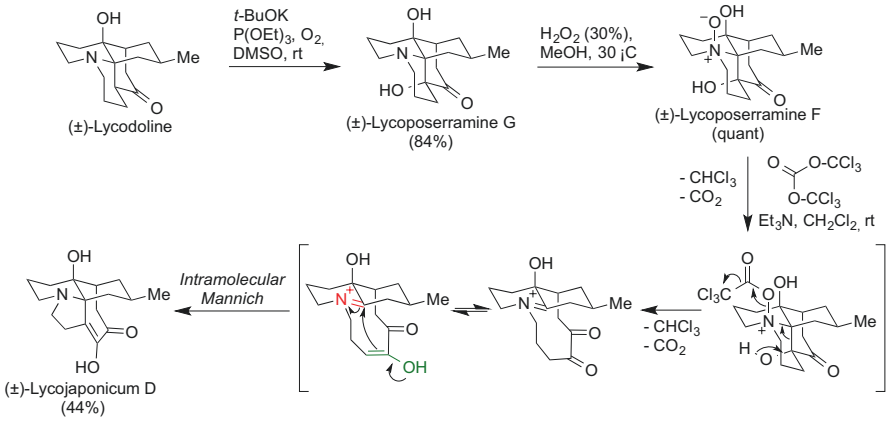

Fig. 7 Collective synthesis of some *Lycopodium* alkaloids including lycojaponicumin D

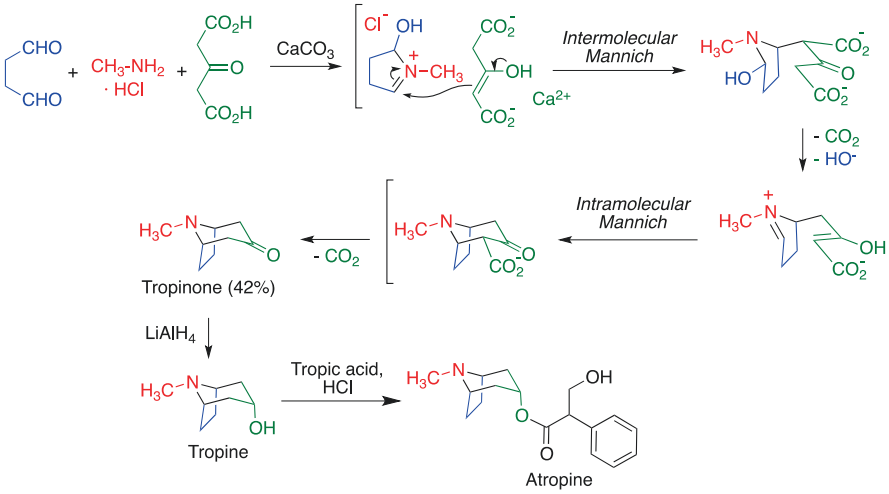

Fig. 8 The Robinson tropinone synthesis and its application to the preparation of atropine

developed by Schneider. A vinylogous Mukaiyama–Mannich reaction from *p*-anisidine, succinic hemialdehyde ethyl ester, and the silyldienolate **16** (Hoppmann and García-Mancheño 2021) in the presence of the chiral phosphoric acid catalyst **17** afforded intermediate lactam **18**, which was readily transformed into several indolizidine alkaloids including coniceine, indolizidine 167B, and monomorine (Abels et al. 2014).

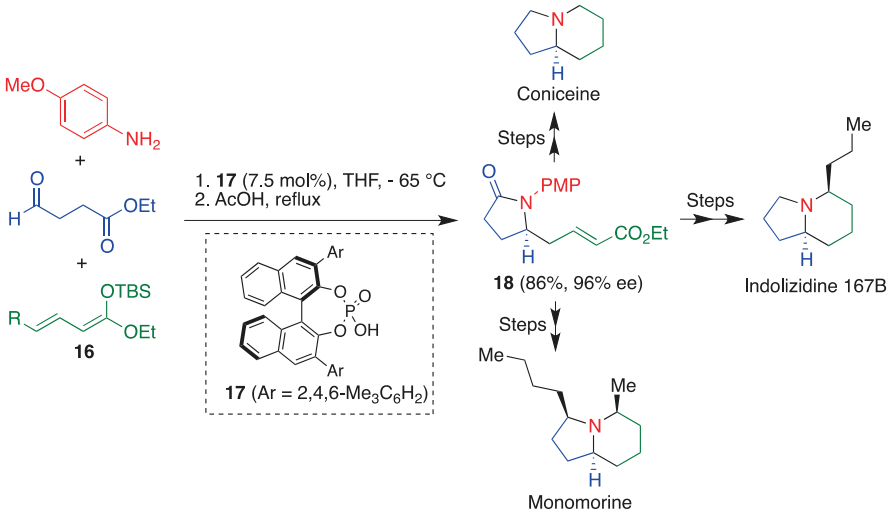

Fig. 9 Unified organocatalytic synthesis of some indolizidine alkaloids

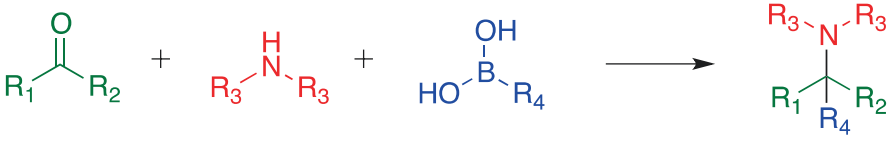

Fig. 10 The Petasis (Borono–Mannich) reaction

2.3 Petasis (Borono–Mannich) Reaction

A variation of the Mannich reaction where the nucleophile is a boronic acid is known as the Petasis reaction (Fig. 10). It is compatible with the presence of hydroxyl and carboxy groups in the molecule core and retains the double bond geometry of vinylboronic acids (Wu et al. 2019). Mechanistically, the Petasis reaction involves the formation of an iminium ion by condensation between the amine and the carbonyl compound, followed by coordination with the boronic acid and migration of the substituent attached to boron to the iminium carbon (Souza et al. 2015).

Pyne and coworkers have shown that the 2,5-dihydropyrrole-derived acetonide **23** is a suitable intermediate for the synthesis of natural compounds with polyhydroxylated 3-hydroxymethylpyrrolizidine-type structures (Ritthiwigrom and Pyne 2008; Ritthiwigrom et al. 2010). Thus, a three-component Petasis reaction between xylose **19**, allylamine and vinylborane **20** afforded compound **21**. Protection of the amino group as a Boc carbamate and the terminal diol as an acetonide led to diene **22**. Finally, a ring-closing metathesis reaction using Grubbs' first-generation ruthenium catalyst provided the common intermediate **23**, which was transformed into

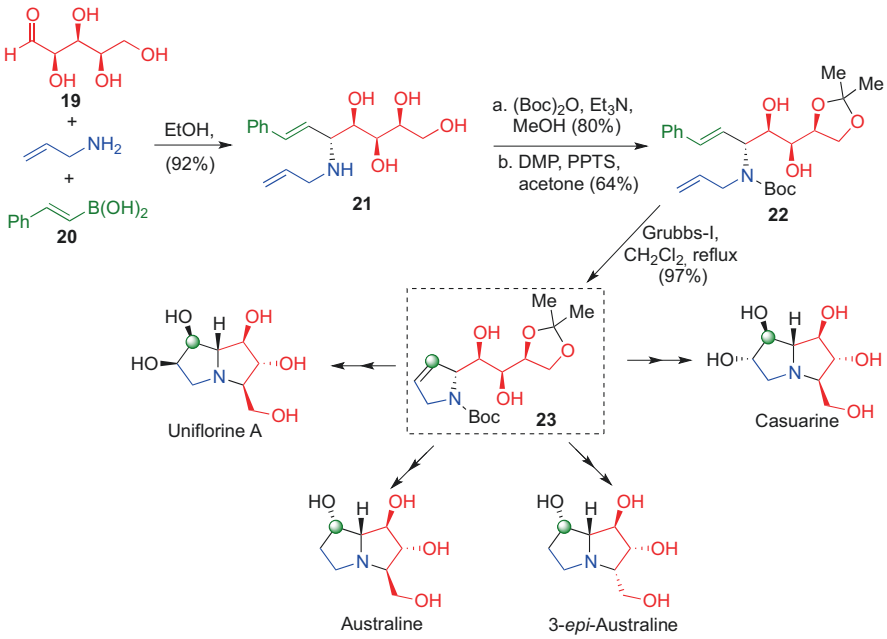

Fig. 11 Synthesis of pyrrolidine alkaloids through a Petasis-type reaction

polyhydroxylated alkaloids such as uniflorine A, casuarine, and australine (Fig. 11). These compounds are of interest as inhibitors of enzymes such as α-glucosidase and intestinal maltase (Kato et al. 2003; Li et al. 2022).

2.4 Povarov Reaction

The Povarov reaction is a versatile and efficient method that gives access to the tetrahydroquinoline scaffold via an inverse electron-demand hetero Diels–Alder cycloaddition between *N*-arylamines and electron-rich olefins, generally catalyzed by Lewis or Brönsted acids (Ghashghaei et al. 2018). A large variety of dienes and dienophiles are suitable substrates for this reaction, and enantioselective variations have been thoroughly explored (Clerigué et al. 2022; Lemos et al. 2022). Two types of mechanism are being considered for the Povarov reaction starting from the initial formation of an imine (Palacios et al. 2010; Ríos-Gutiérrez et al. 2015), namely a Mannich/Friedel Crafts stepwise sequence via cationic intermediate **24**, or an asynchronous concerted process proceeding through transition state **25** (Fig. 12).

Extracts of the root of the vine *Martinella iquitosensis* have been traditionally used by Amazonic indigenous peoples to treat eye inflammation and conjunctivitis. This root contains two guanidine alkaloids derived from the hexahydropyrrolo[3,2-*c*]quinoline skeleton, namely martinellic acid and martinelline. The Povarov

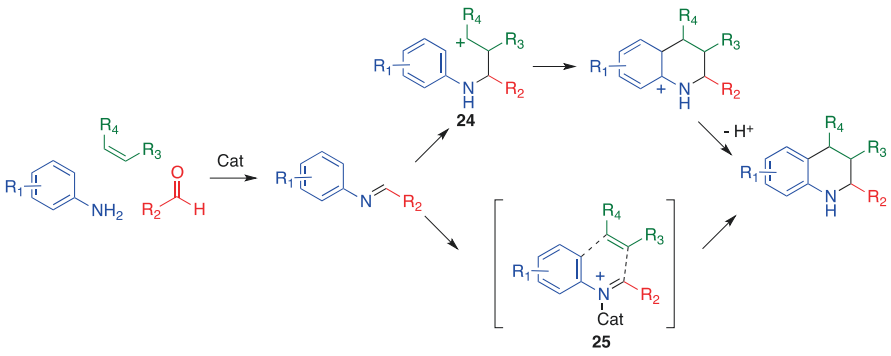

Fig. 12 The Povarov reaction and its mechanism

reaction can be readily adapted to yield fused tetrahydroquinolines by employing cyclic olefins as the dienophile component, and in this context, Powell and Batey developed an ABB'-type three-component process starting from anilines and 2 equivalents of N-protected 2-pyrrolines to give functionalized derivatives of the hexahydropyrrolo[3,2-*c*]quinoline framework. During their optimization work, they discovered that the use of camphorsulfonic acid as catalyst afforded the *exo* relative configuration characteristic of the *Martinella* alkaloids, in contrast with the *endo* configuration obtained in the presence of Lewis acids. As shown in Fig. 13, compound **26** thus obtained was transformed into (±)-martinellic acid in seven additional steps, and finally this alkaloid was also readily converted into (±)-martinelline (Powell and Batey 2002).

In a similar approach, Iwabuchi and coworkers carried out the enantioselective total synthesis of (−)-martinellic acid and (−)-martinelline by application of intramolecular Povarov chemistry (Fig. 14). The chiral cyclic imine **27** was transformed into a diastereomeric mixture of pyrroloquinolines using a Povarov reaction promoted by boron trifluoride and proceeding through the *o*-azaxylylene intermediate **28**. The *exo* major product (**29**) was subsequently transformed into the natural products (Ikeda et al. 2007).

Intramolecular Povarov reactions have also been successfully employed in natural product synthesis. For instance, Batey reported a formal total synthesis of the alkaloid camptothecin, an anticancer compound acting by topoisomerase I inhibition, that relies on an intramolecular Povarov reaction of the in situ-generated imine **30** (Fig. 15). A similar strategy was employed to achieve a brief synthesis of luotonin A, a camptothecin-related quinazoline alkaloid (Twin and Batey 2004), and also of libraries of anticancer compounds related to the latter alkaloid (Almansour et al. 2017).

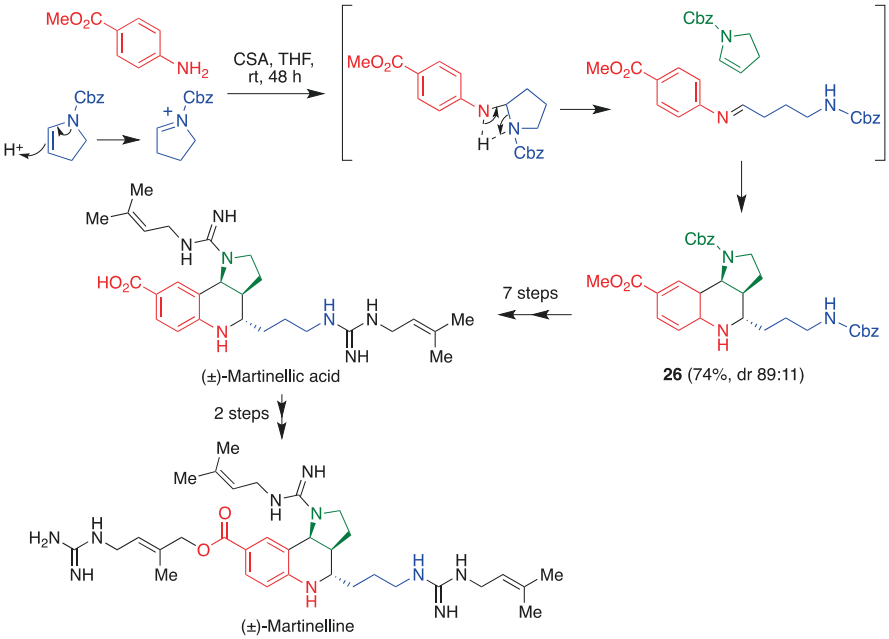

Fig. 13 Synthesis of (±)-martinellic acid and (±)-martinelline developed by the Batey group. CSA camphorsulfonic acid

3 Isonitrile-Based Multicomponent Reactions

Multicomponent reactions involving an isocyanide (isonitrile) as one of the components are particularly important in synthetic chemistry. These isocyanide-based multicomponent reactions (IMCRs) are among the first multicomponent reactions to be discovered and can be regarded as the flagship of modern multicomponent chemistry.

3.1 Passerini Reaction and Its Variants

The first isocyanide-based multicomponent reaction described in the literature was first reported by Mario Passerini in 1921 and involves the combination of an isocyanide, a carboxylic acid and an aldehyde in nonpolar solvents to give an α-acyloxyamide. It is generally accepted that this reaction follows the concerted mechanism shown in Fig. 16, followed by a Mumm rearrangement (Banfi et al. 2021).

Tubulysins are natural bacterial tetrapeptides that disrupt the microtubule spindle and are among the most potent known antimitotic agents. A tubulysin-folate conjugate (EC1456) is under clinical testing against cancer and some tubulysins have been employed as the cytotoxic component of drug-antibody conjugates. It is thus

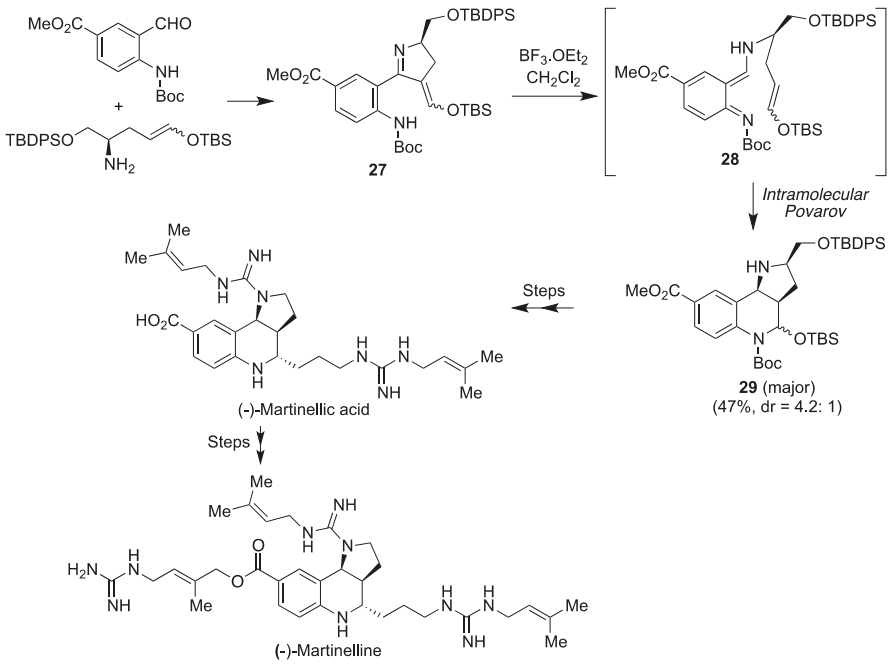

Fig. 14 Synthesis of enantiomerically pure martinellic acid and martinelline using an intramolecular Povarov reaction to generate the hexahydropyrrolo[3,2-*c*]quinoline core

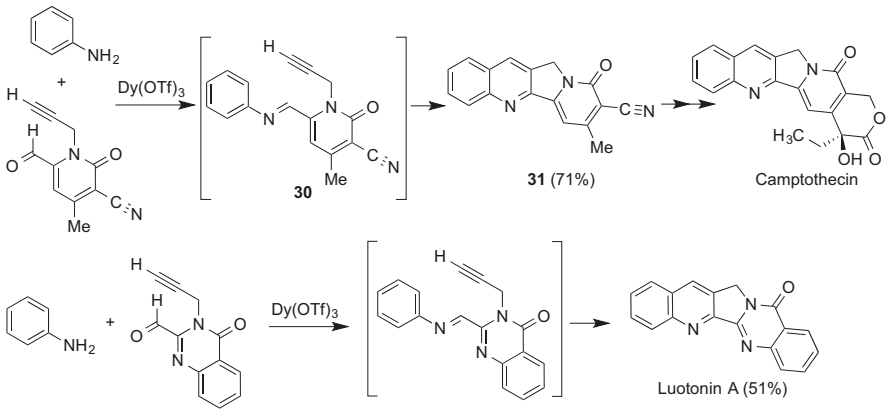

Fig. 15 Formal synthesis of camptothecin and total synthesis of luotonin A by intramolecular Povarov reactions

not surprising that tubulysin synthesis is an active area, although routes based on conventional peptide chemistry require tedious functional group manipulations while also suffering from the need for coupling sterically hindered amino acids. The Passerini reaction has simplified access to these molecules as showcased by the

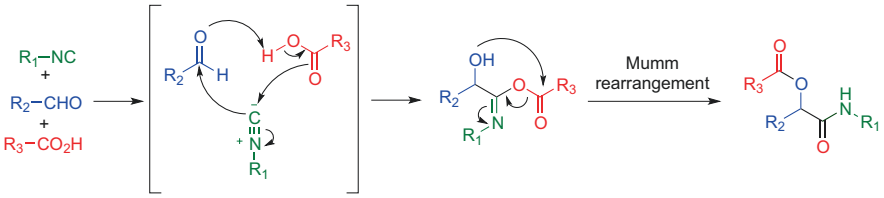

Fig. 16 The Passerini reaction

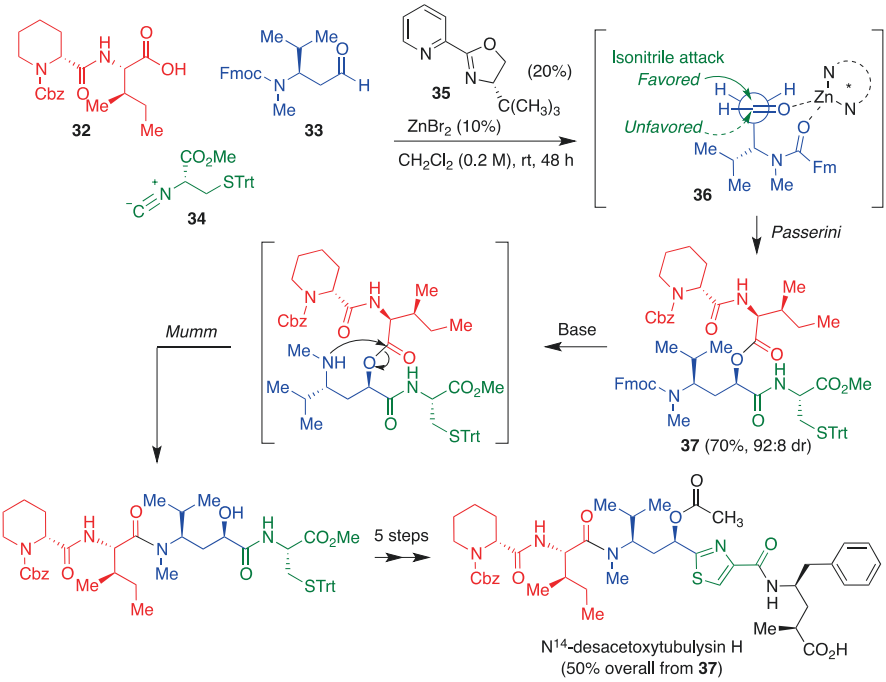

Fig. 17 Synthesis of N^{14}-desacetoxytubulysin H by Dömling, based on a Passerini reaction

concise total synthesis of N^{14}-desacetoxytubulysin H reported by Dömling (Vishwanatha et al. 2020). This route relied on the use of a chiral oxazoline additive **35**, together with zinc bromide, to induce a diastereoselective Passerini reaction of carboxylic acid **32**, aldehyde **33** and isonitrile **34** via the transition state **36**, to yield compound **37**, which was then transformed into the target molecule in six additional steps, starting with a base-promoted Fmoc deprotection/Mumm rearrangement sequence (Fig. 17).

A variation of the Passerini reaction that employs an oxime rather than an aldehyde has been employed by Ichikawa as the key step for the total synthesis of pseudouridimycin, a nucleoside antibiotic from a *Streptomyces* species with potent, broad spectrum antibacterial activity that includes drug-resistant strains (Okawa et al. 2022). The key step of this synthesis was the three-component reaction

between isonitrile **38**, oxime **39** and N-fmoc glycine to furnish compound **40**, which was transformed in three steps into the target natural product or its epimer at the asparagine center (Fig. 18).

A truncated variation of the Passerini reaction developed by Denmark which, moreover, can be made enantioselective, allows the mild one-carbon homologation of aldehydes to α-hydroxyamides or α-hydroxyesters (Denmark and Fan 2005). It involves the use of silicon tetrachloride instead of the usual carboxylic acid as the third Passerini component. This compound, acting as a weak Lewis acid, interacts with a strong Lewis base (e.g., HMPA, pyridine *N*-oxide), forming a silicon cation that activates the aldehyde for nucleophilic attack of the isocyanide to give an imidoyl chloride **41**. Aqueous workup affords α-hydroxy *tert*-butyl amides **42,** whereas a low-temperature methanol quench followed by basic workup furnishes α-hydroxy methyl esters **43** (Fig. 19).

The lapidilectine and grandilodine alkaloids have an unusual structure comprising a common pyrroloazocine indole core where the azocine ring is embedded in a rigid [4.2.2]azabicyclic structure and some of them are able to reverse multidrug resistance in vincristine-resistant cancer cells. Echavarren has reported the collective total synthesis of seven members of this family (Miloserdov et al. 2018) using an approach where the pyrroloazocine indole core was built via a gold-catalyzed 8-*endo*-dig hydroarylation that furnished aldehyde **44** after functional group manipulation. The homologation of the aldehyde to an ester-containing precursor suitable for the subsequent cyclization was carried out using the Denmark variation of the Passerini reaction (compound **45**). Finally, the azabicyclo system **46** was created via a radical 6-*exo*-trig photoredox cyclization, using again as catalyst an Au complex (Fig. 20).

Another variant of this reaction includes the Passerini reaction-amine deprotection-acyl migration (PADAM) sequence (Banfi et al. 2000), often used in the preparation of peptidomimetic compounds such as eurystatin A (Owens et al. 2001) and norstatine derivatives (Shaw et al. 2012). The process involves a Passerini condensation between a N-protected enantiomerically pure α-aminoaldehyde **47**, an

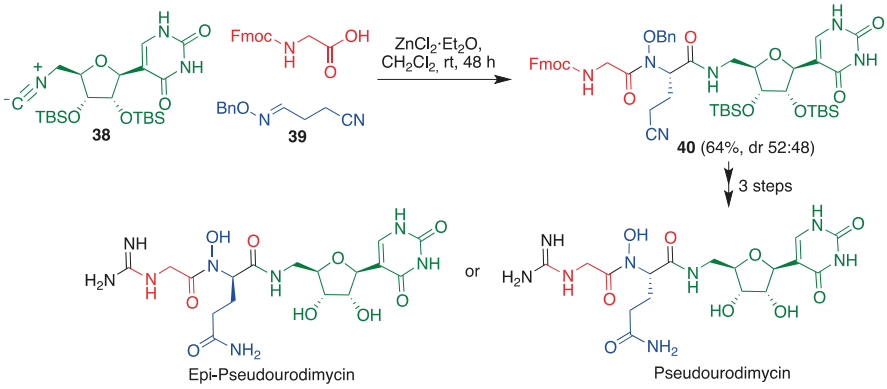

Fig. 18 Total synthesis of pseudouridimycin and an epimer

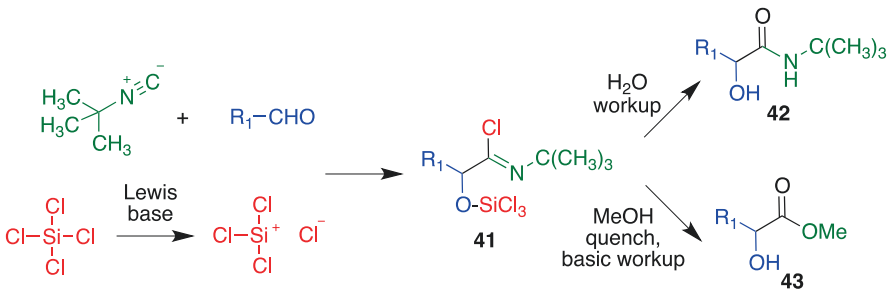

Fig. 19 Truncated Passerini reaction developed by Denmark, leading to α-hydroxyamides or α-hydroxy esters

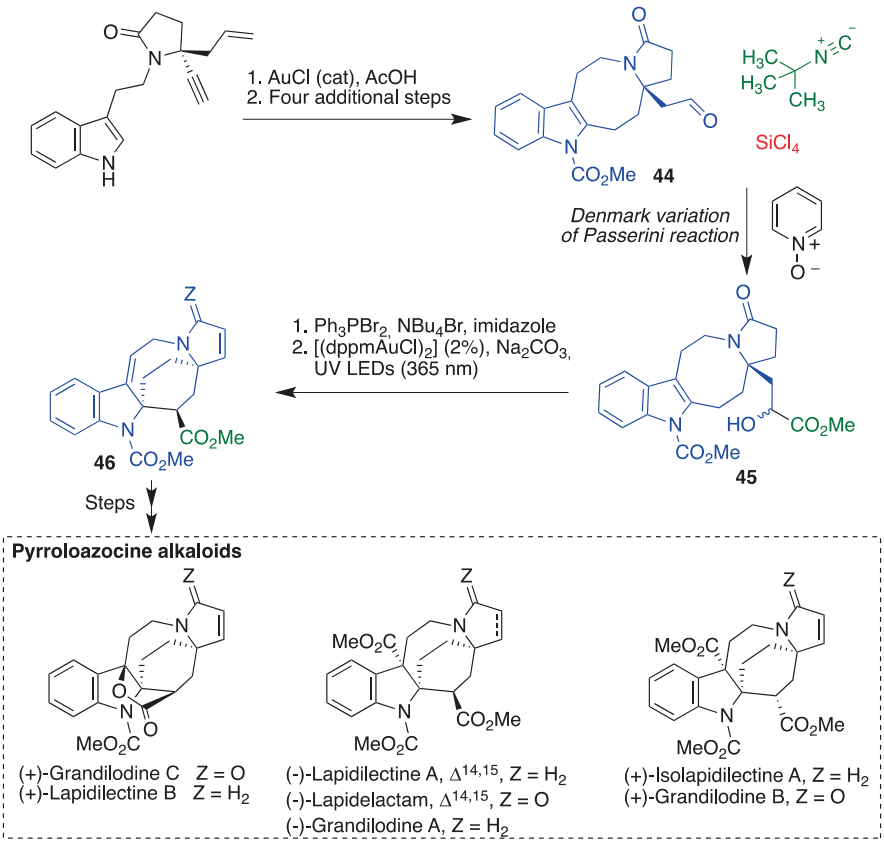

Fig. 20 Collective synthesis of seven pyrroloazocine alkaloids by Echavarren. Dppm bis(diphenylphosphanyl)methane

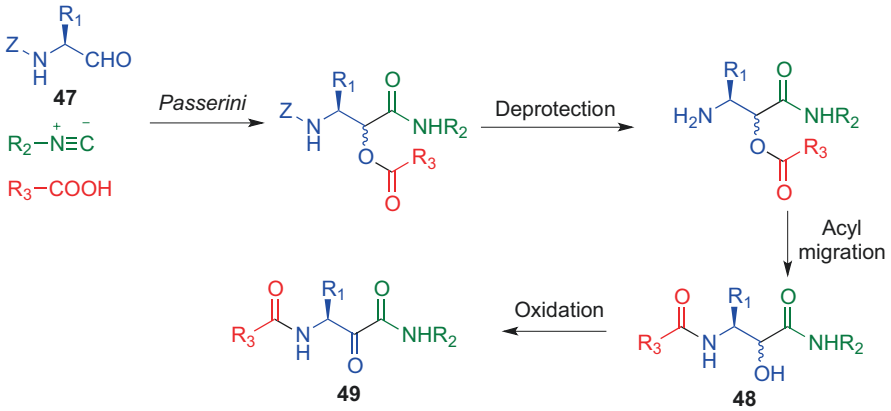

Fig. 21 The Passerini reaction-amine deprotection-acyl migration (PADAM) strategy

isonitrile and a carboxylic acid, followed by one-pot *N*-deprotection and intramolecular acyl migration to give α-hydroxy-α-acylaminoamide **48**, which can be oxidized to provide compounds **49** containing α-oxoamide and α-amino acid moieties (Fig. 21).

An interesting application of this methodology has been described by Aitken and coworkers (Faure et al. 2009) as the key step for the synthesis of cyclotheonamide C, a cyclic pentapeptide isolated from marine sponges *Theonella ircinia* and *Theonella swinhoei* that possesses potent inhibitory activity against serine proteases. The synthetic sequence involves an initial Passerini reaction of protected chiral amino acid **50**, isonitrile **51** and Fmoc-protected α-amino aldehyde **51** to afford α-acyloxyamide **52**. N-Boc deprotection induced an acyl migration that furnished pentapeptide **53**. Three additional steps that included simultaneous N/C deprotection, pentapeptide cyclization, and oxidation completed the synthesis of cyclotheonamide C (Fig. 22).

3.2 Ugi Reaction

Ivar Ugi, starting in 1959, expanded the synthetic possibilities of the Passerini reaction by introducing a primary amine as the fourth component. This process is known as the Ugi four-component reaction (U-4CR) or simply the Ugi reaction and affords dipeptide-like scaffolds, greatly increasing the potential of multicomponent reactions as tools for natural product synthesis (Fouad et al. 2020).

The mechanism of the classical 4CR Ugi reaction (Dömling 2006) starts with the condensation between the amine and the carbonyl component to give an imine that is then protonated by the carboxylic acid. The activated iminium (**54**) reacts with the isocyanide leading to an α-aminonitrilium **55**, which reacts with the carboxylate to

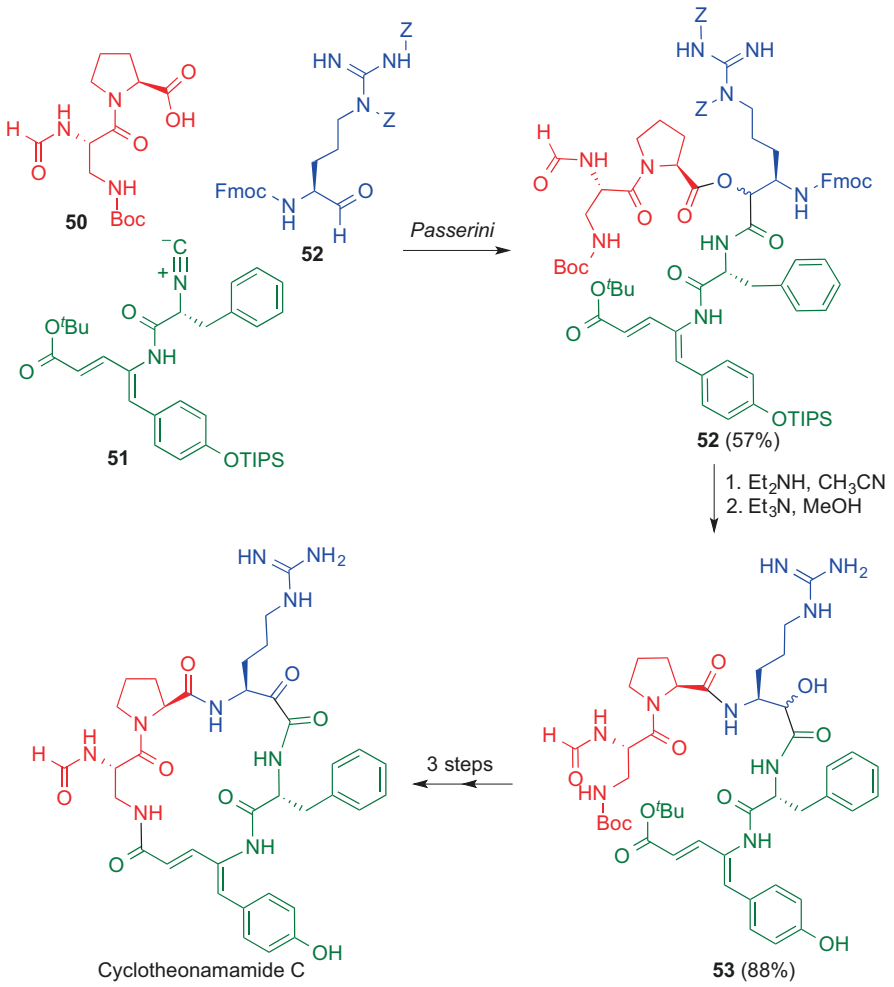

Fig. 22 Aitken's total synthesis of cyclotheonamide C. TIPS triisopropylsilyl

give **56**. Finally, the amino group promotes the Mumm rearrangement, which is irreversible and drives the equilibria to the formation of the final product (Fig. 23).

Several natural products containing glutarimide (2,6-piperidinedione) moieties show interesting bioactivities. For instance, julocrotine has in vitro antiproliferative effects against *Leishmania amazonensis*. Dömling has described an efficient synthesis of this framework and the total syntheses of representative alkaloids via an Ugi four-component reaction (Konstantinidou et al. 2018). Thus, julocrotine was obtained in four steps starting with the Ugi reaction between aldehyde **57**, chiral acid **58**, chiral amine **59** and isonitrile **60**. The reaction product was hydrolyzed to **61**, cyclized via mixed anhydride formation to **62** and finally N-deprotected to the natural product. A similar strategy was employed to obtain crotinimides A-C (Fig. 24).

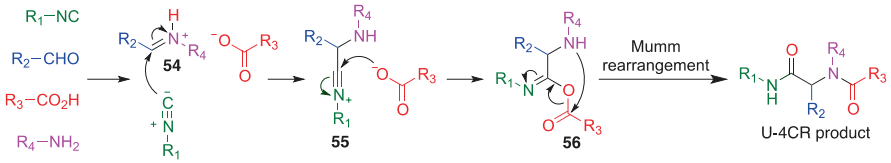

Fig. 23 The Ugi four-component reaction (U-4CR)

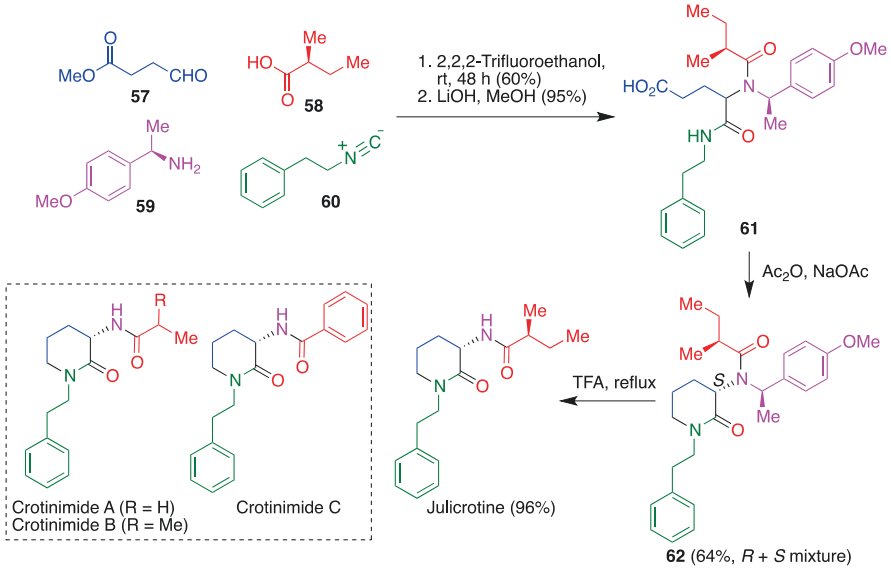

Fig. 24 Total synthesis of julocrotine and crotinimides A–C

Hemiasterlin is an antimitotic marine natural product, binding at the vinca domain between the α- and β-subunits of tubulin, and showing in vitro low- to subnanomolar potency against several cancer cell lines. Taltobulin (HTI-286) is a synthetic hemiasterlin analogue that advanced to Phase II clinical trials for the treatment of nonsmall cell lung cancer (NSCLC), although its subsequent development was halted for business reasons. Spring has described a concise synthesis of both hemiasterlin A and taltobulin based on an Ugi reaction (Fig. 25), together with an investigation of the use of both compounds as cytotoxic payloads in antibody–drug conjugates (Charoenpattarapreeda et al. 2020).

As a third example of a synthetic route to a highly complex natural product based on an Ugi reaction, this time having as an intermediate a 2,5-diketopiperazine, we will discuss a second synthesis of the anticancer agent trabectedin, due to the Fukuyama group (Endo et al. 2002). The Ugi reaction of the chiral starting materials **63** and **64**, *p*-methoxyphenyl isocyanide and acetaldehyde gave dipeptide **65**,

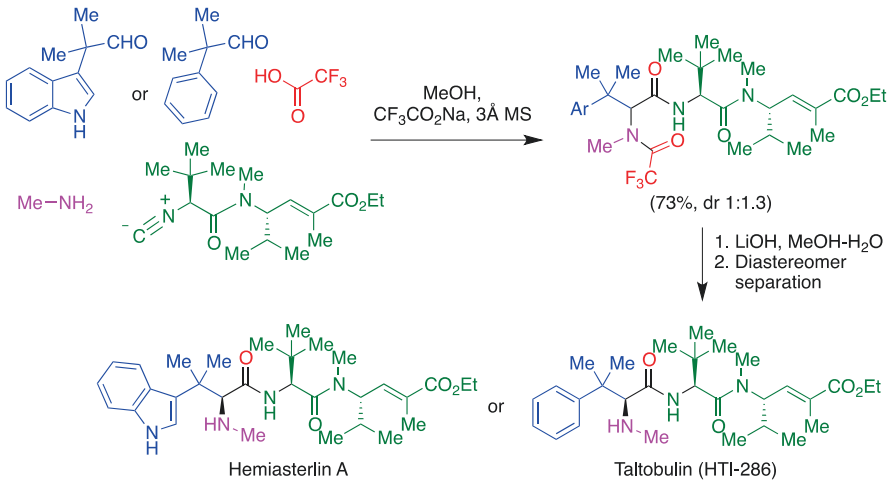

Fig. 25 Total synthesis of hemiasterlin A and taltobulin based on an Ugi reaction

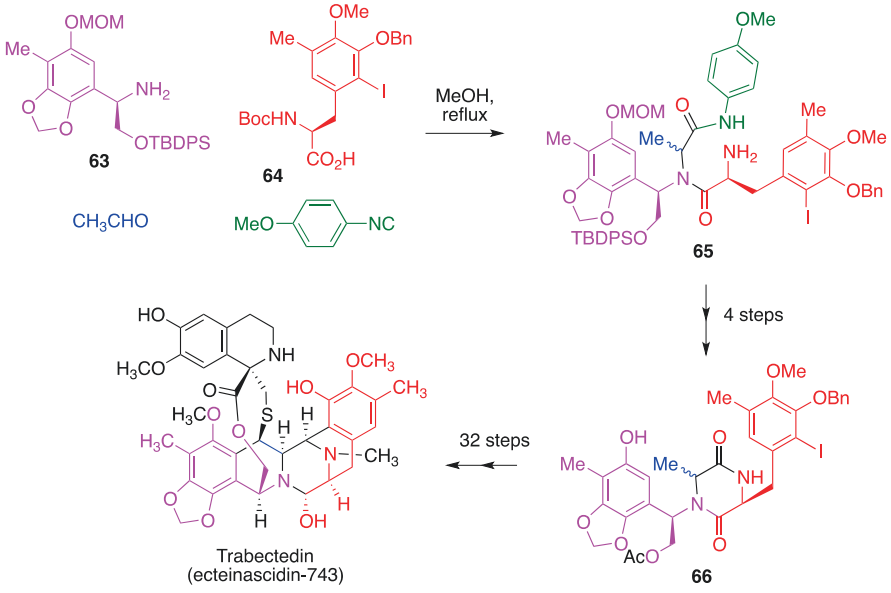

Fig. 26 Synthesis of trabectedin based on a four-component Ugi reaction

containing all the carbon atoms of the pentacyclic core of the alkaloid. This intermediate was cyclized in 4 steps to diketopiperazine **66**, which was transformed into the target natural product by a 32-steps route (Fig. 26).

Syringolin A is a member of the syrbactins, which are 12-membered macrolactams containing an *E* α,β-unsaturated carboxamide moiety and a side chain. These

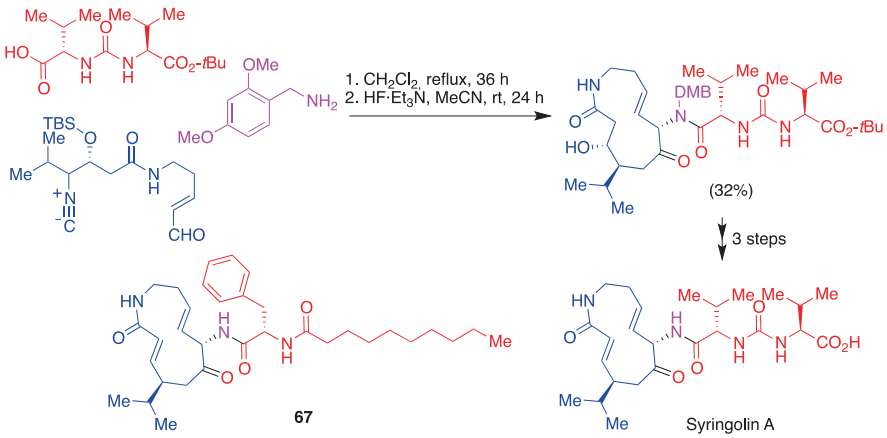

Fig. 27 Total synthesis of syringolin A based on an intramolecular Ugi four-component reaction, and structure of an analogue (**67**) with high activity as a proteasome inhibitor. DMB dimethoxybenzyl

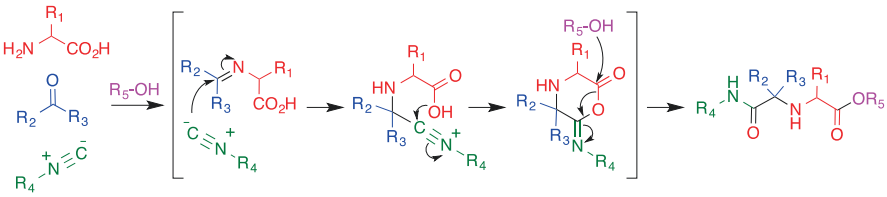

Fig. 28 The Ugi-5-center-4-component reaction (U-5C-4CR)

natural products are irreversible inhibitors of the 20S proteasome, an important anticancer target. The Ichikawa group has employed an intramolecular Ugi reaction as the key step of a total synthesis of syringolin A, as shown in Fig. 27. This chemistry was adapted to the synthesis of analogues of the natural product and yielded, among others, compound **67**, which showed ca. 1100-fold proteasome inhibition activity and ca. 750-fold cytotoxicity over the natural model (Chiba et al. 2014).

One interesting variation of the Ugi reaction is the so-called Ugi-5-center-4-component reaction (U-5C-4CR) that employs α or β-amino acids as bifunctional reagents, leading to a lactone that is finally opened by the alcohol used as solvent (Fig. 28).

An application of this reaction that led to the first total synthesis of the marine natural product exigurin from (+)-menthone is summarized in Fig. 29. The reaction between intermediate (–)-10-epi-axisonitrile-3 **68**, formaldehyde, sarcosine, and methanol constructed the target exigurin in a single step (Hosokawa et al. 2020).

A three-component version of the Ugi reaction (U-3CR or truncated Ugi reaction) starts from an aldehyde or ketone, an amine and an isocyanide to give an α-aminoamide **69**. An acid catalyst is needed, but it is not incorporated into the final

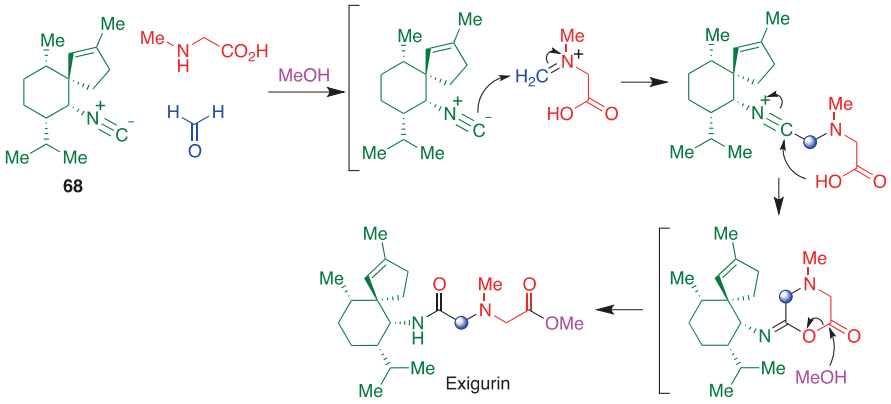

Fig. 29 Synthesis of exigurin using an Ugi-5-center-4-component reaction

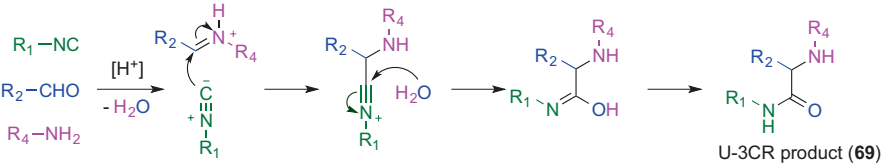

Fig. 30 The Ugi three-component reaction

product. The classical U-3CR starts as the U-4CR but, due to the absence of the carboxylic acid component, the nucleophile is the molecule of water released in the initial amine-aldehyde condensation (Fig. 30) (Flores-Reyes et al. 2021).

An application of this reaction to natural product synthesis (Avilés and Rodríguez 2010) has allowed a one-step synthesis of monamphilectine A, a terpenic α-lactam isolated from the marine sponge *Hymeniacidon* sp. and endowed with potent antimalarial activity (Fig. 31).

4 Cycloaddition-Based Multicomponent Reactions

4.1 [3 + 2] Cycloadditions

Spirotryprostatin B is a cytotoxic agent isolated from the fermentation of *Aspergillus fumigatus* that inhibits the progression of G2/M phase in mammalian tsFT210 cells (Cui et al. 1996). An approach to the synthesis of this alkaloid developed by Williams and coworkers is shown in Fig. 32. Their key step involves the preparation of spirooxoindoline **73** through a diastereoselective 1,3-dipolar cycloaddition between enantiomerically pure morpholine **70**, oxoindole **71,** and aldehyde **72**.

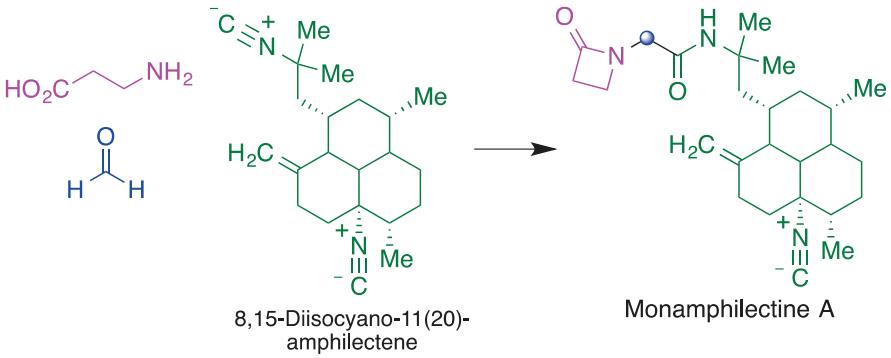

Fig. 31 Total synthesis of monamphilectine A using U-3CR chemistry

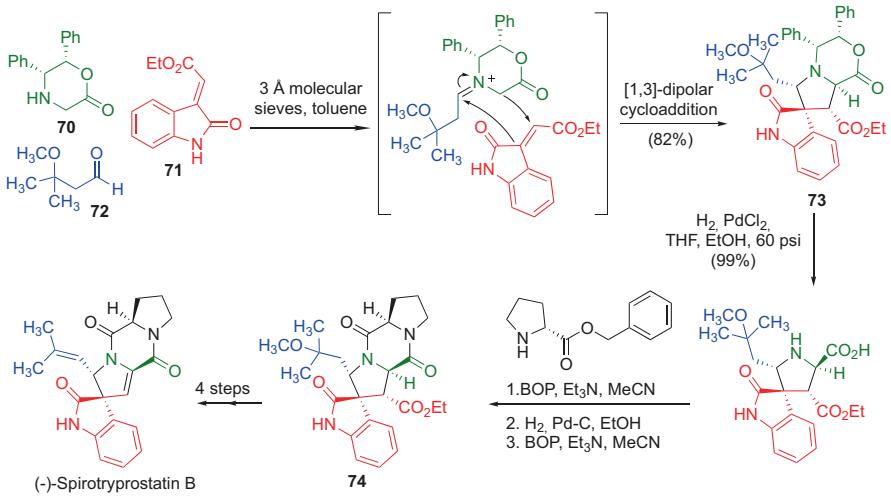

Fig. 32 Synthesis of spirotryprostatin B by Williams

Reductive cleavage of the chiral auxiliary in **73** followed by coupling with D-proline benzyl ester yielded diketopiperazine **74** through a sequence that involves amide bond formation, deprotection of the benzyl group, and intramolecular cyclization. The total synthesis of the natural product was subsequently completed in four additional steps (Sebahar and Williams 2000).

Garner has developed a three-component synthesis of highly functionalized pyrrolidines catalyzed by silver(I), usually described as the [C + NC + CC] coupling (Garner et al. 2006). The starting materials are an aldehyde (the "C" component), an amine (the "CN" component), which also contains Oppolzer's camphorsultam as a chiral auxiliary, and an olefin (the "CC" component). The domino process is initiated by the formation of an imine, which generates an azomethine ylide in the presence of the Ag(I) cation with assistance from the chiral auxiliary. Finally, a

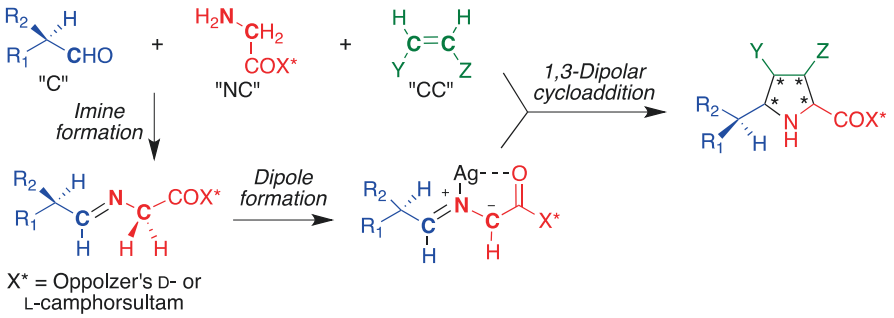

Fig. 33 Garner's Ag^{I}-catalyzed asymmetric [C + NC + CC] coupling reaction

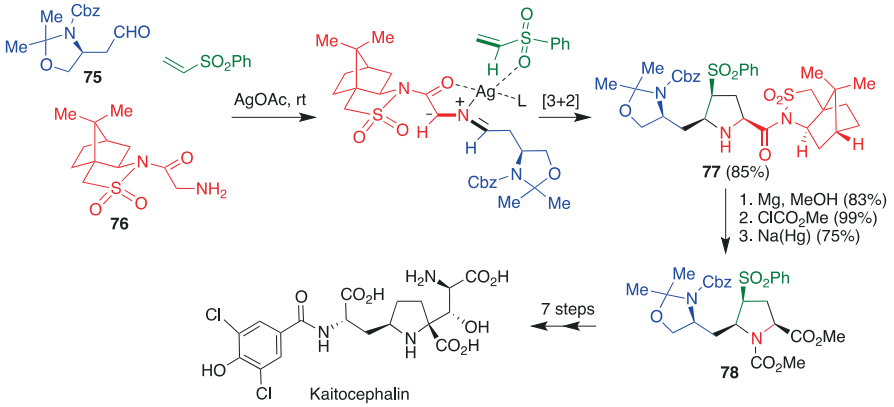

Fig. 34 Total synthesis of kaitocephalin based on the [C + NC + CC] coupling

1,3-dipolar cycloaddition with the olefin yields the pyrrolidine product (Fig. 33). The advantage of this reaction over alternative [3 + 2] cycloaddition methodologies is that it allows the use of aliphatic aldehydes without side processes resulting from enolization or enamine formation.

Kaitocephalin, isolated from the fungus *Eupenicillium shearii*, is a potent competitive antagonist of ionotropic glutamate receptors that was efficiently synthesized using the route summarized in Fig. 34. The [C + NC + CC] coupling of the aspartic acid-derived aldehyde **75**, (*S*)-glycyl Oppolzer's sultam **76** and vinyl sulfone was carried out at room temperature in the presence of Ag(I) acetate and afforded pyrrolidine derivative **77** in 85% yield. Subsequent methanolysis of the acyl sultam using $Mg(OMe)_2$, *N*-carbamoylation, and reductive cleavage of the phenylsulfonyl group with sodium amalgam afforded compound **78**, which was transformed into kaitocephalin in seven additional steps (Garner et al. 2014).

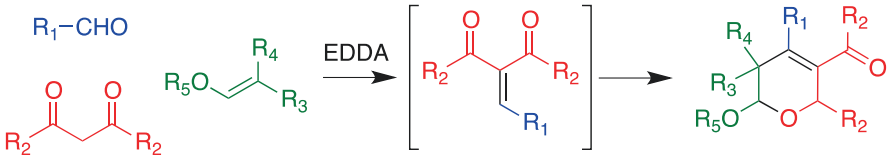

Fig. 35 The Knoevenagel/Hetero Diels–Alder domino sequence. EDDA ethylene diammonium diacetate

4.2 Knoevenagel/Hetero Diels–Alder Domino Sequence

The Tietze group (Tietze and Rackelmann 2004, 2005) developed a three-component base-promoted reaction of aldehydes, 1,3-dicarbonyl compounds and enol ethers, affording functionalized dihydropyran derivatives through a Knoevenagel condensation/oxa Diels–Alder cycloaddition domino sequence (Fig. 35).

Using this chemistry, the Tietze group has achieved the synthesis of representatives of several families of bioactive alkaloids. Thus, the synthesis of emetine, an *Ipecacuanha* alkaloid employed as an antiprotozoal and an emetic, and the *Alangium* alkaloid tubulosine has been achieved using a three-component domino Knoevenagel–hetero Diels–Alder reaction as one of the key steps (Tietze et al. 2004). Enantiomerically pure aldehyde **80**, prepared by enantioselective hydrogenation of the imino group in the dihydroquinoline derivative **79**, was treated with Meldrum's acid and enol ether **81** in the presence of a catalytic amount of ethylene diammonium diacetate led to **82** after a Knoevenagel–hetero Diels–Alder sequence and thermal fragmentation of the dioxinone ring. The cycloadduct **82** was treated in crude state with methanolic potassium carbonate and a catalytic amount of Pd/C, first in an inert atmosphere and afterward under hydrogen to remove the carbobenzoxy protection to give the benzoquinolizidine derivative **83** (together with two additional diastereomers), which was transformed into the target alkaloids using straightforward sequences of reactions (Fig. 36).

5 Multicomponent Reactions Based on Aryne Intermediates

Dehydroaltenuene B is an antibacterial compound isolated from marine fungal species from the *Tubeufiaceae* family. Drawing on their expertise in aryne chemistry, Barrett and coworkers described the first total synthesis of this natural compound through an elegant route that involved a four-component aryne coupling as the key step (Soorukram et al. 2008). Their route starts with the generation of benzyne **84** from 1-fluoro-3,5-dimethoxybenzene by lithiation-elimination. Then, Grignard reagent **85** was added to form regioselectively the arylmagnesium derivative **86**, on which a carboxylation reaction followed by a diastereoselective iodolactonization of the arylcarboxylate **87** was carried out in a one-pot procedure to obtain

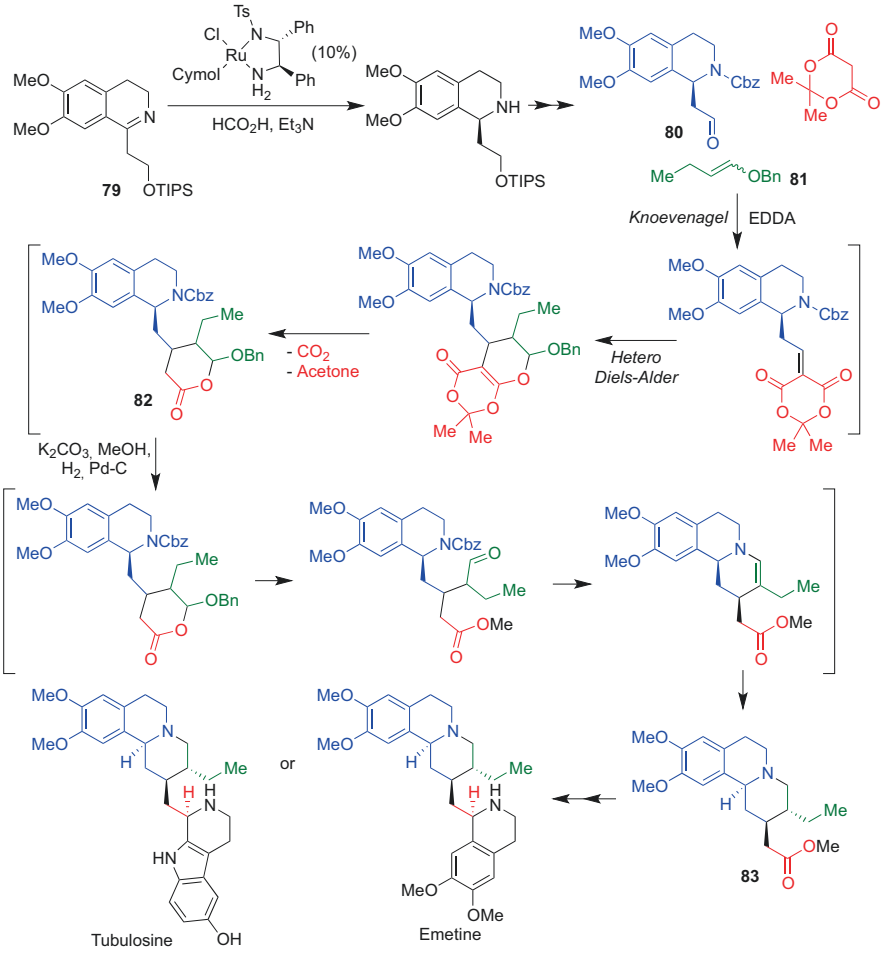

Fig. 36 Total synthesis of emetine and tubulosine by Tietze, featuring a domino Knoevenagel–hetero Diels–Alder reaction. EDDA ethylene diammonium diacetate

compound **88**. The tricyclic product was then subsequently transformed into dehydroaltenuene B over six steps (Fig. 37).

The same approach was employed for the synthesis of the enantiomer of clavilactone B, a potent natural kinase inhibitor. As shown in Fig. 38, the three-component aryne coupling of compounds **89**, **90**, and **91** afforded the advanced intermediate **92**, which was transformed into the target compound, with the complete synthesis comprising only ten steps (Larrosa et al. 2006).

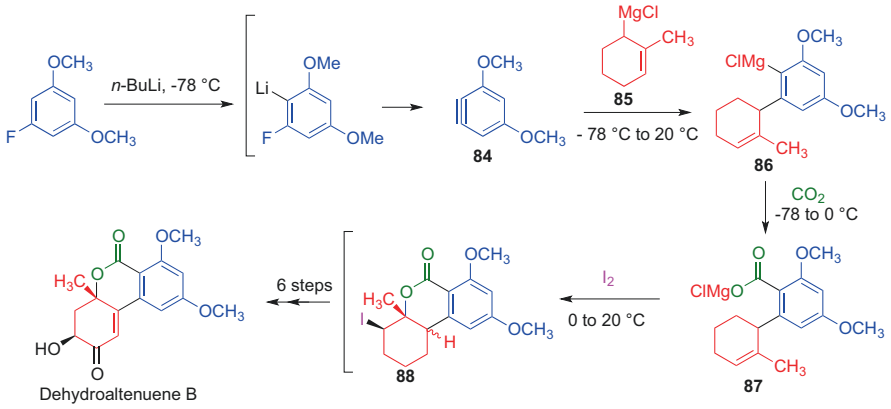

Fig. 37 Barrett's synthesis of dehydroaltenuene B

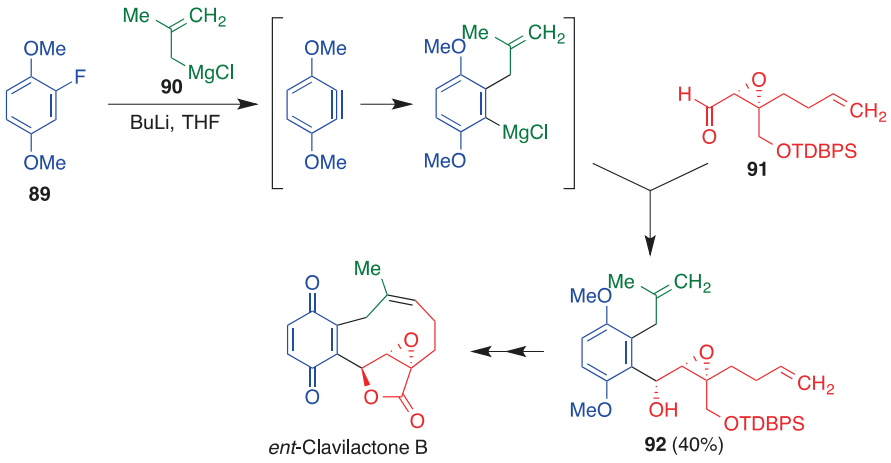

Fig. 38 Total synthesis of *ent*-clavilactone B

6 Anion Relay Chemistry

Anion relay chemistry (ARC) is a powerful synthetic tool to prepare complex structures in a single operation. According to the classification proposed by Smith, anion relay reactions can be divided into two classes, involving negative charge migration "through-bonds" or "through-space" (Smith and Wuest 2008). Through-bonding anion relay involves a charge transfer through the bond system of the molecule, as in the case of 1–4-conjugate addition reactions where anionic charge is transferred through the unsaturated α-system of the molecule (Fig. 39a).

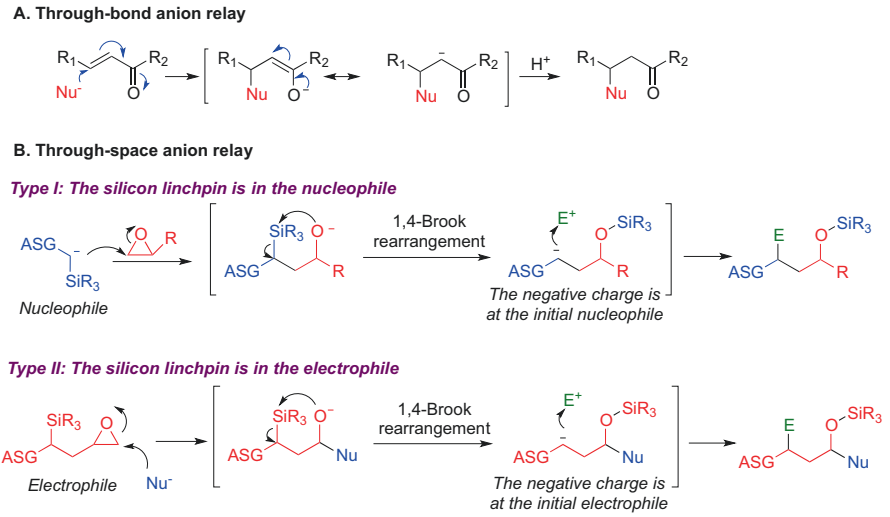

Fig. 39 Classification of anion-relay reactions: A. Through-bond anion relay processes. B. Through-space anion relay processes

On the other hand, through-space anion relay involves the migration of a group resulting in the breaking of a σ-bond and development of anionic charge at the adjacent carbon where the σ-bond was broken, as happens in the Brook rearrangement wherein negative charge of the generated alkoxide is transferred to the adjacent carbon via silyl group migration through the formation of a hypervalent pentacoordinate silicate species. Through-space anion relay reactions are further divided into types I and II, differentiated by type of linchpin used (the coupling partner containing the migrating group) and placement of the anionic charge after the relay. Thus, in a type I anion relay process, the linchpin is the nucleophile that initiates the attack to an electrophile, and after the coupling the negative charge goes back to its initial position on the linchpin. In type II additions, a nucleophile is added to a linchpin derivative, which acts as an electrophile, forming an anionic intermediate. On the anion formed, the transfer of the silyl group and the migration of the negative charge to a new locus on the linchpin takes place (Fig. 39b).

Anion relay chemistry has many desirable features, including the ability to build complex molecular architectures and a good control of their stereochemical features, especially in the case of through-space anion relay processes. For this reason, it has special importance in the synthesis of natural products and has been exploited by many groups, most notably that of Smith (Deng and Smith 2020). We will describe some representative examples, classified according to the above-mentioned scheme.

6.1 Type I Anion Relay Chemistry

Mycoticins are polyene macrolides isolated from *Streptomyces ruber* that show antibiotic activity. The first total synthesis of mycoticin A was described by Schreiber in 1993 (Poss et al. 1993) through a route that had acetonide **101** as a key intermediate. Smith and Pitram approached the synthesis of this intermediate by means of anion-relay chemistry (Smith and Pitram 1999), beginning with the deprotonation of silyl dithiane **93** with ^{t}BuLi, followed by alkylation with epoxide **94**. The alkoxy intermediate **95** thus generated was treated in situ with HMPA to promote the migration of the silyl group and afford carbanion **96**. Then, 1 equivalent of bis-epoxide **97** was added giving adduct **98** through a one-pot, five-component coupling process. The transformation of **98** into the Schreiber intermediate **101** required 7 additional steps, including the protection of the diol as an acetonide followed by the elimination of the silyl group and the hydrolysis of the dithiol to obtain compound **99**. Its reduction with sodium borohydride and subsequent reaction with 2,2-dimethoxypropane yielded triacetonide **100**, which by hydrogenolysis of the O-benzyl groups and subsequent protection of one of the alcohols as a silyl derivative afforded the target **101** (Fig. 40), thus completing a formal total synthesis of the natural product with an improved step count over the original route.

The type-I anion relay chemistry developed by Smith and coworkers has led to the synthesis of several additional natural products such as spongistatin 1 (Smith et al. 2001a, b, 2008), spongistatin 2 (Smith et al. 2009), enigmazole A (Ai et al. 2015), indolizidine 223A, and alkaloid 205B (Smith and Kim 2006) (Fig. 41).

6.2 Type II Anion Relay Chemistry

Secu'amamine A is an indolizidine alkaloid isolated from *Securinega suffruticosa* var. *amamiensi* by Ohsaki (Ohsaki et al. 2003). Its chemical structure consists of an unsaturated azabicyclo[3.3.1]nonane core fused with piperidine and butanolide rings. In view of their atypical skeleton and the varied biological activities of this family of alkaloids, several research groups, including that of Weinreb (Liu et al. 2008), have reported the total synthesis of secu'amamine A. In this connection, the Smith group reported a four-component Type II anion relay reaction allowing the synthesis of tetracyclic compound **110**, an intermediate of Weinreb's route (Haan and Smith 2015). The synthesis begins with alkylation of dithiol **102** by reaction with (*R*)-linchpin **103** in the presence of potassium *tert*-butoxide and lithium *tert*-butyl. The alkoxide **104** formed undergoes a solvent-mediated Brook rearrangement and gives rise to a new nucleophile **105** which initiates a controlled nucleophilic attack on the aldehyde **106** via Felkin-Anh control to give **107**. Its in situ reaction with methoxymethyl bromide leads to the four-component adduct **108** in one pot. The sequence continued with the cyclization of **108** to indolizine **109**, followed by five additional steps that yielded Weinreb's intermediate **110** and completed the formal synthesis of the alkaloid (Fig. 42).

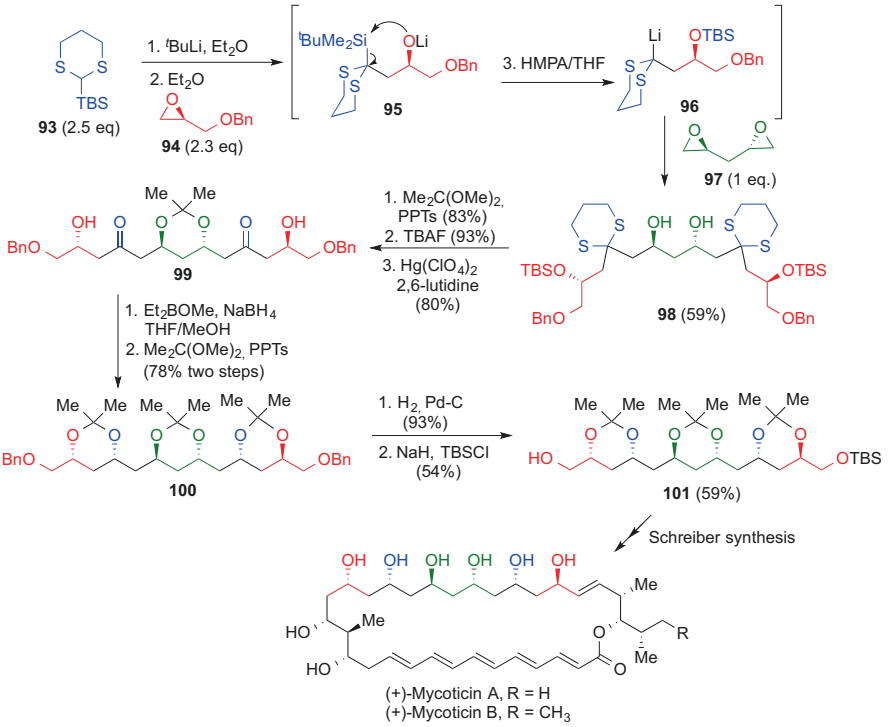

Fig. 40 Formal total synthesis of (+)-mycoticin A. HMPA hexamethylphosphoramide, PPTS pyridinium *p*-toluenesulfonate, TBAF tetrabutylammonium fluoride, TBSCl *tert*-butyldimethylsilyl chloride

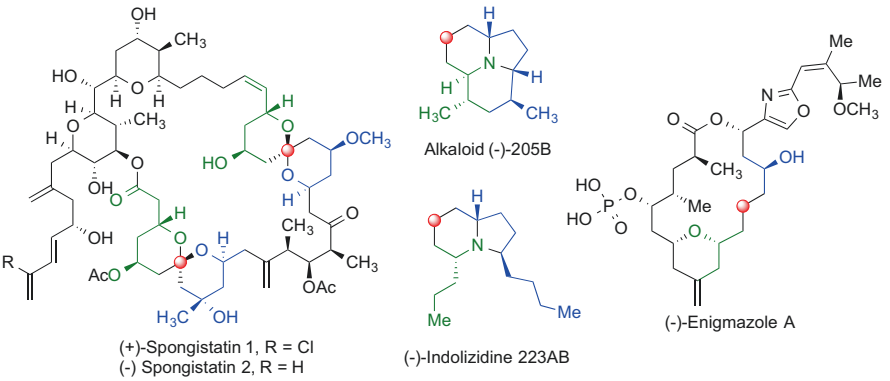

Fig. 41 Some representative examples of natural products obtained by type I anion relay chemistry

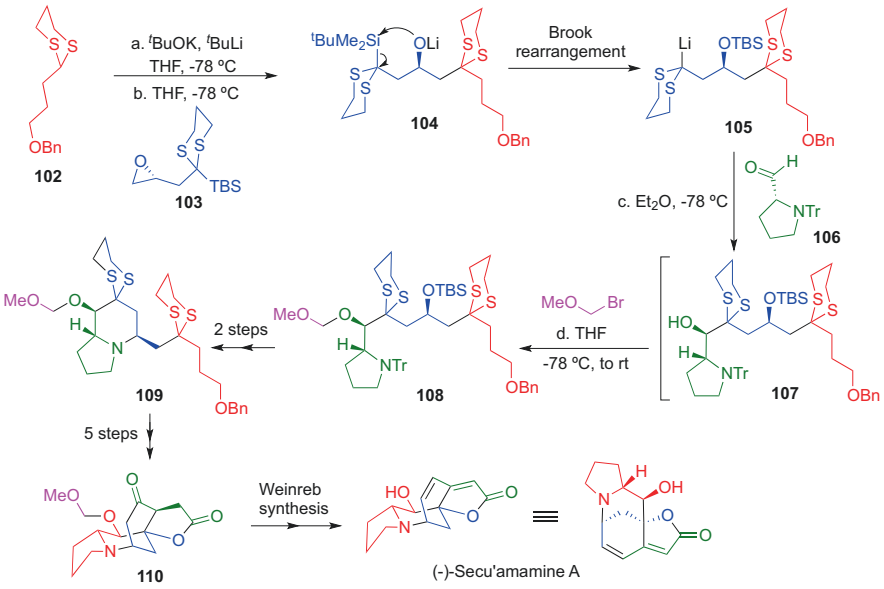

Fig. 42 Formal total synthesis of (−)-secu'amamine A reported by Smith

6.3 Combination of Type I and Type II Anion Relay Chemistry

Mandelalide A is a glycosylated macrolide isolated from a species of *Lissoclinum* that exhibited a potent cytotoxic activity (Sikorska et al. 2012). For its synthesis, Smith and coworkers applied a route that relied on the construction of both hemispheres of the molecule using type I and type II anion relay chemistry, respectively (Nguyen 2016). Thus, the preparation of fragment **117** involved a three-component type II anion relay chemistry reaction between dithiol **111,** vinyl epoxide **112** and acetal **114** via formation of the intermediate anion **113** followed by Brook rearrangement. Nucleophilic attack onto electrophile **114** leads to the formation of intermediate **115** through a cyanide-mediated cross-coupling reaction. Then, deprotection of **115** with tetra-n-butylammonium fluoride (TBAF) yields the unprotected alcohol **116**, which was transformed into iodovinyl derivative **117** in ten additional steps (Fig. 43).

Using four-component type I anion relay chemistry, an intermediate corresponding to the southern hemisphere of the molecule (compound **122**) was obtained through a sequence of reactions involving the alkylation of dithiane **93** by reaction with the epoxide **118**, (S)-epichlorohydrin **119**, and vinylmagnesium bromide **120** in a single step. The preparation of tetrahydropyran **122** was completed by a 10-step sequence of reactions (Fig. 44).

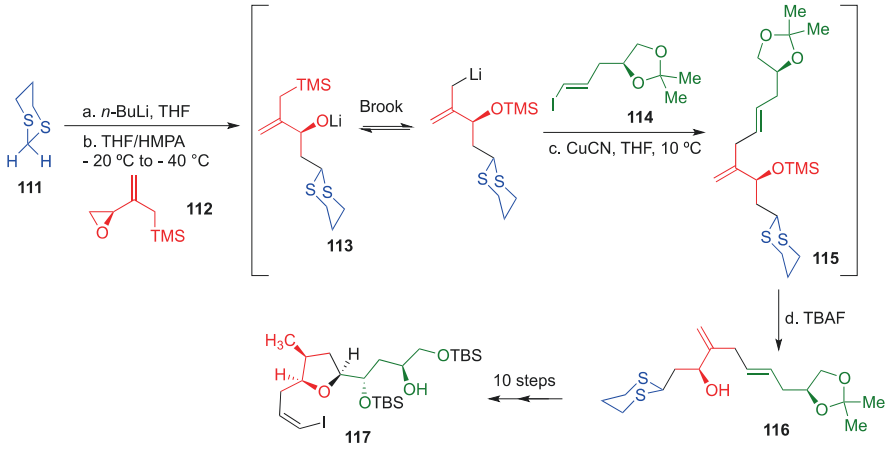

Fig. 43 Preparation of the mandelalide A northern hemisphere (**117**) using type II anion relay chemistry. TBAF tetrabutylammonium fluoride

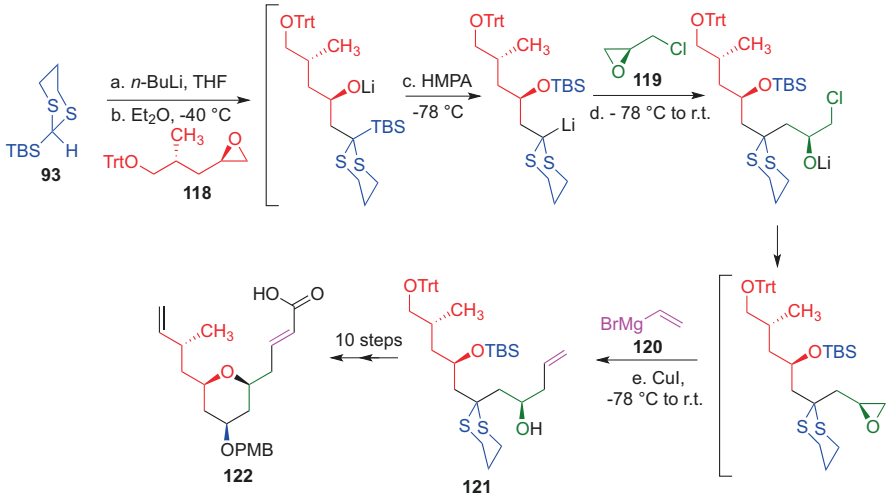

Fig. 44 Preparation of the mandelalide A southern hemisphere (**122**) through type I anion relay chemistry. Trt trityl (triphenylmethyl)

With both hemispheres of the natural product in hand, the final steps involved their coupling via a Yamaguchi esterification to give compound **123**, followed by Kahne glycosylation with sulfoxide **124** and macrocyclic ring closure by an intramolecular Heck reaction (Fig. 45).

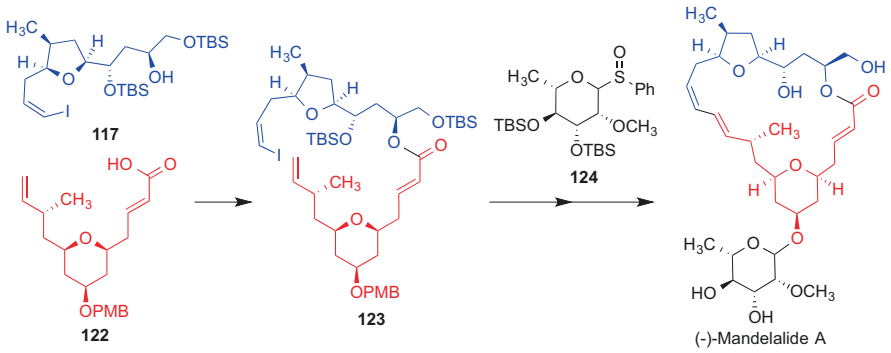

Fig. 45 Final steps of the Smith synthesis of (−)-mandelalide A

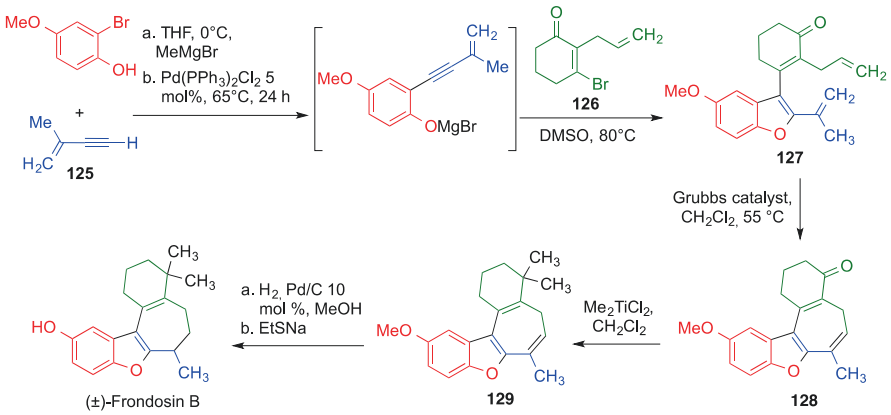

Fig. 46 Total synthesis of (±)-frondosin B described by Flynn

7 Transition Metal-Catalyzed Multicomponent Reactions

7.1 Palladium-Catalyzed Reactions

Frondosin B is a sesquiterpenoid isolate from marine sponge *Dysidea frondosa* which behaves as an antagonist of the interleukin-8 (IL-8) receptor. Although several groups of chemists have achieved its total synthesis, in the context of multicomponent reactions it is noteworthy the strategy developed by Flynn and coworkers and summarized in Fig. 46 (Kerr et al. 2004). The route begins with the preparation of benzofuran **127** in a palladium-catalyzed Kumada-type coupling between 2-bromo-4-methoxyphenol and enyne derivative **125**, followed by the addition of bromocyclohexanone **126**. A metathesis reaction using the Grubbs ruthenium catalyst yields tetracycle **128**. The final steps to obtain the natural compound include the

transformation of the ketone into the dimethyl derivative **129**, the subsequent selective hydrogenation of C_6-C_7 double bond and a final *O*-demethylation.

The Catellani reaction, described in 1997, is an interesting method to achieve the synthesis of highly substituted arenes. The typical reaction, summarized in Fig. 47, involves an aryl iodide **130**, an alkyl halide **131** and a terminal olefin **132** in the presence of norbornene and palladium as cocatalysts to furnish the *ortho* and *ipso* functionalized derivate **133** in one pot (Fig. 47a) (Catellani et al. 1997). The accepted mechanism of this reaction includes numerous steps including the formation of C,C-palladacycle **134**, which allows the C-H bond activation at *ortho* position for its subsequent alkylation, and a Mizoroki–Heck reaction to incorporate the olefin (Fig. 47b). Since its discovery, numerous variants of this method have been developed, greatly increasing its versatility (Dong and Luan 2021).

Lauten and coworkers reported the total synthesis of the lignan linoxepin, isolated from *Linum perenne* and structurally similar to anticancer lignans such as etoposide and podophyllotoxin (Motyka et al. 2023). This achievement represented the first synthesis of a complex natural product in enantiomerically pure form using

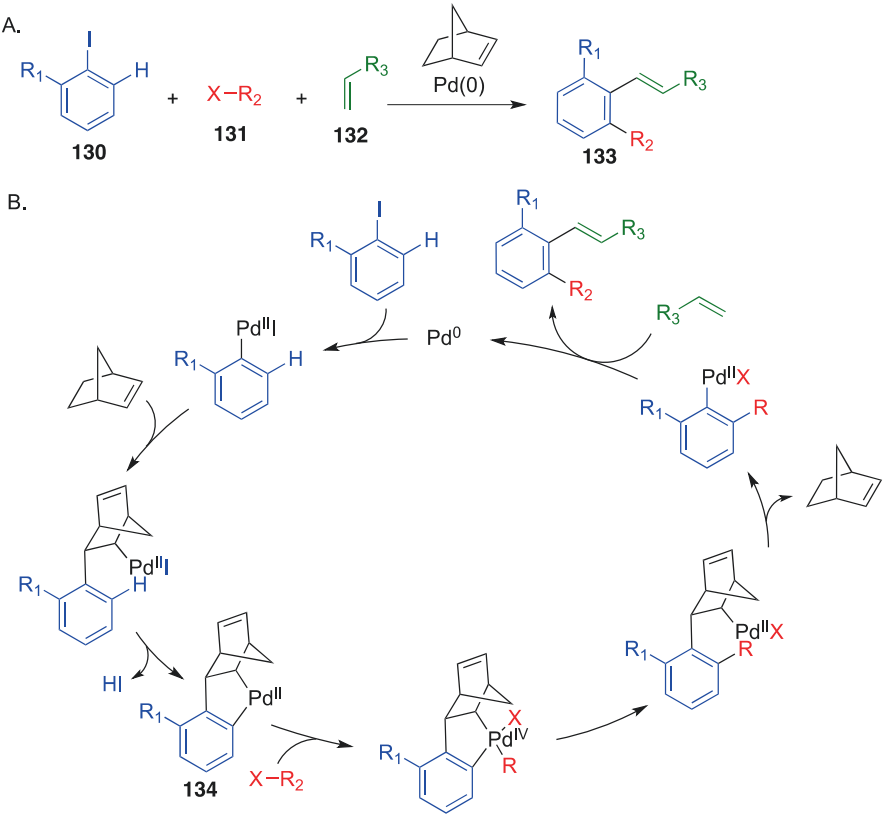

Fig. 47 A. The Catellani reaction and B. its proposed mechanism

a Catellani reaction, and is summarized in Fig. 48. The preparation of unsaturated ester **137** was attained through a direct three-component Catellani coupling reaction between aryl iodide **135**, iodolactone **136** and *tert*-butyl acrylate. The next step involved the preparation of oxidative cleavage of the olefin to give **138**, which was followed by intramolecular aldol condensation to give **139**. Finally, the total synthesis of linoxepin was completed by generation of the seven-membered oxepane ring via an intramolecular Mizoroki–Heck reaction (Weinstabl et al. 2013; Qureshi et al. 2014).

More recently, a three-component Catellani type reaction, combined with an oxa-Michael reaction, was applied by Qu, Zhou, and coworkers to achieve a concise synthesis of (−)-berkelic acid, a spiroketal isolated from a *Penicillium* fungus that exhibits a high cytotoxicity against the ovarian cancer cell line OVCAR-3 (Cheng et al. 2021). To this end, a mixture of iodobenzoate **140**, chiral epoxide **141**, and enone **142** was heated at 60 °C in the presence of palladium and norbornene as catalysts. Cesium carbonate was then was added in order to promote the intramolecular oxa-Michael reaction that closed the chromane system, yielding compound **143** as a mixture of diastereoisomers. Consecutive deprotection of the benzyl groups by catalytic hydrogenation and of the acetonide in acidic conditions induced the formation of the spiroketal **144** as the major diastereoisomer, which was transformed into berkelic acid in three steps (Fig. 49).

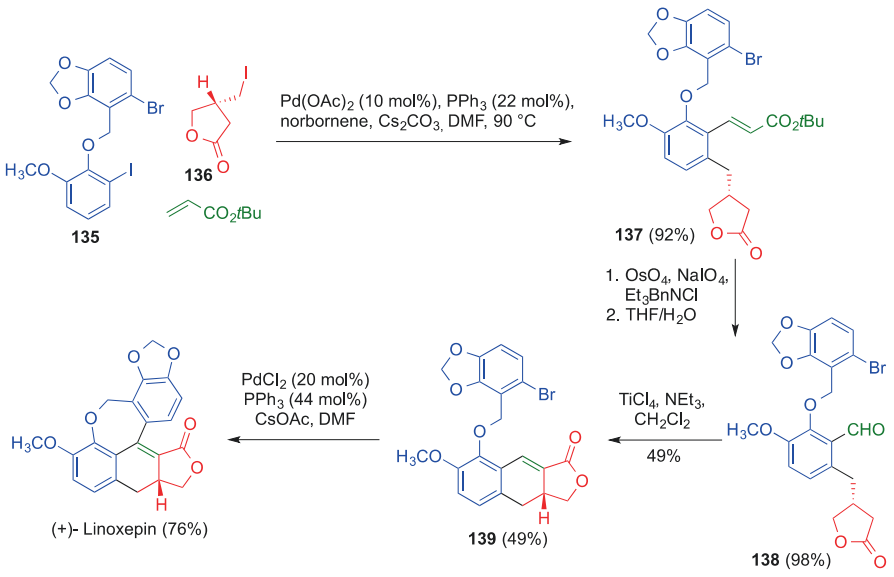

Fig. 48 Total Synthesis of (+)-linoxepin using a Catellini coupling as the key step

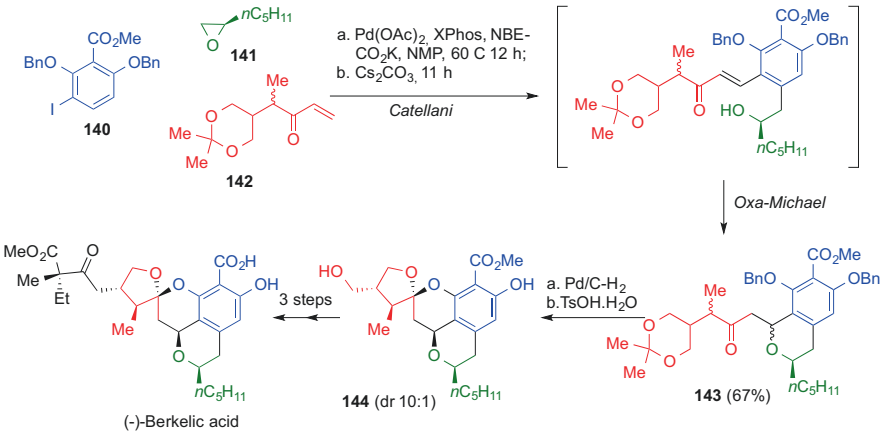

Fig. 49 Synthesis of (−)-berkelic acid developed by Qu, Zhou and coworkers

7.2 Copper-Catalyzed Reactions

Rottnestol, a cyclic hemicetal isolated from marine sponges of the genus *Haliclona* sp., was synthesized by Hoveyda and coworkers using a copper-catalyzed sequential three-component reaction as the key step (Meng et al. 2014). Thus, bis(pinacolato) diboron ($B_2(pin)_2$) **145** was mixed with chiral ligand **146**, copper chloride and potassium *tert*-butoxide as a base to afford the N-heterocyclic carbene-Cu-B(pin) complex **147**. Then, allene **148** and allylic phosphate **149** were added to the same reaction medium, yielding the *Z* adduct **150** as the major product (98:2 er). Treatment of **150** with methyllithium and iodine resulted in the substitution of the C-B bond by a C-Methyl, with complete isomerization of the double bond (>98% *E*), as shown in Fig. 50. On the other hand, the reaction between allene **153**, aldehyde **154**, and bis(pinacolato) diboron ($B_2(pin)_2$) in presence of chiral ligand **155** and cooper chloride, followed by silicon protection of the hydroxy substituent, afforded silyl derivate **156**, from which an additional four-step sequence led to the advanced intermediate **157**. Then, a N-heterocyclic carbene-Cu-catalyzed protoboration of the alkyne group in **157** provided compound **158**, which was finally coupled with the iodo derivative **152** previously obtained under palladium catalysis, followed by acidic acetal hydrolysis, to afford the natural compound (Fig. 51).

The Hoveyda group used a similar protocol in their synthesis of herboxidiene (Fig. 52). This polyketide, isolated from a *Streptomyces chromofuscus* A7847 cluster, exhibits a wide range of activities such as antitumor, herbicidal, and anticholesterol (Thirupathi and Zilla 2019). For its synthesis, a three-component reaction cooper-catalyzed reaction was carried out between allene **159**, allyl derivate phosphonate **160**, and bis(pinacolato) diboron ($B_2(pin)_2$) **145** in the presence of ligand **146** and cooper chloride to achieve compound **161**. Its reaction with methyllithium

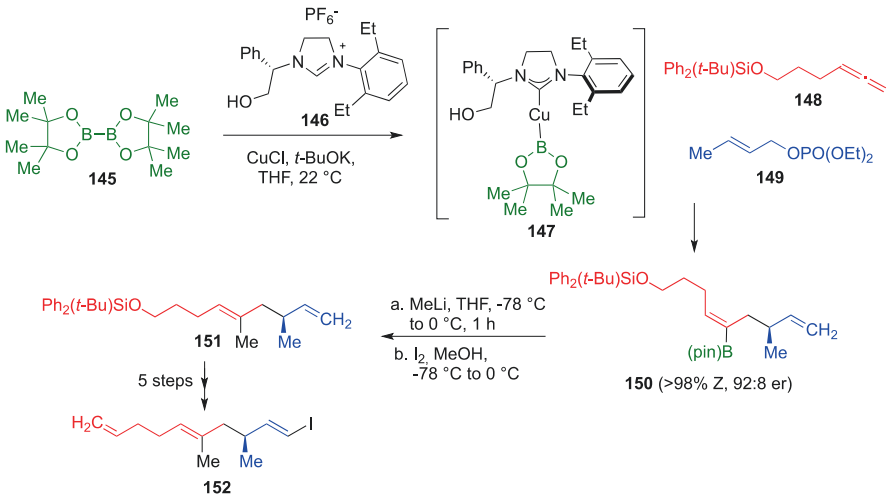

Fig. 50 Preparation of fragment **152** based on a copper-catalyzed three-component reaction; er, enantiomeric ratio

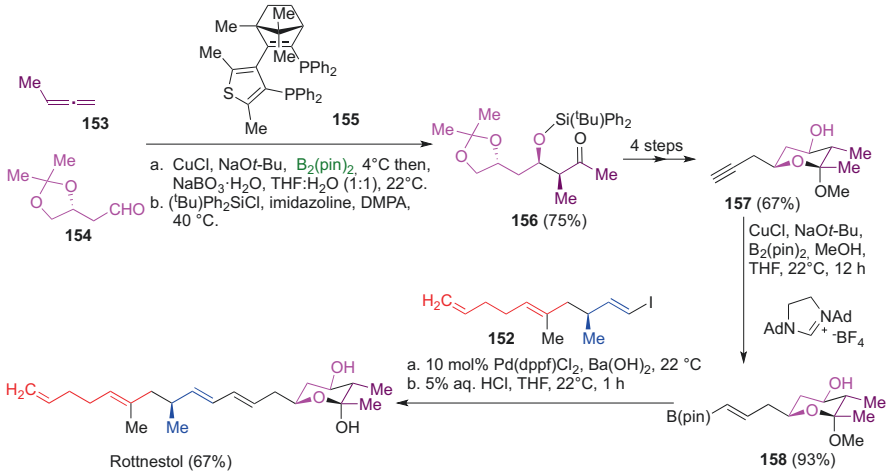

Fig. 51 Preparation of fragment **158** and final coupling with **152** to complete the total synthesis of rottnestol

and iodine followed by cross metathesis with vinyl-B(pin) **162** resulted in the formation of diene **163** (>98:2 *E*,*E*). In the next step, compound **163** was treated with tetrahydropyran **164** under palladium-catalyzed conditions to provide compound **165**. The synthesis of herboxidiene was completed in three additional steps (Meng et al. 2014).

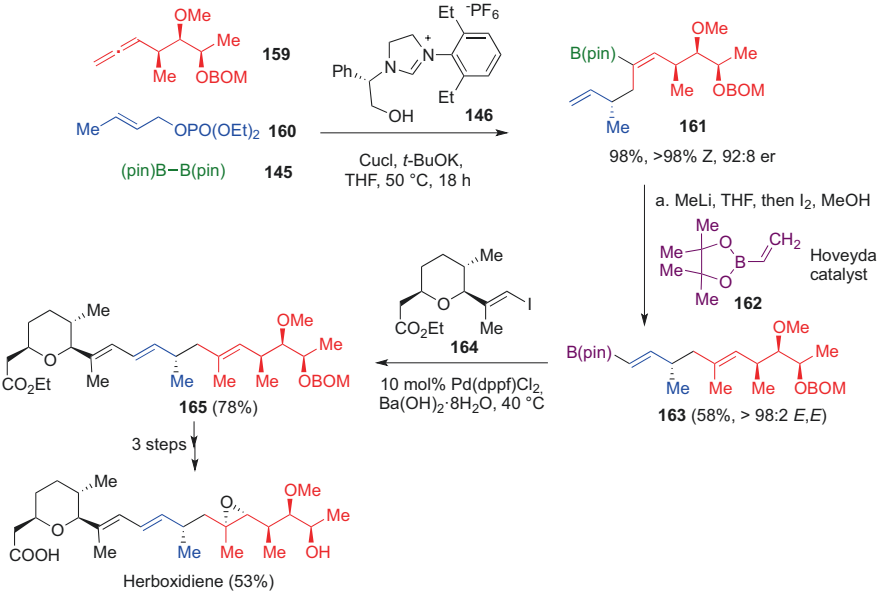

Fig. 52 Hoveyda's total synthesis of herboxidiene. B(pin), (pinacolato)boron

8 Miscellaneous Multicomponent Reactions

Marine butenolides of the cadiolide family are broad spectrum antibacterials and some of them are inhibitors of *Candida albicans* isocitrate lyase. In an effort to contribute to structure–activity relationships in this area, cadiolides A–C and a library of synthetic analogues were synthesized by application of a three-component reaction from condensation of aryldioxinones **166**, hydroxyketones **167**, and aldehydes **168** under thermal conditions (Boulangé et al. 2015). At high temperatures, the dioxinones **166** undergo a retro Diels–Alder reaction that transforms them into acylketones **169**, which react with the hydroxy group in compounds **167** to provide **170**, whose cyclization by an intramolecular Knoevenagel reaction to give **171**, followed by a second Knoevenagel reaction with aldehyde **168** led to the butenolides **172**. A final demethylation of the methoxy groups with boron tribromide afforded the library of natural products and their analogues (Fig. 53).

Liu et al. developed a method for the preparation of quinazolines through a one-pot sequential three-component condensation promoted by microwave irradiation (Liu et al. 2005). This procedure allowed the synthesis of bioactive quinazoline alkaloids such as glyantrypine, fumiquinazoline F, and fiscalin B (Fig. 54). The sequence begins with the condensation of anthranilic acid with the suitable N-Boc-L-amino acid in the presence of triphenyl phosphite. Subsequently, tryptophan methyl ester is added, and the system is heated under microwave conditions to afford the target alkaloids.

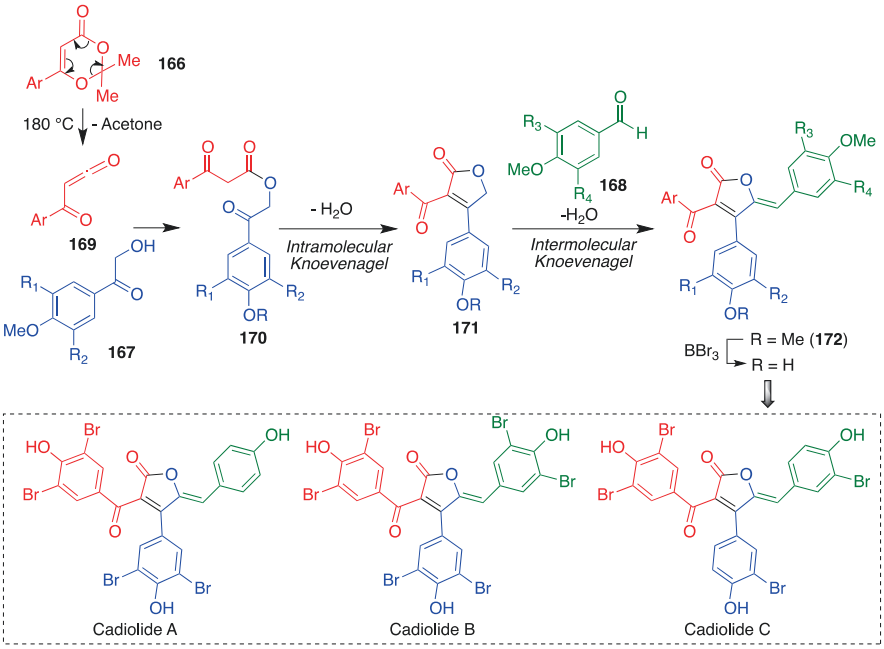

Fig. 53 Three-component synthesis of cadiolides A, B, and C

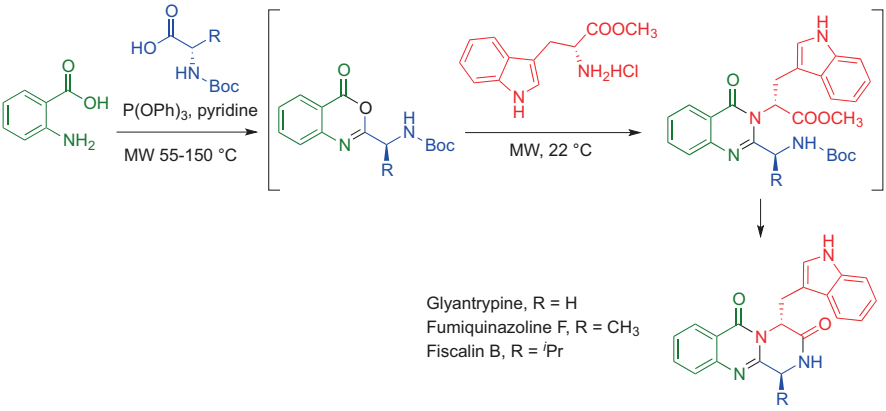

Fig. 54 Synthesis of quinazoline alkaloids based on a sequential three-component reaction

The same strategy was employed to achieve a concise total synthesis of alantrypinone, an insecticidal alkaloid isolated from the fungus *Penicillium thymicola* that acts as a selective GABA antagonist. Nishida's group approached the synthesis of this alkaloid and a library of derivatives thereof using the three-component strategy summarized above followed by a hetero Diels–Alder reaction to construct the pentacyclic system (Watanabe et al. 2009). In this case, the condensation of anthranilic

acid, Boc-L-alanine, and glycine methyl ester under microwave conditions afforded quinazolinone **173** in one pot. Its reaction with boron trifluoride etherate, followed by DDQ dehydrogenation, gave the aromatic quinazoline **174**, which served as the Diels–Alder 2-azadiene against the dienophile **175**, affording the natural product after a final lactim ether hydrolysis step (Fig. 55).

Many bioactive alkaloids isolated from skin extracts of dendrobatid or manteline frogs have a decahydroquinoline as its key structural fragment. These natural products often have potent pharmacological activities, particularly as nicotinic receptor antagonists. Our group has developed (Maiti and Menéndez 2011) a diastereoselective synthesis of one of these alkaloids, pumiliotoxin C (Fig. 56), based on an in-house-developed (Sridharan et al. 2009) four-component reaction between 1,3-cyclohexanedione, benzylamine, crotonaldehyde, and ethanol in the presence of indium triflate that gave octahydroquinoline derivative **176** through the generation of one C-C, two C-N and one C-O bonds. The three-carbon chain at C-2 was incorporated by allylation of **176** with allyltrimethylsilane in the presence of boron trifluoride, presumably via an iminium intermediate, to give **177**. From this point, a linear sequence of seven steps completed the synthesis of the natural product (Fig. 56).

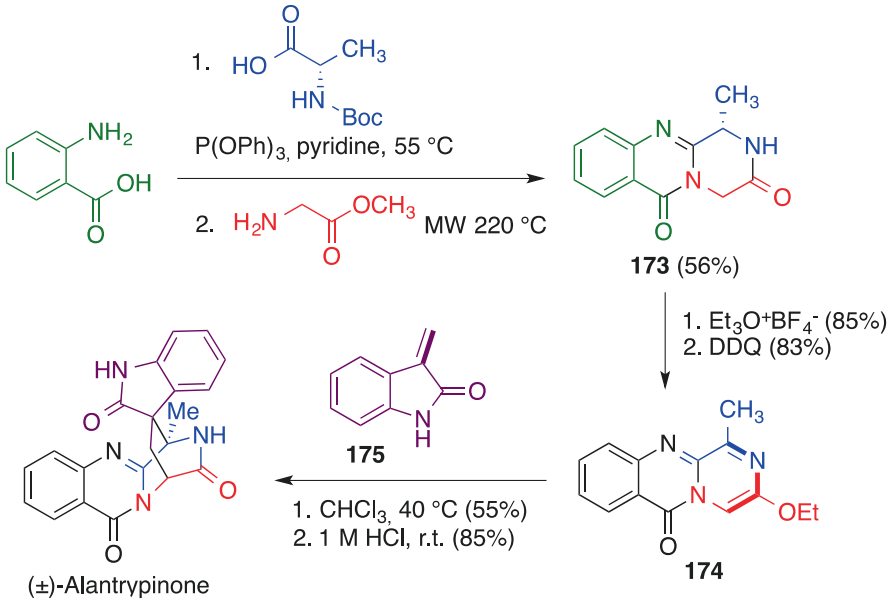

Fig. 55 Total synthesis of (±)-alantrypinone by the Nishida group

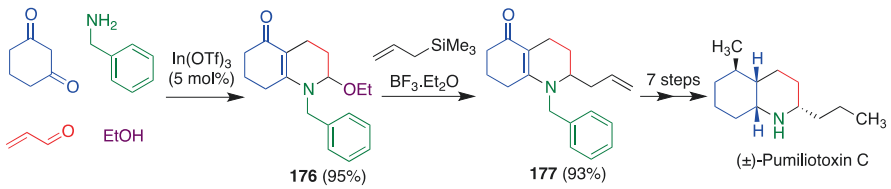

Fig. 56 Total synthesis of the dendrobatid alkaloid pumiliotoxin C based on a four-component reaction

9 Combinations of Multicomponent Reactions in Natural Product Synthesis

The combination in the same synthetic route of two or more multicomponent reactions can provide fast access to structurally complex molecular architectures. Some examples of the application of this strategy to the synthesis of natural products and their analogues are given below.

9.1 Passerini, Ugi-3CR, and Ugi-4CR

Tubugis (also called tubUgis) are synthetic tubulysin analogues designed for a lower energy barrier between the s-*cis* and s-*trans* configurations by amino acid N-alkylation. This relatively minor structural change increases the hydrolytic stability of the peptide toward amidases.

Wessjohann has described a route that provides ready access to tubugis by combination of three multicomponent reactions (Pando et al. 2011). Thus, the thiazole derivative **178** was obtained by a variation of the Passerini reaction described by Dömling, while compound **179** was obtained in parallel using an Ugi three-component reaction. Finally, an Ugi four-component reaction allowed the combination of both fragments (Fig. 57). A similar strategy has given access to derivatives of pretubulysins, which are slightly simplified analogues of the tubulysin (Hoffmann et al. 2015).

9.2 Combination of Two Joullié–Ugi Reactions

The Joullié–Ugi three-component reaction (JU-3CR) starts from a cyclic five- or six-membered imine, a carboxylic acid and an isocyanide and yields an *N*-acetylpyrrolidine or piperidine carboxamide. The group of Ichikawa has used this reaction to prepare two key fragments of plusbacin A_3, a cyclic lipodepsipeptide with potent antibacterial activity against a wide range of Gram-positive bacteria (Fig. 58). They discovered that the reaction of isonitrile **180**, carboxylic acid **181**

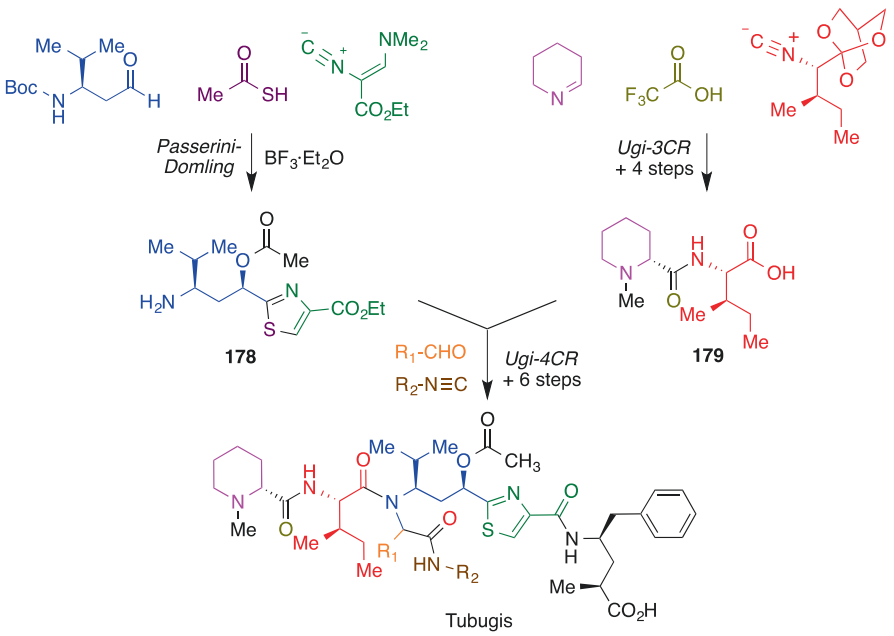

Fig. 57 Synthesis of tubulysin analogues (tubugis) by combination of Passerini–Domling, Ugi-3R, and Ugi-4-CR multicomponent reactions

and imine **182** in 1,1,1,3,3,3-hexafluoroisopropanol as solvent gave the required *trans* compound **183**, whereas a *cis* isomer was the predominant product in toluene (Katsuyama et al. 2016). Compound **183** was then transformed into the required fragment **184** in two additional steps. Fragment **186** was obtained similarly via intermediate **185**, and **184** and **186** were coupled with two additional fragments to give plusbacin A_3 in nine steps (Katsuyama et al. 2017). The same group has adapted this chemistry to the use of solid-phase peptide synthesis (Takashina et al. 2022).

9.3 Combination of a C + NC + CC Coupling and a Strecker Reaction

The silver acetate-catalyzed [C + NC + CC] reaction of aldehyde **187**, (*S*)-glycyl Oppolzer's sultam **188** and methyl acrylate, used as solvent, furnished pyrrolidine **189**, which was transformed into compound **190** in six steps. Its Swern oxidation of the primary hydroxyl to aldehyde in the presence of trimethylsilyl cyanide allowed an intramolecular Strecker reaction that afforded compound **191**. Four additional functional group manipulation steps allowed the formation of **192**, thus completing a formal total synthesis of cyanocycline A and bioxalomycin (Fig. 59) (Kaniskan

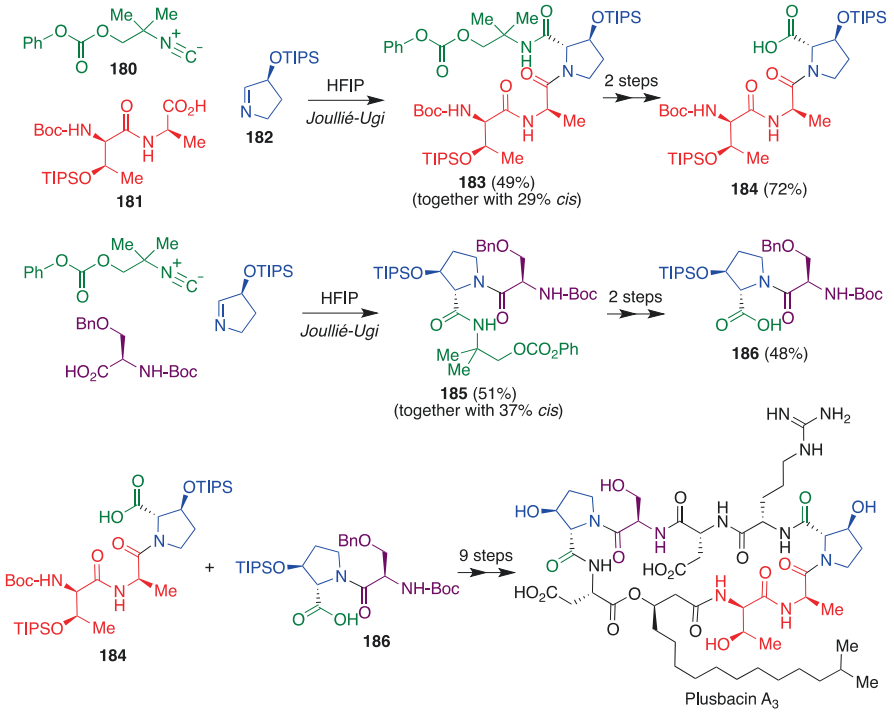

Fig. 58 Total synthesis of plusbacin A_3 using a combination of two Joullié-Ugi reactions. HFIP 1,1,1,3,3,3-hexafluoroisopropanol

and Garner 2007). Both fungal metabolites are of considerable biological interest because they are endowed with broad-spectrum antibacterial activity.

10 Natural Products as Substrates for Multicomponent Reactions

One way to achieve the rapid generation of natural product-like libraries is the use of a natural product as a substrate for multicomponent reactions. Curcumin, having a relatively simple polyfunctional structure, is well suited to this approach (Nelson et al. 2017; Ajavakom et al. 2017). Some multicomponent transformations that take advantage of the α-dicarbonyl structure of curcumin are summarized in Fig. 60, and include:

(a) The synthesis of curcumin-based pyrano[2,3-*d*]pyrimidines **193** from curcumin, aromatic aldehydes, and barbituric acid in the presence of a new catalyst prepared by surface modification of magnetic nanoparticles with sulfanilic acid (Panahi et al. 2017).

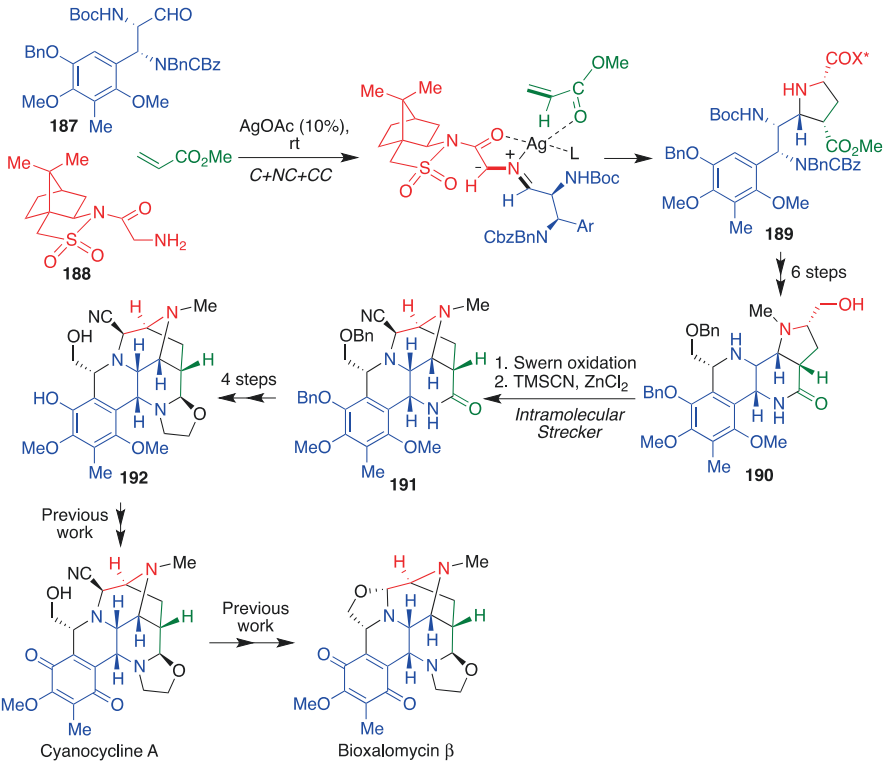

Fig. 59 Formal synthesis of cyanocycline A and bioxalomycin based on a C + NC + CC coupling. TMSCN trimethylsilyl cyanide

(b) The synthesis of a library of curcumin-derived 1,4-dihydropyridines **194** from curcumin, aromatic aldehydes, malononitrile, and aromatic amines in ethanol, in the presence of *p*-toluenesulfonic acid (Dangolani et al. 2020) while, in the absence of the amine component, curcumin-derived 4*H*-pyrans were obtained, both with toluenesulfonic acid or sodium formate (Brahmachari and Mandal 2019) as catalysts. The latter compounds have shown interesting properties against diabetes, being inhibitors of α-glucosidase and α-amylase as well as showing antioxidant activity (Tavaf et al. 2020).

(c) The synthesis of curcumin-derived 4*H*-pyrimido [2,1-*b*] [1,3]benzothiazoles **195** from curcumin, aromatic aldehydes and 2-aminobenzothiazoles in ethanol, with pyridine as catalyst (Agarwal et al. 2018).

(d) Finally, we will mention the Biginelli reaction between curcumin, urea or thiourea, and aromatic aldehydes that give antioxidant and anti-inflammatory 3,4-dihydropyrimidinones/thiones **196** (Lal et al. 2016).

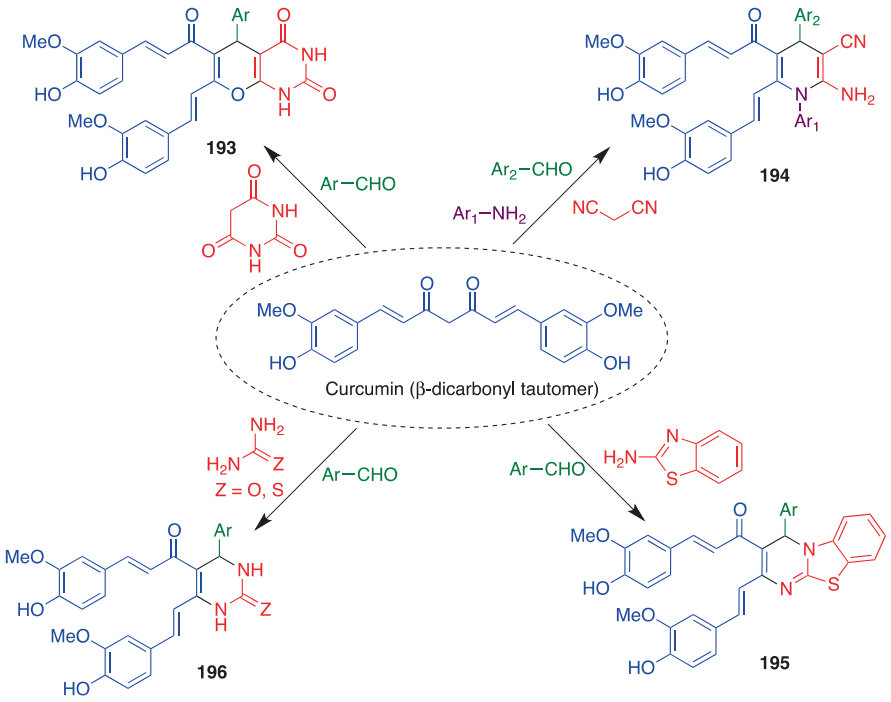

Fig. 60 Some multicomponent reactions having curcumin as one of the substrates

11 Conclusions

Multicomponent reactions, the main class of multiple bond-generating processes, are one of the most promising approaches to the goal of achieving improved efficiency and sustainability in synthesis due to their high atom- and step-economy and the avoidance of intermediate isolation and purification steps, which allows for a lower consumption of solvents and chromatographic stationary phases. Due to these features, multicomponent reactions provide a highly efficient alternative to sequential multistep procedures and are therefore ideally suited for simplifying routes aiming at the total synthesis of natural products. This area is in constant evolution, and we hope to have given a representative cross-section of the main methods.

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Applications of (Nano)encapsulated Natural Products by Physical and Chemical Methods

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Abstract Natural products that originate from fungal, bacteria, plant, marine, and animal sources have a wide variety of applications. Numerous studies have highlighted natural products in different areas for medicinal purposes, such as antimicrobial, antioxidant, anti-inflammatory, and anticancer agents. Although they are fascinating from an applied point of view, natural products can be unstable and fragile. A key issue for using these natural products and biomolecules is their bioavailability and stability, depending on the context in which they will be applied. In this context, encapsulation is a viable alternative to protect active compounds against the deterioration of environmental conditions, maintaining their natural compounds. Many encapsulation methods can be used, whether physical or chemical, and their use is intrinsically linked to their application. Among them, we can highlight electrospinning methods and micelles' formation. These encapsulation methods allow their application internally or externally to living organisms, bringing a series of distinct benefits. In an increasingly efficient search for new drugs, the

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encapsulation of natural products can enhance delivery and use. This chapter will emphasize physical and chemical methods and the characterization of nanoencapsulated natural products for different applications.

Keywords Encapsulation · Natural products · Biomolecules

1 Introduction

Biomolecules are biological compounds derived from cells, comprising nucleic acids, lipids, and structural proteins that lead to important antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial properties, being available in fruits and vegetables, yeasts, bacteria, animals, and even marine and freshwater organisms that have gained notoriety due to its potential of interacting with designed systems to enhance therapeutic activity and/or technological applications (e.g., drug delivery and bioremediation) (Rajput et al. 2022; Saeed et al. 2022).

However, biomolecules extracted from their natural matrices exhibit some degree of instability when exposed to changes in temperature, pH, light, and oxygen concentration once their native structures do not protect them. As the biomolecules can deteriorate by polymerization (Jakobek 2015) with other molecules and oxidation reactions, the challenge for Science and Industry for scaling up the technological production lies in preserving the desired biological effects of bioactive compounds, mainly, their antioxidant activity and bioavailability by improving its stability. In this context, the literature has drawn attention to (nano)encapsulation as an important prospective method for addressing this challenge (Giaconia et al. 2020).

Encapsulation consists of an active substance known as core material entrapped into a coating material (Gupta et al. 2016). This combination allows changes in solubility (Chaari et al. 2018), chemical and thermogravimetric stability (Centurion et al. 2021; Schmatz et al. 2020), and inhibition of bacterial growth (Malheiros et al. 2016), corroborating to several specific purposes. Encapsulating biomolecules has several benefits, such as maintaining their functionality, preventing degradation through processing and storing, controlling its release in living tissues, and keeping food safe along shelf-life (Pereira et al. 2022). Along these lines, this chapter is underpinned by examining nanoencapsulated natural products from microbes, plants, animals, and marine and freshwater organisms over physical and chemical methods and their characterization for different applications.

2 Encapsulation Methods

Bioactive compounds have several biological properties such as anti-inflammatory, anticarcinogenic, and antimicrobial antioxidant; however, these properties are affected when these biomolecules are exposed to changes in physical and chemical

factors such as temperature, pH, light, and oxygen concentration, these conditions being frequent in processes industrial and biological (Ramos et al. 2020). Faced with these limitations for the application of these compounds, encapsulation is a powerful tool to overcome these drawbacks as it provides protection to a wide variety of compounds by coating the molecules with a matrix (Ramos et al. 2022).

Due to the diversity of properties that bioactive compounds present, different techniques can be used to guarantee protection and maintenance of the biomolecule, the performance of the selected matrix, and mainly the bioavailability to exert the necessary action. Each technique for forming encapsulated materials (nano- or microencapsulation) depends on a series of factors that related to the material to be protected, since the matrix-forming substance and the conditions in which the techniques will be applied are also fundamental in the process. Choosing the most appropriate methodology includes analyzing several parameters such as physicochemical characteristics of the substances for encapsulation, particle size (or structure) and release rate, in addition to the economic aspect (Giaconia et al. 2020).

Nanoemulsions are kinetically stable solutions composed of two dispersed insoluble liquid phases and in the presence of surfactants, in which it is possible to guarantee the bioavailability of lipophilic bioactive compounds in aqueous medium (oil-in-water (o/w)) or hydrophilic compounds in oil phase (water-in-oil (w/o)). The formation of nanoemulsions can occur by high energy, such as homogenization under high pressure and sonication, or low energy, such as phase inversion temperature or solvent displacement, the latter being more applied since it ensures greater control in particle formation and low cost (Giaconia et al. 2020). Nanoemulsions consist of the formation of nanometric droplets that have excellent stability, which is why they have been widely used as a delivery system due to their protective effect on bioactive compounds against hostile environments provided, mainly in biological systems and industrial processes, improving their stability, solubility, and bioavailability (Saini et al. 2019).

The nanoemulsion formation by solvent evaporation is a traditional technique that involves the emulsification of a polymer in the liquid phase and the consequent evaporation of the solvent, resulting in precipitation of the polymer as nanoparticles with a spherical shape and its size is related to properties such as viscosity of the solution polymer used, agitation rate and temperature (Sabjan et al. 2019). Researchers evaluated the bacterial activity of lavender essential oil before and after incorporation into a nanoemulsion using ethanol and acetone as organic solvents, and subsequent dispersion in an aqueous phase, under agitation. The nanoemulsion of lavandula essential oil formed after solvent evaporation showed greater activity against bacteria when compared to its crude form (Garzoli et al. 2020). Nanoemulsions are commonly used for delivery systems, and thus, pesticide microcapsules have been synthesized using solvent evaporation system from dichloromethane as solvent, polylactic acid as carrier material, and polyvinyl alcohol as emulsifier. The microcapsules containing β-cypermethrin as a pesticide had a smooth, spherical shape with an encapsulation efficiency greater than 80%, in addition to an effective release mechanism (Feng et al. 2018).

Nanoemulsions is associated with the change in the surfactant spontaneous curvature during the emulsification process. This change can be achieved in two different ways: maintaining a constant composition while varying the temperature (phase inversion temperature method—PIT method) and maintaining a constant temperature while varying the composition (phase inversion composition method—PIC method). Both methods imply phase inversion (Ren et al. 2019).

In the PIT method, at low temperatures, temperature-sensitive surfactants are water soluble and the spontaneous curvature of the surfactant layer at the micelle interface is positive, and at high temperatures, these surfactants are oil soluble and the spontaneous curvature of the surfactant layer at the micelle interface becomes negative. At an intermediary temperature (PIT), the surfactants have the same affinity for the oil and aqueous phase, and the spontaneous curvature of the surfactant layer at the micelle interface is zero (Jintapattanakit 2018). Nanoemulsions of cinnamon oil were produced by PIT method using nonionic surfactant and water. The mixture was heated above the PIT of the system, and then rapidly cooled with continuous stirring, which led to the spontaneous generation of small oil droplets (107.30 nm and 100.70 nm) were formed in the condition using 40:60 wt% of cinnamon oil and surfactant in total lipid phase (Chuesiang et al. 2018). The PIC method does not have restriction on surfactant characteristics, as in the PIT method which requires thermosensitive surfactants. The procedure for forming the emulsion in the PIC method consists of adding water, at constant temperature, to a mixture containing the oil phase and surfactant. As the oily mixture (w/o) is added to water, generally slowly or stepwise, there is a change in the spontaneous curvature of the surfactant and consequent change of the mixture to o/w (Feng et al. 2020).

The spontaneous emulsification technique is used to obtain nanoemulsions with properties similar to obtained by physical methods, but this method, in addition to being simpler and faster, has a lower cost. This technique takes place through spontaneous emulsification of an oil phase with surfactant that has affinity for the organic phase. An organic solvent is used to dissolve the oil phase and then this phase is poured into an aqueous phase, consisting of water and hydrophilic surfactant, under agitation. The next step consists of removing the solvent through evaporation under reduced pressure. After adding the oil phase to the water phase, the diffusion of solvents promotes the formation of droplets (Bouchemal et al. 2004). Carotenoids-loaded nanoemulsions were prepared by spontaneous emulsification, and the optimized conditions of the process produced particles with diameters of 50 nm (Zhang and Li 2022).

One of the most used methods for nanoencapsulation is the electrospun technique, in which a polymeric solution subjected to a high voltage, promotes the evaporation of the solvent from the solution and the formation of nanostructures. Electrospun does not require specific reagents or extreme temperatures and therefore is closely related to food and pharmaceutical applications (Ramos et al. 2021).

The electrospun technique can lead to the formation of two types of structures: nanoparticles (electrospray) or nanofibers (electrospinning). In both techniques, a

polymeric matrix is used that will protect the bioactive compound and, consequently, increase its properties and application viability. The polymeric solution is subjected to a potential difference when it is ejected through a metal needle until it reaches a collector that is also metallic. The solid nanostructure is deposited in the collector since the solvent evaporated during the process. The diameter size of the structures formed depends on the flow rate and the applied voltage. However, the polymer type, solute concentration, and nozzle to collector distance are also important regulators of the process (Walia et al. 2019).

To attest to the use of nanoencapsulation of natural pigments in the face of digestive processes, nanofiber composite with incorporated anthocyanins from jussara pulp using polyethylene oxide was developed through electrospinning. The polymeric solution and composites produced maintained the antioxidant activity, showing their protective effect on bioactive compounds and the composite improved thermal stability of the anthocyanins (Kalsoom et al. 2020). Nanofibers containing hydroxyapatite dispersed in polycaprolactone/chitosan were developed by electrospinning aiming application in tendon and ligament tissue engineering. The nanofibers showed significant mechanical and biological properties (Wu et al. 2018).

3 Nanoencapsulation of Biomolecules from Microbes

Biological, ubiquitous, and diverse organisms classified as viruses, bacteria, archaea, fungi, or protists, considered pathogenic, beneficial, or neutral, are called microbes. They can be found in almost all habitats and are adapted to survive extreme conditions (Berg et al. 2020). Microorganisms are critical cellular factories for synthesizing proteins, small and large metabolites, and the production of single-cell proteins. Microbial biotechnology includes methods and strategies for producing and using prokaryotic and eukaryotic microorganisms in essential applications such as industrial, pharmaceutical, medical, agricultural, energy, food and feed, biocatalysis, mining, and biomaterials (Amaning Danquah et al. 2022; Kalsoom et al. 2020).

The recombinant DNA technology made it possible to modern microbial biotechnology to include fermentation, microbial physiology, screening of new metabolites and strain improvement, bioreactor design and processing, cell immobilization, cell fusion, metabolic engineering, and directed evolution of enzymes (Adrio and Demain 2010). However, for these microbial cells to function optimally, they must find suitable growth and metabolic conditions while being protected from the threatened environmental conditions to which they are exposed. Furthermore, in the case of microbial cells producing commercially essential substances, it is often desirable to achieve high cell densities for higher product yields, retention of activity over more extended periods, and ease of cell recovery from the product (Cho et al. 2022). The encapsulation of microbial cells or their products has been proposed to attain these goals for different commercially advantageous applications (Fig. 1).

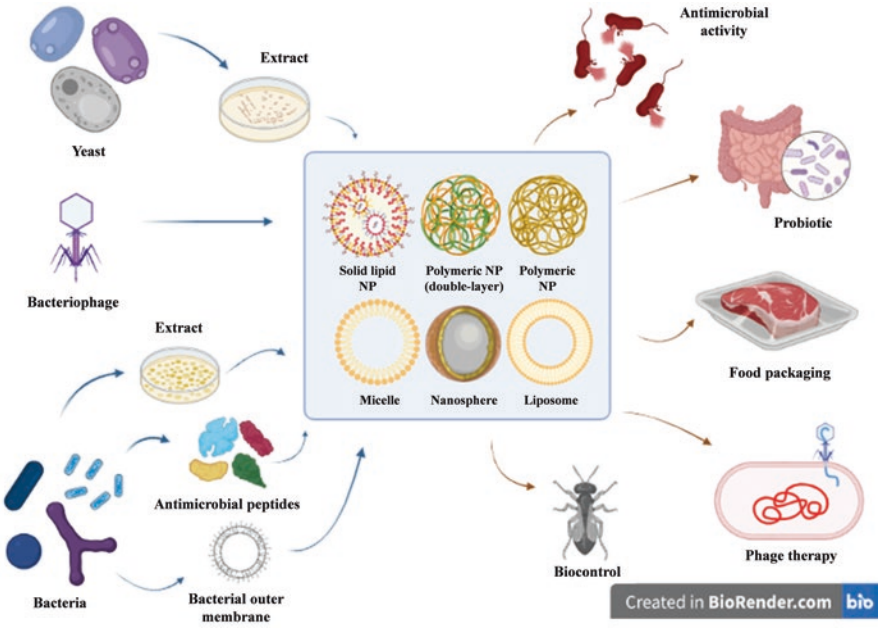

Fig. 1 Nanoencapsulation of biomolecules from microbe. Created in Biorender

3.1 Nanoencapsulation of Biomolecules from Bacteria

Bacteria are unicellular prokaryote organisms, generally classified into five groups according to their basic shapes: spherical (cocci), rod (bacilli), spiral (spirilla), comma (vibrio), or corkscrew (spirochaetes). They can be found as single cells, in pairs, chains, colonies, or biofilms. They are rich in bioactive compounds such as carbohydrates, proteins, enzymes, genetic material, antimicrobial peptides, virulence factors, resistance genes, etc. Due to the facility of manipulation and engineering, they can display specific functions for desired applications. This way, bacterial secondary metabolites, extracts, or biomolecules are potential bioactive compounds that can be encapsulated and used in various industries. In the pharmaceutical field, they can be applied in probiotic delivery, improving survival, resistance, and targeted release of sensitive microorganisms. In the food industry, encapsulation increases important molecules' functional properties and antimicrobial activities to avoid product spoilage (Bagheri Darvish et al. 2020; Freschlin et al. 2022). The results presented in this section are summarized in Table 1.

In medical research, a vaccine delivery system is a novel application for encapsulated bacterial products. Outer membrane vesicles (OMV), a spherical, nonreplicant structure naturally produced by Gram-negative bacteria, were encapsulated in sodium alginate nanoparticles using the unique gelation method. OMVs extracted from Bordetella pertussis are used as an adjuvant molecule to induce a stronger immune response in mice (Rami et al. 2021). Alginate is a hydrophilic linear

Table 1 Nanoencapsulation of bacterial products and its application

Source	Encapsulated compound	Encapsulation system	Encapsulation method	Application	Target	References
Bordetella pertussis	Outer membrane vesicles (OMV)	Sodium alginate	Unique gelation process	Immunogenicity, respiratory challenge for vaccine application	BALB/c mice	Rami et al. (2021)
Lactobacillus sakei	Bacteriocins	Phosphatidylcholine and 1,2-dioleoyloxy-3-trimethylammonium-propane (DOTAP)	Thin-film hydration	Antimicrobial activity against *L. monocytogenes*	UHT Goat Milk	Malheiros et al. (2016)
Commercial	Nisin	Liposomes	Thin-film hydration	Proteomic profile of *L. monocytogenes* under treatment	In vitro tests	Pinilla et al. (2021)
Commercial	Nisin	Microemulsions	W/O microemulsion	Antimicrobial activity against *S. aureus, L. monocytogenes,* and *B. cereus*	In vitro tests	Chatzidaki et al. (2019)
Commercial	Nisin	Alginate + resistant starch	Encapsulator	Antimicrobial activity against *P. acidilactici* and *C. tyrobutyricum*	Cheddar cheese	Hassan et al. (2020)
Commercial	Nisin	Soy soluble polysaccharide (SSPS)	Titration method	Stability and antimicrobial activity against *S. aureus, L. monocytogenes,* and *B. cereus*	Tomato juice	Luo et al. (2019)
Commercial	Nisin	Alginate-chitosan	Alginate ionic gelation and complexation with chitosan	Antimicrobial activity against *Listeria monocytogenes*	Vacuum-sealed, refrigerated beef	Zimet et al. (2018)

(continued)

Table 1 (continued)

Source	Encapsulated compound	Encapsulation system	Encapsulation method	Application	Target	References
Commercial	Nisin	Multicomponent colloidosomes + chitosan and alginate	Ionotropic pregelation	Antimicrobial activity against *S. aureus, Listeria monocytogenes,* and *E. faecalis*	In vitro tests	Niaz et al. (2018)
Commercial	Nisin	Poly-γ-glutamic acid (γ-PGA) and poly-L-lysine (PLL)	Electrostatic self-assembly	Antimicrobial activity against *S. aureus* and effect on quality and shelf life	Pork meat	Cui et al. (2018)
Commercial	Nisin	Chitosan (CS) or chitosan modified + monomethyl fumaric acid (CS-MFA)	Ionic cross-link	Physiochemical structures and antimicrobial activity against *S. aureus, L. monocytogenes, E. coli,* and *S. enterica*	Orange Juice	Khan et al. (2018)
Bacillus amyloliquefaciens	CAMT2	Soybean phosphatidylcholine	Reverse-phase evaporation	Physiochemical structures and antimicrobial activity against *L. monocytogenes*	Skim and whole milk	Jiao et al. (2020)
Pediococcus pentosaceus	Pediocin	Liposome		Physiochemical structures and antimicrobial activity against *Listeria* sp.		Suganthi et al. (2021)
Enterococcus faecalis	Enterocin Gr17	Essential oil nanoemulsion	Nanoemulsion	Quality characteristics and antimicrobial activity against several bacteria and fungi	Liquid-smoked salmon	Duan et al. (2023)

Lactobacillus sakei	Sakacin-A	Cellulose nanofibers	Absorption	Antimicrobial activity against *Listeria* spp.	Smoked salmon	Mapelli et al. (2019)
Lactobacillus lactus	Lacticin 3147	Solid Lipid Nanoparticles	Nanoprecipitation	Optimization of the encapsulation method and stability of the biomolecule	In vitro tests	Ryan et al. (2022)
Commercial	Lysozyme	Polyamide membranes	Electrospinning	Enzymatic kinetics and antimicrobial activity against *Pseudomonas* spp.	Chicken slices	Bugatti et al. (2018)
Commercial	Lysozyme	Carboxyl Carbon Nanotubes	Electrospinning	Enzymatic activity and Cytotoxicity	In vitro tests	Liu et al. (2018)
	Lactoperoxidase System (LPOS)	Chitosan	–	Physicochemical, sensorial and antimicrobial activity against *S. putrefaciens* and *P. fluorescens*	Trout fillets	Jasour et al. (2015)
Commercial	Lactic acid	Chitosan + Pectin + Starch	Film-formation and casting	Antioxidant and antimicrobial activity against *Bacillus subtilis, Listeria monocytogenes,* and *Escherichia coli*	In vitro tests	Akhter et al. (2019)

(continued)

Table 1 (continued)

Source	Encapsulated compound	Encapsulation system	Encapsulation method	Application	Target	References
Lactobacillus plantarum	Extract	Alginate-gelatin	Extrusion	Bacteriocin production and antimicrobial activity against *S. aureus, L. monocytogenes, B. subtilis, E. coli* and *S. enterica*	Pork meat	Le et al. (2019)
Lactobacillus lactis	Extract	Corn starch or carboxymethylcellulose film	Film-formation and casting	Bacteriocin production and antimicrobial activity against *S. aureus*	In vitro tests	Lan et al. (2021)
Pseudomonas fluorescens	Extract	Sodium alginate	Unique gelation process	Biocontrol activity	Potato	Pour et al. (2019)
Lactobacillus acidophilus	Extract	Sodium alginate or carrageenan	Encapsulator	Viability in GIT conditions	Ice cream	Afzaal et al. (2019)
Lactobacillus casei	Extract	Alginate, carrageenan, locust bean gum, gellan gum or xanthan gum + starch, lactulose or lactosucrose	Extrusion	Viability in GIT conditions	In vitro tests	Ta et al. (2021)
Lactobacillus reuteri	Extract	Sodium alginate or galacto-oligosaccharides	Spray-drying	Viability in GIT and storage conditions	Camel milk infant formula	Algaithi et al. (2022)
Lactobacillus curvatus	Extract	Liposome	O/W emulsion	Stability, cytotoxicity and antimicrobial activity against *C. albicans* and *A. niger*	Lotion emulsion	Kim et al. (2021)

Lactobacillus paracasei	Extract	Alginate-based nanofiber	Electrospinning	Viability in GIT conditions	Kefir	Yilmaz et al. (2020)
Lactobacillus gasseri	Extract	Sodium alginate	Ionic gelation and emulsification	Viability in GIT and storage conditions	Apple juice	Romero-Chapol et al. (2022)
Lactobacillus acidophilus	Extract	Sodium alginate, chitosan, maltose, hydroxyethyl-cellulose, hydroxypropyl-methylcellulose or calcium lactate	–	Viability in GIT conditions	Reishi mushroom	Mirmazloum et al. (2021)
Lactobacillus acidophilusLactobacillus rhamnosusBifidobacterium bifidumBifidobacterium animalis	Extract	Corn starch or sodium alginate	Electrospinning	Viability in GIT conditions	In vitro tests	Atraki and Azizkhani (2021)
Pseudomonas fluorescens Bacillus subtilis	Extract	Silica or carbon nanotubes	Emulsification/ internal gelation	Viability of plant growth-promoting bacteria	Pistachio	Pour et al. (2019)
Bacillus subtilis	Extract	Alginate + chitosan	–	Viability in GIT conditions	White shrimp	Adilah et al. (2022)

polysaccharide obtained from brown algae, composed of two interconnected monosaccharide chains: 1–4 β-D-mannuronic (M) and α-L-guluronic (G). The gelling mechanism of alginate occurs when the G chains link on opposite sides to form a hydrophilic cavity that binds Ca2+ using oxygen atoms from the carboxyl group. The ratio and sequence of G and M residues achieved the physical properties of alginate hydrogels: the more G chains, the more rigid and porous the gel will be. In comparison, the more M chains form soft gels that disintegrate more easily. Alginate has become an excellent support for the administration of biomolecules, mainly due to the processing under mild conditions by crosslinking ions at room temperature and the good permeability of alginate gels, which facilitates the exchange of air, nutrients, and the release of metabolites (Wang et al. 2022).

In the food industry, strict control of food safety and microbiological quality is one of the fundamental requirements present in all stages of production, storage, and distribution. Bacteria and fungi contaminate different food products and cause several adverse effects on the sensory properties of foods, such as taste, color, and texture, as well as nutritional and economic losses (Delshadi et al. 2021). They are responsible for food deterioration, causing a decrease in shelf life and disease transmission and impairing food safety and quality. Microbial growth in food products can be affected by internal factors such as pH, oxygen, and amount of water present in the food; and external factors such as light, temperature, and humidity. Despite the recent exploration of chemical or artificial preservatives to prevent these losses, their growing association with adverse health implications, such as carcinogenic effects and allergies, has resulted in arisen of the bio-preservatives, which includes the use of microorganisms or their natural products (Kaur and Kaur 2021).

Among microbial compounds capable of controlling undesired microorganisms in food systems, bacteriocins naturally produced by some bacterial species are the best studied. Bacteriocins are antimicrobial peptides capable of prolonging the shelf life of food by inhibiting spoilage by pathogenic Gram-positive and Gram-negative bacteria (Kumariya et al. 2019). Bacteriocins are mainly bactericidal, acting by forming pores in the membranes of microorganisms. At the same time, some are bacteriostatic, making them useful in the food and pharmaceutical sectors, especially where fermentation is undesirable. Although the producer organisms are generally recognized as safe, as proteolytic enzymes degrade them in the human intestinal tract, the direct incorporation of bacteriocins to the surface of food has some limitations, generally associated with the interaction with food compounds, which result in partial or total loss of antimicrobial activity (Mokoena et al. 2021; Kaur and Kaur 2021). Thus, the encapsulation of antimicrobial peptides in nanostructures has been used to protect them against degradation and improve their bioavailability. Encapsulated bacteriocins produced by *Lactobacillus sakei* subsp. *sakei* 2a in phosphatidylcholine and cationic 1,2-dioleoyloxy-3-trimethylammonium-propane (DOTAP) nanovesicles were characterized and evaluated for their effect against *Listeria monocytogenes,* in vitro and UHT contaminated goat milk (Malheiros et al. 2016). The method used for encapsulation was the thin-film hydration method. The encapsulation showed high efficiency of encapsulation (95%), low

polydispersion index, and excellent stability, and was able to delay bacterial growth in 5 days in UHT goat milk stored at 7 °C.

Among the existing bacteriocins, the most used is nisin, "Generally recognized as safe" (GRAS) by the US Food and Drug Administration (USFDA) and approved as a suitable additive for food applications by the European Food Safety Authority (ESFA). In Pinilla et al., nisin-loaded liposomes showed better results for the treatment against *L. monocytogenes* compared to free nisin. The encapsulation obtained by the thin-film hydration method presented a bacteriostatic effect on *L. after* 30 min and reduced the expression of proteins that contribute to infection and resistance to nisin due to the stress imposed on the cells (Pinilla et al. 2021).

Another nisin encapsulation was developed to evaluate the biopreservative effect of nanoparticles using poly-γ-glutamic acid (γ-PGA) and poly-L-lysine (PLL) using the self-assembled electrostatic method. Based on the microbial, chemical, color, and texture analysis, nanoparticles could effectively control the growth of *S. aureus* in pork meat and have no impact on the quality of the product samples (Cui et al. 2018). Furthermore, the antimicrobial activity of nisin encapsulated in olive oil-based microemulsions enriched with essential oils was investigated against *S. aureus, L. monocytogenes,* and *B. cereus.* Rosemary, thyme, oregano, and dittany essential oil-contained microemulsions were formulated by the W/O microemulsion method. This technique increases the membrane's flexibility and facilitates the diffusion of nisin to the outer environment, enhancing the antimicrobial effect of the system (Chatzidaki et al. 2019).

Finally, nisin-loaded alginate nanoparticles were encapsulated by ionic gelation and further complexed with chitosan. Nanoparticles were prepared by ionic gelation of alginate upon dropwise addition of nisin, followed by complexation with dropwise addition of chitosan, under stirring. The nanocapsules were tested for antimicrobial activity against *L. monocytogenes* culture and as biopreservative agents of refrigerated, vacuum-packaged lean beef meat. The encapsulated nanoparticles not only delayed the growth of *L. monocytogenes* but also displayed sustained activity over time, both in vitro and in vivo assays (Zimet et al. 2018).

Other bacteriocins can be used as an antimicrobial agent in the food industry. CAMT2, a bacteriocin with antilisterial activity, was encapsulated in phosphatidylcholine nanovesicles by the reverse-phase evaporation method, which guaranteed an encapsulation efficiency of about 70% (Jiao et al. 2020). Liposome-encapsulated pediocin (extracted from *Pediococcus pentosaceus*) also showed an improved antibacterial effect compared with free purified pediocin (Suganthi et al. 2021). Enterocin Gr17 is a bacteriocin that presents inhibitory effects on many pathogens found in food and has the potential for application as a natural food additive, such as liquid smoked fish. However, bacteriocins with a high content of hydrophobic amino acids, such as Enterocin, easily bind to charged or hydrophobic macromolecules in food products. As salmon has a fat content of about 8% to 14%, the antimicrobial activity of bacteriocins is impaired. Thus, Duan et al. proposed the encapsulation of bacteriocins and EOs in a nanoemulsion, which can be used as a delivery system to protect the effects of bioactive compounds. In addition, the controlled release of the biomolecule allows for a longer duration of action of the active

substances, maintaining their antimicrobial activity for a more extended period. The nanoemulsion system incorporating Enterocin Gr17 and cinnamaldehyde essential oil was able to significantly inhibit microbial growth and maintain better color and texture sensory profiles during the storage of smoked salmon at 4 °C. From a microbiological, physical–chemical, and sensory point of view, the treatment can extend the product's shelf life to 42 days (Duan et al. 2023).

Sakacin-A is a bacteriocin produced by *Lactobacillus sakei*, which plays an antimicrobial effect, especially against *Listeria* sp. In Mapelli et al., sakacin-A was adsorbed on cellulose nanofibers without chemical modifications to obtain an antimicrobial material. Cellulose nanofibers have been widely used to improve mechanical and barrier properties when applied to packaging materials, which can help extend shelf life and enhance food quality (Mapelli et al. 2019).

In the same line of work, Lacticin is a noncytotoxic bacteriocin produced by *Lactococcus lactis*, comprised of two peptides that work synergistically to kill even antimicrobial-resistant bacteria such as *Staphylococcus aureus* and *Clostridioides difficile*. In addition, solid lipid nanoparticles (SLNs) are an excellent alternative transport system to traditional lipid-based methods, as they are biocompatible and biodegradable and can increase the stability, solubility, and, subsequently, the bioavailability of a variety of drugs. In Ryan et al., seeking to optimize the encapsulation method and increase the molecule's stability, the two peptides of Lacticin were encapsulated individually in SLNs using a modificated nanoprecipitation method. The nanomaterial showed a higher encapsulation efficiency, cytotoxic activity, and protection from proteases in the duodenum (Kaur and Kaur 2021).

The biopreservative effect on food packaging can also be done by bacterial enzymes, organic acids, or bacteria. Lysozyme is an enzyme naturally produced by bacteria capable of decomposing the cell wall of other species, acting as an antimicrobial peptide. Bugatti et al. proposed a preparation of bio-based membranes composed of Polyamide 11 from renewable sources and lysozyme encapsulated into halloysite nanotubes through the electrospinning process (Bugatti et al. 2018). On the other hand, lactoperoxidase (LPO), an enzyme included in the lactoperoxidase system (LPOS), has been widely used in food packaging due to its bactericidal or bacteriostatic properties, which inhibit microbial growth by transmitting sulfhydryl groups (SH) to microbial enzymes and other proteins. The research of Jasour et al. was based on an active packaging system for specific microorganisms of the fish industry. A chitosan solution was solubilized in acetic acid under stirring. Then, the solution was heated to add glycerol as a plasticizer. With the pH adjusted, LPOS was added to the chitosan solution. After antimicrobial, physical–chemical, and sensory tests, the authors could maintain the trout's quality and guarantee the storage time extension (Jasour et al. 2015).

Lactic acid can also be used as an antimicrobial agent, especially when combined with other compounds with the same properties. A biocomposite film made of chitosan, pectin, and starch was loaded with nisin, acid lactic, rosemary, and mint essential oils to prevent lipid oxidation and microbial spoilage of foods. After incorporating the compounds in a film-forming solution, it was placed in glass plates and dried for a few hours. The films were tested for antioxidant and antimicrobial

activity against *Bacillus subtilis, Listeria monocytogenes* and *Escherichia coli* and showed that the incorporation positively influenced not only these properties but also the barrier and mechanical quality (Akhter et al. 2019). Since the bacterial antagonists are sensitive to environmental conditions such as competition, pH fluctuations, and temperature changes, the colonization process gets challenging, and they can rapidly disappear. Le et al. developed an alginate-gelatin compound that could protect *Lactobacillus plantarum* bacteria from these threats so it could survive and produce bacteriocins directly in refrigerated pork meat. The bacterial cells were extruded inside the nanomaterial and found to increase the percentage of antimicrobial activity. Bacteriocins activity was enhanced with high temperatures and was not affected by pH. The formulation also inhibited the growth of pathogenic bacteria in pork meat throughout the 12 h period (Pour et al. 2019). A similar study was carried out by encapsulating *Lactobacillus lactis* in a film of corn starch and carboxymethylcellulose to improve nisin production and application on food packaging. The films showed the best performance and the lowest water vapor transmission while preserving the antibacterial activity against *Staphylococcus aureus* for 8 days (Lan et al. 2021).

The World Health Organization (WHO) defines probiotics as live microorganisms that confer various health benefits to the host when administered adequately to the desired target site (Food and Agriculture Organization and World Health Organization and others 2006). These advantages encourage their wide use in sectors such as the agricultural, food, pharmaceutical, and cosmetics industries. The most frequent genera are *Lactobacillus, Bifidobacterium, Bacillus, Saccharomyces,* and *Escherichia coli*, which are particularly sensitive to the harsh conditions of many foods and the human intestine (Yao et al. 2020a). Its preventive and therapeutic properties against infectious diseases, metabolic, anticancer, and antimutagenic activities come mainly from the production of nutrients and cofactors, competition with pathogens for nutrients or adhesion sites, and stimulation of the host's immune response (George Kerry et al. 2018). Although the potential for probiotics in treating or even preventing gastrointestinal diseases is high, their clinical efficacy still needs to improve due to conflicting results in clinical trials for many diseases. This is partly due to the lack of viability of probiotics using traditional manufacturing and packaging methods (Centurion et al. 2021). Several factors significantly affect the viability and survival rate of probiotics during processing, storage, and consumption, such as harsh conditions within the upper human GI. such as the presence of antimicrobial lysozyme in the mouth, low pH conditions in the stomach, bile salts and digestive enzymes in the small intestine, and other complex factors, including osmotic pressure and oxidative stress throughout the gastrointestinal tract (Techo et al. 2019). Thus, probiotics must survive the hostile gastric environment, remain metabolically active, and be released in sufficient quantities and controlled at the site of action in the lower gastrointestinal (GI) tract to confer beneficial health effects (Razavi et al. 2021).

Nanoencapsulation of probiotics has been proposed as an effective solution to improve survival, resistance, and targeted release of sensitive microorganisms in the GI tract, as it can trap small amounts of bioactive compounds and/or

microorganisms in small nanostructured compounds (Centurion et al. 2021). In essence, the goal of nanoencapsulation is to create a microenvironment that protects bacteria from exposure to external factors (such as low gastric pH) during digestion and subsequently reduces cell injury or death before their release to the target site (Pateiro et al. 2021). There are many reports on nanoencapsulation of probiotic bacteria resulting in highly increased viability of probiotics during storage and administration. Due to unique physical and chemical properties, nanostructured materials show great promise for protecting microorganisms from the acidic conditions of the stomach and therefore allow the successful release of trapped probiotic cells into the intestinal lumen at natural pH (Centurion et al. 2021; Sharma et al. 2019).

In the food industry, the so-called "functional foods" have gained importance since consumers have been looking for foods with properties that go beyond nutrition. A functional food can be defined as one that provides beneficial effects to the human body in addition to its basic nutritional properties, as is the case with probiotic foods (Atraki and Azizkhani 2021). The dietary supplemented probiotics market is predicted to grow at a compound annual growth rate of 7% compound annual growth rate through 2027 (2022). Accordingly, studies have been conducted to increase the viability of these products both in the production stages and through the GI passage. Azfaal et al. used sodium alginate and carrageenan to encapsulate Lactobacillus acidophilus in ice cream to evaluate the viability of probiotic bacteria under simulated GI conditions. The nanocapsules could provide protection and enhanced survival of probiotics in food, guaranteeing the health recommended level for the best benefits (Afzaal et al. 2019). In Yilmaz et al., the authors used the electrospinning method to produce alginate-based nanoparticles with *Lactobacillus paracasei* KS-199. The viability in simulated GI conditions was improved, as well as the survival in kefir and the protection against thermal degradation (Yilmaz et al. 2020). Similarly, cells of *Lactobacillus gasseri* were encapsulated in sodium alginate capsules using ionic gelation and emulsification methods. The encapsulated probiotics retained 100% of their viability, compared with the free cells, and enhanced the viability in stored apple juice for 21 days (Romero-Chapol et al. 2022).

As already mentioned, probiotics are beneficial in different sectors of production. In the pharmaceutical industry, probiotics are used as supplements and can be added to other health products, such as infant milk formulas. Probiotic agents are becoming a vital part of the tools against GI problems, especially in formula-fed infants (Putta et al. 2018). Algaithi et al. fortified camel milk infant formula with *Lactobacillus reuteri* encapsulated in sodium alginate and galactooligosaccharides via spray drying. The nanocapsules were evaluated for stability in simulated infant GI, storage conditions, physicochemical properties, and *L. reuteri* viability and proved to be an excellent delivery system of probiotics for kids (Algaithi et al. 2022).

In addition, probiotics have beneficial effects on the skin, not only when taken orally but also when applied topically. Oral consumption of probiotics improves the overall metabolic content of the human body by inhibiting harmful intestinal microflora. Similarly, inhibiting harmful microbial growth by topical application of probiotics alters the epithelial microbiome by lowering surface pH and generating an

amino acid layer, preserving skin moisture (Puebla-Barragan and Reid 2021). Moreover, probiotics produce valuable metabolites with antioxidant and tyrosinase inhibitory activity, which induce skin-lightening effects. Therefore, these extracts could be exploited as multifunctional natural preservatives in the cosmetics industry. In this scenario, *Lactobacillus curvatus* cells were encapsulated in liposomes using the O/W emulsion technique. Characterization and cytotoxic tests indicated great stability, permeability, and functionality in lotion emulsion and inhibitory effect against *Candida albicans* and *Aspergillus niger* (Kim et al. 2021).

Lastly, the agricultural sector is one of developing countries' most important economic sectors. Using soil microorganisms as biofertilizers for different agricultural products has grown daily. When interacting with plants, these bacteria stimulate plant growth and health through mechanisms such as nitrogen fixation and phytohormone production. In addition, antagonistic bacteria play an essential role in the biocontrol of pathogens by producing substances that inhibit the growth of other microorganisms. However, the fundamental challenge to the success of biocontrol is the survival of the antagonist bacteria and the provision of the necessary conditions for the production of inhibitors in the correct amount and the correct place (de Souza Vandenberghe et al. 2017). Two crucial plant growth-promoting bacteria were encapsulated in silica and carbon nanotubes to work as a delivery system in pistachio micropropagation. In a comparison of the free bacteria, the nanoformulation with *Pseudomonas fluorescens* and *Bacillus subtilis* successfully enhanced root length and proliferation and phytohormone production by rhizobacteria after three days (Moradipour et al. 2019).

3.2 Nanoencapsulation of Biomolecules from Yeasts

Yeasts are eukaryotic, unicellular microorganisms classified as members of the fungal kingdom. With their unicellular growth habit, yeasts can be contrasted with molds, which develop hyphae. Fungal species that can assume both forms (depending on temperature or other conditions) are called dimorphic fungi (Coradello and Tirelli 2021). The beneficial physiological properties of yeast have led to its use in biotechnology. The yeast species *Saccharomyces cerevisiae* converts carbohydrates into carbon dioxide and alcohol through fermentation. The products of this reaction have been used in baking and making alcoholic beverages for thousands of years. *S. cerevisiae* is also an important model organism in modern cell biology research and is one of the most studied eukaryotic microorganisms. In addition, yeast is used as an ingredient in foods for its umami flavor, which contains free glutamic acid (Hittinger et al. 2018). Other yeast species, such as *Candida albicans,* are opportunistic pathogens and can cause human infections. Some yeasts may find potential applications in the field of bioremediation. Yeasts such as *Yarrowia lipolytica* are known to degrade hydrocarbon contaminants such as alkanes, fatty acids, fats, and oils and have been investigated for their potential as a heavy metal biosorbent (Saeed et al. 2022). Yeasts are also used as probiotic supplements to maintain and

restore the natural microbiota of the gastrointestinal tract (Tamang and Lama 2022). In this way, for all these applications, it is vital to improve yeast bioavailability and reduce its degradation during storage to develop efficient nutraceutical additives and biocontrol agents based on fungi cells or their products. The results presented here are summarized in Table 2.

Like bacteria, yeasts can also face the same threats to survive during food processing and passage through the human GI system. Some studies with this purpose include *Sacharomycopsis flibugera* encapsulated in electrospun wheat bran fiber and exopolysaccharide combined with polyvinylpyrrolidone (Ragavan and Das 2020); *Pichia barkeri, Yarrowia lipolytica, Wickerhamomyces anomalus,* and *Saccharomyces cerevisiae* in sodium alginate nanoparticles or combined with chitosan or starch (Suvarna et al. 2018); *Saccharomyces boulardii* extruded in calcium alginate nanocarriers (Morales-Amparano et al. 2019); and *Kluyveromyces lactis* in a gelatin hydrogel complexed with graphene oxide and glutaraldehyde (Patarroyo et al. 2021).

Alginate is an anionic polysaccharide mainly found in the cell wall of brown algae, which includes two copolymers, guluronic acid, and mannuronic acid. The use of alginate hydrogels for biomolecule delivery is being widely used (Angra et al. 2021). Alginate-encapsulated dextranase exhibited maximum stability and activity in toothpaste for three months. Dextranase, generally extracted from *Chaetomium gracile* or *Penicillium* spp., is GRAS in cosmetics and drug formulations for oral care products because it has excellent antibiofilm activity. Alginate beads were able to protect dextranase activity from harsh conditions, offer a higher release in toothpaste during brushing, and improve stability after long-term storage (Juntarachot et al. 2020). In Nguyen et al., sodium alginate and β-lactoglobulin were used to formulate a nanocapsule loaded with *Saccharomyces cerevisiae* by the layer-by-layer method. This technique facilitates the survival of the yeast since it avoids significant chemical changes in the cell caused by dehydration at the high processing temperatures of some food products (Nguyen et al. 2020).

Saccharomyces cerevisiae is a unicellular eukaryotic organism that belongs to the Fungi kingdom. It is the yeast used in the production of bread and beer, in addition to being used for the production of fuel alcohol. In the case of fermented alcoholic beverages, *Saccharomyces cerevisiae* converts sugar into ethyl alcohol and can also contribute to the formation of secondary constituents responsible for flavor, as is the case with beer, rum, and whiskey (Vanderwaeren et al. 2022). This way, microencapsulated *S. cerevisiae* was used to promote a continuous fermentation process on a green beer. Chitosan-calcium alginate double-layer microcapsules reached 91% of encapsulation efficiency. They maintained physicochemical parameters such as pH, color, alcohol content, and bitterness, demonstrating an excellent approach for beer production (Benucci et al. 2021). Similarly, the same formulation was made to produce a sparkling wine, which displayed increased pressure and oxygen consumption compared to the free yeast. After six months, few differences in sensory properties were observed, similar to those produced with free cells (Benucci et al. 2019).

Table 2 Nanoencapsulation of fungi products and its application

Yeast	Encapsulation system	Encapsulation method	Application	References
Saccharomycopsis fibuligera	Wheat bran fiber or exopolysaccharide + polyvinylpyrrolidone (PVP)	Electrospinning	Probiotic delivery	Ragavan and Das (2020)
Pichia barkeri; Yarrowia lipolytica; Wickerhamomyces anomalus; Saccharomyces cerevisiae;	Sodium alginateSodium alginate + chitosanSodium alginate + starch	ExtrusionExtrusion + emulsification	Probiotic delivery	Suvarna et al. (2018)
Unknown yeasts	Polyacrylamide nanofiber	Electrospinning	Immobilized efficiency	Fan et al. (2021)
Penicillium roquefortii	Alginate	Encapsulator	Dextranase production in toothpaste	Juntarachot et al. (2020)
Trichoderma harzianum	Nanocellulose and/or Carboxymethylcellulose		Biocontrol activity	Brondi et al. (2022)
Yarrowia lipolytica	Organogel	Photopolymerization	Stability and physicochemical properties	Zhang et al. (2022)
Unknown yeast	Silica	Boron hydroxide click reaction	Cell protection and stability	Geng et al. (2019)
Saccharomyces cerevisiae	B-lactoblobulin Sodium alginate	Layer by Layer	Cell protection and stability	Nguyen et al. (2020)
Saccharomyces cerevisiae	Chitosan-calcium alginate	Microencapsulation	Production of sparkling wine	Benucci et al. (2019)
Saccharomyces boulardii	Calcium alginate	Extrusion	Probiotic delivery	Morales-Amparano et al. (2019)
Saccharomyces pastorianus	Calcium alginate	Encapsulator	Biosorption performance	Rusu et al., (2022); Webster et al. (2022)
IM7-displaying yeast cells	Calcium alginate	Crosslink reaction	Protein purification	Yin et al. (2022)
Saccharomyces cerevisiae	Chitosan-calcium alginate	Encapsulator	Optimization of bear production	Benucci et al. (2021)
Meyerozyma caribbica	Resistant maltodextrin	Electrospraying	Biocontrol activity	Aguirre-Güitrón et al. (2022)
Kluyveromyces lactis	Gelatin hydrogel + graphene oxide + glutaraldehyde	–	Probiotics and bioreactor packings	Patarroyo et al. (2021)

Despite their relevance as a eukaryotic model organism for medical and biotechnological applications, the potential use of antagonistic yeasts as biocontrol agents still needs to be explored. However, in addition to powerful antifungal activities, yeasts also show intense antagonistic activity, culture ability, formability, applicability, and stress resistance and are therefore promising for developing biological plant protection agents. In addition, because they are extensively studied organisms, it is possible to take advantage of the molecular tools and the infinity of data developed for these organisms for basic and application-oriented studies in biocontrol yeasts (Freimoser et al. 2019). Aguirre-Guitrón et al. encapsulated *Meyerozyma caribbica* cells in whey protein processed with resistant maltodextrin to act as a biocontrol nanomaterial against *Colletotrichum gloeosporioides*. The electrospraying process showed efficiency in increasing cell viability and stability in storage at 4 °C, as well as a great antagonist effect (Aguirre-Güitrón et al. 2022).

Still, in the environmental field, persistent organic pollutants in different environmental matrices are a primary concern worldwide. Over the past two decades, pharmaceuticals have been detected in surface water, seawater, groundwater, drinking water, and effluent from sewage treatment plants. To avoid the negative impact of pharmaceutical products, it is necessary to develop technology for their complete removal from wastewater before being discarded in the environment. Although these conventional procedures have exciting characteristics such as efficiency, sustainability, and cost, they cannot eliminate pharmaceuticals from water (Brazesh et al. 2021). Biosorption using natural polymers as support for biomass can represent an alternative to these methods because they are efficient, cheap, nontoxic, and readily available. The ability of microorganisms to remove pharmaceutical products from aqueous solutions has been studied and shows limited efficiency due to their separation from effluents after treatment. In this sense, researchers developed a calcium-alginate matrix encapsulating *Saccharomyces pastorianus* cells, used as a biosorption microorganism for ethacridine lactate contamination. The authors tested the influence of the main parameters on the biosorption process, and the best removal efficiency obtained for ethacridine lactate was over 85%, demonstrating to be a great low-cost material for environmental treatment (Webster et al. 2022).

Yeast cells can also act as encapsulating agents, particularly for fat- and water-soluble compounds. It is a simple and highly economical process. The species most used for encapsulation are *S. cerevisiae, Torulopsis lipoferrina, Saccharomyces bayanus, Endomyces vernalis,* and lactic yeasts such as *Candida utilis* and *Kluyveromyces fragilis* (Coradello and Tirelli 2021). In general, two parts of yeast cells can be used for encapsulation: the cell wall and the plasma membrane. The cell wall maintains the cell's shape, protects the cytoplasm from cell lysis, and surrounds certain enzymes that prevent unwanted activities. The plasma membrane is composed of two phospholipid chains with excessive steroids and neutral lipids. It has a liposome-like structure and makes the yeast cell suitable for use as a coating in encapsulation. These materials have been applied to encapsulate poorly soluble actives, such as flavoring agents, antioxidants, and biocides, resulting in increased water dispersibility, thermo/oxidative stability, mechanical protection, and release control (Dadkhodazade et al. 2021).

3.3 Nanoencapsulation of Bacteriophages

Bacteriophages are viruses that exclusively infect bacterial cells that have been known for over a century. They are harmless to all organisms, including humans, except their target bacterial hosts and are the leading ones responsible for infections in bacteria. They interact with bacterial cells that express specific surface membrane receptors. However, suppose a bacterial cell does not have the specific receptor for a bacteriophage on its surface. In that case, the bacteriophage cannot infect it, which is why it is a mechanism of very high specificity. When a phage infects a bacterial cell, it replicates, and the new virions are released into the extracellular milieu and can infect other cells (Furfaro et al. 2018).

As a consequence, phages control bacterial population growth and, at the same time, contribute to moving genes from one cell to another. Their genomes encode proteins useful for biotechnological applications, including food safety, diagnostics, antibiotic therapy of infections caused by antimicrobial-resistant bacterial strains, DNA delivery vehicles, and many other relevant technologies. The study of bacteriophage particles provides information about the evolution of genomes, adaptive bacterial evolution, and the way DNA is expressed and copied, and potentially sheds light on the development of new biotechnological products (Harada et al. 2018).

However, these products need careful formulation development, and an assessment of the chemical and physical stresses bacteriophages may encounter during processing and storage. Phage inactivation and long-term reduction after storage are highly undesirable. Delivery of high levels of phages and their controlled release to the treatment site affects pharmacokinetics and treatment efficiency. Phages are composed primarily of proteins and are therefore susceptible to factors known to denature proteins, such as exposure to organic solvents, high temperature, pH variations, ionic strength, and interfacial effects (Malik 2021). Incorporating bacteriophages in therapeutic formulations usually involves encapsulation within a stabilizing substance. Through this approach, various antimicrobial materials can be produced, offering effective delivery to the site of infection and, consequently, better patient outcomes. Table 3 summarizes all the results presented in this section (Rosner and Clark 2021).

In Rahimzadeh et al., bacteriophages isolated from three different bacteria strains (*Salmonella enterica, Shigella flexneri,* and *Escherichia coli*) constituted a phage cocktail to be encapsulated in chitosan nanoparticles using the ion gelation method. The study aimed to use this novel nanocapsule to treat bacterial diarrhea in rats. The authors demonstrated that the encapsulation protected the phage from enzymes and stomach acid, thus effectively transporting it to the site of action. It prevented the rats from weight loss when submitted to gastrointestinal infection (Rahimzadeh et al. 2021). In another study, PEV2 (*Podovirus*) and PEV40 (*Myovirus*), two types of *Pseudomonas* phages, were encapsulated in a liposome matrix of soy phosphatidylcholine and cholesterol by two different techniques. The authors compared the microfluidic and conventional thin film hydration methods, followed by extrusion. PEV2-derived nanoparticles showed the smallest, and liposomes got the highest

Table 3 Nanoencapsulation of bacteriophage and its application

Bacteriophage	Encapsulation system	Encapsulation method	Bacteria target	Application	References
Phage cocktail	Chitosan	Ionic gelation	*S. enterica, S. flexneri* and *E. coli*	Biocontrol agent	Rahimzadeh et al. (2021)
T3	W/O emulsion	Encapsulator	*E. coli*	Animal feed and biocontrol	Richards and Malik (2021)
Podovirus PEV2 *Myovirus* PEV40	Liposome	Thin-film hydration + extrusion and microfluidic	*P. aeruginosa*	Encapsulation analysis	Leung et al. (2018)
UFV-AREG1	Alginate and alginate + chitosan, carrageenan or whey protein	Extrusion	*E. coli*	Encapsulation analysis	Silva Batalha et al. (2021)
ISP	Hydrogel	Emulsion	*S. aureus*	Bacteriophage therapy in rabbits	Onsea et al. (2021)
Paer4, Paer14, Paer2, W2005A	Hydrogel	Crosslink reaction	*P. aeruginosa*	Bacteriophage therapy in mice	Wroe et al. (2020)
phiIPLA-RODI	Liposome	Encapsulator	*S. aureus*	Food industry	Menéndez et al. (2018); Webster et al. (2022)
S1	Alginate	Ionotropic gelation	*Salmonella* spp.	Bacteriophage therapy in chicken	Gomez-Garcia et al. (2021)
pAh-6C	PLGA/alginate	W/O/W double-emulsion	*A. hydrophila*	Bacteriophage therapy	Kim et al. (2022)
Podoviridae T7	PolyHIPE	W/O/W triple emulsion	*E. coli*	Bacteriophage therapy	Kopač et al. (2021)
ZCEC5	Chitosan-alginate	Extrusion	*E. coli*	Animal feed and biocontrol	Abdelsattar et al. (2019)

encapsulation efficiency (59% and 50%, respectively) and minimal titer reduction with the microfluidic formulation (Leung et al. 2018). Gomez-Garcia et al. used ionotropic gelation to formulate alginate nanoparticles loaded with lytic bacteriophage S1 for *Salmonella enterica*. The phages were encapsulated at a rate of 70% and were protected from pH changes in the chicken (*Gallus gallus domesticus*) gastrointestinal system for phage therapy for 3 h (Gomez-Garcia et al. 2021). Phage-loaded alginate matrices were used in PLGA microspheres for application in bacteriophage therapy against resistant pathogens. The encapsulation ensured a higher bacteriophage concentration than the PLGA matrix alone and showed a diminished immune response. The controlled release of the infective particles was extended for

60 days in vitro and 28 days in vivo after the W/O/W double emulsion (Kim et al. 2022). The same technique was used to formulate nanocellulose-based hydrogels with lytic T7 bacteriophage isolated from *E. coli*. The encapsulation acted as a mechanical protection and a fast phage delivery system, providing improved patient compliance and reducing drug administration frequency. Finally, Abdelsattar et al. formulated *E. coli* phage ZCEC5 chitosan-alginate nanoparticles by extrusion for the delivery system in oral administration to farm animals (Kopač et al. 2021). The capsules demonstrated an efficient protective effect against pH changes and sustained particle release and lysis activity for an extended period, proving to be an excellent application in the animal feed industry (Abdelsattar et al. 2019).

4 Nanoencapsulation of Biomolecules from Plants

The secondary metabolism of plants is directly related to the primary metabolism, which is responsible for plant growth and development, energy production, and production of small molecules that directly affect secondary metabolism, photosynthesis, citric acid pathway, and other pathways (Xu et al. 2021). Although most plant-derived bioactive compounds are produced as part of their secondary metabolism, they can also be found in leaves, fruits, stems, and roots. Bioactive compounds from the secondary metabolism of plants have been used for thousands of years due to their medicinal properties. Although its exact mechanism of action was unknown, its positive effects in promoting health and combating disease were noted. Bioactive compounds have a major disadvantage since they are susceptible to degradation when exposed to various factors such as oxygen, humidity, light, and heat, which compromise their bioactivity (Zambrano-Zaragoza et al. 2017). In this context, nano/microencapsulation techniques present an alternative to extend the useful life of compounds obtained through plant extracts (Hosseini and Jafari 2020; Rahaiee et al. 2020). Although encapsulation is advantageous for maintaining the integrity of the BCs, its functional characteristics will directly depend on the choice of materials used in the encapsulation, which need to be able to prevent the degradation of the BCs and, at the same time, maintain their properties without modifying them.

Bioactive compounds originating from plants are grouped, in general, into terpenes, nitrogen-containing compounds, and phenolic compounds (Montiel-Sánchez et al. 2021; Maccelli et al. 2020). The methods and materials used in nano/microencapsulation, as well as the applications, characterizations, and limitations of the technique for each compound, will be discussed below.

Terpenes are one of the largest classes of inorganic compounds produced by plants. They are responsible for the taste, fragrance, and pigment of plants, being the major constituents of essential oils (Hosseini and Jafari 2020; Diniz et al. 2021). Terpenes are widely known for their anti-inflammatory effects and anticancer, insecticidal, fungicidal, bactericidal, and antiviral properties. However, their low water solubility and high susceptibility to oxidation limit their use (Diniz et al. 2021; Tunç and Koca 2019).

The terpenes present in black pepper essential oil are volatile, and their properties can be reduced under certain conditions. Encapsulation can protect the EO and preserve its terpenes. Complex coacervation was chosen due to several advantages. Bastos et al. analyzed the composition of black pepper essential oil, determining the most suitable conditions for forming the complex between gelatin and sodium alginate. The primary terpene identified in black pepper essential oil was β-caryophyllene, followed by limonene and sabinene. The ratio of 6:1 (gelatin/sodium alginate) at pH 4.0 was the ideal condition found by the authors for encapsulation. The encapsulation efficiency ranged from 49.13% to 82.36%, and the chemical composition of the encapsulated EO was identified by gas chromatography. GC analysis indicated good core protection with the materials used. These biopolymers can serve as a potential delivery system for black pepper EO (Heckert Bastos et al. 2020).

Terpenes can be efficiently encapsulated within YPs by passive diffusion through porous cell walls, as YPDs are hollow, porous microspheres, a by-product of some yeast extract manufacturing processes. The first generation YP terpene materials were developed with a <2:1 terpene:YP weight ratio. Soto et al. reported methods to increase terpene carrying capacity in YPs up to a 5:1 terpene:YP weight ratio. A mixture of geraniol, eugenol, and thymol (GET) previously used to develop YP-GET 1.1:1 was used as a model terpene composition to prepare hyperloaded YP-GET. Hypercharged YP terpenes extend payload release kinetics by up to three times compared to commercially available terpene YP formulations. The hypercharged YP-terpene compositions were optimized to achieve high terpene storage encapsulation stability from −20 °C to 54 °C. The development of hypercharged YP terpenes has a wide range of potential agricultural and pharmaceutical applications with terpenes and other compatible active substances that can benefit from a delivery system with high payload capacity combined with increased payload stability and sustained release properties (Soto et al. 2022).

Tackenberg et al. study aim to improve the understanding of a counter rotating twin screw extrusion process. Orange terpenes as model flavor, maltodextrin, and sucrose as matrix materials, were encapsulated by extrusion, amorphous and partly crystalline samples were obtained. The loss of crystalline sucrose was linked to a dissolution process of the sugar in the available water amount. Melting of the excipients did not arise, resulting in a plasticization extrusion process. Maximally 67% of the flavor was retained (corresponding to a 4.1% product flavor load). The flavor loss correlated with insufficient mixing during the process and flavor evaporation after extrusion. Based on these results, recommendations for an improved encapsulation process are given (Tackenberg et al. 2015).

The encapsulation of essential oils from fruit juices and the effect of delivery on the antimicrobial activity of terpenes were investigated by Donsi et al. to increase the antimicrobial capacity and increase the quality of the final product. A terpene mixture and d-limonene were encapsulated into nanoemulsions based on food-grade ingredients. The sunflower oil or essential oil-in-water nanoemulsions were prepared using a high-pressure homogenization (HPH) technique. Three different microorganisms were tested: *Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, and *Escherichia coli*. The increase in antimicrobial activity depended on the

formulation and average diameter of the delivery systems as well as on the microorganisms class. The nanocapsules with the greatest antimicrobial capacity were pear and orange juices inoculated with *L. delbrueckii*. Due to the higher antimicrobial activity of the nanoencapsulated compounds, lower antimicrobial concentrations are required for a bactericidal action under accelerated aging at 32 °C, with a minimal alteration of the organoleptic properties of the juice (Donsì et al. 2011).

Propolis extract is a bioactive compound with several properties and potent pharmacological efficacy. Casein-maltodextrin nanocomplexes loading propolis was successfully synthesized by Soleimanifard et al. The characterization of the resulting nanocomplexes was carried out by monitoring the average size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), color properties, and morphology. FT-IR and XRD analysis showed that propolis extract had been located adequately inside nanoparticles. Most of the particles were between 500 and 3800 nm: particle size higher than 1000 nm could be due to particle aggregation or an increase in the amount of larger molecules of sodium casein. The results of DLS and EE showed that smaller nanoparticles with less polydispersity index and less coral material/encapsulant amount had better size distribution and stability. The authors demonstrated that the encapsulated propolis extract could be used in several applications by the pharmaceutical industry (Soleimanifard et al. 2021).

The use of essential oils from *Origanum glandulosum* Desf. has been used as an alternative to antibiotics due to the various bioactive compounds present. To overcome the drawbacks of using oils as the hydrophobicity and negative interaction with the environmental conditions, in addition to increasing their activity, encapsulation for the oil was performed using high-speed homogenization (HSH) into nanocapsules and high-pressure homogenization (HPH) into nanoemulsion (Bouaouina et al. 2022). The antimicrobial activity of the essential oil, nanoemulsion, and nanoencapsulation were tested against *E. coli, S. aureus*, and *A. baumannii*. Nine components were identified in the essential oil, thymol (48.52%), carvacrol (16.13%), p-cymene (27.56%), and γ-terpinene (5.59%) were the predominates. A considerable change in composition was observed in oil nanocapsules concerning the essential oil. The mean particle size of the nanoemulsion was 54.24 nm, while that of nanocapsules was 120.60 nm. The antimicrobial activity assays demonstrated that the nanocapsules were more effective than the nanoemulsion, but both showed lower effectiveness in relation to the essential oil. The nanoencapsulation using intensive-energy techniques is responsible for the changes in aroma profiles discussed above and, consequently, for the antibacterial activities. Both formulations have shown relatively significant action against biofilm state at subinhibitory concentrations, where nanoemulsion was more potent than nanocapsules due to the higher thymol concentration.

Betalains are the leading group of compounds containing hydrogen atoms found in fruits. They are water-soluble and responsible for the fruits' color, which varies between red and yellow (Montiel-Sánchez et al. 2021). To develop new products and applications, recent studies have been carried out to explore new alternatives for optimizing bioactive compounds. The antioxidant capacity, elimination of free

radicals and reactive oxygen species, inhibition of lipid peroxidation, and anti-inflammatory and antimicrobial activities are among the biological properties of interest (Coy-Barrera 2020; Yao et al. 2020b).

To encapsulate caffeine, a highlighted compound of the alkaloid family, the ideal conditions for manufacturing chitosan-coated liposomes were investigated. The morphological properties of the developed nanochitosan were investigated by FESEM, TEM, and AFM analyses. FT-IR analysis was also conducted to evaluate the possible interaction between chitosan and caffeine (Seyedabadi et al. 2021). Nanoliposomes were synthesized by dissolving in deionized water and heating at 50 °C. 0.09 g lecithin, 0.01 g cholesterol, and 0.02 g Tween® 80 were dissolved in absolute ethanol using a magnetic stirrer and dropped into the aqueous solution containing caffeine. The prepared solution was sonicated for 20 min (1 s on and 1 s off) at different ultrasonication power levels. Morphology evaluation revealed the formation of uniform spherical nanoliposomes with a size of 100 nm. Furthermore, the zeta potential of the sample was 31.9 mV after adding chitosan and −25.5 mV before chitosan addition. The FTIR analysis demonstrated that the inclusion of caffeine took place at the polar sites of phosphatidylcholine of the nanostructures, which are present on the internal surface of nanoliposomes. The increase in sonication power resulted in increased stability of chitosomes due to the size reduction of the developed chitosomes and homogenous distribution of the nanoparticles in the solution. It was demonstrated that chitosan nanoliposomes efficiently encapsulate caffeine and could be used in the pharmaceutical and food sectors.

The effects of microencapsulation of phenolic components obtained from grape pomace extract were investigated by Tolun et al. (Tolun et al. 2020). The polyphenols obtained from grape pomace were encapsulated using the spray drying technique. After manufacturing the microcapsules, they were stored in two different humidity conditions (33% and 52%) for 75 days. Analyzes were performed every 15 days to measure total phenolic content, antioxidant activity, and individual phenolic compounds. The combination of maltodextrin and gum arabic as a microencapsulating material resulted in improved stability of the polyphenols when compared to the microcapsules obtained only with maltodextrin. The most promising results were obtained with encapsulation with maltodextrin DE4-7 prepared by adding gum arabic to the material in a ratio of 8:2. Stable polyphenols microencapsulated have great importance for several areas like the food industry to contribute to human health.

Curcumin is a phenolic component found in the turmeric plant, the best known of which is *C. longa*, native to India and tropical Asia. In addition to being widely used as a food spice, turmeric is also known for its orange and yellow dye. Due to its pharmaceutical properties, it has been used for thousands of years to treat common ailments such as arthritis, wounds, acne, digestive tract problems, and infections. Such functional properties have been mainly related to the unique active compounds called curcuminoids in the rhizome, the most important of which is curcumin (Maheshwari et al. 2006).

5 Nanoencapsulation of Biomolecules from Marine and Freshwater Organisms

Of the total water available on the planet, 97% is in the seas and oceans, and only 3% is fresh water. Of that small percentage, just over 2% is in glaciers, so less than 1% is available for consumption. The seas and oceans make up approximately 70% of the coverage of the entire planet Earth, which is the largest natural habitat on Earth (Grosberg et al. 2012). Marine and freshwater ecosystems represent a high and complex biological and chemical diversity, being a potential carrier of biomolecules of interest for developing new technologies, especially in the health field (Irfan and Alatawi 2019). Exploring the potential of aquatic habitats began with the cultivation of algae, sponges, and corals, later expanding to the study of cyanobacteria and fungi and, at the latest, to large organisms such as fish, crustaceans, and some aquatic mammals (Fig. 2). Through these pioneering studies, it was observed that these organisms are unique sources of unique bioactive compounds. Among these biomolecules, polyunsaturated fatty acids (PUFAs), polysaccharides, minerals and vitamins, enzymes, and bioactive peptides stand out (Nova et al. 2020). Currently, these compounds are being widely studied, as in many cases, they have various molecular targets, which may be potential biomolecules for developing essential drugs.

5.1 Nanoencapsulation of Biomolecules from Algae

Algae are critical aquatic organisms in both marine and freshwater ecosystems. An infinity of algae is cataloged and subdivided into macro- or microalgae, differing by their cellular structure and the number of cells that form them. Macroalgae are

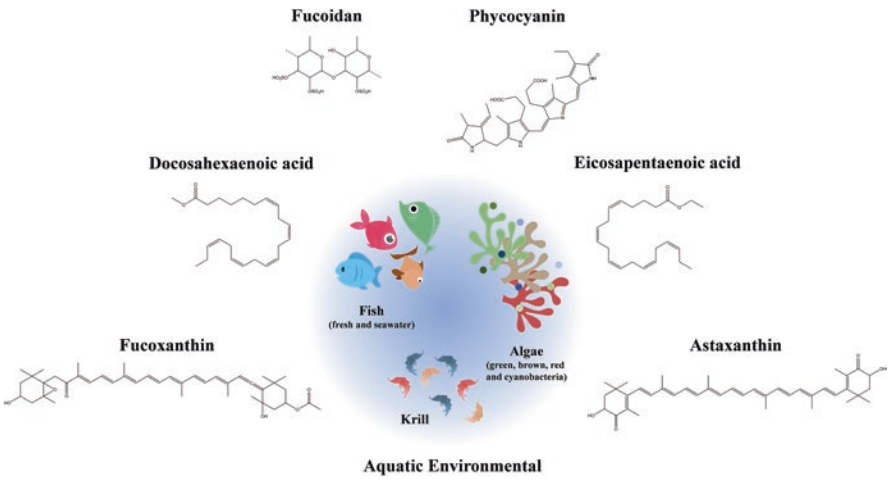

Fig. 2 Aquatic environment and its primary sources of biomolecules

eukaryotic and multicellular organisms that do not have the specialized structures and forms of reproduction of true plants (Menaa et al. 2020). The different photosynthetic pigments can distinguish them in their cells (green, brown, and red). Microalgae are unicellular/multicellular eukaryotic algae (green algae) or prokaryotic (cyanobacteria), the main base of the aquatic trophic chain. They are rich in bioactive compounds such as carbohydrates, proteins, minerals, polyunsaturated fatty acids, fatty acids, amines, amides, antioxidants, and pigments such as carotenoids, chlorophylls, carotene, xanthophylls, and phycobilins (Harwood 2019). The production of these compounds is totally influenced by the algae species and by the cultivation conditions (availability of nutrients, temperature, pH, salinity, luminosity, etc.) (Menaa et al. 2021). This way, metabolites and extracts from algae are potential biomolecules for their encapsulation and subsequent use with drugs, stabilizers, and food supplements. The results presented below are summarized in Table 4.

Commercial algal oils rich in PUFAs, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been widely encapsulated to improve their stability. Wang et al. encapsulated commercial algal oils in different proportions through the microfluidization method, using stearic acid as a solid lipid and poloxamer 188 as a surfactant (Wang et al. 2014). The EE obtained in all proportions was greater than 88%, with a maximum loading capacity of approximately 18%, stable between 4 °C and 25 °C and at acidic pH. In another work, Prieto et al. analyzed the effect of whey protein purity on the encapsulation of commercial seaweed oils using the electrospraying method assisted by pressurized gas (Prieto et al. 2022). It was found that the whey protein's purity interferes with the encapsulated material's morphology, which is reflected in its oxidative stability. Chen et al. investigated nanoemulsions obtained by ultrasound using commercial seaweed oil with phytosterols stabilized by quillaja saponin to reduce the number of oxidized compounds from the oil that cause unpleasant odors to food (Chen et al. 2016). An excellent reconstruction of these nanoemulsions was observed 30 days after being dried and sprayed. In addition, compared to pure oil, there was a decrease in oxidized compounds that cause unpleasant odors, revealing that the nanoemulsion decreases the oxidation of this oil.

As previously mentioned, algae have a very rich composition of biomolecules, making their extract possess unique nutritional and pharmacological properties. The in vitro phytochemical release profiles of *Jania rubens* extract encapsulated with chitosan were analyzed using the ionic gelation method (Maghraby et al. 2022). A high EE was obtained for these nanoparticles (99.7%), observing that the release of the extract occurred in a controlled manner, maintaining its high antioxidant power. In another work, the same authors observed that the same extract encapsulated with chitosan and tripolyphosphate by the ionic gelation method considerably reduces the rancidity of vegetable oils, making this extract a natural alternative to the use of synthetic antioxidants (Maghraby et al. 2021). *Sargassum boveanum* extracts were encapsulated in lecithin using the Mozafari method and used as antimicrobial agents in mayonnaise formulations (Savaghebi et al. 2021). It was observed that the encapsulated extract delayed the lipid oxidation of mayonnaise and considerably reduced

Table 4 Type of algae, target biomolecule(s), encapsulation technique, encapsulating agent, size, morphology, and application of nanoencapsulated bioactives from algae

Source	Encapsulated compound	Encapsulation method	Encapsulation system	Application	References
Chlorella pyrenoidosa	Hydrolyzed polypeptides	Complex coacervation and ionotropic gelation methods	Chitosan	Antitumor	Wang and Zhang (2013)
Commercial microalgae oil	–	Microfluidization method	Stearic acid and polaxamer 188	Stability	Wang et al. (2014)
Commercial microalgae oil	–	Electrospraying assisted by pressurized gas	Whey protein	Stability	Prieto et al. (2022)
Commercial microalgae oil	–	One-pot ultrasound emulsification	Phytosterol	Antioxidant and food additive	Chen et al. (2016)
Jania rubens	Extract	Ionic gelation	Chitosan	Antioxidant	Maghraby et al. (2022)
Dunaliella salina	Extract	Serials injections	Magnetic nanoparticles grafted with gum arabic	Antioxidant and antitumor	Zamani et al. (2019)
Ulva ohnoi	Extracts	Ionic gelation	Chitosan	Immunostimulant	Fernández-Díaz et al. (2017)
Jania rubens	Extract	Ionic gelation	Chitosan	Food additive	Maghraby et al. (2021)
Sargassum boveanum	Extract	Mozafari method	Lecithin	Food additive	Savaghebi et al. (2021)
Archaea	Carotenoids	Nanoelmulsion	Triton X100 and tween 80	Antioxidant	Chaari et al. (2018)
Haematococcus pluvialis	ATX	Hot homogenization	Stearic acid and soy lectihin	Stability	Salatti-Dorado et al. (2019)
Commercial	ATX	High-pressure homogenization	Soybean oil	Stability	Khalid et al. (2017)

(continued)

Table 4 (continued)

Source	Encapsulated compound	Encapsulation method	Encapsulation system	Application	References
Commercial	ATX	Solvent displacement	Polycaprolactone	Stability	Tachaprutinun et al. (2009)
Commercial	ATX	Supercritical antisolvent	Poly (l-lactic acid)	Stability	Liu et al. (2019b)
Commercial	ATX	Emulsification-solvent evaporation	Potato protein	Bioavailability	Abuhassira-Cohen et al. (2020)
Commercial	ATX	Supercritical fluid technique	Ethyl cellulose	Antioxidant	Tirado et al. (2019)
Commercial	ATX	Antisolvent precipitation and electrostatic deposition	Poly(lactic acid-co-glycolic acid) and chitosan oligosaccharides	Bioavailability	Liu et al. (2019a)
Commercial	ATX	Emulsification-solvent evaporation	Chitosan	Cellular uptake and antioxidant	Wang et al. (2017)
Commercial	ATX	Supercritical fluids	Polyvinylpyrrolidone	Bioavailability and antioxidant	Kaga et al. (2018)
Fucus vesicolus	Fucoidan	Emulsification-solvent evaporation	Lecithin	Anticancer and immunomodulator	Qadir et al. (2008)
Laminaria japonica	Fucoidan	Emulsification-solvent evaporation	Protamine	Antitumor	Lu et al. (2017)
Padina tetrastromatica L	FXT	Ionic gelation	Chitosan and glycolipids	Antioxidant	Ravi and Baskaran (2017)
Phaeodactylum tricornutum	FXT	Ionic gelation and polyelectrolyte complexation	Alginate	Bioavailability	Koo et al. (2023)
Phaeophyce	FXT	Freeze-drying	Maltodextrin and tween 80	Stability	Indrawati et al. (2015)
Spirulina platensis	Hydrolyzed polypeptides	Ionotropic gelation method	Chitosan	Antitumor	Zhang and Zhang (2013)
Spirulina LEB-18	Protein concentrate	Reverse phase evaporation	Rice and soy bean lecithins	Antioxidant	Machado et al. (2019)

Commercial	Phycocyanin	Electrospinning	Chitosan	Antitumor	Wen et al. (2020)
Gracilaria gracilis	Phycobiliprotein	Reverse phase evaporation	Soy Lecithin	Food packaging	Haghdoost et al. (2022)
Spirulina sp. LEB 18	Protein concentrate	Electrospinning	Poly(ethylene oxide)	Antioxidant	Moreira et al. (2019)
Commercial	Phycocyanin	Electrospinning	Poly(ethylene oxide) and polyvinylalcohol	Ph sensor	Moreira et al. (2018)
Spirulina platensis	Protein concentrate	Electrospinning	Gelatin	Antioxidant	Mosayebi et al. (2022)
Spirulina platensis	Protein concentrate	–	Chitosan	Food packaging	Karimzadeh et al. (2023)
Commercial	Phycocyanin	Electrospraying	Polyvinylalcohol	Antioxidant	Schmatz et al. (2020)

the number of microbial colonies without deteriorating the product's sensory properties, extending its shelf life by up to 4 months. Chitosan nanoparticles loaded with *Ulva ohnoi* extract were obtained through the ionotropic gelation method, producing active nanocarriers to be used against *S. senegalensis* macrophages (Fernández-Díaz et al. 2017). A drug delivery system was created using magnetic nanoparticles and gum arabic as carriers of *Dunaliella salina* extract (Zamani et al. 2019). For this system, an EE greater than 91% was observed with a minimum load capacity greater than 79%. This system's antioxidant and cytotoxic analysis was evaluated in MCF-7 and HeLa cells, indicating a high antioxidant and anticancer effect, time-dependent, and dose-dependent. Polypeptides extracted from *Chlorella pyrenoidosa* with antitumor action (HepG2 human liver cancer cells) were encapsulated through complex coacervation and ionotropic gelation methods using chitosan as encapsulating polymer (Wang and Zhang 2013). The encapsulation efficiency (EE) achieved was 74.5% and 30.1% for the complex coacervation and ionotropic gelation method, respectively, despite the polypeptide content being approximately equal (12.7% and 12.3%, respectively). Both encapsulations showed good preservation of gastric enzymatic degradation against in vitro release tests of these polypeptides.

Specific biomolecules from algae are also of extreme interest when it comes to pharmacological control and development (Takaichi 2013) Carotenoids are important liposoluble pigments in bacteria, algae, fungi, and vegetables responsible for the orange, yellow, and red colors (Christaki et al. 2013). Photosynthetic organisms participate as coadjuvants in the photosynthesis process and help to protect against possible damage caused by light (Allen et al. 1964). The main applications of carotenoids involve their antioxidant and anti-inflammatory properties. Chaari et al. extracted carotenoids from *Archaea halophilic,* encapsulating them in oil-in-water dispersions to increase their water solubility and use them as a functional food (Chaari et al. 2018). The encapsulation processes used were high-pressure homogenization and spontaneous microemulsion, using limonene with oil phase, Triton X-100/Tween-80 mixtures as emulsifiers, and water/glycerol solutions. The oxidizing activity of this compound was evaluated by radical scavenging using Electron Paramagnetic Resonance Spectroscopy (EPR) in the stable free radical scavenging Tempol.

Among the carotenoids, a biomolecule of interest is astaxanthin (ATX). ATX is one of the few carotenoids that does not convert to Vitamin A in the human body, a powerful antioxidant (Dose et al. 2016). Due to its antioxidant properties, it has numerous health benefits, especially in the immune system (Anarjan et al. 2010). The primary sources of ATX come from marine organisms such as algae, krill, and shrimp. Salatti-Dorado et al. encapsulated ATX from *Haematococcus pluvialis* using a combination of molecular solvents and the hot homogenization method (Salatti-Dorado et al. 2019). The EE obtained from this process was at least 58%, with a loading capacity of 19%, depending on the surfactant and solid phase used. The oxidizing capacity of this new type of encapsulation was superior to Trolox e and α-Tocopherol standards, in addition to protecting human endothelia from attack by reactive oxygen species (ROS). Similar studies used a combination of DNA and chitosan for the efficient encapsulation of ATX (Wang et al. 2017). These particles

showed good cytoprotective effects against oxidative cell damage and high efficiency in eliminating ROS, being quickly endocytosed by Caco-2 cells.

The influence of the aqueous phase to encapsulate ATX nanoemulsions was evaluated by Khalid et al. using the high-pressure homogenization method (Khalid et al. 2017). Lecithin and sodium caseinate were used as aqueous phases, showing that when lecithin is used, stability at different pHs and temperatures is greater, in addition to its bioavailability. In another work, the influence of the encapsulated polymer was evaluated against EE and its thermal degradation (Tachaprutinun et al. 2009). Poly(ethylene oxide)-4-methoxycinnamoylphthaloylchitosan (PCPLC), poly(vinylalcohol-co-vinyl-4-methoxycinnamate) (PB4), and ethylcellulose (EC) were used as encapsulating agents, showing that only PCPLC presents satisfactory EE (98%) and loaning capacity (40%). The supercritical antisolvent (SAS) process was efficient when encapsulating ATX using poly (l-lactic acid) (PLLA), with EE of 91.5% and stability gain during storage (Liu et al. 2019b). A process similar to SAS is used for the encapsulation of ATX using poly(lactic-co-glycolic acid) (PLGA) nanoparticles coated with chitosan oligosaccharides, and EE greater than 85% and loading capacity greater than 15% are obtained (Liu et al. 2019a). Potato protein also proves to be an efficient way to increase the oral bioavailability of ATX when encapsulated, presenting a nonallergenic and vegan way to encapsulate lipophilic bioactive (Abuhassira-Cohen et al. 2020). The process of enhanced dispersion in solution by supercritical fluids (SEDS) also appears as an efficient alternative for the encapsulation of ATX (Kaga et al. 2018; Tirado et al. 2019). The pressure and temperature parameters used in SEDS are fundamental since they impact the bioavailability and antioxidant activity of the final material.

Another marine carotenoid from brown algae and diatoms is fucoxanthin (FXT) (Nomura et al. 1997). FXT is one of the most abundant carotenoids and is characterized as an orange pigment (Shimoda et al. 2010). The main potential health-promoting effects of FXT are associated with its antioxidant, anti-inflammatory, antitumor, antiobesity, and antidiabetic effects (Peng et al. 2011). This way, encapsulating it to protect its properties and increase its bioavailability and storage time is essential for this compound to be used as a nutraceutical additive or in developing new drugs. Indrawati et al. encapsulated carotenoids (mostly FXT) from *Sargassum* sp., using maltodextrin and Tween-80 as encapsulating agents (Indrawati et al. 2015). EE values greater than 88% were obtained, showing that the Freeze-Drying method effectively encapsulates these carotenoids. Koo et al. encapsulated FXT extracted from *Phaeodactylum tricornutum* using alginate, casein, and chitosan through the ionic gelation method, achieving EE greater than 78% (Koo et al. 2023). It was observed that during simulated gastrointestinal digestion, there was a controlled release of FXT and improvement in the permeability of Caco-2/TC7 cells. In addition, an increase in FTX metabolites was observed by analyzing the plasma of mice after oral ingestion. Chitosan nanogels with glycolipids also proved effective in increasing FXT bioavailability and PPARγ expression (Ravi and Baskaran 2017).

There is currently a growing interest in nutraceutical supplements derived from *Spirulina*. *Spirulina* is a microalgae known to be a superfood, consisting of carbohydrates, lipids, and between 50% and 70% proteins (Stejskal et al. 2020). In

addition, it is rich in fatty acids, vitamins, and some pigments such as phycobiliproteins (Fernández-Rojas et al. 2014). C-phycocyanin has been widely studied among phycobiliproteins due to its antioxidant, antitumor, anti-inflammatory, and COX-2 enzyme inhibitor properties (Eriksen 2008). C-phycocyanin is an important blue pigment, and with carotenoids, it is one of the most studied pigments from algae. Encapsulation of *Spirulina* protein extract with chitosan nanoparticles was carried out by Karimzadeh et al., reaching a maximum EE of 67% and a loading capacity of 14% (Karimzadeh et al. 2023). These particles reduced microbial contamination in the adequate storage of fish. Liposomes with phycobiliproteins extracted from *Gracilaria gracilis* were obtained using soy lecithin as an encapsulating agent (Haghdoost et al. 2022). The results of this work show that the EE obtained was approximately 84%, providing less lipid oxidation and microbial deterioration in the storage of carp burgers. Liposomes of rice and soy lecithins with *Spirulina* phenolic extracts were obtained (with EE greater than 88%) and evaluated against the controlled release of these extracts in a dynamic gastrointestinal system, getting satisfactory results (Machado et al. 2019).

Ultrafine fibers with antioxidant activity from *Spirulina sp.* LEB 18 was obtained from the electrospinning method, aiming to obtain smart packages (Moreira et al. 2019).

In another work, polyvinyl alcohol (PVA) and polyethylene oxide (PEO) fibers containing phycocyanin were obtained using electrospinning (Moreira et al. 2018). The fibers were used as pH sensors to be used in smart packaging since phycocyanin is a natural visible pH sensor. Fast-dissolving fibers were also obtained by electrospinning using gelatin and *Spirulina* protein extract, evaluating the DPPH and ABTS radical scavenging activity (Mosayebi et al. 2022). The electrospray technique is also effective for obtaining particles with encapsulated phycocyanin. Schmatz et al. used PVA to encapsulate commercial phycocyanin through the electrospraying technique with EE above 75%, in addition to high thermal resistance and maintenance of its antioxidant activity (Schmatz et al. 2020).

Spirulina encapsulated materials have shown high potential to be used in developing new antitumor drugs. Wen et al. observed a significant antiproliferative effect on human colon cancer cells HCT116, indicating that electrospinning fibers based on polysaccharides, prebiotics, and phycocyanin have the potential to be used as antitumor agents (Wen et al. 2020). The antitumor activity of the Y2 polypeptide extracted from *Spirulina platensis* and encapsulated with chitosan was evaluated in human breast cancer MCF-7 cells and liver cancer HepG2 (Zhang and Zhang 2013). The EE obtained for this material was 49% with a loading capacity of 15%, with an IC_{50} for both cancer cells of 61 mg/mL.

Fucoidan is a term used to define heterofucan-type polysaccharides that contain less than 90% L-fucose from brown algae (Yoo et al. 2019). This polysaccharide has been extensively studied due to its varied pharmacological activities such as anticoagulant, antiviral, antitumor, antithrombotic, etc. (Luthuli et al. 2019). Qadir et al. obtained fucoidan nanoliposomes encapsulated with lecithin to investigate its antitumor and immunomodulatory activity (Qadir et al. 2008). It was found that after the particles were uptake by the cells, the antitumor activity increased by up to 10%

compared to free fucoidan. In another work, fucoidan was encapsulated with protamine, showing an inhibitory effect against metastatic breast cancer cells (Lu et al. 2017).

5.2 Nanoencapsulation of Biomolecules from Fishes and Krill

In the last decade, several works have been developed on the effects of diets supplemented with fish oil as a source of omega-3 PUFAs in preventing several diseases, especially for the treatment of cardiovascular diseases (Sargent 1997). The fatty acids that form omega 3 are DHA and EPA, have numerous critical pharmaceutical properties, such as antiarrhythmic, antithrombotic, antiatherosclerotic, anti-inflammatory, help to reduce blood pressure and decrease the concentration of triglycerides, etc. (Yetiv 1988; Harris 2004). In this way, improving its bioavailability and reducing its degradation during storage are extremely important for the development of nutraceutical additives and drugs based on fish oil. The results presented below are summarized in Table 5.

One of the most used techniques for fish oil encapsulation is electrospinning since it has high reproducibility and high EE rates. Zein is a polymer widely used to encapsulate fish oil by electrospinning. Yang et al. encapsulated fish oil and ferulic acid with zein using a small portion of glycerol in the encapsulating phase, obtaining EE of 94% and a loading capacity of 20% (Yang et al. 2017). In this case, ferulic acid decreased the oxidation of fish oil without changing its bioavailability. Alcoholic zein solutions can also reduce fish oil oxidation and increase the EE of electrospinning fibers (Moomand and Lim 2014). Torres-Giner et al. produced DHA capsules encapsulated with zein, achieving better results against its degradation in confined environments (sealed packaging situation) (Torres-Giner et al. 2010). Cod liver oil nanofibers encapsulated with PVA were obtained from this technique with EE greater than 92% and a loading capacity of 11%. However, PVA encapsulation did not protect the fish oil from oxidation, with a higher content of oxidized products than pure oil (García-Moreno et al. 2016). Kafirin and mixtures of whey protein with carbohydrates also proved to be good encapsulating biovectors of fish oil by electrospinning, with an EE above 94% (García-Moreno et al. 2018; Cetinkaya et al. 2021).

Drying techniques can also produce stable fish oil encapsulations. Berjrapha et al. compared the stability of fish oil in poly-ϵ-caprolactone (PLCPL) nanocapsules obtained by the emulsion-diffusion method, followed by vacuum freeze drying (VFD) and conventional freeze drying (CFD) (Bejrapha et al. 2010). The sample obtained by CFD showed higher EE, in addition to having greater oxidative stability, since VFD can compromise the PLCPL membrane. Sage polyphenols appear as an alternative for stabilizers of the encapsulating agent's sodium caseinate and gum arabic in the encapsulation of sardine fish oil by spray-drying, presenting the final nanoencapsulated product with high EE, high thermal resistance, and low oxidation (Binsi et al. 2017). Peptides from *Sardina pilchardus* and *Trachurus mediterraneus*

Table 5 Type of fish/krill, target biomolecule(s), encapsulation technique, encapsulating agent, size, morphology, and application of nanoencapsulated bioactives from fishes and krill

Source	Encapsulated compound	Encapsulation method	Encapsulation system	Application	References
Commercial cod liver oil	–	Electrospraying	Polyvinylalcohol	Stability	García-Moreno et al. (2016)
Commercial fish oil	–	Electrospraying	Zein	Stability	Yang et al. (2017)
Commercial fish oil	–	Electrospraying	Zein	Stability	Moomand and Lim (2014)
Commercial fish oil	–	Electrospraying	Kafirin	Stability	Cetinkaya et al. (2021)
Commercial fish oil	DHA	Electrospraying	Zein prolamin	Stability	Torres-Giner et al. (2010)
Commercial cod liver oil	–	Electrospraying	Whey protein and carbohydrates	Stability	García-Moreno et al. (2018)
Menhaden	Fish oil	Freeze drying	Polycaprolactone	Stability	Bejrapha et al. (2010)
Sardinella longiceps	Fish oil	Spray drying	Sodium caseinate and gum arabic	Stability	Binsi et al. (2017)
Commercial fish oil	Omega-3	Gas-Saturated Solutions-dried	Modified starch	Stability	Melgosa et al. (2019)
Commercial fish oil	Omega-3	Microfluidization	Sunflower oil	Stability	Komaiko et al. (2016)
Commercial fish oil	DHA	Emulsification-solvent evaporation	Casein	Stability	Zimet et al. (2011)
Commercial fish oil	Omega-3	Low energy spontaneous emulsification	Tween 80	Stability	Walker et al. (2015)
Sparus aurata	Sea bream scales collagen	Emulsification-solvent evaporation	Soybean lecithin	Stability	Mosquera et al. (2014)
Menhaden	Fish oil	Nanoelmulsion	Palmitic acid and quercetin	Stability	Azizi et al. (2019)
Commercial fish oil	–	High intensity ultrasound method.	Tween 80 and span 80	Stability	Nejadmansouri et al. (2016)
Commercial fish oil	Omega-3	Hot high-pressure homogenization	Tristearin and lecithin	Stability	Salminen et al. (2013)
Engraulis encrasicolus L.	Omega-3 fatty acids	Controlled crystallization and kneading method	B-cyclodextrin	Stability	Ünlüsayin et al. (2016)

(continued)

Table 5 (continued)

Source	Encapsulated compound	Encapsulation method	Encapsulation system	Application	References
Clupeonella cultriventris caspia	Peptídeos	Electrospraying	Chitosan and polyvinylalcohol	Antioxidant	Hosseini et al. (2019)
Sardina pilchardus and *Trachurus mediterraneus*	Fish oil	Spray-drying	Glucose syrup	Antioxidant	Morales-Medina et al. (2016)
Oncorhynchus mykiss	Peptides	Liposome	Chitosan	Antioxidant	Ramezanzade et al. (2017)
Oncorhynchus mykiss	Fish oil	Liposome	1,2-dipalmitoyl-sn-glycero-3--phosphocholine	Antioxidant	Hosseini et al. (2017)
Commercial fish oil	–	Liposome	Soy lecithin and sunflower oil	Food additive	Ghorbanzade et al. (2017)
–	EPA and DHA	Ionic gelation	Sodium caseinate and gum arabic	Food additive	Ilyasoglu and El (2014)
Commercial fish oil	–	Freeze drying	Gum arabic and tween 80	Food additive	Moghadam et al. (2019)
Commercial krill oil	–	Nanostructured lipid carriers	Palm stearin acid and lecithin	Stability	Zhu et al. (2015)
Euphausia superba	–	Emulsion-electrostatic interaction method	Chitosan and tripolyphosphate	Food additive	Haider et al. (2017)
Curcumin	–	Reflux followed by thin drug-lipid film hydration method	Commercial krill oil	Antitumor	Ibrahim et al. (2018)

were also successfully encapsulated by spray-drying using different polymers with high cytocompatibility (Hosseini et al. 2019; Morales-Medina et al. 2016). Obtaining omega 3 nanocapsules with modified starch by the Gas-Saturated Solutions (PGSS)-drying method showed EE above 95% and lowered susceptibility to oxidation (Melgosa et al. 2019).

Nanoemulsions obtained from microfluidization of fish oil in water using sunflower phospholipids as an emulsifier were obtained by Komaiko et al., and they have a series of advantages over artificial emulsifiers, such as high oxidative stability and being hypoallergenic (Komaiko et al. 2016). Surfactant concentration becomes a critical point in optimizing the encapsulation of fish oil in emulsions, reflecting on the emulsions' morphology, size, EE, and stability (Walker et al. 2015). Zimet et al. took advantage of the self-assembly properties of casein to encapsulate DHA with high colloidal stability and bioactive conservation (Zimet et al. 2011).

The emulsification-solvent evaporation obtained peptide nanoliposomes from sea bream scales encapsulated with soy lecithin (Mosquera et al. 2014). The

maintenance of antioxidant activity and ACE inhibitory activity remained constant for 8 days at low temperatures, preserving the biological activities of the extracted peptides. Oncorhynchus mykiss peptides were encapsulated in nanoliposomes with EE above 46%, depending on the polymer concentration used for encapsulation (Hosseini et al. 2017; Ramezanzade et al. 2017). To improve the properties of the encapsulated fish oil, quercetin is an antioxidant additive that may help slow down the oxidation of fish oil (Azizi et al. 2019). Other important factors to be considered when designing new encapsulations are the composition of the encapsulating agent, the surfactant's nature, and the fish oil's composition, which can abruptly alter the stability of nanoemulsions (Salminen et al. 2013; Nejadmansouri et al. 2016; Ünlüsayin et al. 2016).

Due to their excellent nutraceutical properties, fish oils are potent additives for fortifying foods. Ghorbanzade et al. used fish oil nanoliposomes with lecithin and soybean oil to fortify yogurt (Ghorbanzade et al. 2017). After 21 days of storage, the authors reported that liposome-fortified yogurt had higher EPA and DHA content. Gum arabic as an encapsulating agent and tween 80 as an emulsifier were also used to encapsulate fish oil by freeze drying to observe the viability of *Lactobacillus plantarum* in probiotic fermented milk (Moghadam et al. 2019). The results showed EE of 87% with increased probiotic bacterial viability and high content of EPA and DHA, which can be used as a food fortifier. Ilyasoglu et al. also used gum arabic to encapsulate EPA and DHA by ionic gelation to fortify fruit juice, achieving good nutritional values (Ilyasoglu and El 2014).

As an alternative source of omega 3 to fish, krill oil is gaining prominence, as 30% to 65% of the fatty acids in krill oil are in the form of phospholipids (Kolakowska et al. 1994). This factor alters the bioavailability of krill oil, providing it with better bioavailability (Schuchardt et al. 2011). Nanostructured lipid carriers containing high krill oil content were prepared using palm stearin as solid lipid and lecithin as the surfactant, with EE of 97% and a maximum loading capacity of 12%, aiming at their application in functional foods (Zhu et al. 2015). Haider et al. used chitosan and tripolyphosphate to encapsulate krill oil from *Euphausia superba*, obtaining a maximum EE of 59%, a loading capacity of 25%, and a high oxidant capacity (Haider et al. 2017). Liposomes with krill oil enriched with curcumin also showed good EE and loading capacity values used with antitumor agents against A549 lung cancer cells (Ibrahim et al. 2018).

6 Conclusions

Natural products generated from fungal, bacteria, plant, marine, and animal sources have a wide variety of applications with a high global impact. To preserve and maintain the natural molecules properties generated from these sources, it is necessary to apply robust techniques. Due to the diversity of properties that bioactive compounds present, different techniques can be used to guarantee protection and maintenance of the biomolecule, the performance of the selected matrix, and mainly the

bioavailability to exert the required action. Encapsulation is a viable alternative to protect active compounds against the deterioration of environmental conditions, maintaining their natural compounds. Many encapsulation methods can be used, whether physical or chemical, and their use is intrinsically linked to their application. Among them, we can highlight electrospinning methods and micelles' formation with several applications.

Each technique for forming encapsulated materials (nano or microencapsulation) depends on a series of factors that are related to the material to be protected, since the matrix-forming substance and the conditions in which the techniques will be applied are also fundamental in the process. In food, nanoencapsulation application can prolong postharvest shelf life. This process when applied to functional food ingredients can help increase their water solubility and/or dispersibility in foods and beverages, improving their bioavailability. Encapsulation imparts several benefits including improved thermal and chemical stability, preserves or masks flavor, taste, or aroma, controlled and targeted release, and enhanced bioavailability. The biopreservative effect on food packaging can also be done by bacterial enzymes, organic acids, or bacteria.

Nanoencapsulation is a technique based on enclosing a bioactive compound in liquid, solid or gaseous states within a matrix or inert material for preserving the coated substance (food or flavor molecules/ingredients). Nanoencapsulation strategies, including the possibility to deliver controlled natural compounds, synthetic molecules, or other actives (viruses) for the treatment of different human disease could revolutionize conventional medical and food science, pharmaceutical and food industry.

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Part V
Identification of Molecular Targets of Natural Molecules and Biological Potential

Targeted Delivery of Natural Products

Ahsan Ibrahim, Hunaiza Fatima, and Mustafeez Mujtaba Babar

Abstract Natural products have been a precious source of innumerable bioactive chemicals due to which many conventional therapies and remedies have been associated with them. Phytochemicals such as alkaloids, flavonoids, glycosides, terpenoids, and many more have contributed significantly to the drug discovery process. Many phytochemical formulations have aided in improving the health status of patients in clinical settings. However, some problems related to the effective delivery of natural constituents to their site of action still exist. These are mainly attributable to the challenging physicochemical and pharmacokinetic characteristics. To address these issues, a lot of research is being conducted that is based upon the interlinking of natural products with nanotechnology. Many studies have shown encouraging outcomes in terms of the targeted delivery of these phytochemicals to their intended sites for the desired therapeutic response using nanocarriers such as metallic nanoparticles, liposomes, and dendrimers, among others. Due to their better tolerability as compared to synthetic chemical entities, natural products particularly the phytochemicals are being explored for achieving delivery through active and passive targeting. This chapter provides an introduction to the formulation barriers of natural products and the recent advances in nanotechnology and formulation science in improving the overall drug targeting to the intended site of action.

Keywords Natural products · Phytochemicals · Targeted delivery · Nanotechnology · Nanoparticles

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1 Introduction

Natural products have been used for centuries by virtue of their vast medicinal uses. The knowledge of traditional medicine has helped the drug developers in devising means for more efficient drug discovery using the phytochemicals. Of all the drugs approved by the United States Food and Drug Administration (FDA) in 2019, around 24% of the drugs were of natural origin (de la Torre and Albericio 2021). Not only plant-based, but animal and microbial origin natural products have also contributed to the development of an effective drug discovery and development process, leading, thereby, to the clinical applications of a series of efficacious molecules. A number of plant-origin drugs have been employed in clinical settings across various medicinal systems. These include Colchicine from *Colchicum autumnale,* Paclitaxel from *Taxus brevifolia,* Hyocyamine from *Hyoscyamus niger,* Morphine from *Papaver somniferum,* and Mitomycin from *Streptomyces caespitosus,* among others (Shah et al. 2020; Shao et al. 2020; Hemati et al. 2021; Bouabdallaoui and Tardif 2022; Sinawe and Casadesus 2022).

Novel, potentially active therapeutic agents are subject to a number of studies to establish their effectiveness in clinical settings. However, their therapeutic delivery remains an uphill task due to the complications in their pharmaceutical presentations including low dissolution profile, stability issues and subsequent problems associated with their pharmacokinetics and bioavailability. Phytochemicals including terpenes, polyphenols, and alkaloids also undergo a higher metabolic processing by the phase II enzymes. This leads to a lesser amount of drug available in systemic circulation, thereby, leading to a decrease in the pharmacological activity of a potential drug candidate (Bose et al. 2020). To cope with these drawbacks, natural product-based nanocarriers since their advent have bridged huge gaps in the targeted drug delivery of these molecules. Based on the principles of targeted drug delivery, these novel mechanisms have been able to target specific receptors, thereby, ruling out the nonselective actions of some natural products such as cytotoxic phytochemicals. This concept is being applied in the lead discovery with natural products of plant origin. Natural product-based nanocarriers have been successfully tested in animal-based models to target the desired sites such as tumors, leading to effective pharmacological profiling of these natural chemical entities. Computational experiments and tools have also confirmed the pharmacological activity of these compounds in silico (Chen et al. 2020). This chapter covers the scope and significance of targeted delivery of natural product-based drugs for maximizing the utility of these bioactive phytochemicals.

2 Natural Products in Medicinal Use

Numerous secondary metabolites with variable chemical structures are produced by a diversity of medicinal plants. These secondary metabolites have played an important part in the discovery and development of drugs. In brief, the plant-based

systems have given rise to a huge number of lead compounds used in both traditional and modern medicine. Cytotoxic agents, including antimicrobials and anticancer agents developed by the study of floral and microbial metabolites as chemotherapeutics, are the chief examples of these molecules. Similarly, a wide variety of compounds have been discovered from sponges, algae and phytoplankton that have supplied a number of leads for drug discovery and development. Discovery of naturally occurring neurotransmitters and active peptides have also proved to be important milestones in therapeutic research.

Ethnopharmacology forms the basis of the medicinal use of plants and other natural sources. Ethnobotany, conversely, is the study of interaction of local plants with the biological aspects of the native people. This term basically deals with the medical applications of an indigenous plant using the inherent knowledge. This helps in imparting the essential parameters that are a prerequisite for the planting of these indigenous botanical sources and their use in day to day life. On the other hand, the broadest definition of ethnopharmacology is "*the interdisciplinary scientific examination of the biologically active compounds that are customarily utilized*" (Leonti 2022). As a result, the ethnopharmacological approach is based upon the merger of pharmacology, chemistry, and botany for attaining therapeutic outcomes in human population. With a very wide scope, field observations, descriptions of the application and biological effects of traditional remedies, botanical identification of plant material, and phytochemical and pharmacological studies form a part of ethnopharmacology. Many researchers have been interested in studying traditional cures and their potential effects for a long time. This has led to significant discoveries that are still playing a vital part in current pharmacotherapy practices (Verma and Singh 2020).

The use of medicinal plants in various ailments has been established. A number of historical accounts have been found in ancient civilizations that provide a record of medicinal use of plants and other natural products for centuries. The rhizomes of *Glycyrrhiza glabra L.*, for instance, are well known for their traditional use as antitussive effects induced by glycyrrhizin, glycyrrhetinic acid, and other phytochemicals. *Papaver somniferum L.* containing alkaloids including morphine, papaverine, and codeine have been used to relieve intense pain as herbal remedy since ancient times. Morphine, a popular and efficacious opioid, was first isolated by Friedrich Serturner in 1803 from poppy plant and continues to be a part of modern analgesic regimens. *Cinchona officinalis L.*, a rich source of quinine, has been a promising medicinal therapy against malaria for centuries (Tisnerat et al. 2021). In Brazilian indigenous medicine, *Achillea millefolium L.* has been used for diuresis in patients with nephropathy and cardiac disease. *Achillea arabica,* a Mediterranean plant, is known to have lipid-lowering activity and has been used in many cardiovascular disorders. *Bidens pilosa L.*, also known as Spanish needles, is a South American herb used as a decoction or tincture, that aids in lowering blood pressure by vasodilation (Michel et al. 2020). *Foeniculum vulgare Mill.*, belonging to *Apiaceae* family, is a traditional herb, the seeds of which are orally ingested to alleviate gastric acidity, constipation and nonproductive coughs. Similarly, *Argemone Mexicana L., Morus albal, Cassia fistula L.,* and *Mentha longifolia L.* are medicinal flora that

exhibit beneficial therapeutic effects in improving the digestion. *Citrullus colocynthis L.,* containing polyphenols and tannins provides relief from urinary problems and jaundice and is used in the form of pharmaceutical powders to treat these ailments. As per the ethnobotanical approach, the plants used for countering bacterial infections for hundreds of years include *Acacia eriloba*, also known as camel thorn, whose infusion helps to treat bacterial pneumonia. The infusions and decoctions of *Abrus precatorius, Artemesia afra, Asparagus africanus*, and *Chrysanthemum segetum L.* provide adequate antitubercular effect and relieve whooping cough (Cock and van Vuuren 2020). *Terminalia glaucescens* extracts have shown activity against various gram-negative bacteria, while ethanolic extracts of *Azadirachta indica* and *Zingiber officinale* show inhibition of growth in *Salmonella typhi* strains (Ugboko et al. 2020). In brief, these and many other medicinal plants form an essential component of the traditional and modern medicine. Figure 1 represents a summary of the diverse traditional medicinal uses of natural products derived from medicinal plants.

Recently, the advent of SARS-CoV-2 lead to dedicated research efforts for the drug discovery and development process using natural compounds. By employing in silico, in vitro, and in vivo approaches, many natural constituents have been investigated that may act as a potential inhibitor of SARS-CoV-2. A few of these components are summarized in Table 1.

In light of these applications, it can be established that the natural products encompass a vast family of varied chemical substances that may be produced by any organism or may originate from a mineral source. They possess a wide range of

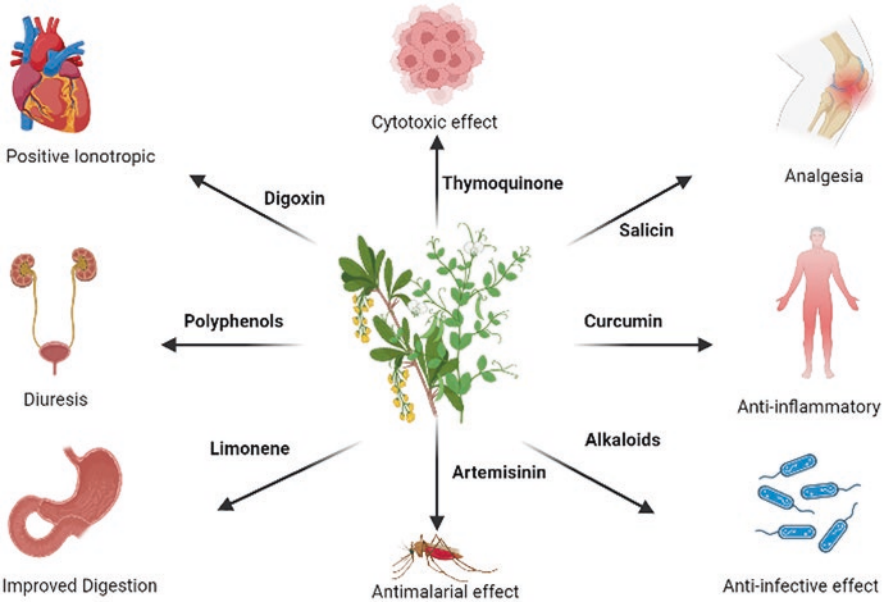

Fig. 1 Medicinal uses of the plant-based natural products and constituents

Table 1 Natural lead compounds having potential activity against SARS-CoV-2

Natural compound	Chemical class	Botanical source	References
Amentoflavone	Flavonoid	*Torreya nucifera*	Orhan and Senol Deniz (2020)
Chrysin	Flavonoid	*Oroxylum indicum*	Shah et al. (2021)
Quercetin	Flavonoid	*Lactuca sativa L.*	Gasmi et al. (2022)
Quercetin-3-O-rutinoside	Glycoside	*Dysphania ambrosioides*	da Silva et al. (2020)
Lycorine	Alkaloid	*Lycoris radiata*	Jin et al. (2021)
Baicalin	Flavonoid	*Scutellaria baicalensis*	Wu et al. (2020)
Berberine	Alkaloids	*Coptis chinensis*	Wink (2020)
3,7-di-O-methyl-kaempferol	Flavanoid	*Siparuna cristata*	Leal et al. (2021)
Lactucin	Sesquiterpene	*Cichorium intybus L.*	Ávila-Gálvez et al. (2022)
Hispidulin	Monomethoxyflavone	*Artemisia sublessingiana*	Jalmakhanbetova et al. (2021)

biological activities and unique pharmacological effects. Natural products have historically been important in the drug discovery process. This importance is further established owing to their use in traditional remedies. Natural products have been used for centuries as common remedies, and in recent years, the scientific community has turned its attention to them as a result of mounting data linking them to health advantages and the prevention of numerous diseases. Recently, their significance has again started to increase as masses are turning toward naturopathy apparently considering the ill-effects of the synthetic chemicals. The major challenge, however, associated with discovery of bioactive compounds is to establish a drug delivery methodology that ensures that adequate amount of the therapeutic agent reaches the site of action. The formulation development of phytochemicals is a laborious process and incorporates a series of steps. Following, the early screening of the crude extract and extraction of specific metabolites, the identification process of natural product is started. This involves the structure elucidation processes including Mass Spectrometry and Nuclear Magnetic Resonance among others. Thereafter, in vitro and in vivo screening of the biological activities of the isolated compound is initiated. Preclinical studies based upon animal models to unveil the early information regarding the pharmacokinetics and pharmacodynamics of the active molecule are carried out. Once validated, formulation engineers develop a diverse series of formulations that can potentially be employed for human testing. During these preclinical trials, not only is the natural compound tested for its effectiveness but the best formulation options available are also selected. Marketing of the drug after approval and subsequent post marketing surveillance is then carried out as the last, yet continuous, step of the process (Mushtaq et al. 2018).

Conventionally, the crude drug from natural sources is subjected to extraction processes using various solvents based upon the physicochemical properties of the

constituents. These mixtures are then processed for fractionation and final formulation. These can be in the form of tinctures, i.e., the extracts of series of diverse compounds based upon their miscibility in water and alcohol like tinctures of *Cannabis sativa*. Similarly, decoctions which are extracts prepared by heating a medicinal crude drug in water for a given time interval to extract out its principal chemical substances like the decoction of *Panax ginseng* and *Glycyrrhiza uralensis*. Pastes, a semisolid formulation of natural products containing about half of solid powdered content of the crude drug levigated in a fatty base are also very common. Herbal teas comprising of infusions of comminuted herbs such as *Camellia sinensis* have been used for symptomatic relief for centuries. Similarly, liniments which are the preparations rubbed on skin for provision of warmth and pain alleviation, for instance of *Capsicum annuum*, are often employed for pharmacological benefits (Pengelly 2020).

3 Contemporary Approaches to Formulation Development of Natural Products

Natural products are accepted as one of the most significant components of both the traditional and modern medicine owing to their benefits and diverse biological activities. However, developing them into clinical candidates has been hampered by a number of problems including pharmaceutical issues like poor solubility, limited permeability, and poor chemical stability. These formulation problems may affect the bioavailability of these compounds and may lead to their therapeutic failure. Stability of a potential drug molecule in the systemic circulation also restricts its efficacy and leads to early degradation of molecule before reaching the target site. In order to get over these obstacles, novel drug delivery systems can be developed to enhance the stability, dissolution, therapeutic efficacy and selectivity of a given natural drug candidate (Almeida et al. 2022; Rego et al. 2022).

The advent of nanotechnology has provided various targeted drug delivery options including metallic nanoparticles, liposomes, micelles, nanotubes, dendrimers and quantum dots among others. These systems have largely improved the pharmacological properties of these natural products. These systems targeted toward a specific receptor site to improve the pharmacological aspects of natural product formulations have been widely accepted both in basic and clinical applications. Of these, passive drug targeting of phytochemicals to the tumor microenvironment (TM) follows the application of nanotechnology in formulation development. It is well known that following an intravenous administration, the enhanced permeability retention (EPR) effect causes macromolecules to reach and accumulate in the solid tumors more than they do in the healthy tissues. This EPR effect has gained popularity over the past years as it has been used to enhance the delivery of medicines by nanoparticles for solid tumor diagnosis and treatment using the concept of passive drug targeting. Due to an imbalance of growth factors and mediators, the

vasculature contains endothelial gaps and becomes quite leaky. These leaky vessels could be used for passively targeting the solid tumor as nanoparticles can reach there and a better retention can be expected due to impaired drainage of lymphatics at tumor sites. Passive targeting involves spontaneous entry or diffusion of drug-loaded nanocarriers into the tumor microenvironment. In comparison, as represented in Fig. 2, the active targeting involves the formulation of nanocarriers decorated with a specific surface ligand that helps reaching the binding site, tumor site or site of injury more readily due to a its higher affinity for it, thereby, producing a targeted extravasation of the drug-loaded nanocarrier at the particular site. A number of tissue injuries have been reported to be relieved after coupling of nanomedicine and EPR effect (Narum et al. 2020).

The widely accepted novel drug delivery systems, employing active or passive targeting approach, that have gained a widespread popularity are nanoparticles. Their size ranges from 1 to 100 nm approximately and may exploit the EPR effect for targeted natural product delivery. The use of nanoparticles in target-specific therapeutic activity may prove promising due to better physical and chemical characteristics. Green synthesis or biogenic synthesis is a method that utilizes the concept of using extracts or chemicals derived from natural sources to produce nanoparticles for the purpose of stabilization via either a bottom-up approach, i.e.,

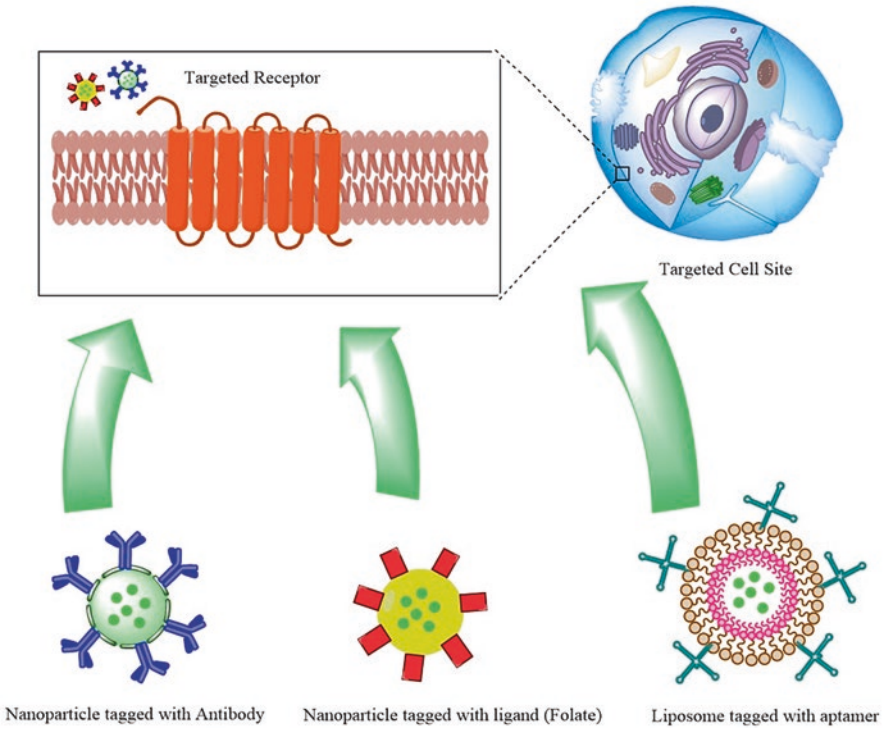

Fig. 2 Active targeting of nanocarriers toward the desired receptor

assembling small atoms or molecules into larger entities or a top-down approach by splitting a larger sized particle into tiny particles. The plant extract containing the phytochemicals, for instance polyphenols, terpenoids or other chemicals, is added in the solution containing metal ions which we want to use for the synthesis of nanoparticles. Several metallic nanoparticles (MNP) could be synthesized through green synthesis including silver, zinc oxide, gold, palladium, platinum and copper nanoparticles (Jadoun et al. 2021). Plant sources reported to contribute effectively in green synthesis of MNPs include, but are not limited to, *Moringa oleifera Lam.* (leaves), *Acorus calamus L.* (rhizome), *Aerva lanata (L.)* Juss. (whole plant), *Allium sativum L., Curcuma longa L.* (powder), *Artemisia haussknechtii Boiss.* (leaf), and *Mirabilis jalapa L.* (leaf) (Maghimaa and Alharbi 2020; Alavi and Karimi 2020; Puthur et al. 2020; Palithya et al. 2021; Paiva-Santos et al. 2021).

Silver (Ag) nanoparticles have been reported to possess efficient cytotoxic activity against cancer cells. Due to the nanosize range, they passively target tumor sites and have been reported to be effective on a number of cell lines including human lung epithelial A549 cells, MCF-7 human cell line, HepG2 cell line, and HCT116 cell line (Sankar et al. 2013; Abootalebi et al. 2021; Raj et al. 2020; Deepika et al. 2020). Their anticancer effect has been related to genotoxicity, cell cycle arrest, and anti-angiogenic properties of Ag nanoparticles. It was also revealed that Ag nanoparticles are involved in the generation of free radical species leading to disruption of mitochondrial processes and, ultimately, cell death (Ratan et al. 2020; Lima et al. 2022).

Recent studies have established the role of Gold (Au) nanoparticles in anticancer therapy. They have been proven to be safe carriers for passive as well as active targeting of natural products toward the binding sites. They have negligible toxicity, high biocompatibility and demonstrate rich surface reduction by phytochemicals. The effect of Hesperidin conjugated Au nanoparticles were studied in human triple-negative breast cancer cell line MDA-MB-231, which emphasized the antitumor potential of these nanoparticles along with their role in activation of macrophages in eradicating tumor cells effectively rendering them as a novel option in anticancer therapy (Sulaiman et al. 2020). Another study unveiled that quercetin-conjugated Au nanoparticles were very functional in case of inducing apoptosis in hormone-dependent MCF-7 cell lines. Furthermore, the epidermal growth factor receptor signaling pathways involved in unregulated cell proliferation were also inhibited (Khan et al. 2021).

Apart from the MNPs, liposomal form of phytochemicals like curcumin has proven to have a significant cytotoxic activity against cancers. Curcumin-loaded liposomes and nanoparticles have been reported to arrest the uncontrolled cell division in prostate cancer cell lines and cervical cancer cell lines respectively (Kashyap et al. 2021). D Kong et al. reported that Resveratrol plus epirubicin-loaded liposomes modified with wheat germ agglutinin (WGA) demonstrated a promising cytotoxic effect, as compared to only epirubicin-loaded liposomes, in the C6 glioma cells cytotoxicity assay after about 48-hour incubation period. The Resveratrol plus Epirubicin liposomal formulation was also targeted in vitro for avascular C6 glioma

spheroids. A notable effect was produced as not only the volume of tumor spheroids was reduced, but most of tumor cells also underwent lysis (Kong et al. 2022).

Antibody–drug conjugates have also been effectively applied for the active drug targeting of cancer cells. This idea is widely adopted for natural products as well. Eribulin, a derivative of Halichondrin B, is a natural constituent obtained from *Halichondria okadai,* a marine sponge. Halichondrin B has been found to be effective against various solid tumors and has been used for the formulation of an antibody–drug conjugate. The formulation is currently under investigation in phase II clinical trials to establish its efficacy (Newman 2021). Similarly, studies are underway to study the active targeting of natural chemical constituents using hyaluronic acid labeled nanocarriers. Hyaluronic acid has affinity for CD-44, a protein overexpressed in many cancers. Hence, CD-44 receptor is a target through which the natural compounds can be specifically delivered in the cancer cells. A recent study exhibited that the hyaluronic acid decorated thymoquinone nanoparticles showed a marked cell death in triple negative breast cancer cell lines MDA-MB-468 and MDA-MB-231. This activity was also confirmed in in vivo study in mice model which recorded a decrease in tumor mass after inoculation of these nanoparticles (Bhattacharya et al. 2020). Figure 3 provides an insight into the process of active and passive targeting of cancer cells. Similarly, another study stated that the hyaluronic acid conjugated nanoparticles with curcumin, actively target the cancer cells. These nanoparticles did not only provide controlled drug release but the cell growth was also inhibited effectively in the human cancer cell lines, i.e., A549, PANC-1, HCT116, and Caco-2 cell lines (Malaikolundhan et al. 2020; Thummarati et al. 2021).

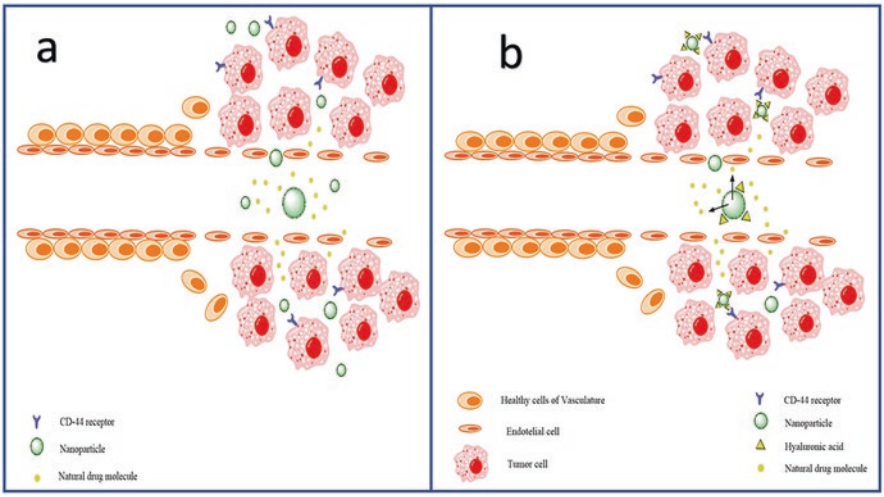

Fig. 3 (**a**) Passive targeting of nanoparticles with natural constituents toward tumor cells exploiting the EPR effect (**b**). Actively targeted natural drug-loaded nanoparticles labeled with a ligand (Hyaluronic acid) reaching the targeted receptor site of tumor cells, CD 44 receptor

For the pharmacotherapy of Alzheimer's disease (AD), dedicated research studies are underway to identify the phytochemicals that can specifically target the mechanisms involved in the disease progression. The major challenge lies in the delivery of the natural products to the target site by crossing the Blood Brain Barrier (BBB). Hence, nanodrug delivery systems have been employed in order to cope with such issues. Exosome-like liposomes (<200 nm size), for instance, have been loaded with curcumin from *Curcuma longa* and tested for their neuroprotective activity in the human neuroblastoma cell lines SH-SY5Y. They halted the oxidative stress, hence exhibiting their antioxidant and anti-inflammatory activities. This has been confirmed in vivo studies using zebrafish embryo model (Fernandes et al. 2021). Similarly, a glycoside, Luteolin-loaded chitosan nanoparticles have been studied for the protective effects in AD. In vivo studies in mice model exhibited the improvement in space-related memory by providing an improved antioxidant effect along with reduction in neuroinflammation by retarding neurofibrillary tangle formation at molecular level (Abbas et al. 2022). In another study, female Swiss mice were injected with amyloid peptide to induce Alzheimer's disease. Lipid-core nanocapsules loaded with Chrysin, obtained from *Passiflora caerulea,* were prepared and the mice were administered with the formulation. The findings exhibited that the neuroinflammation was reduced up to a significant extent. The brain-derived neurotrophic factor (BDNF) level was upheld along with reduced oxidative damage in prefrontal cortex of the brains of mice (Giacomeli et al. 2020). In addition to AD, targeted drug delivery of phytochemicals has also been studied in other neurodegenerative conditions. The extract of plant *Aphanamixis polystachya*, loaded in liposomal formulation has been tested for its anti-Parkinson's activity in mouse model. The principal constituents, including 2-Pentanone, 5-hydroxypipecolic acid, and beta-elemene in liposomal carrier, successfully provided targeted drug delivery and provided pronounced neuroprotective and anti-inflammatory effects. The positive changes in the behavior of mice along with betterment of their locomotion were also observed (Shariare et al. 2020).

The targeted delivery of phytoconstituents also attenuates bacterial infections to a significant level. Recently, the zinc nanoparticles synthesized using the leaf extract of *Aloe socotrina* proved to possess effective antibacterial potential against *Pseudomonas aeruginosa* and *Proteus vulgaris* at concentrations of 50 μg/mL and 75 μg/mL. Bactericidal effect was also observed for *Klebsiella pneumonia* and *Escherichia coli* at higher concentrations. These nanoparticles are described to disrupt the bacterial cell membranes and subsequent killing of bacteria (Fahimmunisha et al. 2020). Similarly, palladium nanoparticles synthesized by using *Rosmarinus officinalis* extract have shown significant antibacterial and antifungal activity both in vitro and in vivo (Rabiee et al. 2020). While considering the clinical and regulatory processes, the natural product-based nanoformulations aimed at targeted drug delivery of these actives have acquired the attention in the global market. They are being employed in routine clinical use at a number of settings internationally. Table 2 summarizes a few marketed brands based upon natural product nanoformulations.

Table 2 Marketed nanoformulations for the targeted drug delivery of phytochemicals

Marketed product name	Natural product formulation	Manufacturer	Actions	References
Abraxane®	Nanosuspension of human serum albumin protein loaded with paclitaxel	American BioScience (USA)	Treats breast cancer after failure of prior chemotherapies	Yuan et al. (2020)
PICN®	Paclitaxel injection mixture for nanodispersion	Sun pharma advanced research co., ltd. (Mumbai, India)	Attenuation of breast cancer	Ma et al. (2021)
Bepanthol ultra facial protect Cream®	Lecithin, ceramides, niacin, Dexpantenol, glycine, glycerine, etc. dispersed in nanoemulsion	Bayer HealthCare (Spain)	Moisturizes the skin, prevents aging of skin	Cardoza et al. (2022)
Nouriva repair moisturizing Cream®	White petrolatum, zinc oxide, lanolin, liquid paraffin, glycerin, lecithin, glycolic acid, allantoin, etc. loaded nanoparticles	Ferndale laboratories, Inc. (United States)	Provides moisturizing effect to the skin	Kaushik and Kumar (2020)
Marquibo®	Vincristine Sulfate loaded liposomes	Talon Therapeutics Inc. (United States)	Attenuates Hodgkin and non-Hodgkin lymphoma	Shahin et al. (n.d.)
Identik masque floral repair®	Seed extract of *Punica granatum* and hydrolyzed yeast	Identik (France)	Provides repair to the hair	Kaul et al. (2018)

4 Opportunities and Challenges in Targeted Delivery of Natural Products

On the landscape of natural drug development, the accumulation of natural products and nanotechnology has made a significant breakthrough over the past decades. This paradigm shift has provided more effective drug delivery at the intended site of action with less hazardous effects, thereby, resulting in the development of efficient therapeutic options especially in neoplastic diseases for specifically targeting the tumor cells. However, there is still a long way to go before it is adequately streamlined for acceptance at both preclinical and clinical phases.

A number of factors determine the in vivo behavior and efficacy of the nanocarriers encapsulating a natural constituent desired for targeting. The physicochemical characteristics of the carrier as well as the charge induced on the its surface, the specific polymer or metallic group used in formulation, and any particular functional group decorated on the surface of the carrier may affect its stability within the bloodstream. One of the major problems is that the circulation half-life of the nanocarrier may not be too sound to reach the desired target due to their rapid clearance

by the reticuloendothelial system. This may lead to early exit of the nanocarriers from the bloodstream of the patient and, hence, failure of the drug delivery system. Efforts are currently underway to optimize the circulation half-life of the nanocarriers for productively reaching the target. A number of techniques including stealthing of nanocarriers by polyethylene glycol (PEG) or related chemicals and nanosizing further to refine the pharmacokinetics are currently being tested clinically (Yadav and Dewangan 2020). In addition, these drug delivery systems intended for active targeting of the cells may contain peptides or proteins that might make them mimic a biological entity. They can, hence, be considered as antigens or immunogens by the immune system, thereafter initiating a hypersensitivity response or related toxicities (Kashyap et al. 2021; Muzammil et al. 2023).

Translation of safety and effectiveness observed during preclinical studies into clinical applications remains one of the most daunting tasks for the drug developers. Many compounds and drug delivery systems may prove to be efficient in reducing the overall disease presentations in preclinical studies; however, when employed to clinical conditions, these pharmacological systems do not show any significant potential. While studying the EPR effect in cancer models, for instance, there may exist a significant difference in EPR effect in human subjects with cancer and in vivo models. Another hurdle is the interindividual genetic and clinical differences, that may also hinder the selective targeting of the nanocarrier loaded with natural compounds. Every tumors environment differs, and hence, the pharmacological properties of various nanocarriers may also vary. Moreover, many studies have revealed that the nanocarriers may require a synchronization of their physicochemical features as per the type of individual or patient in order to ensure maximum bioavailability. Developing natural product-based personalized nanomedicines, hence, needs to be exploited for attaining effective clinical outcomes (Narum et al. 2020).

5 Conclusion and Future Perspectives

The association of natural products and nanomedicine has imparted very promising outcomes in the field of contemporary pharmacotherapy research. Many problems such as the toxicities of synthetic chemical entities and their processing schemes have been overcome by employing phytochemicals having adequate pharmacological activities against various diseased conditions. The recently developed systems have proved to be effective in a number of in vivo studies and ongoing clinical trials and have exhibited targeted delivery of the natural constituent to the desired receptor sites without any notable nonselectivity and toxicity. However, scalability, safety, effectiveness, and cost management still remain major challenges before these natural products can be translated for targeted delivery in clinical conditions. Moreover, more focused research in the field of nanotechnology in tailoring the nanocarriers as per the individual factors may be done for developing improved natural product formulations based on the idea of precision medicine. A large number of natural

products can be made to target specific cell and tissue types by the concerted efforts of pharmacognosists, pharmacologists, and formulation developers by the employment of advanced technologies.

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Quorum Sensing and Quorum Sensing Inhibitors of Natural Origin

Nourhan G. Naga and Mona I. Shaaban

Abstract Now, infectious bacteria represent the worldwide health threat. Treatment with antimicrobial agents becomes ineffective with the time, especially with the massive development of antimicrobial resistance. For instance, there should be alternatives, and one of the main approaches to control bacterial virulence is quorum sensing (QS). QS is a bacterial communication system that controls the expression of bacterial virulence factors including secretion of exoenzymes, bacterial toxins, biofilm, and bacterial motility. Bacteria secret QS signals that control bacterial quorum and associated virulence factors. These signals are mainly acyl homoserine lactones (AHLs) in Gram-negative bacteria, autoinducing peptides in Gram-positive bacteria, and AI-2 signals in both. Therefore, QS is a promising target to control bacterial pathogenicity and enhance bacterial inactivation by the immune system. Many quorum sensing inhibitors have been developed that either block QS receptors, inhibit the biosynthesis of QS signals, or degrade QS signals. Various quorum sensing inhibitions (QSI) have been identified from natural sources such as plant extracts, pure compounds, natural enzymes, marine organisms, fungi, bacteria, and herbs. Plants are considered as a rich source of QSI inhibitors either, edible plants, fruits, spices, essential oils, medicinal plants. Also, several pure extracts exhibited QSI activity, such as terpenoids, flavonoids, and phenolic acids. This chapter highlights the QSI activities of natural products and how they affect QS-regulated virulence. Also, the influence of natural products on the expression of QS-regulatory network will be discussed, with focus on their advanced applications in the elimination of microbial virulence and suppression of bacterial pathogenicity.

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1 Introduction

The number of different microorganisms in the adult human body was evaluated to be at least ten times more than the number of human cells (Walter et al. 2011). A majority of these microorganisms are commensal and may even play an important role in maintaining our health and well-being (Gerritsen et al. 2011). They can live inside the human body and silently work, but they can turn on us and become "pathogenic" with too many virulence factors and cause diseases if our immune systems are weakened. Additionally, pathogenic bacteria in our environment frequently infect us. Our immune system successfully destroys microorganisms in most cases; however, at other times, our defenses cannot. Antibiotic use has been the only treatment choice for bacterial infections that for almost a century (Davies et al. 2006). Firstly, antibiotics were identified as substances produced by microorganisms that inhibit the growth of other microorganisms. With continuous and excessive use of antibiotics through the years, antibiotics were abused and overused, and this led to a serious consequence: multiple-drug resistance (MDR). The World Health Organization (WHO) identified multiple-drug resistance (MDR) as one of the top ten global public health challenges facing humanity as they lost their efficacy in the treatment of pathogenic infections (Rather et al. 2017). Therefore, the pharmaceutical industries need to develop new approaches to combat bacterial pathogens. Many pathogens that affect people, plants, animals, and aquatic life rely on bacterial communication between cells (Bruhn et al. 2005). These communication systems are called "quorum sensing" (QS) which is considered to be the key regulator of virulence factors (Williams et al. 2007). Therefore, any disruption of QS will prevent the release of virulence factors which consequently affect the pathogenicity of microorganisms. This is an innovative and effective strategy to control infectious bacterial diseases (Dong et al. 2007; Muzammil et al. 2023).

QS controls the virulence factors by regulating gene expression through autoinducer (AI) production. AIs are small organic signaling molecules that are primarily produced during the stationary phase (Czajkowski and Jafra 2009). Once the growth reaches a certain threshold level, these molecules act as mirrors that reflect the inoculum size density and control the expression of associated genes (Elgaml et al. 2014). AIs can be categorized into three classes: autoinducing peptides (AIPs), autoinducer-1 (AI-1), and autoinducer-2 (AI-2). AI-1 is known as N-acylated L-homoserine lactones (AHLs) which are the most prevalent class of QS signaling molecules in Gram-negative bacteria (Geske et al. 2008). In Gram-positive bacteria, AIPs are the main autoinducers (Sturme et al. 2002). AI-2 is used by both Gram-negative and Gram-positive bacteria and is produced in intraspecies, so it is known to be a "universal" AI (Lowery et al. 2008; Alves et al. 2023).

Quorum sensing inhibition (QSI) is achieved by too many pathways; blocking bacterial receptors, inhibiting the biosynthesis of QS signal, and degrading of QS signal in the extracellular environment. QSI strategy is an innovative and potent alternative to antibiotics use and it is thought to be less likely result in the emergence of resistance (Miller and Bassler 2001). However, according to the latest studies, it is difficult to predict this consequence, and it is probably influenced by too many factors (Cornforth et al. 2014). Designing de novo quorum sensing inhibitors (QSIs) can be opportune to draw inspiration from nature as it has long been believed that natural products are a good source of vital antibacterial agents that can be utilized to treat a variety of pathogenic diseases (Howes et al. 2020). In this review, we highlight natural QSIs from many different sources and how they affected QS-regulated virulence genes expression.

Everything Starts in Nature

Nature is always the key; it introduces a massive source of drugs. More than half of all prescribed drugs are originated from natural sources (Harper 2001; Marris 2006). Similarly, many QSIs were isolated from many natural sources such as marine organisms, fungi, plants, and herbs due to the natural competition. They exhibited a high potency in inhibiting and disrupting the bacterial QS mechanism (Rasmussen and Givskov 2006). Here, we provide a list of the most potent naturally occurring anti-QS that have been identified from a variety of diverse habitats.

1.1 Plants

Plants harbor a high density of microbial communities. So, they developed many defense mechanisms against pathogenic organisms. They display an extensive range of therapeutic purposes in conventional medicine. The therapeutically effective plant-isolated active ingredients should be safe for human cells. Toxicological studies on these active substances must be carried out to avoid their toxicity. The aim to detect and study the biological processes and mechanisms behind their therapeutic effects has increased. Biologically active components of natural resource, especially those produced from plants, have thus far prompted the creation of brand-new medicines for the treatment of a variety of diseases. QS system manipulation by plants is thought to be a form of protection against microbial pathogens because plants lack an immune system, unlike animals and humans. This forced researchers to hypothesize additional defense mechanisms to overcome the pathogenic strains infection (Koh et al. 2013). Plant extracts were reported to act as QSI. Plant chemicals often target the bacterial QS system in three different pathways (Fig. 1)**:** by degradation of the signaling molecules, blocking the synthesis of AIs, or by targeting the receptors of the signals (Koh et al. 2013).

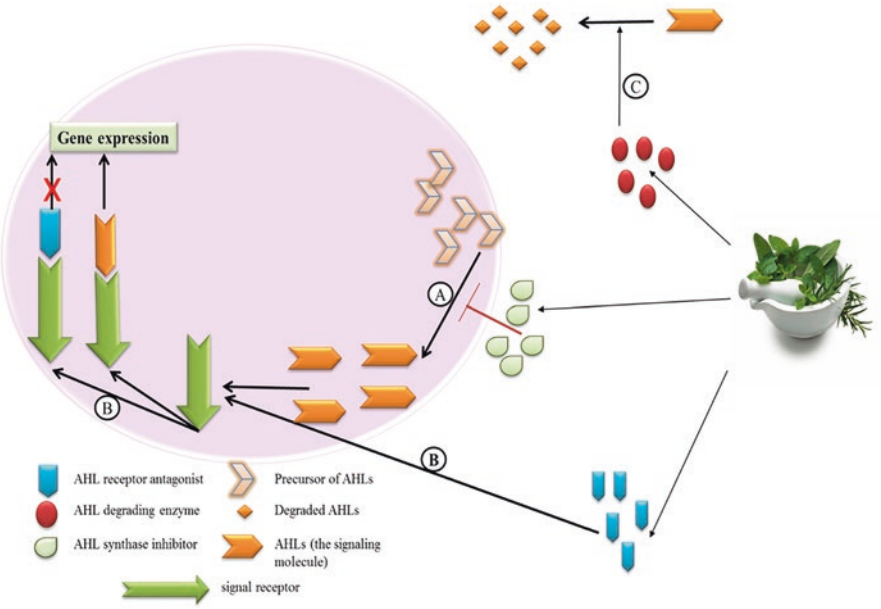

Fig. 1 Mechanisms of quorum sensing inhibition by plants secondary metabolites through blocking the synthesis of AIs (A), targeting the receptors of the signals (B), and degradation of the signaling molecules (C)

1.1.1 Edible Plants

All plant's diversity approved efficacy against QS signaling systems of pathogenic bacteria. For example, some plants used for nutrition exhibited QSI potency as *Medicago truncatula Gaertn* plant extract could inhibit the QS against *Chromobacterium violaceum* CV026, *Escherichia coli* JM109, *Pseudomonas aeruginosa*, and *Sinorhizobium Meliloti* (Gao et al. 2003). Also, *Pisum sativum* was reported to reduce violacein pigment in *C. violaceum* and swarming and motility in *P. aeruginosa* PA01 (Fatima et al. 2010)**.** Methanolic extract of *Capparis spinosa* inhibited QS and virulence in *E. coli*, *C. violaceum, S. marcescens*, *P. mirabilis*, and *P. aeruginosa* PA01 (Abraham et al. 2011). Erucin and sulforaphane compounds isolated from *Brassica oleracea* (broccoli) plant inhibited *P. aeruginosa* PA01 virulence factors (Ganin et al. 2013). *Phaseolus vulgaris* (bean) and *Oryza sativa* (rice) inhibited the biofilm formation in *Sinorhizobium fredii* SMH12 and *Pantoea ananatis* AMG501 (Pérez-Montaño et al. 2013). Additionally, myristic acids and pantolactone isolated from *Allium cepa* (onion) inhibited *P. aeruginosa* virulence factors (Abd-Alla and Bashandy 2012).

1.1.2 Fruits

Fruits also showed potent QSI activity against QS-regulated virulence genes. For example, the methanolic extract of *Mangifera indica* (mango) reduced the pyocyanin, elastase, chitinase, total protease, swarming motility, and exopolysaccharide (EPS) production by 89% 76%, 55%, 56%, 74%, and 58%, respectively, in *P. aeruginosa* PAO1 at 800 μg/mL (Kim et al. 2019). *Vitis* sp. (grape), total extracts of *Rubus idaeus* (raspberry), and *Vaccinium angustifolium* Aiton (blueberry) inhibited violacein production in *C. violaceum* (Kalia 2013). The limonoids in orange seeds including deacetyl nomilinic acid glucoside, ichangin, and isolimonic acid inhibited the biofilm formation in *V. harveyi* (Vikram et al. 2010)**.** Similarly, aqueous extracts of edible fruits such as *Musa paradisiaca*l (banana), *Ananas comosus* (pineapple), and *Manilkara zapota* (sapodilla) showed QSI activity against violacein pigment in *C. violaceum*, pyocyanin, biofilm formation, and protease in *P. aeruginosa* PA01(Musthafa et al. 2010). Biofilm formation of *Yersinia enterocolitica* was inhibited by the peel extract of *Punica granatum* (pomegranates) (Oh et al. 2015). *Psidium guajava* (guava) could reduce the biofilm production in *P. aeruginosa* PAO1 and violacein pigment synthesis in *C. violaceum* (Vasavi et al. 2014). Similarly, it inhibited quorum sensing mediated virulence factors of *Staphylococcus aureus* (Divyakolu et al. 2021).

1.1.3 Spices

Spices exhibited to be a potent source of QSIs. For instance, curcumin, which is produced from *Curcuma longa* inhibited the expression of virulence genes in *P. aeruginosa* PA01 (Rudrappa et al. 2008). Furthermore, curcumin was evaluated for its ability to disrupt mature biofilms in uropathogenic strains. It was discovered to reduce QS-dependent virulence factors such as extracellular polymeric substance formation, alginate production, and swarming motility. Curcumin was found also to make *P. aeruginosa* PA01 more susceptible to common antibiotics (Packiavathy et al. 2014). Besides, the effects of cinnamaldehyde and its derivatives were reported to be effective QSI in QS-regulated processes, including biofilm formation in *P. aeruginosa* and AI-2-mediated QS in several *Vibrio* species (Brackman et al. 2008). Additionally, it was discovered that extracts from various plant components including the leaves, flowers, fruit, and bark of *Combretam albiflorum*, *Laurus nobilis*, and *Sonchus oleraceus* had anti-QS properties (Al-Hussaini and Mahasneh 2009). *Allium sativum* (garlic) extract inhibited β-galactosidase in *Agrobacterium tumefaciens* NTL4 and violacein production in *C. violaceum* (Bodini et al. 2009). Moreover, *Vanilla planifolia* aqueous methanolic extract inhibited violacein pigment in *C. violaceum* CV026 (Choo et al. 2006).

1.1.4 Essential Oils

Essential oils showed some anti-QS properties, and the production of violacein in *C. violaceum* CV026 was significantly affected by the QSI properties of the essential oils extracted from *Piper brachypodon Benth*, *P. caucasanum Bredemeyer*, and *P. bogotense* (Olivero V et al. 2011). Similarly, methanol and hexane extracts of clove inhibited violacein pigmentation in *C. violaceum* CV026. Chloroform and methanol clove extracts dramatically decreased the amount of bioluminescence in *E. coli* [pSB1075] that is produced when cultivated with N-3-oxododecanoyl-L-homoserine lactone. While virulence factors of *P. aeruginosa* PAO1, such as pyocyanin pigment synthesis, were suppressed by the hexane extract (Krishnan et al. 2012). Eugenol is the key component of clove extract as it exhibited anti-QS properties and inhibited the virulence factors of *P. aeruginosa* and *E. coli* biosensors at subinhibitory concentrations (Zhou et al. 2013).

1.1.5 Medicinal Plants

Recent studies revealed that medicinal plants are a very potent source of QSIs. This potency is modulated by the secondary metabolites production. These metabolites are classified mainly into three main classes; terpenoids, phenolic acids, and flavonoids (Bouyahya et al. 2022).

Terpenoids

Terpenoids demonstrated remarkable antibacterial activity through a variety of pathways, including QS inhibition. Many terpenoids, including eugenol, carvacrol, linalool, D-limonene, and -pinene, have inhibitory effects via various QS mediators. For example, eugenol showed significant effects on methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from food handlers (Al-Shabib et al. 2017), as well as biofilms of clinical isolates of *P. mirabilis*, *S. marcescens*, and *P. aeruginosa* (Packiavathy et al. 2012). Interestingly, an additional study showed that eugenol hindered *P. aeruginosa* from producing its virulence factors such as elastase, pyocyanin, and the development of biofilms (Zhou et al. 2013; Al-Shabib et al. 2017; Rathinam et al. 2017). Moreover, eugenol had a notable impact against (AIs) and significantly reduced the formation of biofilm of *P. aeruginosa* PAO1 by 65.6% (Rathinam et al. 2017). Recently, other studies demonstrated that eugenol decreases the production of N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) and C4-HSL N-acyl homoserine lactone signal molecules, pyocyanin, and swarming motility in *P. aeruginosa* by 50% at sub-MIC (Lou et al. 2019). Besides, eugenol reduced the expression of QS-regulated genes by 65%, 61%, and 65% for lasI, rhlI, and rhlA, respectively, and by 36% for biofilm formation (Lou et al. 2019).

Similar to this, carvacrol displayed a QSI activity against QS and biofilm development. Recent research demonstrated that carvacrol inhibited the development of biofilms in *P. aeruginosa* at very low concentrations (0.9–7.9 mM) and reduced the synthesis of pyocyanin by 60% (Tapia-Rodriguez et al. 2017). Furthermore, another study reported that subinhibitory concentrations (<0.5 mM) of carvacrol inhibited biofilm formation in *S. aureus* 0074, *Salmonella enterica* subsp., and *S. Typhimurium* DT104 (Burt et al. 2014).

Phytol is a well-known diterpene and was reported as QSI. Specifically, this substance inhibited the biofilm formation in *S. marcescens* and *P. aeruginosa* PAO1 (Pejin et al. 2015; Srinivasan et al. 2016, 2017). Phytol inhibited prodigiosin, protease, and biofilm formation by 92%, 68%, and 64%, respectively in *S. marcescens* at a concentration of 10 μg/mL (Srinivasan et al. 2016).

Another terpene that has demonstrated anti-QS action is called sesquiterpene lactone. This substance inhibited the activity of QS mediators in *C. violaceum* and *P. aeruginosa* ATCC 27853 (Amaya et al. 2012; Aliyu et al. 2021). It was reported that sesquiterpene lactones belonging to goyazensolide and isogoyazensolide chemical families approved QSI activity and inhibited the production of AHL. Also, oleanolic aldehyde coumarate inhibited biofilm formation in *P. aeruginosa* and all lasI/R, rhlI/R regulated genes (Rasamiravaka et al. 2015). Other terpenoids as linalool inhibited the biofilm formation of *A. baumannii* (Alves et al. 2016; Wang et al. 2018).

Flavonoids

The second classes of secondary metabolites found in medicinal plants are flavonoids. Recent studies revealed that this chemical group has an antibacterial impact through various mechanisms of action, including inhibition of QS and its main traits, like the development of biofilm. Epigallocatechin is one of the flavonoids, it showed antibiofilm activity against *S. typhimurium* (Wu et al. 2018; Hosseinzadeh et al. 2020) and disrupted the QS activity of *Streptococcus mutans* biofilms. It also reduced motility and decreased AI-2-regulated virulence factors activity (Castillo et al. 2015). Additionally, epigallocatechin inhibited QS and the formation of biofilm in *S. aureus* and *Burkholderia cepacia* (Huber et al. 2003), *Listeria Monocytogenes* (Nyila et al. 2012), and *Eikenella corrodens* (Matsunaga et al. 2010). Besides, naringenin inhibited biofilm formation in *S. mutans* and downregulated mRNA expression of luxS, gtfC, gtfB, comE, and comD (Yue et al. 2018). Moreover, this compound inhibited the swarming and motility in *C. violaceum* (Truchado et al. 2012).

Quercetin exerts antagonistic effects on bacterial signaling systems, and has been shown to have an important role as QSI (Vikram et al. 2010). For instance, it inhibited the biofilm formation of *E. coli* and *V. harvei* (Vikram et al. 2010). Also, it inhibited the violacein pigment production in *C. violaceum* and QS-regulated phenotypes in *P. aeruginosa* PAO1 (Al-Yousef et al. 2017). Other flavonoids like naringenin showed QSI activity against *P. aeruginosa* and inhibited elastase and

pyocyanin virulence factors (Hernando-Amado et al. 2020). Meanwhile, morin flavonoids inhibited EPS production, biofilm formation, and motility in *S. aureus* (Chemmugil et al. 2019). In addition, methoxyisoflavone inhibited the violacein pigment in *C. violaceum* and pyocyanin, protease, hemolysin, and biofilm in *P. aeruginosa* clinical isolates, PAO1, and PA14 (Naga et al. 2022). On the other side, kaempferol inhibited adhesion-related gene expression (Ming et al. 2017). Taxifolin flavonoids also showed a significant QSI activity on *P. aeruginosa* and reduced elastase and pyocyanin production (Vandeputte et al. 2011).

Phenolic Acids

Several natural resources, including medicinal plants release phenolic acids as secondary metabolites. Numerous studies showed that these phenolic compounds have anti-QS properties. In two *Pectobacterium* species, *P. carotovorum* and *P. aroidearum*, salicylic acid has been found to interfere with the QS system, influence QS machinery, and changed the expression of bacterial virulence factors (Joshi et al. 2016). Additionally, it decreased the intensity of the AHL signal and reduced the expression of several QS genes. Salicylic acid treatment significantly decreased the biofilm formation of *P. aeruginosa* as well as twitching, swarming, and motility (Chow et al. 2011). Similarly, salicylic acid modulated 103 virulence-related gene families and decreased AHL production and biofilm formation in *A. tumefaciens* (Yuan et al. 2007). On the other hand, rosmarinic acid (RA) at 750 μg/mL decreased elastase, hemolysin, and lipase production in *Aeromonas hydrophila* and inhibited the development of biofilms. The virulence genes ahh1, aerA, lip, and ahyB were also downregulated (Rama Devi et al. 2016). Also, RA inhibited the QS-regulated virulence factors in *P. aeruginosa*, it inhibited elastase, pyocyanin, and biofilm formation (Walker et al. 2004; Corral-Lugo et al. 2016; Fernández et al. 2018). Cinnamic acid is another phenolic acid with known biofilm and QS inhibitory properties. It effectively prevented *P. aeruginosa* from producing the QS-dependent virulence factors and biofilm formation at sublethal concentrations without any effect on viability (Rajkumari et al. 2018). Additionally, research revealed that cinnamic acid inhibited the virulence gene expression of *P. aroidearum* and *P. carotovorum* (Joshi et al. 2016). Cinnamic acid also decreased the intensity of the AHL signal and suppressed the production of QS genes. Similar effects were reported when *C. violaceum* ATCC12472 was exposed to two cinnamic acid derivatives, 4-dimethylaminocinnamic acid (DCA) and 4-methoxycinnamic acid (MCA) (Cheng et al. 2020). DCA and MCA reduced the production of violacein, chitinase, and hemolysin in *C. violaceum* and decreased the levels of N-decanoyl-homoserine lactone (C10-HSL).

Researchers reported that chlorogenic acid (CA) significantly reduced *P. aeruginosa* virulence factors such as biofilm formation, swarming, elastase, protease, pyocyanin, and rhamnolipid (Wang et al. 2019). Also, p-coumaric acid inhibited the QS-related virulence genes of *P. chlororaphis*, *C. violaceum* 5999, and *A. tumefaciens* NTL4 (Bodini et al. 2009)**.** In addition, it inhibited violacein pigmentation in

C. violaceum (Chen et al. 2020). Another QSI phenolic acid is caffeic acid which showed antibiofilm activity in *S. aureus* in addition to hemolysin inhibition activity (Luís et al. 2014). Besides, phenylacetic and ellagic acid were reported to be efficient against the biofilm-forming bacteria *B. cepacia* (Huber et al. 2003) and *P. aeruginosa* (Musthafa et al. 2012).

2 Fungal Quorum Sensing Inhibitors

Fungi inhabit a wide range of ecosystems and interact with other organisms, such as microorganisms, animals, and plants. They are almost cosmopolitan in nature. Additionally, they can live in extreme habitats. Organisms that cohabit in nature as partners have evolved tools to fight one another, including chemicals, enzymes, and metabolites (Sharma and Jangid 2015; Almeida et al. 2022). In soil, bacteria and mycorrhizal fungi work together closely. Fungi have inherent defenses against a bacterial population that have formed or evolved as a result of their close association. These could be for space, nutrition, or pathogenicity. Furthermore, they are known to produce a number of secondary metabolites such as enzymes, chemicals, and mycotoxins (Pitt 2000; Frisvad et al. 2008). Even so, there is little information available on fungal QSIs. So, finding fungal QSI potency isolated from varied habitats, such as endophytes and marine fungi may help.

Fungi are well-known to produce a variety of quorum sensing molecules (QSMs). For example, *Candida albicans* produces farnesol and tyrosol. Farnesol is also produced by a majority of dimorphic yeasts with a significant impact on their morphogenesis (Shirtliff et al. 2009; Weber et al. 2010). It exhibited antimicrobial activity against *Fusarium graminearum* (Semighini et al. 2006), *Paracoccidioides brasiliensis* (Derengowski et al. 2009), *Staphylococcus epidermidis*, *S. aureus* (Cerca et al. 2012), and other bacteria (Pammi et al. 2011). It was reported to act as an adjuvant against *S. epidermidis* when combined with antibiotics (Pammi et al. 2011). On the other hand, farnesol produced by *C. albicans* was reported to inhibit biofilm formation, which is regulated by QS (Ramage et al. 2002). It showed efficacy in protecting mice from candidiasis (Hisajima et al. 2008). A comparable study on *C. parapsilosis* and *C. tropicalis* revealed that farnesol at high concentrations reduced the formation of biofilms (Laffey and Butler 2005; Zibafar et al. 2015).

Additionally, many fungal secondary metabolites showed QSI activities. For instance, secondary metabolites of *Tremella fuciformis*; *Tremella* is a member of the Basidiomycota family *Tremellaceae*, also known as "jelly fungi." *T. fuciformis* inhibited QS in *C. violaceum* CVO26 and inhibited the production of violacein pigment. This pigment is regulated by QS and AHL signaling molecules. It was inhibited by different concentrations (0.2%–0.8%) of *T. fuciformis* extracts without any effect on viability and growth (Zhu and Sun 2008). Also, *Phellinus Igniarius* which is classified as a plant pathogen was reported to have anti-QS activity (Zhu et al. 2012) as well as anticancer, antidiabetic, and antioxidant characteristics (Lung et al. 2010). Additionally, heterocyclic compounds that synthesize the pigments of

Auricularia auricula could bind to the active site of receptor proteins and inhibit the AHL-regulated signaling mechanism (Zhu et al. 2011; Almeida et al. 2022). Similarly, its total extract reduced the biofilm formation of *Escherichia coli* by 73% (Li and Dong 2010).

Mycotoxins were reported to have QSI activity. Penicillic acid mycotoxin which is produced by *Penicillium radicola* and patulin which is produced by *P. coprobium* inhibited QS in *P. aeruginosa* by targeting the LasR and RhlR proteins (Rasmussen et al. 2005b). Additionally, a mouse with *P. aeruginosa* infection recovered faster after receiving patulin treatment, and it was more susceptible to tobramycin antibiotic (Rasmussen et al. 2005b). Also, a lot of promises exist for metabolites with antibacterial activity in endophytic fungi that inhabit a plant host. So, some endophytic fungi were isolated from *Ventilago madraspatana* plant (Rajesh and Rai 2013; Lima et al. 2022).

3 Marine Organisms Are a Potent Source of QSIs

Before the emergence of the first plants on the land about half a billion years ago, life existed primarily in the oceans for almost three billion years and it was at this point when QS molecules and their inhibitors started to perform their distinct roles. Numerous marine bacteria, fungi, algae, and bryozoans have been identified as QSIs, in addition to corals and sponges. For example, marine cyanobacteria are one of the richest sources of physiologically active and structurally distinct natural compounds. The family of halogenated furanones that were isolated from the marine alga *Delisea pulchra* has attracted a lot of attention and is considered to be one of the most effective and widely used natural QSI.

3.1 Algae

In the aquatic environment, beneficial and pathogenic bacteria coexist in close contact with eukaryotes including algae, protozoa, fungi, and plants. Eukaryotes have inevitably evolved several defense mechanisms for interacting with bacteria, such as creating secondary metabolites like as QSIs (Kjelleberg and Steinberg 2002; Rasmussen et al. 2005a; Dudler and Eberl 2006). For example, the red macroalga *Delisea pulchra* was the source of the first identified QSI and it exhibited a strong antifouling activity (Givskov et al. 1996). A variety of secondary metabolites like halogenated furanones were detected at the algae surface and were approved to be the main cause of the QSI activity (Dworjanyn et al. 1999). They are similar in structure to AHL, these halogenated furanones differ in having a furan ring rather than a homoserine lactone ring. The crude extract of *D. pulchra* approved efficacy against the human pathogenic bacteria; *Proteus mirabilis* and inhibited the motility

and swarming activity (Gram et al. 1996). The natural compound that has received the greatest attention to date is the halogenated furanones as it exhibited high QSI activity in AHL-controlled expression in various Gram-negative bacteria (Rasmussen et al. 2000; Hentzer and Givskov 2003) and also inhibited AI-2 signaling molecules (Ren et al. 2001). The disruption of AI-2 QS by natural and synthetic brominated furanones has been shown to protect *Artemia franciscana* shrimp from pathogenic isolates of the species *Vibrio Harveyi*, *V. campbellii*, and *V. parahaemolyticus* (Defoirdt et al. 2006). Furthermore, it was demonstrated that natural furanone inhibited the pathogenic *V. harveyi* strain from producing the toxin T1 and luminescence, both of which are QS-regulated against farmed shrimp (Manefield et al. 2000). Besides, it was shown that the natural furanone attenuated the adverse effects of various pathogenic *V. harveyi* strains in the rotifer *Brachionus plicatilis* (Tinh et al. 2007b; Tinh et al. 2007a). These findings demonstrated the ability of furanones to function as antivirulence compounds in several microbial marine ecosystems.

3.2 Bacteria

According to studies, a variety of bacteria can suppress the QS of other bacteria by producing quorum-quenching enzymes (QQEs) such as acylase and lactonase enzymes (Kalia 2013). A bacterial flora was isolated from the gut of white shrimp *Penaeus vannamei*. Then, it was cultivated with AHLs as the sole nitrogen and carbon source. It was discovered that the enrichment cultures accelerated the growth of rotifers in vitro exposed to pathogenic *V. harveyi* and degraded its signaling molecules in vitro (Tinh et al. 2007b). Similarly, other bacterial QSIs were isolated from the gut of *Lates calcarifer* and *Dicentrarchus labrax* fish (Van Cam et al. 2009). Some bacteria can serve as antagonists by releasing substances that interfere with QS signaling systems. For instance, 35 out of 88 actinomycetes stains prevented biofilm formation of *V. vulnificus*, *V. harveyi*, and *V. anguillarum* without any effect on their growth (You et al. 2007). Similarly, borrelidin, behenic acid, and 1H-pyrrole-2-carboxylic acid isolated from *Streptomyces coelicoflavus* KJ855087 inhibited QS-regulated virulence factors of *P. aeruginosa* PAO1(Hassan et al. 2016). In a cocultivation study, phenethylamine compounds were produced by *Halobacillus salinus* C42 inhibited *V. harveyi* bioluminescence. Also, these compounds inhibited several QS regulated phenotypes in Gram-negative bacteria, including luminescence in *V. harveyi,* violacein pigment in *C. violaceum* CV026, and fluorescence in *E. coli* JB525 reporter strain (Teasdale et al. 2009).

Similarly, 11 bacterial strains that were isolated from Palk Bay sediments inhibited the QS signaling systems in *C. violaceum* ATCC 12472 and *C. violaceum* CV026 (Nithya et al. 2010). Moreover, the marine isolated bacteria *Bacillus pumilus* significantly inhibited *P. aeruginosa* PAO1 virulence factors (Nithya et al. 2010). It inhibited LasB elastase by 84%, LasA protease by 76%, caseinase by 70%, pyocyanin by 84%, and pyoverdine, as well as biofilm formation by 87%. *Bacillus*

pumilus S8-07 approved QSI activity against virulence factors of *Serratia marcescens*. It exhibited a highly significant reduction in biofilm formation by 61%, hemolytic activity by 73%, prodigiosin by 90%, and caseinase by 92% (Nithya et al. 2010).

Another example of marine *Bacillus* sp. strain was isolated from the coastal region of Calimere showed a potency as QSI was reported by Musthafa and coauthors (2011). *Bacillus* sp. SS4 inhibited the violacein pigment production in *C. violaceum* by 86% and reduced the virulence factors of *P. aeruginosa* PAO1 by 88%, 68%, 65%, 68%, and 86% for biofilm, LasA protease, total protease, elastase, and pyocyanin, respectively.

3.3 Other Marine Organisms as QSIs

Aquatic invertebrates and sponges as well as marine algae and bacteria can produce QSIs that may hinder QS systems (Husain and Ahmad 2015). For example, the bryozoan *Flustra foliacea* from the North Sea excretes brominated alkaloids that lowered the signal intensity of various QS phenotypes by 20% to 50%. Additionally, the metabolites suppressed QS-regulated phenotypes of *P. aeruginosa* such as protease production (Peters et al. 2003). Furthermore**,** the sponge *Luffariella variabilis* exhibited a potent QS inhibition in LuxR-regulated systems. The inhibitory effect of this sponge was discovered to be mediated by manoalide, monoacetate, and secomanoalide secondary metabolites production (Skindersoe et al. 2008). Expression of virulence gene in *S. marcescens* and the violacein synthesis in *C. violaceum* were used to test the QSI activity of marine sponges which were collected from Palk Bay, India. Among 29 tested marine sponges, methanol extract of *Clathria atrasanguinea, Aphrocallistes bocagei*, and *Haliclona* (*Gellius*) *megastoma* inhibited the violacein production in *C. violaceum* ATCC 12472 and CV026. Besides, these sponge methanol extracts inhibited the virulence factors of *S. marcescens* PS1 such as biofilm formation, protease, hemolysin, and prodigiosin pigment production (Annapoorani et al. 2012)**.**

4 Natural Enzymatic Degradation of QSMs

Another major class of natural QSIs is enzymes. All organisms; mammals, plants, fungi, archaea, and bacteria have all been reported to participate in the production of QQEs. So, enzymatic degradation has arguably received the most attention to date (Romero et al. 2015). Many species of bacteria with enzymatic QSI activity have been identified so far (Table 1). The widespread enzymatic QSI activity among bacteria shows that disrupting bacterial communication is essential to giving bacterial populations a strategic advantage over the competition. There are now three primary groups of AHL QQEs based on the modification process. The first is the

Table 1 Quorum quenching enzymes produced by bacterial strains

Organism	Activity	Enzyme	Reference
Agrobacterium tumefaciens	Lactonase	AttM	Zhang et al. (2002)
	Lactonase	AiiB	Carlier et al. (2003)
Anabaena sp.	Acylase	AiiC	Romero et al. (2008)
Arthrobacter nitroguajacolicus	PQS	Hod	Pustelny et al. (2009)
Anabaena sp.	Acylase	AiiC	Romero et al. (2008)
Bacillus megaterium	Oxidoreductase	CYP102A1	Chowdhary et al. (2007)
Bacillus sp.	Lactonase	AiiA	Dong et al. (2001)
Brucella melitensis	Acylase	AibP	Terwagne et al. (2013)
Chryseobacterium sp.	Lactonase	AidC	Wang et al. (2012)
Geobacillus kaustophilus	Lactonase	GKL	Chow et al. (2010)
Kluyvera citrophila	Acylase	KcPGA	Mukherji et al. (2014)
Klebsiella pneumoniae	Lactonase	AhlK	Park et al. (2003)
Mesorhizobium loti	Lactonase	MLR6805	Funami et al. (2005)
Microbacterium testaceum	Lactonase	AiiM	Wang et al. (2010)
Mycobacterium avium	Lactonase	MCP	Chow et al. (2009)
Ochrobactrum sp.	Acylase	AiiO	Czajkowski et al. (2011)
	Lactonase	AidH	Mei et al. (2010)
Pseudoalteromonas byunsanensis	Lactonase	QsdH	Huang et al. (2012)
Rhodococcus erythropolis	Lactonase	QsdA	Uroz et al. (2008)
Rhizobium sp.	Lactonase	DlhR	Krysciak et al. (2011)
	Lactonase	QsdR1	
Solibacillus silvestris	Lactonase	AhlS	Morohoshi et al. (2012)
Sulfolobus solfataricus	Lactonase	SsoPox	Merone et al. (2005)

lactonase enzyme, which breaks down the ester linkage in the homoserine lactone ring of metalloproteins AHL (Dong et al. 2000, 2001) (Fig. 2). These enzymes break down all signals regardless of acyl side chain substitutions and size, making them the ones with the widest diversity of AHL specificity. The second category is the acylase enzyme which breaks down the AHL amide linkage, releasing the corresponding homoserine lactone ring and free fatty acid (Lin et al. 2003). Acylases exhibit more substrate selectivity than lactonases, which could be a result of their ability to detect the signal's acyl chain. The oxidoreductases are the third class of known AHL QQEs; unlike acylase and lactonase activities, they oxidize or reduce the acyl chain of the AHLs instead of destroying them. The signals are not degraded by these reactions, but the alterations change the specificity and this consequently affects signal and receptor interaction.

Fungi are well known for producing extracellular enzymes such as cellulases, proteases, amylases, and others that can be used to degrade bacterial biofilms. For example, some enzymes extracted from *Trichoderma viride*, *Aspergillus niger*, and *Penicillium* species approved their efficacy as QSIs and degraded the biofilm of *P. aeruginosa* (Gautam et al. 2013).

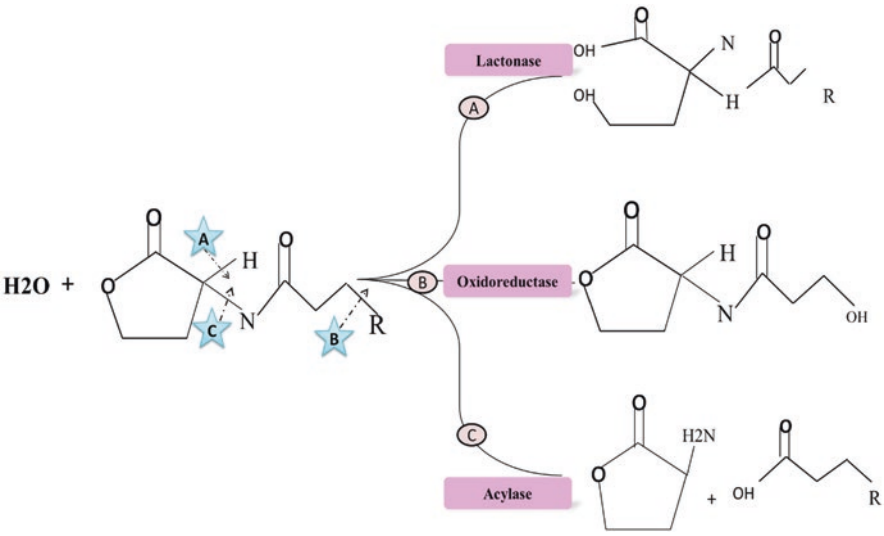

Fig. 2 Mechanisms of action of lactonase enzyme; A, oxidoreductase enzyme; B, and acylase enzymes; C

5 Conclusions

This review shows how we might draw inspiration from nature to focus on bacterial communication networks in the battle against diseases. Many other molecular entities that can interfere with bacterial virulence have been found in recent research, and many more are expected to be found in the near future. Anti-QS is crucial for combating infections because it does not put selection pressure on the population and is unlikely to lead to a resistance issue. For a better understanding of the processes involved, in vivo investigations in relevant animal models are required. It is crucial to thoroughly examine the organism's pathogenicity mechanisms, including their relationship to QS.

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Bioactive Natural Products from Medicinal Plants

Athar Ata

Abstract Natural product chemistry has provided many lead compounds to the drug discovery process. Approximately 50% of the commercially available pharmaceuticals on the market are of natural product origin. This high success rate of this class of organic chemistry in providing new bioactive compounds is due to its enormous structural diversity compared to other sources, including synthetic chemistry, combinatorial chemistry, and genomic approaches. We have identified natural products exhibiting antimicrobial and health-related antienzymatic activities. These health-related enzymes include glutathione S-transferase, α-glucosidase, acetylcholinesterase, and renin–angiotensin system, which are involved in the pathogenesis of cancer drug resistance, type 2 diabetes, Alzheimer's disease, and hypertension, respectively. This chapter describes the results obtained from our bioassay-directed phytochemical studies on medicinally important plants. Additionally, structure–activity relationship studies on some potent bioactive natural products have also been discussed.

Keywords Glutathione S-transferase inhibitors · α-glucosidase inhibition · Acetylcholinesterase inhibition · Antimicrobial natural products · Antirenin activity · Phytochemistry

1 Introduction

Natural product chemistry plays a key role in providing lead compounds for drug discovery, especially in treating cancer, infectious diseases, cardiovascular diseases, and neurodegenerative diseases (Atanasov et al. 2021; Newman and Cragg 2016; Waltenberger et al. 2016; Barnes et al. 2016). This is mainly due to two reasons: (i)

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the enormous diversity in scaffolds; and (ii) the complex structural features of natural products. These compounds contain more sp3 carbon and oxygen atoms; fewer nitrogen and halogen atoms have also been reported. The presence of oxygen, nitrogen, and halogen atoms in this class of organic compounds provides higher numbers of hydrogen bond acceptors and donors and hydrophilic properties to these compounds, whereas many carbon atoms provide higher hydrophobic features to natural products.

Additionally, these structural features have greater molecular rigidity (Atanasov et al. 2021). These features are required for discovering lead compounds for drug discovery which are difficult to achieve in compounds obtained from traditional organic synthesis (Lawson et al. 2018; Shultz 2019; Lachance et al. 2012). Though these features mentioned above make natural product chemistry an attractive source for discovering lead compounds for the drug discovery process, research in this area has several challenges. These challenges led pharmaceutical companies to reduce their natural products-based drug discovery programs (Henrich and Beutler 2013). These challenges are multifold and are summarized as follows.

1.1 Screening of Crude Extracts

The starting point for identifying lead bioactive compounds for drug discovery is to achieve a library of bioactive extracts by screening crude extracts obtained from natural sources. Two types of bioassays, cell- or enzyme-based, are used to screen crude extracts/fractions and pure natural products. The latter bioassay is frequently used in modern drug discovery to discover small molecules with enzyme-inhibiting activities. This bioassay is safe to perform in medicinal chemistry labs and to provide bioactive lead compounds against targeted diseases as enzymes perform all biochemical processes of human life, including metabolism, catabolism, cellular signal transduction, cell cycling and development. Malfunctioning in these biochemical processes is responsible for several diseases, including cancer, diabetes, cardiac problems, neurodegenerative diseases, etc. These malfunctions are associated with the dysfunction of enzymes/overexpression or hyperactivation of enzymes involved (Ata et al. 2011a, b). The detailed studies of biochemical processes have led to understanding diseases at the molecular level that resulted in the discovery of effective enzyme inhibitors against several diseases used in clinics (Ata et al. 2011a, b). The enzymes involved in these bioassays are purified human enzymes. The measurement of product formation is obtained by spectroscopic methods, and the results are compared using reference compounds. The crude extracts are colored and contain polyphenols such as lignans in plants that could lead to false-positive results in antienzymatic assays. This problem can be overcome by decolorizing crude extracts by passing through charcoal or removing lignans by filtering extracts through commercially available resins (Ata 2012).

1.2 Bioassay-Directed Isolation of Lead Compounds

For the drug discovery process, bioassay-directed investigation of bioactive extracts has a higher chance of providing lead compounds. This process is labor intensive and has several problems, including replication (isolation of known compounds) and bioactivity of crude extracts due to the synergetic effect. The former problem can be overcome by analytical techniques, including LC-MS and NMR-based metabolomics of bioactive extracts and correlations of these results with bioactivity profiling (Atanasov et al. 2021). NMR analysis of crude extracts is a reliable approach for studying this aspect of natural product chemistry as the data is reproducible. Also, it provides direct quantitative and structural information on constituents of crude extracts. This method has relatively low sensitivity as it is helpful in profiling major constituents only (Hubert et al. 2017; Wolfender et al. 2019; Stuart et al. 2020). The LC-MS approach, especially the high-resolution mass spectrometer (HR-MS), is routinely used in natural products lab for metabolomics of bioactive extracts as it can separate and identify numerous isomers present in very minor quantities in bioactive extracts (Wolfender et al. 2015). HR-MS provides molecular mass and formula as well as MS/MS data that are cross-searched in the literature or databases, including Dictionary of Natural Products, METLIN, and Global Natural Products Social (GNPS) molecular networking platform, developed in the Dorrestein laboratory (Wang et al. 2016; Atanasov AG et al. 2021). The identification of natural products in bioactive extracts can provide dereplicated bioactive extracts. Comparing metabolomics data with the biological activities of bioactive extracts using chemometric methods can help overcome the replication problem during bioassay-directed chemical studies on bioactive extracts. The bioactive compounds can be traced using multivariate data analysis by correlating the determined bioactivity with NMR and MS spectra signals.

Another problem in natural product chemistry is the bioactivity of the crude extract due to the synergetic effect. Bioassay-directed chemical investigation of crude extracts exhibiting bioactivity due to synergetic effect results in the isolation of moderately bioactive natural products. The bioactivity of crude extracts due to synergetic effect may be determined early in bioassay-directed chemical studies using bioautographic assays. These assays not only help to inform about the bioactivity, qualitatively, of chemical constituents present in bioactive crude extracts but also provide chromatographic information on bioactive compounds. The latter information can help purify bioactive chemical constituents from bioactive crude extracts. These bioautographic assays can give false-positive results. We must perform detailed in-vitro bioassays on pure natural products isolated based on the bioautographic information to overcome this problem. The bioautographic assays do not rule out the possibility of synergetic effect in bioactive extracts, and bioassay-directed chemical studies on these extracts provide moderately bioactive natural products. These scaffolds can help to design new chemical entities using synthetic organic chemistry.

Our research group is involved in discovering new health-related natural products from medicinally important plants. Plants produce a wide range of structurally diverse natural products, which is why plants have provided a significant number of bioactive lead compounds to drug discovery (Erdogan et al. 2021). Approximately over 370,000 species of plants are used as folk medicines in India, China and African Countries (Orhan et al. 2007). Plants for identifying new bioactive natural products are selected using different approaches, including previously reported bioactivities, ethnomedical knowledge, bioinformatic, and phylogenetic approaches (Ata et al. 2007a, b, c; Ata 2012). The latter two methods have so far shown limited success in selecting plants for drug discovery for two reasons: (i) very limited genomic data is available on medicinal plants, and (ii) correlate the bioactivity of plants with their major metabolites. The ethnomedical approach has shown a relatively high success rate (over 70%) in discovering new pharmaceutically active compounds from medicinally important plants (Lobbens et al. 2007). We also select medicinally important plants using ethnomedicinal reports, and our chemical studies on these plants have yielded bioactive natural products described as follows.

2 Antimicrobial Natural Products

Plants are used to treat wounds in folk medicines and have been ignored for discovering natural antimicrobial products. It would be worthwhile to explore medicinally important plants for discovering antimicrobial compounds as they produce these compounds for their survival. These compounds might help overcome microbial drug resistance problems, which significantly threaten human life. Few research groups are working on discovering new antimicrobial compounds as only a few antimicrobial candidate molecules are in the pipeline to develop them as antibiotics for clinics (Taubes 2008). One of our research group's projects is identifying antimicrobial compounds from medicinally important plants.

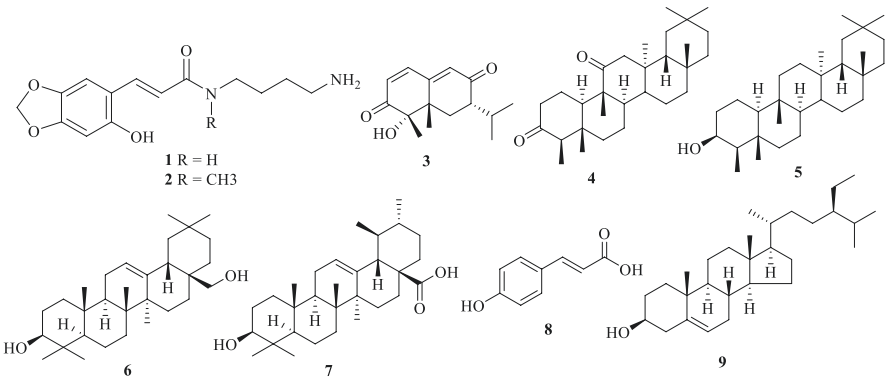
During our screenings of the crude extracts of medicinally important plants for antimicrobial activity, we identified two plants *Drypetes staudti* and *Sphaeranthus indicus* exhibiting potent antimicrobial activity in our bioassays. These results prompted us to perform antimicrobial-directed chemical studies on the crude extracts of these two plants.

Drypetes staudti was collected from Nigeria, where this plant is used to heal wounds by traditional healers. Our bioassay-directed chemical studies on the methanolic extract of *D. staudti* afforded nine antimicrobial compounds. These compounds were named 4,5-(methylenedioxy)-*o*-coumaroylputrescine (**1**), 4,5-(methylenedioxy)-*o*-coumaroyl-4′-*N*-methylputrescine (**2**), 4a-hydroxyeremophila-1,9-diene-3,8-dione (**3**), drypemolundein B (**4**), friedelan-3b-ol (**5**), erythrodiol (**6**), ursolic acid (**7**), *p*-coumaric acid (**8**), and b-sitosterol (**9**) (Grace et al. 2016. Compounds **1**–**9** showed antimicrobial activity against Gram-positive and Gram-negative bacteria with minimum inhibitory concentration (MIC) in the 8–128 mg/ml range. Compounds **1**–**2** were moderately active against *Candida albicans* with a

Table 1 Antibacterial activity data (MIC in μg/ml) of compounds **1–18**

Compounds	*S. aureus*	S. *agalactiae*	*E. coli*	*P. aeruginosa*
1.	8	8	16	16
2.	8	8	16	16
3	64	64	64	64
4	32	32	64	64
5	16	16	32	32
6	32	32	64	64
7	32	32	64	64
8	128	128	128	128
9	128	128	128	128
10	32	32	128	128
11	8	8	64	64
12	8	8	64	64
13	128	128	128	128
14	128	128	128	128
15	128	128	128	128
16	128	128	128	128
17	128	128	128	128
18	128	128	128	128
Thymol	8	8	16	16
Penicillin G	1	1	1	8

MIC value of 32 μg/ml. The bioactivity data of compounds **1–9** are shown in Table 1.

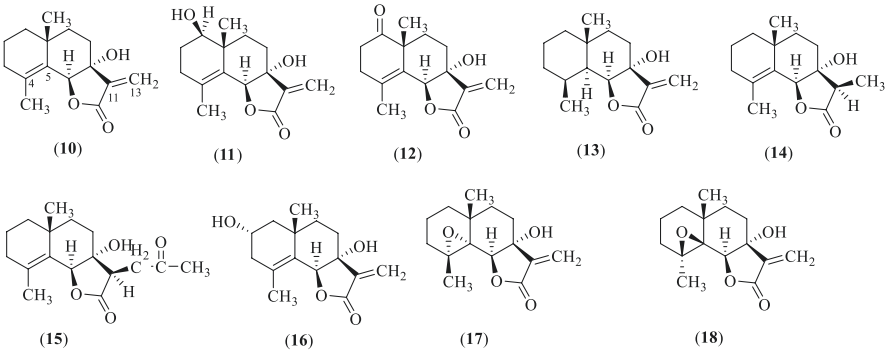


During our α-glucosidase inhibition-directed studies on *D. gossweileri* (discussed in the anti-α-glucosidase section), we have identified compounds **47** and **53** exhibiting antifungal activity against *Candida albicans* with minimum inhibitory concentrations (MIC) of 8.0 and 16 μg/ml, respectively. In order to study the structure–activity relationships of compound **53** for antifungal activity, it was reacted with *m*-chloroperbenzoic acid to afford 12,13-epoxy analogues of **53** (**54** and **55**). Both of these compounds were further reacted with 20% ammonium hydroxide solution to give compounds **56** and **57** (Ata et al. 2011a, b). Compounds **55–57**

showed antifungal activity against *C. albicans* with MIC values of 4.0, 8.0, 8.0, and ≤2.0 μg/ml. The bioactivity data of compounds **54–57** suggested that the enhanced bioactivity of these compounds might be due to the presence of *β*-oriented C-12/C-13 epoxy functionality and an amino group at C-12 in these compounds. Furthermore, it was also observed that the presence of C-12/*α*-amino and C-13/*β*-OH groups significantly increased the bioactivity in this bioassay.

Sphaeranthus indicus was collected from Sri Lanka. This plant has been reported to treat wounds and exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria. We perfumed antibacterial activity-guided fractionations of the crude extract to isolate sesquiterpenoid, 7α-hydroxyfrullanolide (**10**), exhibiting strong antibacterial activity against Gram-positive bacteria with a MIC value of 32 μg/ml. This compound was isolated in a large quantity from this plant (700 mg). We decided to study its structure–activity relationships by using a combination of chemical and microbial reactions. For microbial reactions, we used whole-cell cultures of fungi. Microorganisms are capable of performing oxidation, aldol condensation, Michael addition, and umpolung-type reactions on organic compounds (Ata et al. 2007a).

The whole-cell fungal catalyzed microbial reactions are helpful in predicting the fate of new chemical entities as the metabolites obtained from whole-cell microbial culture reactions are quite often similar to those obtained from mammal biotransformations. This relationship between mammal and fungal biotransformation is due to the presence of a common enzyme, cytochrome P-450 monooxygenase (Ata et al. 2009a). Microbial reactions on compound **10** using the whole-cell cultures of *Cunninghamella echinulata* and *Curvularia lunata* resulted in the production of three compounds, 1β,7α-dihydroxyfrullanolide (**11**), 1-oxo-7α-hydroxyfrullanolide (**12**), 7α-hydroxy-4,5-dihydrofrullanolide (**13**). Compound **10**, upon incubation with the *Aspergillus niger* and *Rhizopus circinans* gave three metabolites, namely 17α-hydroxy-11,13-dihydrofrullanolide (**14**), 13-acetyl-7a-hydroxyfrullanolide (**15**), and 2α,7α-dihydroxysphaerantholide, (**16**) (Ata et al. 2009a). While 4α,5α-epoxy-7α-hydroxyfrullanolide (**17**), and 4β,5β-epoxy-7α-hydroxyfrullanolide (**18**) were prepared by performing an epoxidation reaction on **10** using *meta* chloroperbenzoic acid. Compounds **10–18** were also active in the antibacterial assay. We used thymol and penicillin G as positive controls in our bioassays. These structure–activity relationship studies indicated that the double bonds Δ^{4-5}, Δ^{11-13}, and a γ-lactone moiety in compounds **10–18** are required pharmacophores for the expression of the antibacterial activity of compound **10**.

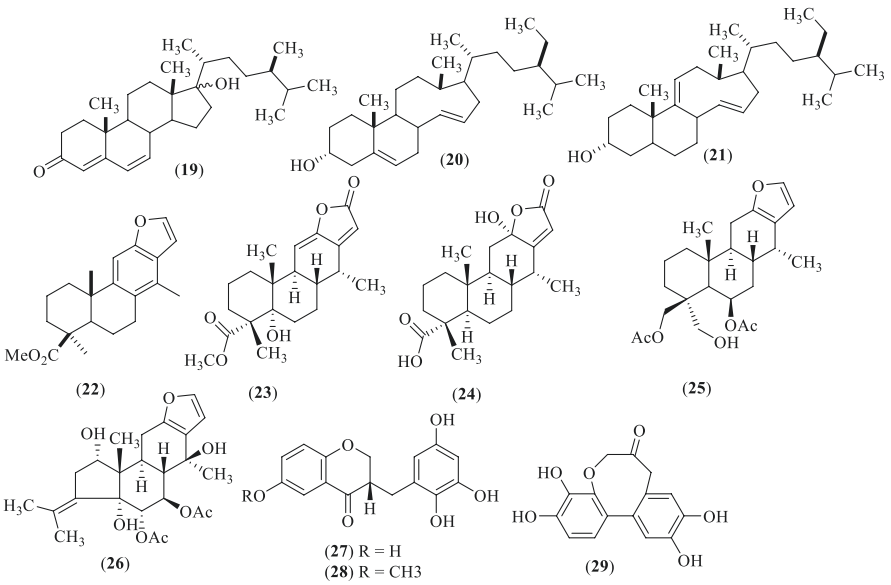


3 Antiglutathione *S*-Transferase Natural Products

Glutathione *S*-transferase (GST) is a multifunctional enzyme that protects cells from cytotoxic and genotoxic stresses. GST acts as a catalyst between the reaction of the electrophilic center of cytotoxic agents (xenobiotics) and glutathione to form an inert water-soluble adduct to excrete from the body. This nature of GST classifies this enzyme as a phase II detoxification system and is believed to be involved in the acquired drug resistance for curing cancer and parasitic diseases. Pharmaceuticals with anticancer and antiparasitic properties contain electrophilic centers in their structures and act as xenobiotics by the human body. GST converts them into water-soluble adducts by reacting with glutathione, and hence, they are excreted from the body. This lowers the concentrations of these pharmaceutical agents in the body and results in the inefficiency of anticancer and antiparasitic chemotherapeutic agents (Ata et al. 2007c; Ata and Udenigwe 2008). The over-expression of GSTs has been observed in various human cancer cells than normal tissues (Douglas 1987; Adang et al. 1990). Lymphocytes isolated from chronic lymphocytic leukemia (CLL) patients that were resistant to chlorambucil A exhibit a two-fold increase in GST activity compared to untreated CLL patients (Schisselbauer et al. 1990). These reports suggest that GST inhibitors can be used as adjuvants during cancer and parasitic chemotherapy to overcome acquired drug resistance problems.

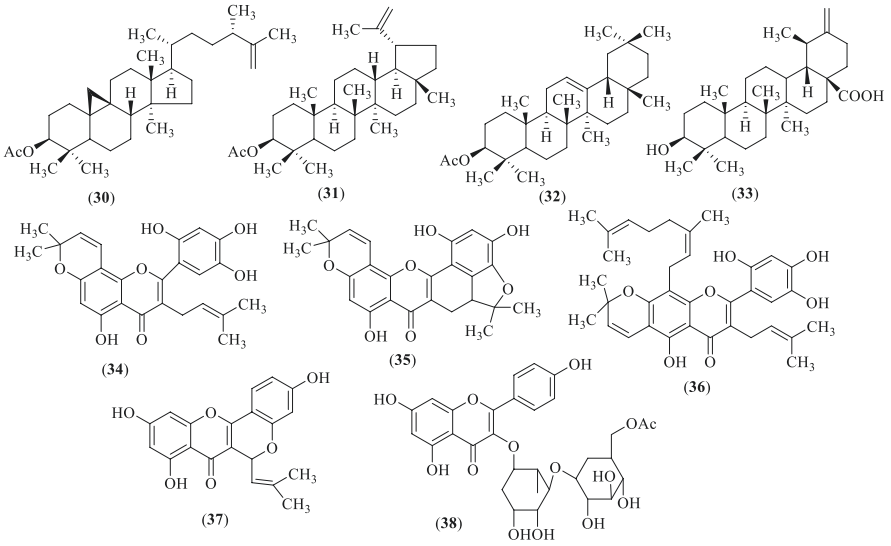
Natural products have yet to be explored for discovering new GST inhibitors, as synthetic compounds are currently used. These compounds exhibit either severe in-vivo toxicity or are inactive in vivo. Toward this end, we have screened several medicinally important plants collected from various parts of the world in our anti-GST assay. This screening process led us to identify the crude extracts of medicinally important plants (*Caesalpinia bonduc*, *Artocarpus nobilis,* and *Nauclea latifolia)* exhibiting anti-GST activity with IC_{50} values of 83.0, 125, and 10.5 μg/ml, respectively. We carried out GST inhibition-directed phytochemical investigation of these bioactive extracts to isolate anti-GST natural products. The results of these phytochemical studies are described as follows.

Our phytochemical studies on bioactive fractions of *Caesalpinia bonduc* afforded nine compounds, namely, 17-hydroxycompesta-4,6-dien-3-one (**19**), 13,14-*seco*-stigmasta-5,14-dien-3*a*-ol (**20**), 13,14-*seco*-9(11),14-dien-3a-ol (**21**), caesaldekarin J (**22**), neocaesalpin P (**23**), neocaesalpin H (**24**), cordylane A (**25**), caesalpinin B (**26**), caesalpinianone (**27**), 6-*O*-methylcaesalpinianone (**28**), and hematoxylol (**29**) (Udenigwe et al. 2007; Ata et al. 2009b; Iverson et al. 2010). Compounds **19**–**29** exhibited anti-GST activity with IC_{50} values of 380, 230, 248, 259, 200, 218, 250, 350, 16.5, 17.1, and 23.6 μM, respectively. Among these isolates, compounds **27**, **28**, and **29** were significantly active in this assay, and their IC_{50} values were comparable to ethacrynic acid (IC_{50} = 16 μM), a standard GST inhibitor which was used as a positive control in our bioassays, Furthermore, it was also observed that compounds **27** and **28** are homoisoflavonoids and have more or less the same potency of inhibiting GST activity as that of ethacrynic acid.

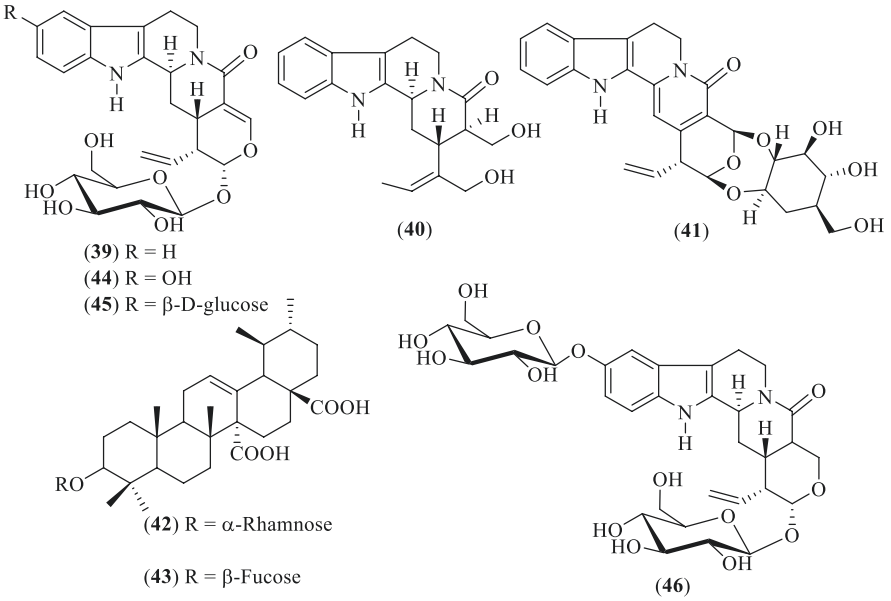


Similarly, GST inhibition-directed chemical investigation of ethanolic extract of *Artocarpus nobilis*, collected from Sri Lankan, afforded four known triterpenoids, cyclolaudenyl acetate (**30**), lupeol acetate (**31**), β-amyrine acetate (**32**), and zizphursolic acid (**33**). Additionally, we have also identified five known flavonoids from bioactive fraction. These compounds are named as artonins E (**34**), artobiloxanthone (**35**) artoindonesianin U (**36**), cyclocommunol (**37**), and multiflorins A (**38**). Compounds **30–38** showed a wide range from weak to strong anti-GST activity with IC_{50} values of 195.1, 146.1, 251.0, 68.5, 2.0, 1.0, 6.0, 3.0, and 14.0 μM, respectively. Again, flavonoids (**34–38**), especially **34–27**, were significantly more active than the rest of the isolates. The higher potency of **34–37** might be due to the presence of the prenyl group in these compounds (Zahid et al. 2007).

Nauclea latifolia has been reported *to* exhibit GST inhibitory activity. Additionally this plant also shows antimalarial and antihypertensive activities. This plant was also active in our anti-GST screening assay. Based on the anti-GST activity data, we decided to perform GST inhibition-directed chemical studies and these studies resulted in the isolation of five known compounds. These compounds were identified as strictosamide (**39**), naucleamides A (**40**), naucleamide F (**41**), quinovic acid-3-*O*-β-rhamnosylpyranoside (**42**), and quinovic acid 3-*O*-*β*-fucosylpyranoside (**43**). Phytochemicals **39–43** showed anti-GST activity with IC_{50} values of 20.3, 27.2, 23.6, 143.8, and 53.5 μM, respectively. Compound **39** was a major metabolite of *N. latifolia,* and was significantly active in our GST inhibition assay. Therefore, it was decided to carry out structure–activity relationships studies by generating its derivatives using microbial reactions. Incubation of this compound with the liquid culture of *Rhizopus circinans* afforded three derivatives that were characterized as 10-hydroxystrictosamide (**44**), 10-β-glucosyloxyvincoside lactam (**45**), and 16,17-dihydro-10-β-glucosyloxyvincoside lactam (**46**) (Ata et al. 2009d) Compounds **40–43** were also active in our anti-GST assay with IC_{50} values of 18.6, 12.3, and 16.6 μM, respectively.



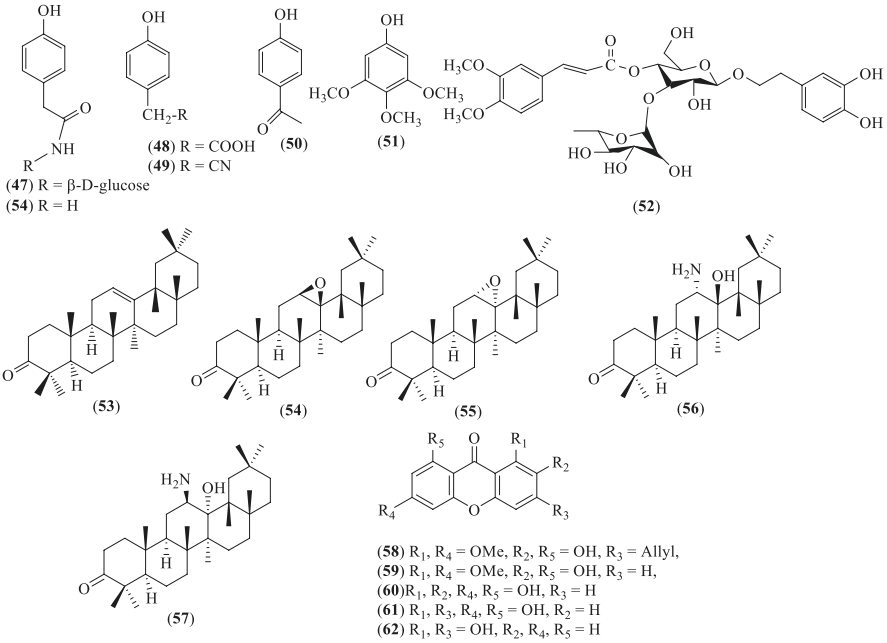
Our structure–activity relationships studies revealed that among compounds **19–46** were significantly active in anti-GST assay. A common functional group, α, β–unsaturated carbonyl group, is present in these compounds. This functional group is believed to be a required pharmacophore for the expression of this bioactivity. The α, β-unsaturated carbonyl group may form a glutathione adduct of these compounds through Michael addition to inhibit the activity of GST.



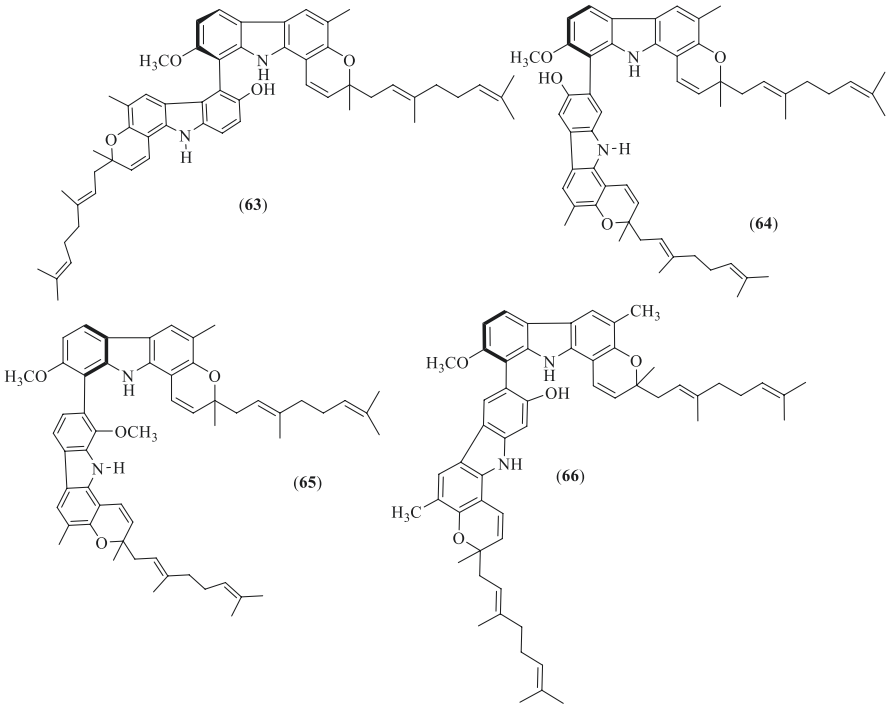
4 Anti-α-Glucosidase Natural Products

α-Glucosidase is a membrane-bound intestinal enzyme that helps to digest carbohydrates by hydrolyzing the glycosidic bonds to liberate free glucose. The glucose produced from this enzymatic reaction causes a significant rise in blood sugar level. This is known as postprandial hyperglycemia and causes type 2 diabetes mellitus affecting over 21 billion people worldwide (Wilcox 2005; Atkinson et al. 2014; Dirir et al. 2022). This ailment can be managed by using potent α-glucosidase inhibitors, as these inhibitors slow down the breakdown of carbohydrates during their digestion to control the blood glucose level. These compounds may also be a therapeutic target for other carbohydrate-mediated diseases, including viral infections, cancer, HIV, obesity, and hepatitis (Berrino et al. 2009; Roglic and Unwin 2010). Our phytochemical studies on medicinal plants for discovering new naturally occurring α-glucosidase inhibitors resulted in the identification of a few bioactive compounds, summarized as follows.

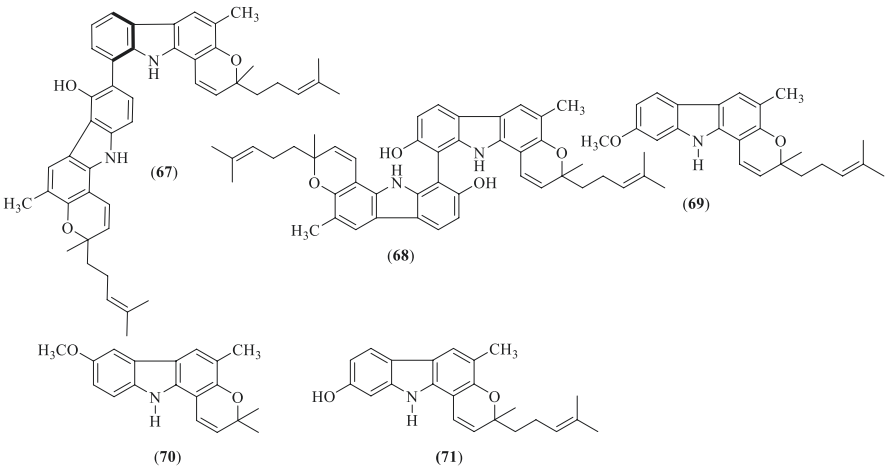
Our α-glucosidase inhibition-directed phytochemical studies on the methanolic extract of *Drypetes gossweileri*, collected from South African based on its folk medicinal history, yielded seven compounds. These compounds were identified as *N*-β-D-glucopyranosyl-*p*-hydroxy-phenylacetamide (**47**), *p*-hydroxyphenylacetic acid (**48**), *p*-hydroxyphenylacetonitrile (**49**), *p*-hydroxyacetophenone (**50**), 3,4,5-trimethoxyphenol (**51**), dolichandroside A (**52**), and β-amyrone (**53**). Among these phytochemicals, compound **47** was the first example of the plant natural products containing *N*-glucose moiety incorporated in its structure. These compounds (**47**–**53**) were active in our α-glucosidase inhibition assay with IC_{50} values of 12, 50, 48, 50, 56, 20, and 25 μM, respectively (Ata et al. 2011a, b). Among all isolates, compound **47** was significantly active in this bioassay and we performed the acidic hydrolysis of this compound to afford compound **54** which was very weakly active in our α-glucosidase inhibition assay (IC_{50} = 60.0 μM). These structure–activity relationships studies revealed that the higher potency of compound **47**, compared to the rest of the isolates, was due to the presence of the *N*-glucose moiety.



Swertia corymbosa was collected from India and our chemical studies on the chloroform extract gave five bioactive xanthones that were identified as 3-allyl-2,8-dihydroxy-1,6-dimethoxy xanthen-9-one (**58**), xanthones gentiacaulein (**59**), norswertianin (**60**), 1,3,6,8-tetrahydroxy xanthone (**61**), and 1,3-dihydroxy xanthone (**62**). Compounds (**58–62**) exhibited anti-α-glucosidase activities with IC_{50} values of 26.3, 44.5, 23.2, 39.0, and 35.2 μM, respectively (Uvarani et al. 2015).

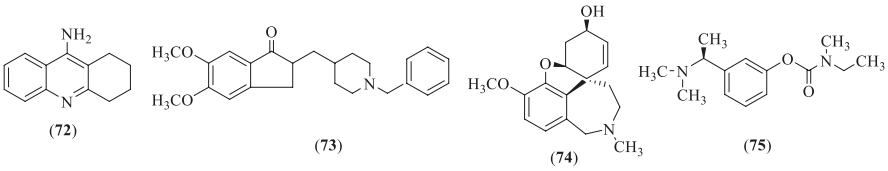


Muraya koenigii was collected from India and our chemical studies on the crude extract of this plant resulted in the isolation of nine carbazole alkaloids which were characterized as bisgerayafolines A-D (**63**–**66**), bismahanimbinol (**67**), bispyrayafoline (**68**), *O*-methyl mahanine (**69**), *O*-methyl mukonal (**70**), and mahanine (**71**). Compounds **63**–**66** belong to dimeric class of carbazole alkaloids, and compounds **67**–**71** are members of monomeric class carbazole alkaloids. Compounds **63**–**71** showed anti-α-glucosidase activity with IC_{50} values of 45.4, 41.2, 69.0, 38.7, 51.3, 29.1, 46.1, 77.5, and 21.4 μM, respectively (Uvarani et al. 2013, 2014). The higher potency of compounds **63**, **64**, and **66** in this bioassay was due to *a*R configuration of biphenyls. Compounds **68** and **71** were also significantly active with IC_{50} values of 29.1 and 21.4 μM. Their higher potency might be due to presence of C-9/OH that can help to bind these compounds with α-glucosidase via hydrogen bonding.

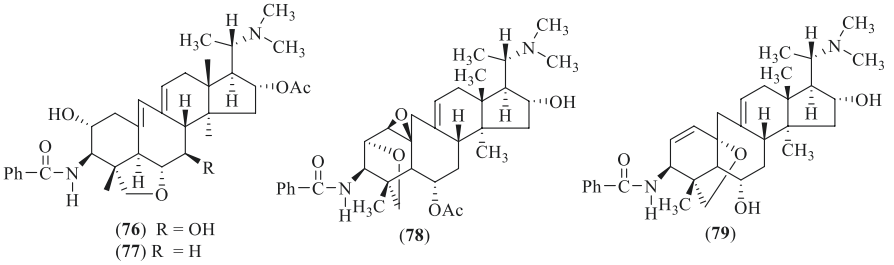


5 Antiacetylcholinesterase Natural Products

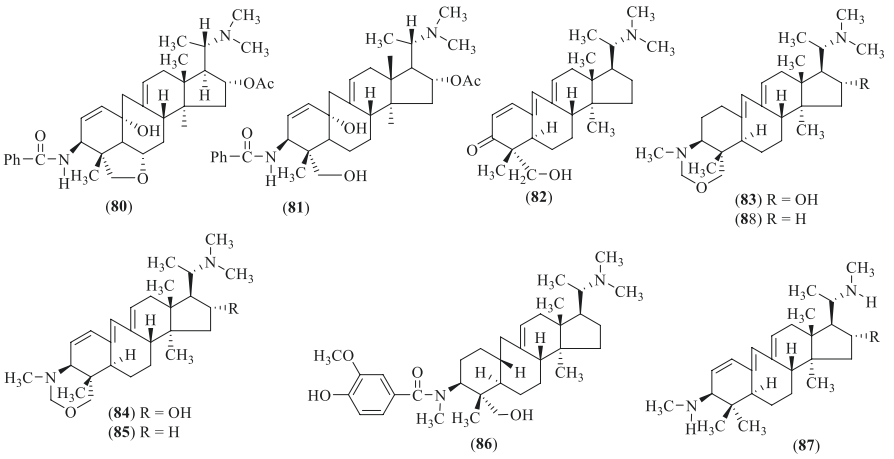
Alzheimer's disease (AD), a neurodegenerative disorder, is a source of severe health disorders. One of these disorders is memory loss in elderly people, which is believed to be due to the decrease in the level of acetylcholine, an important neurotransmitter required for the proper functioning of brain, by hydrolyzing it into acetic acid and choline by an enzyme, acetylcholinesterase (AChE) (Kumar et al. 2017). One of aspects of managing AD is the use of potent acetylcholinesterase inhibitors (AChE). These inhibitors also prevent the pro-aggregating activity of AChE leading to the deposition of β-amyloid, another cause of AD (Orhan et al. 2004). AChE inhibitors have also applications in treating senile dementia, ataxia, myasthenia gravis and Parkinson's disease (Singhal et al. 2012). Currently, four AChE inhibitors, tacrine (**72**), donepezil (**73**), galanthamine (**74**), and rivastigmine (**75**), are approved by the FDA, USA, to be used in clinics for the treatment of AD in its early stage (Orhan and Sener 2003). All approved drugs have limited effectiveness and a several side effects. For instance, tacrine exhibits hepatotoxic lability. Similarly, rivastigmine has a short half-life.



Our phytochemical investigation of the crude extract of *Buxus hyrcana* resulted in the identification of steroidal alkaloids exhibiting anti-AChE activity (Babar et al. 2006; Ata et al. 2010). These compounds include O^6-buxafurandiene (**76**), and 7-deoxy-O^6-buxafurandiene (**77**), exhibiting this bioactivity with IC_{50} values 17.0 and 13.0 μM, respectively.



These preliminary results led us to collect *Buxus natalesis* and *B. macowanii* from South Africa based on their ethnomedicinal use. These plants are being used to enhance memory in elderly people by local traditional healers (Ata et al. 2007c). Both plants were also active in our anti-AChE assay with IC_{50} values of 28 and 30 μg/ml, respectively. We decided to perform anti-AChE-guided phytochemical studies on *B. natalensis* and isolated four bioactive steroidal bases, O^2-natafuranamine (**78**), O^{10}-natafuranamine (**79**), buxafuranamide (**80**), and buxalongifolamidine (**81**). These alkaloids showed AChE inhibitory activity with IC_{50} values of 3.0, 8.5, 14, and 30.2 μM, respectively (Matochko et al. 2010). Similarly, chemical investigation of *B. macowanii* yielded seven bioactive natural products, 31-hydroxybuxatrienone (**82**), macowanioxazine (**83**), 16α-hydroxymacowanitriene (**84**), macowanitriene (**85**), macowamine (**86**), N_b-demethylpapillotrienine (**87**), and moenjodaramine (**88**). Compounds **82–88** were active in the anti-AChE assay with IC_{50} values of 17, 32.5, 11.4, 10.8, 45, 19, and 27 μM, respectively (Lam et al. 2015). Compound **78** was more potent in this bioassay, and its potency is almost identical to huperzine A.

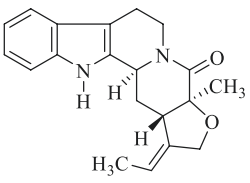


Furthermore, it was also observed that steroidal bases (**76–80**) were equally potent in AChE inhibition assay, suggesting the bioactivity of these compounds might be due to the presence of tetrahydrofuran incorporated in their structures. The location of an ether linkage in these compounds does not play any role in enzyme inhibition activity, as natural product **79** contains an ether linkage between C-31 and

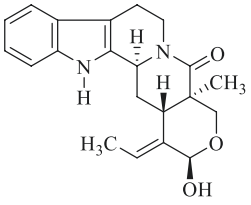
C-10 while compounds **76**, **77**, and **80** contain linkage between C-31 and C-6, whereas an ether linkage between C-31 and C-2 is present in compound **78**. The latter compound also contains an epoxy functionality at C-1/C-10. These two functionalities might be responsible for its higher potency.

6 Antirenin Natural Products

The hypertension is mainly caused by abnormal blood pressure. A renin–angiotensin system (RAS) has been reported to be involved in controlling and maintaining blood pressure in mammals. The enzyme Renin (EC 3.4.23.15), produced by the epithelial cells of the kidney and released into the circulation system by various stimuli, generates the deca-peptide angiotensin I (AI). Angiotensin converting enzyme (ACE) converts AI into angiotensin II (AII). AII lies on the arterial smooth muscle cells to maintain blood pressure and stimulates the synthesis and releases aldosterone from the adrenal cortex. Its overexpression leads to the abnormalities of blood pressure causing hypertension. Inhibition of the activity of RAS system is an important target for discovering new chemical entities against hypertension. *Nauclea latifolia* exhibits antihypertensive activity (Akubue and Mittal 1983) and it was active in our antirenin assay. Our antirenin-guided chemical investigation of methanolic extract of *N. latifolia* resulted isolation of five bioactive indole alkaloids. These compounds were identified as latifoliamide A-E (**88**–**92**) and were moderately active antirenin activity with IC_{50} values of 32.6, 11.3, 95.0, 94.5, and 16.3 μM, respectively. We used alkisiren, currently used antihypertensive drug (IC_{50} = 0.6 nM) as a positive control. This compound works by inhibiting the RAS activity (Agomuoh et al. 2013) but it has several side effects. It is worthwhile to explore natural products for discovering novel antirenin lead compounds to overcome the hypertension problem as natural products have not been explored for this bioactivity.



(**88**)



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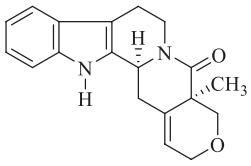
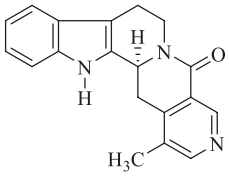


(**90**)



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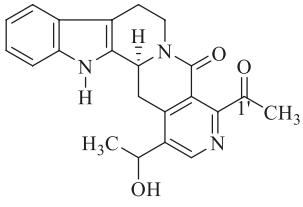

(**92**)

In summary, our phytochemical studies on medicinally important plants have resulted in the isolation of new bioactive natural products. Structure–activity relationships studies on bioactive natural products have provided us information regarding the active pharmacophore required for prescribed bioactivity. This information will provide a rational for designing new lead compounds against these targets. Furthermore, in vivo evaluation and structure–activity relationships studies on potent bioactive compounds are in progress in our lab to determine their potential as new pharmaceutical agents.

Acknowledgments I would like to thank all of my undergraduate and graduate students as well as my collaborators who were involved in discovering new antienzymatic compounds, and their names are cited in the references.

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Natural Product Formulations to Overcome Poor ADMET Properties

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Abstract Many of the human needs, including medicines, have been fulfilled by nature. Medicinal plants have been a source of myriad natural therapeutic agents. Chemically referred to as plant secondary metabolites, each class of these compounds exerts therapeutic effects in different ways. They may act as antioxidants, anti-inflammatory, antibacterial, anticancer, and wound-healing agents. Therapeutic activities of botanic medicines are dependent on pharmacokinetic profile of these compounds. Hydrophilicity and lipophilicity of molecules affect solubility and membrane permeability. Similarly, first pass metabolism reduces the circulation half-life and, hence, the bioavailability of therapeutic agents. In addition, drug–drug interactions may be synergistic or antagonistic and also affect the bioavailability of these active pharmaceutical ingredients. These parameters of drugs hamper their use clinically. Conventionally used strategies to overcome these challenges are micronization, use of surfactants and solvents, and complexation. Recently, nanotechnology-based drug delivery systems in the form of nanoparticles, liposomes, nanoemulsions, and bioconjugates have also provided efficient alternative to the conventional techniques. Nanocarriers ensure targeted delivery of medicines and, hence, can overcome toxicity-related challenges. Besides this, nanocarriers ensure intact drug delivery of all sorts of drugs to the target site, thus producing efficient outcomes even in smaller quantities. The current manuscript reviews the conventional and contemporary technologies for improving the pharmacokinetic parameters of natural products.

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1 Introduction

Humans are dependent on mother nature to fulfill their requirements of food, shelter, and clothes. Among the natural resources, plants not only serve these purposes, but additionally they have been used to treat ailments since times immemorial. Archeologists have found records dating back to thousands of years mentioning the use of plant-based treatments in *Ebers Papyrus*, Latvian folk medicine, Traditional Chinese medicine, and Ayurveda, among others. According to Royal Botanic Gardens, Kew, UK, there are 391,000 vascular plant species on earth. Of these, more than 50,000 species have been reported to be used for medicinal purpose (Chen et al. 2016). Many flowering, aromatic, and culinary plant species including basil, mint, parsley, chamomile, and ginger are commonly used medically. With advances in analytical technology, scientists have been able to successfully extract and isolate thousands of natural compounds. Most synthetic and semisynthetic drug molecules are based on these phytochemicals. However, the potential adverse effects of synthetic medicines shift the focus back to the employment of phytomedicines for medicinal purposes. It has been reported that more than 80% of world population uses plant-based medicines to treat their ailments in one form or another (Ekor 2014).

The pharmaceutical importance of plants is attributed to the metabolites produced by them that serve various therapeutic functions in the body. For example, polyphenols have antioxidative and antibacterial activity. Curcumin, a polyphenol from *Curcuma longa*, has wound-healing, antidiabetic, and antiobesity activity (Rathore et al. 2020). Alkaloids impart anesthetic, anti-inflammatory, cardioprotective, and antitumor role. Paclitaxel, a plant alkaloid extracted from *Taxus brevifolia* (Pacific yew), is effective against multiple cancers (Zhao et al. 2022). To ensure the stability and safe delivery to consumer, phytoproducts are formulated in different dosage forms including tablets, capsules, powders, and syrups, among others. However, clinical therapeutic efficacy of formulated products based on the pharmacokinetic profiles does not align with in vitro results. This chapter will discuss the challenges with natural product formulation development associated with their pharmacokinetics and recent technologies to overcome them.

2 Phytochemicals and Medicinal Uses

Any chemical compound produced by plant is a phytochemical. The word phytochemical is derived from Greek word "*Phyton*" meaning "plant." These are mainly the compounds produced by primary and secondary metabolism. Phytochemicals

are defined as biologically active plant derived chemical compounds which provide health benefits as medicine and nutrients (Prakash et al. 2020). Phytochemicals are found in natural food sources like fruits, vegetables, legumes, cereals, and other plant-based foods. In plant body, these compounds are involved in various primary and secondary metabolic pathways and may help plant to fight against biotic and abiotic stressors. Plant's color, aroma, and flavor also attributed to these phytochemicals.

Primary metabolites are those chemical compounds that are involved in plant growth, development, and reproduction. Primary plant metabolites include carbohydrates, proteins, amino acids, and chlorophylls, among others. While secondary metabolites are the plant chemicals that are produced under stress conditions and help plants to stand against them. Most of these secondary metabolites have therapeutic significance as well and may include compounds including alkaloids, phenols, flavonoids, steroids, and terpenoids. Plant secondary metabolites are reported to have anti-inflammatory, immunomodulatory, antidiabetic, anticancer, antimicrobial, and wound-healing properties (Tiwari and Rana n.d.).

As potential antimicrobial agent phytochemicals use different ways to kill bacterial cells. Flavonoids are reported to disrupt bacterial cell wall by making pores in it (Górniak et al. 2019; Alves et al. 2023). Phenolic compounds in plant extracts may stick to cell membrane and change the hydrophobicity. This disrupts the potassium channels and K^+ leaks out of the bacterial cell (Ergüden 2021). Conversely, alkaloids intervene with cell wall synthesis and cellular DNA machinery and inhibit the protein synthesis (Yan et al. 2021). Phytochemicals, like naringenin, anacardic acids, ursolic acid, and salicylic acid, regulate the genes for quorum sensing and inhibit biofilm formation (Asfour 2018). All these mechanisms eventually halt the bacterial growth and kill them. Not only the pure compounds but plant extracts also show very effective pharmacological activity. Phytochemical-rich extracts of *Ocimum basilicum* showed antimicrobial potential against three fungal species including *Aspergillus flavus*, *Aspergillus niger,* and *Candida albicans*, gram-positive bacteria including *Clostridium difficile*, *Bacillus subtilis,* and *Staphylococcus aureus,* and gram-negative bacteria like *Escherichia coli*, *Salmonella typhi,* and *Klebsiella pneumoniae* (Rubab et al. 2021). Flavonoids from *Carex meyeriana* showed inhibition of *Bacillus pumilus, Bacillus subtilis,* and *Escherichia coli* (Cheng et al. 2020).

Free radical scavenging ability of phytochemicals, similarly, makes them potential antioxidants. Free radicals are electron accepting entities that cause oxidation of compounds. An imbalance in the generation of these reactive oxygen species (ROS) can cause damage to cellular components like carbohydrates, proteins, lipids, and DNA, ultimately ensuing cell death. Plant extracts are abundant in natural antioxidants. *Camellia sinensis* extract have catechins, benzoic acids, cinnamic acids, and flavanols that act as potent antioxidants (Falla et al. 2021). *Atractylis gummifera* extract is rich in polyphenols, flavonoids, and tannins that prevent lipid peroxidation (Bouabid et al. 2020). Similarly, *Ikonnikovia kaufmanniana* extract having dihydroflavanonol, flavanol, isoflavone, and flavanol skeletons prevents oxidative damage of DNA (Baiseitova et al. 2021).

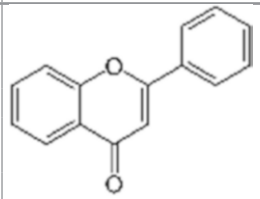
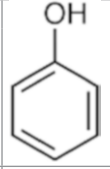
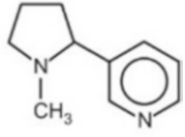
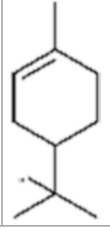
In addition, phytochemicals induce anti-inflammatory action by modulating inflammatory cytokines. Inflammation is a host defense mechanism against cellular stressor. Chronic inflammation may lead to complex presentations including autoimmune diseases, neurodegenerative disorders, and metabolic indications. Phytochemicals are known to attenuate proinflammatory cytokines signaling pathways including interferon (IFN) α and γ, Nuclear factor κB (NF-κB), mitogen-activated protein kinase (MAPK), signal transducers and activators of transcription (STAT), and nuclear factor erythroid 2-related factor 2 (Nrf-2). Phenolics, terpenoids, and alkaloids are main phytochemicals involved in reducing proinflammatory cytokines in disorders like diabetes, rheumatoid arthritis, and Alzheimer's disease (Shin et al. 2020). Phenolic rich *Thymus* species have anti-inflammatory, anticancer, and antioxidative properties (Afonso et al. 2020). *Curcumin longa* extracts have established in vitro and in vivo anti-inflammatory, antioxidative, and immunomodulatory effect (Memarzia et al. 2021). Therapeutic potential of plant phytochemicals also includes anticancer properties. Plant secondary metabolites attenuate cell signaling pathways, e.g., MAPK, NF-κB, and ROS to regulate autophagy, apoptosis, and pyroptosis (Zheng et al. 2022). The anticancer potential of polyphenols, terpenoids, and alkaloids on NF-κB signaling is well-established as well (Chauhan et al. 2022). *Punica granatum* phytochemicals also have potential applications in oncology. Their potential role in breast cancer and lung cancer treatment has been reported recently (Toda et al. 2020).

Apart from these antineoplastic, anti-inflammatory, and antimicrobial properties, plant secondary metabolites play crucial role in wound healing by scavenging ROS, cell proliferation, and re-epithelialization (Addis et al. 2020). Wound healing is a sequential process of homeostasis, inflammation, proliferation, and remodeling and, hence, provides an appropriate target for therapeutic intervention. *Curcuma longa* containing Curcumin that has been effectively used in wound healing. In inflammatory phase, it causes apoptosis of inflammatory cells, reduces ROS, inhibits NF-κB, and reduces cytokines (TNF-α and IL-1) to shorten the inflammatory processes. During proliferation, it aids tissue remodeling, granulation, and collagen deposition, hence leading to wound contraction (Urošević et al. 2022). *Senna auriculata, Piper betle,* and *Phlomis viscosa* leaf extracts have been reported to possess promising wound-healing properties (Lien et al. 2015). Chingwaru and colleagues have recently identified 20 plant species rich in flavonoid, alkaloid, phenol, and saponin content to promote wound healing (Chingwaru et al. 2019). Table 1 provides a brief overview of some of the phytochemical classes and their therapeutic applications.

3 Pharmacokinetic Parameters of Natural Products

Though natural products have been used to treat ailments since the start of civilization, scientific evidence on their efficacy had been lacking. Modern analytical technologies aided in determining how much and how fast the administered product is absorbed in body. Further elucidation of metabolism and drug excretion helped to

Table 1 Classes of phytochemicals and medicinal use

Class	Structure	Subclass	Representative example	Biological role	References
Flavonoids		Isoflavones	Genistin	Anticancer, antioxidative, neuroprotective, and antimicrobial activities	Islam et al. (2020)
		Flavanones	Eriodictyol	Anti-inflammatory and antioxidative	Deng et al. (2020)
		Flavones	Apigenin	Anticancerous	Rahmani et al. (2022)
		Flavonols	Quercitin	Anti-inflammatory antidiabetic, and anticancerous	Azeem et al. (2023)
		Anthocyanidins	Delphinidin	Anticancerous	Mottaghipisheh et al. (2022)
		Flavan-3-ols	Catechins	Antimicrobial, anticancerous, anti-inflammatory	Stanislaus et al. (2021), Baranwal et al. (2022)
Phenols		Phenolic acids	Salicylic acid	Skin treatment	Santos et al. (2022)
		Stilbenes	Resveratrol	Anti-inflammatory	Malviya et al. (2022)
		Tannins	Tannic acid	Antiviral	Wang et al. (2022)
Alkaloids	At least 1 nitrogen in structure  Nicotine		Berberine	Antimicrobial, proapoptotic, anti-inflammatory, anticancerous, antidiabetic	Och et al. (2020), Song et al. (2020)
Terpenoids			Menthol	Wound-healing, anti-inflammatory	Rozza et al. (2021)

link in vitro data with clinical results. The therapeutic activity in the human body is dependent on its pharmacokinetic parameters even if the drug is highly potent. Pharmacokinetics (PK) deals with the characterization of drug in terms of absorption, distribution, metabolism, excretion, and toxicity (ADMET). Absorption or bioavailability of a product largely depends on physiochemical properties. Lipophilic drugs easily pass through the lipid membrane of the cells, but solubility is a major concern for lipophilic drugs. Hydrophilic drugs are more soluble, but passing through biomembrane is of concern. Therefore, an adequate balance of solubility and permeability of drug molecules is essential.

Regarding the natural products, plant phenolic compounds, for instance, are divided into soluble and nonsoluble categories based on their solubility and interaction with the solvents. Soluble phenolic compounds are flavonoids, simple phenolics, and hydrolysable tannins, while nonsoluble phenolic compounds include phenolic acids and condensed tannins. Curcumin, a polyphenolic, has diverse pharmacological activity, but its poor bioavailability is a major challenge in its general acceptability. Poor bioavailability is attributed to its hydrophobic nature, rapid metabolism, and low absorption (Sohn et al. 2021). Quercetin is a flavonoid having promising therapeutic activities as an antioxidant, antibacterial, anticancer, and antiaging agent (Yang et al. 2020). Apart from their effect as individual therapeutic entities, the combined administration of curcumin and quercetin can provide a synergized anticancer effect (Mansourizadeh et al. 2020). Contrarily, combining two drugs may hinder each other's activity, for example, 9-*epi*-artemisinin and artemisitene both are components of *Artemisia annua,* but they antagonize one another against *Plasmodium* (Caesar and Cech 2019).

Among the pharmacokinetic parameters, drug distribution is referred to the availability of a pharmaceutical agent to different tissues and organs. Most of the drugs do not distribute evenly in the body tissues. Water-soluble drugs prefer body fluids, while lipophilic drugs tend to stay in fatty tissues or organs. Digoxin, for example, binds to skeletal muscles while its main target is myocardium. Such nonspecific bindings hinder actual efficacy of product (Ziff and Kotecha 2016). Metabolism of a product converts it into more excretable products to aid the elimination from the body. Gut bacteria and drug-metabolizing enzymes mediate the metabolism of therapeutic agents. Metabolism of a compound, generally, occurs in 2 phases. Primarily occurring in the liver, the phase I, enzymatic oxidation or reduction of drug adds polar groups. Commonly used enzyme in phase I belongs to cytochrome P-450 family. Conversion of paclitaxel to 6α-hydroxy paclitaxel may be considered as an example. Sometimes, phase I metabolism converts drug into its active form, such drugs are known as "prodrugs." For example, codeine and heroin weakly bind to their receptors, but their metabolizing into morphine produces analgesic effects. In phase II, enzymatic conjugation of polar groups to nonpolar compounds converts it into large molecular weight yet less toxic molecule. Glutathione-*S*-transferases conjugate glutathione with many therapeutic compounds to make them less toxic compound so that they can be easily excreted from the body (Kaur et al. 2020).

Metabolizing makes a drug more hydrophilic to eliminate from body. Though the main elimination organ is kidney, excretion through other means including bile,

sweat, breast milk, saliva, and lungs also occurs. Polar metabolites get filtered in kidney and are generally not reabsorbed, while nonpolar drugs absorb back and get stored in fatty tissues (Garza et al. 2022). If a drug is not eliminated appropriately, it can be toxic to the body. It is a general perception that natural products are safe. However, any natural product itself may contain toxic substances like pyrrolizidine alkaloids that can cause liver damage, while aristolochic acid can cause renal toxicity (Gertsch 2011). Moreover, sometimes interaction among natural products is harmful and can lead to allergy, serious illness, or high blood pressure. Optimizing these diverse contributors of pharmacological action needs to be considered while developing a safe and effective natural product-based formulation.

4 Challenges in Natural Product Formulation

A natural product formulation may be delivered through any of the available dosage forms including capsules, tablets, injection, cream, or gel to ensure safe and effective use by patients. Adequate formulations keep the product stable under diverse environmental conditions. Oral formulations are generally available in the form of tablets, infusions, decoctions, tinctures, teas, syrups, and capsules. Major challenge in the oral dosage forms of natural products is their bioavailability. Active ingredients in formulation may be poorly soluble in water or less permeable to biological membranes. Diterpenoid paclitaxel, for instance, obtained from *Taxus brevifolia,* is a broad spectrum cytotoxic therapeutic agent belonging to class IV of biopharmaceutical classification system (BCS) (Jahadi et al. 2021). Compounds with low membrane permeability and less water solubility are classified in this category. They do not easily reach the receptors for therapeutic effects and become inactive before reaching to systemic circulation because of enzymatic breakdown or highly acidic pH in gastrointestinal tract. Another example of poor bioavailability is an alkaloid, berberine isolated from *Berberis vulgaris*. Its clinical applications in cancer, diabetes, hypertension, and polycystic ovarian syndrome make it a pharmaceutically valuable natural product, but its poor bioavailability of 0.68% only poses a major challenge in its clinical use. Several mechanisms of poor berberine bioavailability have been summarized by Khoshandam et al. and include demethylenation, reduction, and cleavage of the dioxymethylene in phase I. Phase II reactions include glucuronidation, sulfation, and methylation (Khoshandam et al. 2022). Self-aggregation of berberine in intestine and stomach reduce its solubility in the gastrointestinal tract and hinder its efficacy. Rapid demethylation of berberine in liver and fast excretion of its metabolites in urine, feces, and bile further reduces its bioavailability (Feng et al. 2019).

Another natural product, vincristine, from *Catharanthus roseus*, also has major problems associated with its solubility, the stability within the systemic circulation and overall toxic effects. All of these factors largely affect the bioavailability of the drug molecule. Many natural compounds such as flavonoids get extensively metabolized by the microsomal enzyme system, which leads to early clearance of flavonoids. Podophyllotoxin and its derivatives exhibit toxic effects which led to their

limited applicability in clinical settings. Many of the naturally occurring potential drug molecules are known to undergo substantial metabolism through methylation, sulfation, and other metabolic events. First pass metabolism prior to entering the systemic circulation further decreases the bioavailability of the natural drugs. Similarly, some aglycones of flavonoids have difficulty in dissolution as well as in crossing the membranes due to their unique chemical structures, resulting in decreased availability at the intended site of action and subsequent less therapeutic activity (Kashyap et al. 2021). Many flavonoids also show unacceptable pharmacokinetics due to their enhanced plasma protein binding. For example, quercetin, an abundant dietary flavonoid, has strong affinity to human serum albumin. Some polyphenolic compounds after their administration are absorbed in the small intestine to a little extent, while the rest is metabolized by the microflora of the large intestine. Digoxin and glycyrrhizin are metabolized by intestinal microflora into metabolites that hinder their bioavailability. All of these pharmacokinetic factors lead to unacceptable ADMET profiles. Figure 1 summarizes the pharmacokinetic challenges faced in developing an effective formulation of natural products.

These challenges require the incorporation of novel drug delivery systems containing these natural products to ensure their targeted delivery at the site of action (Kashyap et al. 2021). Nanocarriers such as metallic nanoparticles, liposomes, nanotubes, nanowires, niosomes, and nanoneedles could be potential options for overcoming all the challenges associated with the poor pharmacokinetics of the natural products. Incorporation of phytoconstituents and other natural products in contemporary delivery systems can provide the benefits of enhancing the bioavailability and stability in circulation along with better dissolution and permeability profiles (Teng et al. 2023). Natural origin chemicals such as artemisinin, resveratrol, quercetin and curcumin have been studied by formulating them as nanocarriers, and they have provided very promising results in terms of improvement in ADMET profile as well as enhanced therapeutic efficacy (Idrees et al. 2020).

5 Formulation Technology Aspects of Natural Products

To overcome the technical challenges associated with natural product formulation, several strategies have been employed by researchers. Ever advancing field of nanotechnology is replacing the conventional methods to enhance the bioavailability, stability, solubility, distribution, and therapeutic potential of natural products. Conventional approaches to overcome PK challenges of natural products are size reduction, addition of surfactant/solubilizing agents, salt formation, and solid dispersion. Nanoparticles, nanoemulsions, dendrimers, liposomes, micelles, phytosomes, coordination polymer, and bioconjugates of natural products are targeted drug delivery systems that have recently been developed to improve the efficacy of phytomedicines. These approaches have been discussed to present the avenues that can be exploited for addressing the issues associated with the ADMET of natural products.

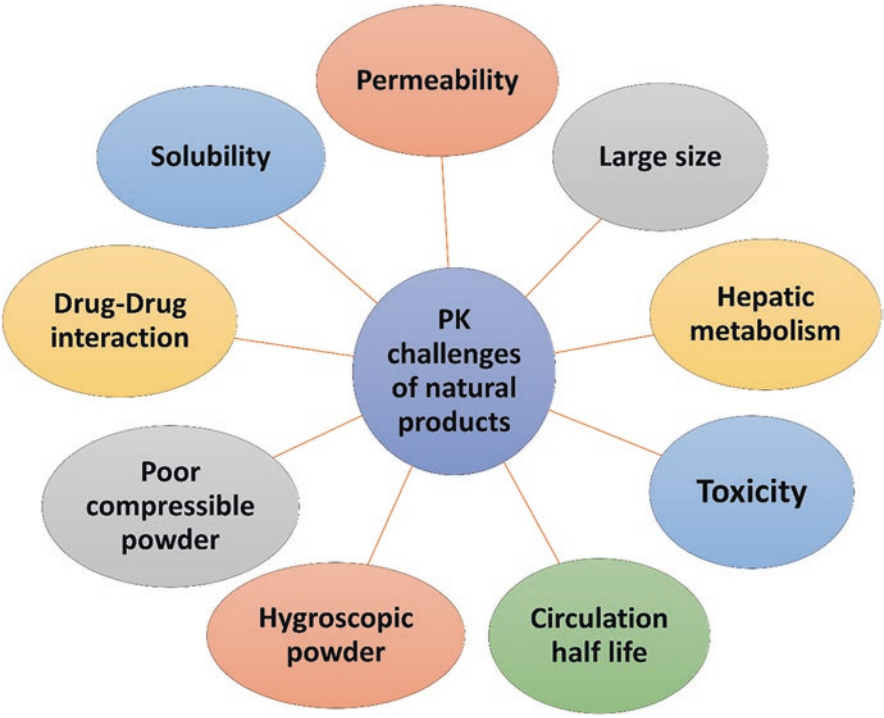


Fig. 1 Pharmacokinetic challenges of natural products

5.1 Conventional Approaches

5.1.1 Size Reduction

Natural products are usually large sized molecules; this inherent property reduces the solubility and ultimately bioavailability. Micronization is a technique of reducing size of such pharmaceutical products to the micrometers range. Reduced size increase the surface area of molecules leading to increased dissolution that favors an increase in bioavailability of the product. *Salvia miltiorrhiza* is a therapeutical herb, the extract of which is used in traditional Chinese medicine (TCM). Its extract has a mixture of both hydrophilic and lipophilic compounds. Tanshinones are the main lipophilic diterpenoids that have pharmaceutical importance. However, their low bioavailability in oral dosage diminishes their clinical use. Reduction in size in the form of micronized tanshinones powder increases the surface area and improves the bioavailability. Granular powder of *S. miltiorrhiza* had greater plasma concentration (C_{max}) and a broader area under curve (AUC) that indicates improved bioavailability. Half-life of *S. miltiorrhiza* has been found to improve in case of granular powder as compared to simple extract (Salehi et al. 2021; Muzammil et al. 2023).

5.1.2 Surfactant and Solubilizing Agents

Hydrophobic metabolites depend on surfactants and solvents to get dissolved in water. One such example is paclitaxel. Its brand Taxol® sold for cancer has Cremophor® as a surfactant. While docetaxel's marketed formulation Taxotere® uses hydroalcoholic solvent mixture with Tween-80 as a surfactant (Paroha et al. 2020). Tween 80 is also employed as a surfactant for digoxin to improve its solubility. In gastrointestinal tract, P-glycoprotein (P-gp) limits digoxin's permeability. Nonionic surfactant Tween-80 also inhibits P-gp and makes digoxin bioavailable while producing significant results in vivo (Rathod et al. 2022).

5.1.3 Salt Formation

Ionizable natural compounds dissolve at a specific pH. Among phytomedicines, weak acids and weak bases become well soluble by the modification of the pH of the solution. Different acids or bases are used as pH modifiers, e.g., lactic acid and magnesium trisilicate. Acidic and basic salts of phytochemicals make them more acceptable pharmaceutically. Berberine with no ionizable groups is sold as berberine chloride and can change to different hydrates according to humidity. To make berberine more stable, cocrystals of berberine chloride with citric acid and fumaric acid are developed that increase the solubility and stability of berberine (Lu et al. 2019).

5.1.4 Polymer Complexation

Conjugating the active ingredient with a polymer can also help in improving the solubility and, hence, absorption profile of the phytochemicals. Cyclodextrin are ring shaped oligosaccharides in which the sugar units are joined by α-1,4 glycosidic linkage. These are cone shaped molecules with inner side of cone being hydrophobic while outer side is hydrophilic. Hydrophilic covering of cyclodextrin increases the solubility of lipophilic molecules. Curcumin, an unstable and hydrophobic drug, is loaded in a conjugate of β-cyclodextrin and methacryloyl. This drug encapsulation improves its stability and solubility. Increased therapeutic activity, deeper penetration, biocompatibility, and degradation in vitro and in vivo can, thereafter, be observed (Zhou et al. 2020). Mitosis inhibitor PTX is a potential antitumor candidate that presents applications in breast cancer, lung cancer, liver cancer, prostate cancer, ovary cancer, and cervical cancer therapy. However, its water-insoluble nature limits its efficacy in vivo. PTX loaded in cyclodextrin in the form of nanoparticles increases its bioavailability by 80% in breast cancer cells (Almeida et al. 2022; Velhal et al. 2022).

5.2 Nanotechnological Approaches

5.2.1 Polymer/Lipid Nanoparticles

A phytoproduct *Cuscuta chinensis* extract has a higher content of water-insoluble flavonoids and lignans that hinder its bioavailability. To overcome the solubility issues, nanoparticles of *C. chinensis* have been prepared and tested in vivo that effectively prevented hepatotoxicity in rats (Akbar 2020). Diminished pharmacological activity of *Salvia miltiorrhiza* extracts limits its clinical effectiveness. Synthesized nanoparticles of *S. miltiorrhiza* showed high antioxidative potential and oral availability (Mishra et al. 2022). Genistein, the chief antioxidant in the extract, had poor bioavailability but in the form of nanoparticles it increased by 241.8%. *Maerua oblongifolia*, another therapeutic plant, has active constituent maerua that otherwise has poor solubility, and its bioavailability limits its practical use. *Maerua oblongifolia* nanoparticles, however, showed controlled release with superior bioavailability (Nisar et al. 2021).

5.2.2 Nanocapsules

Nanocapsules are hollow shells encapsulating a therapeutic agent. Drug delivery through nanocapsules improves the pharmacokinetic parameters of a drug. *A. annua,* a powerful antimalarial therapy, undergoes first pass metabolism and has a very short half-life. To achieve its sustained release, nanocapsules have been developed and evaluated in vivo that increased the half-life and delayed the drug clearance of the bioactive compounds. Increased hydrophilicity and sustained release of *Artemisia* were also observed in nanocapsules and liposomes (Lyu et al. 2021; Rego et al. 2022). *Centella asiatica*, another medicinal plant, finds its applications in dermatology. It initiates collagen synthesis and ROS scavenging that makes it a wound healer, skin moisturizer, and antiwrinkle agent. But poor stability and bioavailability limit its use. To improve its physical stability and bioavailability, *C. asiatica* extract has been encapsulated in polymeric colloidal nanocapsules and tested for entrapment and stability. Encapsulated *C. asiatica* extract was found to be 97.7% entrapped and stable for 60 days under harsh environmental conditions (Perez et al. 2020).

5.2.3 Nanoemulsions

Nanoemulsions are prepared for increased permeability of hydrophilic drugs for dermal applications. Emulsions are prepared by mixing two immiscible liquids with the help of a surfactant. These are best carriers of irritants and lipoidal compounds because interior of emulsion has lipids. Camptothecin, a natural antitumor, has limited applications because of poor solubility, high toxicity, and rapid clearance.

However, oral delivery of camptothecin in the form of nanoemulsion increases its bioavailability up to 17-folds (Galatage et al. 2022). Gingerol is phenolic compound isolated from ginger that has therapeutic efficacy against cerebral ischemia, but its unfortunate solubility and absorption limit its use. Ahmad et al. tried a 6-gingerol nanoemulsion for nasal permeation and were able to successfully achieve improved intracranial bioavailability of the compound (Ahmad et al. 2021).

5.2.4 Dendrimers

Dendrimers are branched polymeric macromolecules that have central cavities to aid the carrying of drugs to the target site. They can efficiently carry hydrophobic molecules such as ursolic acid. Ursolic acid is a triterpenoid with remarkable antibacterial, antioxidative, antidiabetic, and neuroprotective activities. However, its low solubility, less stability, and inappropriate absorption decrease its efficacy. To increase its solubility, Silvana Alfei and colleagues recently prepared polyester-based dendrimers carrying ursolic acid. Consequential solubility was increased by up to 392 times more than free ursolic acid with sustained release (Alfei et al. 2021; Lima et al. 2022).

5.2.5 Phytosomes

Phytosomes are phospholipid complexes containing phytochemicals. Compounds from plant origin interact with hydrophilic parts of phospholipids by hydrogen bonding. Hydrophilic phytomedicines become part of the phospholipid membrane and pass through the cell membrane thereby becoming available at the target site. Curcumin phytosomes, for example, showed increased bioavailability, anti-inflammatory, and antioxidative properties (Al-Kahtani et al. 2020) (Baradaran et al. 2020) (Barani et al. 2021). Phytosomes of other medicinal plant extract, e.g., green tea, panax ginseng, olive oil, maidenhair tree, milk thistle, and grape seeds proved to have an enhanced pharmaceutical activity in comparison to free drug. Similarly, silymarin phytosomes showed high antihepatotoxic activity than silymarin alone (Shriram et al. 2022).

5.2.6 Micelles

Micelles are nanostructures that have hydrophilic shell and lipophilic core. Micelles can carry macromolecules for sustained release to ensure enhanced bioavailability. Paclitaxel micelles have been proven to possess enhanced antitumor efficacy as these micelles change the PK properties of paclitaxel. In the form of micelles, PTX became more soluble, more permeable, more bioadherable, and resistant to liver

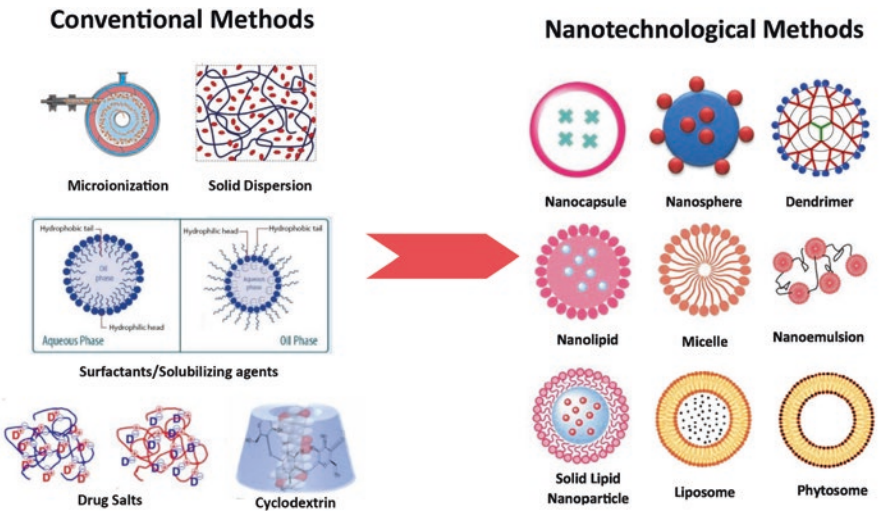


Fig. 2 Formulation interventions to overcome pharmacokinetic challenges

metabolism. These properties aided in increasing the bioavailability by around 3.8 times more than Taxol® (Yang et al. 2020). Curcumin loaded in chitosan/lignosulfonate micelles increased thermal and pH stability of curcumin, increased retention by 6.6 folds, and increased antioxidative potential (Lin et al. 2022) (Fig. 2).

6 Conclusion and Future Perspectives

Phytomedicines may be considered as the nature's gift to humans. Use of plant secondary metabolites in the form of pure compounds or extracts needs a targeted delivery system to ensure their safe and effective delivery to the site of action. Targeted delivery and bioavailability of medicine define its efficacy in vivo. Traditionally used natural product formulations face challenges related to solubility, permeability, metabolism, and bioavailability. Use of herbal nanomedicines can be an effective alternate to conventional approaches to increase therapeutic outcomes. Nanodrug carriers ensure drug bioavailability by overcoming pharmacokinetic challenges. The developments in the field of nanotechnology can, in future, pave the way to the development of phytonanotherapeutics that can be combined with a targeting molecule to ensure on-site delivery and reduced cytotoxicity. Furthermore, nanoencapsulation of drugs in natural polymers can avoid immune response. To translate these outcomes in clinical practice, further studies on the pharmacokinetic profiling of phytonanomedicines shall be carried out.

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Antioxidants in Oral Cavity Disorders

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Abstract Natural products can be used as therapeutic agents for several types of diseases, including those that affect the oral cavity. Among these products, antioxidants stand out, which are substances capable of delaying or neutralizing substrates generated from chemical and biochemical processes, in addition to modulating inflammatory and bacterial processes commonly present in oral diseases such as caries and periodontitis. Caries is the most common disease of the oral cavity, in which it develops from dental biofilm composed of different microorganisms adhered to the tooth surface. Periodontitis is a chronic inflammatory disease linked to the destruction of tooth support structures as a result of the accumulation of bacterial biofilm. Several studies demonstrate that grape seed extract, green tea, vitamin C, lycopene, and resveratrol have important antioxidant, antimicrobial, and anti-inflammatory properties capable of reducing the production of proinflammatory cytokines, thus preventing the appearance of these diseases or their development. The purpose of this chapter is to present the antioxidant effects of natural products on diseases of the oral cavity, caries and periodontitis.

Keywords Natural products · Antioxidants · Therapeutic · Oral cavity · Caries · Periodontitis · Green tea · Vitamin C · Lycopene · Resveratrol

1 Introduction

Natural products can be used as basic raw material for new therapeutic agents. In dentistry, one of the main research topics with these products is the prevention and therapy of oral diseases. Among these products, antioxidants can be mentioned. According to Kaur et al. (2016), antioxidant is any substance that is capable of

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significantly delaying or preventing the oxidation of a substrate as long as it is in concentrations lower than that of it. In other words, Yang et al. (2018) define antioxidants as molecules that can act to reduce and neutralize the products caused by a wide range of chemical and biochemical processes, which are called reactive oxygen species (ROS). When used alone or in combination effectively, antioxidants can provide natural protection against exposure to these free radicals or neutralize them in disease states. Several compounds biologically necessary for the body have important antioxidant properties, such as vitamins, minerals, enzymes, hormones, as well as food supplements and herbal medicines (San Miguel et al. 2011).

Some studies have tested in vitro each antioxidant separately, and some of these can modulate inflammatory, carcinogenic, and bacterial processes. Nonetheless, Yang et al. (2018) point out that the real effect of an antioxidant can only be evaluated taking into account some factors, such as its bioavailability, the concentration of the compound that can effectively reach a specific tissue, cytotoxicity, and whether this antioxidant can perform the same function obtained in vitro.

In this chapter, we will present the role of main antioxidants studied in two commons oral diseases: dental caries and periodontitis.

2 Antioxidants in Caries

Dental caries is one of the most common diseases that affect the oral cavity. Machiulskiene et al. (2020) define caries as "a biofilm-mediated, diet modulated, multifactorial, non-communicable, dynamic disease, that results in mineral loss of dental hard tissues. It is determined by biological, behavioral, psychosocial and environmental factors." Even today, dental caries remains a public health problem. So, preventing this disease remains one of the greatest challenges of dental practice. From this perspective, many products have been sought that can prevent it. To understand how antioxidants can act on caries, it is necessary to briefly understand how it develops.

The development of caries is associated with the formation of dental biofilm, a complex three-dimensional structure composed of different microorganisms adhered to the tooth surface and embedded in an extracellular polymeric matrix. The bacteria *Streptococcus mutans* (*S. mutans*) is identified as the primary etiologic agent, and it performs an important role in the formation of oral biofilm. *S. mutans* has some cariogenic properties, for example ability to adhere to solid surfaces, colonize the oral cavity, and survive the acidic condition of the oral cavity. Also, *S. mutans* can leads to acidic destruction and demineralization of the tooth enamel, and consequently, inducing dental caries by the carbohydrate producing acidic metabolites. *S. mutans* is capable of producing an extracellular enzyme responsible for the formation of glucan from sucrose from the diet, which is called glycosyltransferase B. The function of this synthesized glucan is to promote the adhesion of S. mutans to the tooth enamel, and even, the adhesion of other

microorganisms to each other, which causes increased protection against mechanical forces and various antimicrobial agents (Machiulskiene et al. 2020; Zayed et al. 2021).

Although dental caries has well-established prevention and treatment protocols over the last few decades and antioxidants are not actually able to treat it, the effects of these agents have shown promise in modulating both the prevention and progression of this disease. Below two antioxidants most commonly studies in the prevention and treatment of caries will be described.

2.1 Grape Seed Extract

Grape seed extract (GSE) is rich content of proanthocyanidins (PACs) that have hydrophobic and hydrophilic properties able to irreversibly attach to minerals, proteins, and carbohydrates (Delimont and Carlson 2020). As natural antioxidants, free-radical scavengers, and a bioflavonoid, PACs contains a benzene–pyran–phenolic acid molecular nucleus, that is named as flavin. Also, PACs are a mixture of monomeric flavanols, oligomers including the so-called oligomeric proanthocyanidins; and polymers, known as catechins, a scavenging free radical essential for calcium absorption, epicatechin, and epicatechin-3-O-gallate (Xie et al. 2008; Pavan et al. 2011; Zhao et al. 2014).

In general, GSE plays a role on caries in different ways. At first, it binds to carbohydrate substrates, which are indispensable for bacterial proliferation, and impairs biofilm formation on the tooth surface. PACs prevent enzymatic activity of glucosyltransferase, F-ATPase and amylase. Specifically, the inhibition of glucosyltransferase by PACS inhibits the formation of caries, whereas the glycosyltransferase polymerizes the glycosyl portion of sucrose and starch carbohydrates into glucans. This represents the sucrose-dependent pathway for *S. mutans* to attach to the tooth surface and is necessary for both the formation of dental plaque and the development of caries. In addition, adherent glucans contribute to the formation of dental plaque, and this acid accumulation results in localized decalcification of the enamel surface, as it facilitates bacterial adhesion to the teeth, among themselves and, finally, the accumulation of biofilms (Jawale et al. 2017; Delimont and Carlson 2020).

At second, GSE is grouped with collagenases and other enzymes responsible for the degradation of the dentin surface that occurs after enamel demineralization and performs the crosslinking of collagen-rich dentin surfaces, favoring strengthening and remineralization. In cases of artificial root caries lesions experimentally induced and treated with a minimally invasive approach, GSE inhibits the demineralization and/or promotes the remineralization under dynamic pH cycling conditions, and this remineralization effect appears to be distinct from that of fluoride treatment. Possibly, it can supports mineral deposition on the superficial layer of the lesion and, when they are mixed with the remineralizing solution at pH 7.4, they give rise to insoluble complexes, which can combine with the Ca^{2+} of the remineralizing

solution and increase remineralization. Furthermore, through the PA-collagen bond, GSE interconnects with the organic portion of root dentin, strengthening the exposed collagen matrix. This relationship between PA and matrix proteins appears to involve covalent, ionic, hydrogen bonding, or hydrophobic interactions. So, under the experimental circumstances, the remineralizing effect of GSE can be attributed to changes in the organic matrix, particularly the presence of newly formed collagen cross-links (Xie et al. 2008).

In addition to, in an experimental model of caries progression induced by pH cycling, it has been hypothesized that GSE maintains the integrity of the dentin organic matrix similar to that of the internal carious layer, increasing remineralization and decreasing demineralization. The formation of hydrogen bonds between the amine, carboxyl, and phenolic hydroxyl groups may be the main mechanism for interactions between PA and collagen. The formation of hydrogen bonding between amine, carboxyl, and the phenolic hydroxyl groups may be the primary mechanism for PA and collagen interactions. Moreover, GSE improves the mechanical properties and reduces the degradation rates of sound and caries-affected dentin, indicating the ability of this natural agent to bond with and alter dentin collagen. GSE may perform a role in decreasing collagen digestibility by inhibiting proteases, such as intrinsic metalloproteinases. The collagen network can function as a mechanical barrier to acid diffusion and mineral release, as well as facilitate mineral precipitation in the remineralization process (Pavan et al. 2011).

2.2 Green Tea

Green tea (GT) is a popular nonfermented product of the *Camellia sinensis* leaf consumed all over the world. Historically, it has been used as a natural medicine for oral diseases. GT has a unique composition that includes proteins, carbohydrates such as cellulose, pectin, glucose, fructose and sucrose, and lipid components; vitamins B, C, E; xanthic bases such as caffeine and theophylline; pigments such as chlorophyll and carotenoids; volatile components such as aldehydes and alcohols; minerals and trace elements such as Ca, Mg, Cr, Mn, Fe, Cu, Zn, Mo, Se, Na, P, Co, Sr, Ni, K, F, and Al. Moreover, GT is a rich source of polyphenols, which has a wide range of biological proprieties as antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic. The major polyphenols in GT are epicatechin, epigallocatechin, epigallocatechin-3-gallate (EGCG), and epicatechin-3-gallate. EGCG is the most abundant polyphenol in GT, accounting for 50% to 70% of total components (Wang and Ho 2009; Narotzki et al. 2012).

Some in vivo and in vitro studies have shown that GT consumption can decrease dental caries progression. There are several mechanisms to explain this action. One of these is based on a property of the EGCG, that is the strong inhibition it exerts on the activity of glycosyltransferase (GTF) in *S. mutans*, through reduction of the expression of three genes (gtfB, gtfC, and gtfD), which encode these enzymes. Glycosyltransferases are responsible for converting sucrose to glucan, the building

block of the biofilm-associated exopolysaccharide matrix. The activity of α-amylase was also inhibited by EGCG. Another explanation relies on the fact the EGCG is able to significantly suppress *S. mutans*-induced biofilm formation; suppress the sucrose-induced acid production of *S. mutans* and pH reductions. But what is the importance of these properties mentioned? With every pH unit decrease, the solubility of hydroxyapatite of dental enamel raises. So, EGCG given before sucrose administration protects tooth enamel, as it induces reduction in acid production and impaired the pH decrease by a mechanism inhibiting the enzyme lactate dehydrogenase that leads to the formation of lactic acid from pyruvate. Likewise, EGCG inhibits the uptake of glucose into bacterial cells and suppresses bacterial metabolism and bacterial growth. Finally, it is important to point out that GT has a greater effect in both gram-positive and gram-negative bacteria (Narotzki et al. 2012; Han et al. 2021; Zayed et al. 2021).

3 Antioxidants in Periodontitis

According Papapanou et al. (2018), periodontitis is "a chronic multifactorial inflammatory disease characterized by progressive destruction of the teeth-supporting apparatus. When left untreated, it can lead to tooth loss." Periodontitis is a public health problem of high prevalence and one of the most common chronic diseases of the oral cavity that is associated with the accumulation of bacterial plaque microorganisms, i.e., oral biofilm (Tóthová and Celec 2017; Castro et al. 2019).

Periodontitis can be influenced by many risk factors, for example, oral hygiene, alcohol, stress, smoking, genetic and epigenetic factor, systemic health, nutritional status of patient, diabetes, and hormonal alteration status. The main features of periodontitis that can be cited are gingival inflammation, clinical attachment loss, radiographic evidence of alveolar bone loss, presence of periodontal pocket, and gingival bleeding. All these factors impair the quality of life of patients, as they interfere with the function and aesthetics of patients (Tóthová and Celec 2017; Kwon et al. 2021).

The role of oxidative stress has been discussed through several hypotheses and would be related to the recruitment of predominantly polymorphonuclear leukocytes (PMLs) to the site of infection. These cells are responsible for releasing reactive oxygen species (ROS). According to Sczepanik et al. (2020), a complex interplay between the subgingival biofilm and the magnitude of the host immune response is essential to establishing the pathogenesis of periodontitis.

Dietary antioxidants have a defensive and protective effect on the periodontium since they can reverse the free radicals (FRs), ROS, and reactive nitrogen species. Antioxidants work in periodontal health by three mechanisms: reduce the production of cytokines, chemokines, and proinflammatory proteins by leukocytes; neutralize ROS, and therefore, both safeguard fibroblasts from toxic ROS-emitting substances and assist in reversing the effect of oxidative damage; and facilitates wound healing (Kaur et al. 2016).

Even though there is not enough scientific evidence to indicate the use of antioxidants as a single therapy in periodontal diseases, over the last years, several studies have investigated the role of these antioxidants in the health of the periodontium and as an adjuvant in the treatment of periodontal diseases. One systematic review (Né et al. 2020) evaluated the effect of nutritional intervention in periodontitis, having as a reference point the action of macronutrients and micronutrients as modulators of pro- and anti-inflammatory cascades. Other systematic review (Varela-López et al. 2018) investigated the different vitamin type, periodontal risks, and periodontal health improvement. In the aforementioned study, results were found for various types of vitamins, especially for the most studied, vitamin C. Finally, the results of another systematic review (Castro et al. 2019) suggest that antioxidants, especially lycopene and green tea, may work as good adjuvants in the nonsurgical therapy of periodontitis, acting in the modulation of oxidative stress and, therefore, favoring the maintenance of periodontal health.

Below, some of the main antioxidant agents most studied and cited in cases of periodontitis will be presented.

3.1 Vitamin C

One of the antioxidants that is often associated with periodontium is vitamin C, also known as L-ascorbic acid, a potent antioxidant radical that belongs to the scavenging group of antioxidants. It also scavenges free radicals and possesses antioxidant and immune-modulatory properties, which can control excessive ROS produced. Vitamin C supports the bactericidal activities of PMLs and macrophages and also raises the synthesis of nitric oxide. The supplementation of this vitamin can mitigate the production of proinflammatory cytokines in infected periodontal tissue, and therefore, attenuate gingival oxidative stress. In general, patients with periodontal disease ingest little Vitamin C and therefore have low blood levels of this vitamin (Tada and Miura 2019; Aytekin et al. 2020; Fageeh et al. 2021).

Finally, vitamin C can contribute to reducing the risk, preventing and slowing down the rate of progression of periodontal disease. This last property seems to be associated with the fact that, in vitro, vitamin C inducing, even in the absence of other osteogenic agents, the osteogenic differentiation, and maturation of periodontal ligament (PDL) progenitor cell (Yan et al. 2013).

3.2 Lycopene

Another antioxidant commonly used as an adjuvant approach to nonsurgical periodontal therapy is lycopene, a most efficient biological hydrocarbon carotenoid and one of the primary effective natural antioxidants in the diet present. It is a free radical scavenger that exhibit highest physical quenching rate with singlet oxygen and

is able to reverses the DNA damage promoted by hydrogen peroxide. According to Di Mascio et al. (1989), lycopene is "two times more effective than β-carotene, 100 times more potent than α-tocopherol and 47 times stronger than vitamin E" (Tripathi et al. 2019).

As suggested by Castro et al. (2019), the main biological effects of lycopene are related to its antioxidant and nonoxidizing actions, such as anti-inflammatory and cell signaling activities. The first, that is, antioxidant activity, is associated with binding to ROS through three different mechanisms, namely, transfer of electrons and hydrogen atoms or formation of adducts. It is also associated with the scavenging of other free radicals, which causes a reduction in intracellular and extracellular ROS levels, less MDA formation in plasma and tissue, an increase in glutathione (GSH) levels and in the hepatic activities of GSH-Px, SOD, and CAT. Finally, it is important to highlight that it prevents NF-κB activation, DNA fragmentation, caspase-3 activation, and cytochrome c release; activates the factor 2 (Nrf2)/HO-1 pathway related to NF-E2 p45; and activates kinases that release and translocate Nrf2 to the nucleus.

3.3 Green Tea (GT)

GT has antibacterial and antifilm properties; besides, it has antioxidant properties and can act to reduce the risk or control gingival inflammation. Melo et al. (2021) highlight that for the treatment of periodontitis, sachets for infusion, HPC (hydroxypropyl cellulose) strips, gel, and toothpastes can be used.

As mentioned before, the main active ingredients of GT are polyphenols, most of which are catechins (flavan-3-ols) with greater antioxidant activity than vitamins C and E. But how polyphenols and catechins can exert their antioxidant function? The first, i.e., polyphenols, through enzymes with antioxidant action, such as glutathione S-transferase and superoxide dismutase. Thus, they can not only inhibit growth, cell adhesion, and virulence factors by periodontal pathogens, but also restore alveolar bone. In turn, catechins can act by binding to iron and copper ions; preventing the activation of redox-sensitive transcription factors, suppressing nitric oxide synthase, cyclooxygenase 2 (COX-2), lipoxygenase 2 (LOX-2), and xanthine oxidase; and inhibiting periodontal pathogens and preventing periodontal tissue destruction. It is worth noting that GT also contains other antioxidants, such as carotenoids, ascorbate, and tocopherols (Kaur et al. 2016; Tripathi et al. 2019; Gartenmann et al. 2019).

3.4 Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) is a pleiotropic molecule, antifungal plant-derived substance, polyphenol not flavonoid stilbene, that is found in red wine, peanuts, fruits like apples, vegetables, and berries. It has various biological effects, such

as antioxidant, anti-inflammatory, antiaging, anticancer, antimicrobial, cardioprotective, and neuroprotective properties. It was described as a scavenger of superoxide radicals, hydroxyl radicals, and peroxynitrite (Corrêa et al. 2018; Andrade et al. 2019; Kugaji et al. 2019).

A systematic review (Andrade et al. 2019) investigated the effects of resveratrol administration on periodontal disease control in preclinical studies and concluded that resveratrol treatment can prevent periodontitis progression, possibly by modulation of oxidative stress and inflammatory profile.

Also, Resveratrol can prevent biofilm formation and suppress the expression of virulence factors from *Porphyromonas gingivalis* (*P. gingivalis*), the main one involved in periodontal disease. But how specifically can Resveratrol inhibit biofilm formation? It can inhibit biofilm formation through some mechanisms: by blocking the expression of the Fimbriae gene and, therefore, bacterial adhesion and colonization; by its antimicrobial activity, with bacteriostatic and bactericidal effects; and by inhibiting the expression of the gingipain gene, which prevents connective tissue destruction and alveolar bone loss (Kugaji et al. 2019).

Resveratrol plays an important antimicrobial role against periodontal pathogens by acting directly on oxidative pathways important for the decrease of local oxidative stress, such as the reduction of reactive oxygen species (ROS) and the increase of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which have important enzymatic activity by metabolizing ROS in the region where periodontal disease is installed, thus promoting acceleration in the healing process (Ballini et al. 2019; Tsang et al. 2016).

The response of the organism, on the surface of osteoblasts, against periodontal pathogens, promotes the increase of proinflammatory cytokines that promote changes in the expression of activating receptor kappa B-factor ligand (RANKL). RANKL responds for activating osteoclasts by promoting interaction with the activating receptor of nuclear factor kappa B (RANK), thus resulting in the initiation of bone resorption present in periodontitis. When the concentration of ROS is at high levels, the process of signal transduction begins within the cell, thus resulting in autophagy. This process plays a dual role in periodontitis as it promotes cell death or blocks apoptosis in infected cells. Furthermore, ROS can influence the activation of nuclear signaling factor-κB (NF-κB), causing the elevation of proinflammatory cytokines, thus stimulating osteoclast differentiation (Cochran 2008; Liu et al. 2017).

4 Conclusion

The use of natural products such as antioxidants is already a reality in dentistry. Several studies prove its effectiveness and its role in the maintenance of oral health. Meantime, it is important to point out that some results from the use of them come from in vitro experiments and that their use in patients still requires the performance of randomized clinical studies. Further studies are necessary to establish some parameters for the prescription and large-scale use of it, such as the best way of

administration of the antioxidants (diet, infusions, gels, varnishes, toothpastes, mouthwash, gum, lozenges, and oral sprays); safe dose; and the time and the frequency of application/use of the product. In addition, it is possible that the combination of antioxidants is more effective and promotes greater protective and therapeutic effects against the damage caused by free radicals to oral diseases than the use of antioxidants individually.

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Cotula cinerea as a Source of Natural Products with Potential Biological Activities

Fatima-Ezzahrae Guaouguaou and Nour Eddine Es-Safi

Abstract *Cotula cinerea* of the Asteraceae family is a traditional plant which grows in desert area. The plant is endowed with various biological activities due to the presence of several secondary metabolites. Owing to its use in traditional folk medicine and its interesting biological effects, the plant has been subjected to many scientific explorations resulting in the publication of a multitude of papers in a wide range of scientific fields including phytochemistry, biological activities, and toxicology. The objective of this chapter is to report and gather previous studies on *Cotula cinerea* regarding its botanical description, geographic distribution, bioactive compounds, toxicology, and in vivo and in vitro biological properties. The phytochemical analysis was carried out by several spectroscopic methods, and the obtained results showed the richness of this plant in several phytochemicals including phenolic compounds, volatile compounds, sesquiterpene lactones, and others. Studies on *Cotula cinerea* showed the harmlessness of this plant since its tested extracts were not toxic even at higher doses. The evaluation of the pharmacological activities of the essential oil and the extracts of *Cotula cinerea* have shown that the plant has significant antibacterial, antifungal, antioxidant, anticancer, analgesic, anti-inflammatory, and antipyretic effects. This review showed that even if a number of publications have been reported on the plant, research on *Cotula cinerea* remains an open research area of good interest. It is hoped that the information presented here will be beneficial and useful for further studies that will eventually lead to the development of therapeutic agents from this plant.

Keywords *Cotula cinerea* · Traditional use · Phytochemistry · Toxicity · Pharmacological properties

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1 Introduction

For several years, plants have played a major role in the art of healing throughout the world. The use of medicinal plants or herbal preparations is increasingly popular. Thus, according to estimates, 80% of the world's population depends mainly on traditional medicine (WHO 2012). The use of traditional practices based on medicinal plants is explained by several reasons such as the high cost of pharmaceutical products, the sociocultural habits of the populations, the need to have therapeutic options for resistant pathogens, and the existence of diseases for which there is no effective treatment (Duke et al. 1993; Cox and Balik 1994).

Medicinal plants are extremely numerous. Indeed, estimates indicate that more than 13,000 species of medicinal plants are used as traditional remedies by various cultures around the world (Tyler 1994). Plants used in traditional medicine contain a wide range of chemical substances and compounds, such as phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins, etc.), nitrogen compounds (alkaloids, amines, etc.), vitamins, terpenoids, and certain other endogenous metabolites. Polyphenols are known for their significant antioxidant activities, as they can act by direct scavenging of ROS (reactive oxygen species) (Halliwell and Cross 1994). They are also present as ingredients in several cosmetic preparations used in the treatment of cellular aging and skin protection (Menaa et al. 2014). Thus, the chemical composition of plants can be used to treat chronic and infectious diseases.

According to the World Health Organization (WHO), approximately 80% of the world's population still uses herbal medicines to treat several diseases (World Health Organization 2008).

In order to contribute to the valorization of medicinal plants, we have chosen to establish this review on the *Cotula cinerea* plant which is distributed in the desert regions especially in North Africa.

Cotula cinerea belongs to the Asteraceae family and is widely distributed in sandy and desert soils (Ahmed et al. 1987). It is known as "Gartoufa" and is used in traditional Moroccan medicine used to treat colic, cough, diarrhea, migraine, headaches, and digestive disorders (Bellakhdar 1997).

Moreover, several studies on *Cotula cinerea* have been based not only on the species itself but also on several variations which make the difference of the biological effects namely the geographical origin, the climate, the parts of the plant used, the extraction solvent and the harvest period.

Previous work done on this plant has scientifically proven several biological properties and effects (Fig. 1), such as antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, analgesic, and antipyretic activities.

The study of essential oil and extracts from different parts of *Cotula cinerea* showed the presence of several chemical classes, including phenolic acids, flavonoids, and terpenoids (Mekhadmi et al. 2023; Chlif et al. 2022; Guaouguaou et al. 2020b).

On the other hand, studies on the chemical composition of *Cotula cinerea* have also been analyzed to suggest this species as a new source of medicines, thus justifying its traditional uses.

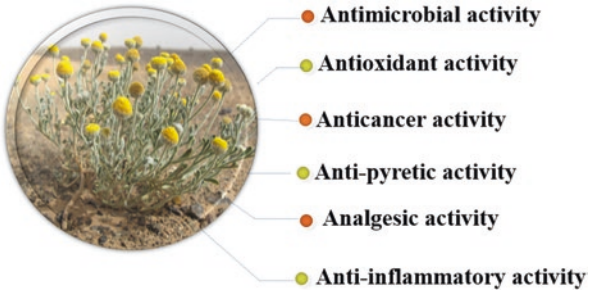


Fig. 1 Pharmacological properties of *Cotula cinerea*

In this review, we tried to find the link between the ethnomedicinal use, the pharmacological properties tested, and the chemical composition which could be the origin of its biological properties.

2 Research Methodology

The literary synthesis on *Cotula cinerea* botanical description, traditional medicinal application, chemical composition, biological activities, and toxicity of *Cotula cinerea* extracts have been collected, analyzed, and summarized in this review.

Scientific search engines such as PubMed, Science Direct, Springer Link, Web of Science, Scopus, Wiley Online, and Google Scholar were used to collect all published papers on this species.

In this work, many key words and scientific terms have been used such as *Cotula cinerea*, essential oil, extracts, chemical composition of *Cotula cinerea*, acute toxicity of *Cotula cinerea*, analgesic effect of *Cotula cinerea*, antioxidant effect of *Cotula cinerea*, cytotoxic activity of *Cotula cinerea*, antimicrobial activity of *Cotula cinerea*, antipyretic activity of *Cotula cinerea*, and anti-inflammatory activity of *Cotula cinerea*. All published papers containing the name *Cotula cinerea* have been cited in this review. To identify other relevant articles, reference lists of retrieved articles were also searched. All data has been discussed in the text and organized in tables to summarize.

3 Results and Discussion

3.1 Botanical Description

The *Cotula cinerea* is a Saharo-Arabic species common throughout the Sahara, in somewhat sandy soils, being very aromatic is used to flavor tea. It is a woolly-looking annual plant with prostrate stems and golden-yellow flowers (Fig. 2). Its stems are 10 to 40 cm in diameter, laid down, and then straightened (Ozenda 1993;

Fig. 2 General morphology of *Cotula cinerea* (Ozenda 1967)

Benhouhou 2005). The leaves are woolly, whitish, and thick, and the upper parts are divided into three to five obtuse. Concerning the flowers, they are flower heads 6 to 10 mm in diameter, woolly involucre, tubular, brown in button then golden yellow when they open (Ozenda 1993; Benhouhou 2005). *Brocchia cinerea* is the synonym of *Cotula cinerea* and has several vernacular names such as Chihia, Chouihia, Robita, and Al gartoufa (Quezel and Santa 1962; Dupont and Guignard 2004).

3.2 Geographic Distribution

The *Cotula cinerea* is a xerophytic plant that grows in desert conditions and requires an average annual rainfall of 100 mm. The *Cotula cinerea* species is widely encountered throughout the Sahara (Djellouli et al. 2013). It grows in ergs and little sandy soils. Geographically, it is widely distributed in North Africa, particularly in the Saharan regions of Morocco, Algeria and Egypt (Ahmed et al. 1987; Boulos 1983; Ozenda 1993; Markouk et al. 1999a, b).

3.3 Ethnobotanical Use

The ethnopharmacological studies have shown that *Cotula cinerea* is widely used to treat colic, cough, diarrhea, migraine, headaches, and digestive disorders (Bellakhdar 1997). In traditional medical practice, it is used as an antiseptic, antipyretic, analgesic, anti-inflammatory, and antibacterial agent, as well as for the treatment of rheumatism (Beloued 2005; Hammiche and Maiza 2006).

The use of *Cotula cinerea* in traditional medicine is generally administered in the form of decoction, maceration, infusion and inhalation (Bellakhdar 1997; Djellouli et al. 2013).

3.4 Phytochemistry

In view of its use in traditional folk medicine and its interesting biological activities, *Cotula cinerea* has undergone several phytochemical investigations. These resulted in the identification and/or the isolation of a huge number of natural products and a wide variety of bioactive secondary metabolites pertaining to different families such as essential oil terpenoids, phenolic compounds, saponins, germacranolides, and other phytochemical compounds (Lakhdar 2018). Among these phytochemical families, the main terpenoids, phenolics, and other phytochemicals previously identified and reported in *Cotula cinerea* are gathered and described below.

3.4.1 Essential Oil Terpenoids

Cotula cinerea essential oils have been subjected to several research studies. The species is widely known as odoriferous and aromatic plant used in the south of Morocco to flavor hot beverages such as tea. The odoriferous and aromatic properties of the plant are due to the presence of essential oil with various volatiles and aromatic compounds. Essential oils of the aerial parts of the plant from different geographical regions in Algeria, Egypt and Morocco have been extracted through hydrodistillation and subjected to qualitative and quantitative analysis through GC-MS techniques. The main detected compounds have been gathered in Table 1 and the structures of some of these compounds are presented in Fig. 3.

Examination of the reported data showed that the obtained essential oils varied both qualitatively and quantitatively according to the corresponding country and even from region to region within the same country. Such variations were thus observed for samples from Algeria (Mekhadmi et al. 2023; Bouziane et al. 2013; Atef et al. 2015; Djellouli et al. 2015), Egypt (Fournier et al. 1989; Fathy et al. 2017), and Morocco (Boussoula et al. 2016; El Bouzidi et al. 2011; Guaouguaou et al. 2020a, b; Chlif et al. 2021; Hamdouch et al. 2022). It should be indicated that this variation could be due to several factors, such as the harvest site and the vegetation stage of the plant of the plant in addition to other exogenous conditions including climate, soil composition, harvesting time and extraction method. It should also be noted that the differences in the phytochemical composition of plants extracts are not specific only to essential oil but usually observed both qualitatively and quantitatively for the major if not any phytochemical metabolite. However, and even if the investigated samples are from different geographical regions, some commonalities could be observed in addition to some disparities in the phytochemical composition of the investigated *Cotula cinerea* essential oils. Thus, on the 14 investigated

Table 1 Major terpenoid compounds detected in various *Cotula cinerea* essential oils

Origin	Used part	Yield (%)	Major compounds (%)	References
Algeria (Sidi Aoun)	Dried aerial parts	0.30	Kessane (34.30), *trans*-chrysanthenyl acetate (20.5), *cis*-chrysanthenol (6.08), terpinene (5.80), liguloxide (5.51)	Mekhadmi et al. (2023)
Algeria (Beni Guecha)	Dried aerial parts	0.58	*trans* thujone (50.10), 1.8-cineole (8.74), sabinene (6.14), terpinen-4-ol (5.84), camphor (4.90), santolinatriene (4.00)	Mekhadmi et al. (2023)
Algeria (Ouargla)	Aerial parts (flowering period)	0.75	Thujone (47.72), camphor (10.54), santolinatriene (8.00), eucalyptol (6.37), lyratyl cetate (4.17), terpinen-4-ol (2.77)	Bouziane et al. (2013)
Algeria (Oued Souf)	Aerial parts (flowering period)	0.080	3-Carene (30.99), thujone (21.73), santolinatriene (18.58), camphor (6.21), eucalyptol (2.79)	Atef et al. (2015)
Algeria (Oued Souf)	Aerial parts (fruiting period)	0.391	Thujone (28.78), 3-carene (15.90), eucalyptol (15.13), santolinatriene (13.38), camphor (7.49), m-cymene (3.34)	Atef et al. (2015)
Algeria (Bechar)	Aerial parts	0.282	(E)-Citral (24.01), *cis*- limonene epoxide (18.26), thymol methylether (15.04), carvacrol (15.03), *trans*-carveol (13.79), carvone (3.06), *trans*- piperitol (2.54).	Djellouli et al. (2015)
Algeria (Hassi Khalifa)	Aerial parts	0.74	*trans*-Thujone (51.86), santalinatriene (10.6), α-pinene (2.02), sabinene (6.17), cineole (5.34), δ-terpinene (1.57), camphor (2.63%)	Larbi et al. (2018)
Algeria (Southwestern)	Aerial parts	–	α-thujone (32.35)	Ghouti et al. (2018a, b)
Egypt (Cairo)	Fresh aerial parts	0.30	Camphor (50), thujone (15)	Fournier et al. (1989)
Egypt	Aerial parts	–	Camphor (65.5), thujone (15.59), 4-terpineol (5.33), camphene (4.76)	Fathy et al. (2017)
Morocco (Smara)	Aerial parts	0.64	Iso-3-thujanol (47.38), santolinatriene (11.67), camphor (10.95), santolina alcohol (7.68), borneol (5.49), neo-iso-3-thujanol (3.74), ß-Pinene (2.98)	Boussoula et al. (2016)
Morocco (Zagora)	Aerial parts	0.87	*trans*-Thujone (41.4), *cis*-verbenyl acetate (24.7), 1,8-cineole (8.2), santolinatriene (7.2), camphor (5.5)	El Bouzidi et al. (2011)
Morocco (Dakhla)	Dried aerial parts	0.92	Thujone (42.12), eucalyptol (12.59), santolinatriene (11.57)	Guaouguaou et al. (2020a, b)

(continued)

Table 1 (continued)

Origin	Used part	Yield (%)	Major compounds (%)	References
Morocco (Tata)	Dried Aerial parts	0.66	Thujone (40.83), camphor (16.58), eucalyptol (10.99), santolinatriene (9.93), 3-caren-4-ol acetoacetate (6.91)	Hamdouch et al. (2022)
Morocco (Al Nif)	Fresh Aerial parts (flowering period)	0.31	Thujone (26.05), *cis* chrysanthenyl formate (15.64), 2-bornanone (15.40), santolinatriene (10.68), 1,8-cineol (8.48), α-phellandrene (3.79)	Chlif et al. (2021)
Morocco (Al Nif)	Dried aerial parts (flowering period)	0.55	Thujone (22.37), santolinatriene (16.45), 1,8-cineol (12.19), *cis* chrysanthenyl formate (12.03), 2-bornanone (11.56), α-Phellandrene (5.02)	Chlif et al. (2021)
Morocco (Akka)	Predried aerial parts	0.39	Thujone (24.9), lyratyl acetate (24.32), camphor (13.55), 1,8-cineole (10.81)	Agour et al. (2022)
Morocco (Zagora)	Aerial parts	0.83	*trans*-thujone (41.4), *cis* verbenyl acetate (24.7), 1,8-cineole (8.2)	Kasrati et al. (2015)

samples, the compound thujone was indicated among the major detected and quantified compounds in 11 samples with percentages up to 50% of the total quantified compounds. Thus, thujone was among the major compounds for *Cotula cinerea* samples from Algeria (Mekhadmi et al. 2023; Ghouti et al. 2018a, b; Bouziane et al. 2013; Atef et al. 2015), from Egypt (Fournier et al. 1989; Fathy et al. 2017; Larbi et al. 2018), and from Morocco (El Bouzidi et al. 2011; Kasrati et al. 2015; Chlif et al. 2021; Hamdouch et al. 2022; Agour et al. 2022). This could also be noted for santolinatriene which was detected among the most abundant compounds in *Cotula cinerea* essential oil from Algeria with percentages ranging from 4% to 18.58% (Mekhadmi et al. 2023; Atef et al. 2015; Bouziane et al. 2013). This was also observed for essential oil samples from Morocco with relative abundance ranging from 7.2% to 16.45% (Boussoula et al. 2016; El Bouzidi et al. 2011; Guaouguaou et al. 2020a, b; Hamdouch et al. 2022; Chlif et al. 2021). This compound was reported among the major compounds in all the Moroccan explored *Cotula cinerea* essential oils. Finally, santolinatriene was reported with a weak percentage or not detected in samples from Algeria (Mekhadmi et al. 2023; Djellouli et al. 2015) and Egypt (Fournier et al. 1989; Fathy et al. 2017). In addition to thujone and santolinatriene, camphor was also detected with a relatively high abundance in the major investigated samples with percentages varying from 4.90% to 65.5%. These higher percentages (50% and 65.5%) were observed for two samples from Egypt (Fournier et al. 1989; Fathy et al. 2017).

Besides these samples which phytochemical compositions were relatively homogenous on the qualitative level with thujone, santolinatriene, camphor as

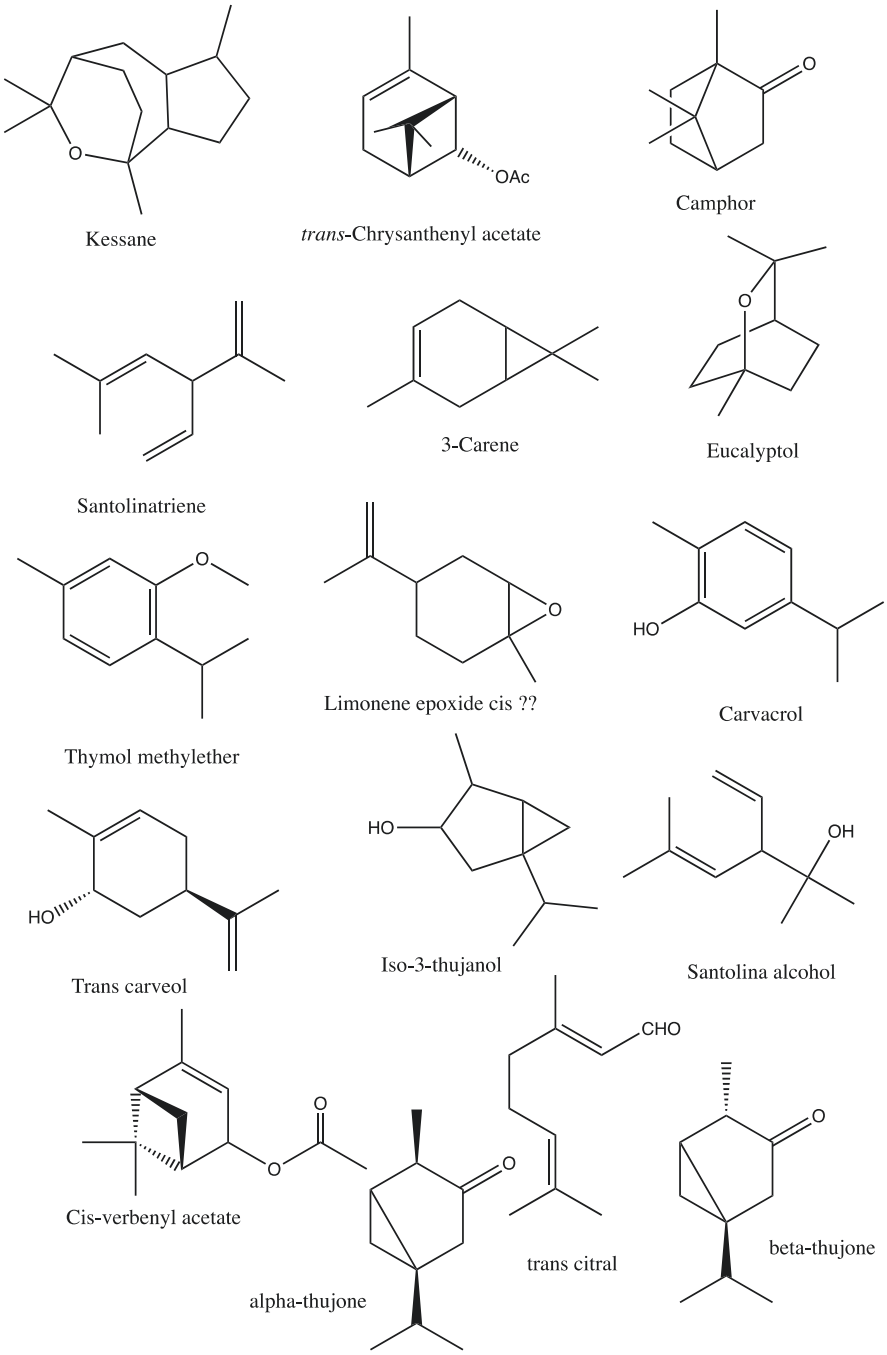


Fig. 3 Structures of some essential oil terpenoids from *Cotula cinerea*

major compounds and for which differences were observed on the quantitative aspect of these compounds, analysis of other *Cotula cinerea* samples showed a peculiar phytochemical composition. This was the case for the essential oil sample from Algeria for which kassane (34.3%) was indicated as major compounds (Mekhadmi et al. 2023). This was also the case for 3-carene which was present as major phytochemicals (30.99%) for the *Cotula cinerea* essential oil from Eastern Algeria (Atef et al. 2015). This especial fact appeared also for trans citral (24.01%) and iso-3-thujanol (47.38%) detected as major compounds in samples from Algeria (Djellouli et al. 2015) and Morocco (Boussoula et al. 2016) respectively.

In addition to the volatile compounds constituting the essential oils of *Cotula cinerea*, its nonvolatile secondary metabolites have also been the subject of several scientific investigations. The different compounds have been separated through various chromatographic techniques including high performance liquid chromatography or column chromatography and have been characterized through hyphenated coupled compounds such as UHPLC-MS and MS^n techniques with the use of powerful high-resolution MS detectors with time of flight analyzers. Some compounds have also been characterized after isolation through one-dimensional (1D) and two-dimensional (2D) homonuclear and heteronuclear NMR spectroscopy techniques. Several identified compounds pertaining to various families such as phenolic acids, flavonoids, and germacranolides are discussed below.

3.4.2 Phenolic Acids

Cotula cinerea was reported to contain some phenolic acids. The phytochemical analysis of the plant summarized in Table 2 showed the presence of various phenolic compounds pertaining to phenolic acids. The structures of some of these derivatives previously reported in the plant are presented in Fig. 4. Among these, six mono- (chlorogenic acid and its isomers) and di- (3,4; 3,5; and 4,5) caffeoylquinic acid derivatives with different substitution site have been reported in *Cotula cinerea* from Morocco (Khallouki et al. 2015). Similar derivatives have also reported in a sample from Algeria with one mono and two dicaffeoylquinic acid adducts (Ghouti et al. 2018a, b). Another sample of the plant from Dakhla (Morocco) was also found to contain phenolic acids derivatives including caffeic acid, coumaric acids, and some other derivatives (Guaouguaou et al. 2020b).

3.4.3 Flavonoids

A phytochemical investigation on the flavonoids of *Cotula cinerea* from Egypt was carried out since the seventies of the last century where free quercetin and kaempferol in addition to quercetrin and kaempferitrin have been identified (Mahran et al. 1976) as indicated in Table 2.

The phytochemical investigations of various *Cotula cinerea* extracts revealed the presence of several flavonoids. The content of flavonoids reported in the plant was

Table 2 Major compounds detected in *Cotula cinerea* extracts

Origin	Plant part	Extraction solvent	Compounds	References
Algeria Bechar	Aerial parts	EtOH/H_2O Infusion	Phenolic acids: 5-O-caffeoylquinic acid, 4,5-O-dicaffeoylquinic acid, 3,5-O-dicaffeoylquinic acid Flavonoids: luteolin-7-O-glucoside, luteolin-O-malonylhexoside, luteolin-dihexoside, quercetin-O-hexoside, luteolin-O-pentosyl-hexoside, quercetin-O-malonylhexoside, luteolin-O-malonylhexoside	Ghouti et al. (2018a, b)
Southeastern Algeria	Aerial parts (flowering stage)	EtOH/H_2O	Flavonoids: chrysospenol D, chrysosplenetin, oxyayanin-B, axillarin, 3-methylquercetin, pedaletin, isokaempferid, apigenin, luteolin, 6-hydroxyluteolin, 3-glucosylisorhamnetin, 3-methyl-7-glucosylquercetin, 7-O-β-D-glucosylapigenin, 7-O-β-D-glucosylluteolin, 7-O-β-D-glucosyl-quercetin, 7-O-β-D-glucosylaxillarin 7-O-β-D-diglucosylluteolin Germacranolides: 1α,6α-dihydroxygermacra-4E,9Z,11(13)-trien-12,8 α-olide	Dendougui et al. (2012)
Southern Algeria	Aerial parts	CH_2Cl_2	Guaiantrienolides:, 6-acetoxy-1β-hydroxyguaiantrienolide, 6-acetoxy-1α-hydroxyguaiantrienolide, 6-acetoxy-10-β-hydroxyguaiantrienolide Germacrenolides: haagenolide, 1,10-epoxyhaagenolide	Cimmino et al. (2021)
Egypt	Roots		Flavonoids: kaempferitin, quercetrin, quercetin, and kaempferol	Mahran et al. (1976)
Egypt	Aerial parts	MeOH/H_2O	Flavonoids: luteolin, luteolin 7-O-β-D-glucoside, luteolin 7-O-β-D-diglucoside, luteolin 6-hydroxy-7-O-β--D-glucoside, apigenin, apigenin 7-O-L-rhamnoside, apigenin 6-C-arabinosyl-8-C-glucoside, isochaftoside, quercetin 3-O-β-D-glucoside, quercetin 3-O-β-D-galactoside, quercetin 7–3-O-β-D-glucoside, 5,3′,4′-trihydroxy 3,6,7-trimethoxyflavone	Ahmed et al. (1987)

(continued)

Table 2 (continued)

Origin	Plant part	Extraction solvent	Compounds	References
Red Sea Region Egypt	Aerial parts	MeOH-Petrol-Ether	Spiroketal enol-ether, sesquiterpene lactones, germacranolides, guaianolide	Metwally et al. (1985)
Egypt	Roots	Ether-Petrol, Ether	Isofraxidin derivatives: farnochrol, drimartol A, acetyldrimartol A, acetyldrimartol B, pectachol, pectachol B, acetylpectachol B Scopoletin derivatives: scopofarnol, farnesylscopoletin	Greger and Hofer (1985)
Eastern desert Egypt	Aerial parts	Ether-Petrole-MeOH	Spiroketal enolethers, lactones, germacranolides, eudesmanolides, guaianolides, glaucolides	Jakupovic et al. (1988)
Errachidia Morocco	Aerial parts (flowering stage)	MeOH	Phenolic acids: neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid Flavonoids: luteolin-4'-O-glucoside	Khallouki et al. (2015)
Dakhla Morocco	Aerial parts	Ethanol/Water	Phenolic acids: caffeic acid, coumaric acid, caffeic acid derivative, coumaric acid derivative, HCA derivative Flavonoids: chrysospenol D, chrysosplenetin, oxyayanin B, axillarin, 3-methyl quercetin, quercetin 3-O-glucoside, axillarin 7-O-glucoside, chrysospenol isomer, kaempferol, kaempferol 3,7-O-Me, luteolin, luteolin 7-O-glucoside, hydroxyluteolin, pedalitin, dimethoxy hydroxyluteolin, trihydroxy-trimethoxyflavone isomer, apigenin, apigenin derivative Sulfated flavonoids: kaempferol 3-sulfate 7-O-glucoside, kaempferol 3-O-sulfate, luteolin 7-O-sulfate, apigenin 7-O-sulfate Terpenoids: Tatridin derivative, dehydrotatridin derivative dehydrotatridin derivative	Guaouguaou et al. (2020b)

higher either qualitatively or quantitatively than that of phenolic acids. The major detected flavonoids are presented in Table 2 and the structures of some of them are presented in Fig. 5.

In 1987, flavones and flavonols derivatives have been reported in an hydroethanolic extract of *Cotula cinerea* from Egypt (Ahmed et al. 1987). Free and glycosylated adducts with both C- and O-glycosylated adducts have been reported in this study. Among the flavones, free luteolin and apigenin in addition to their glycosides

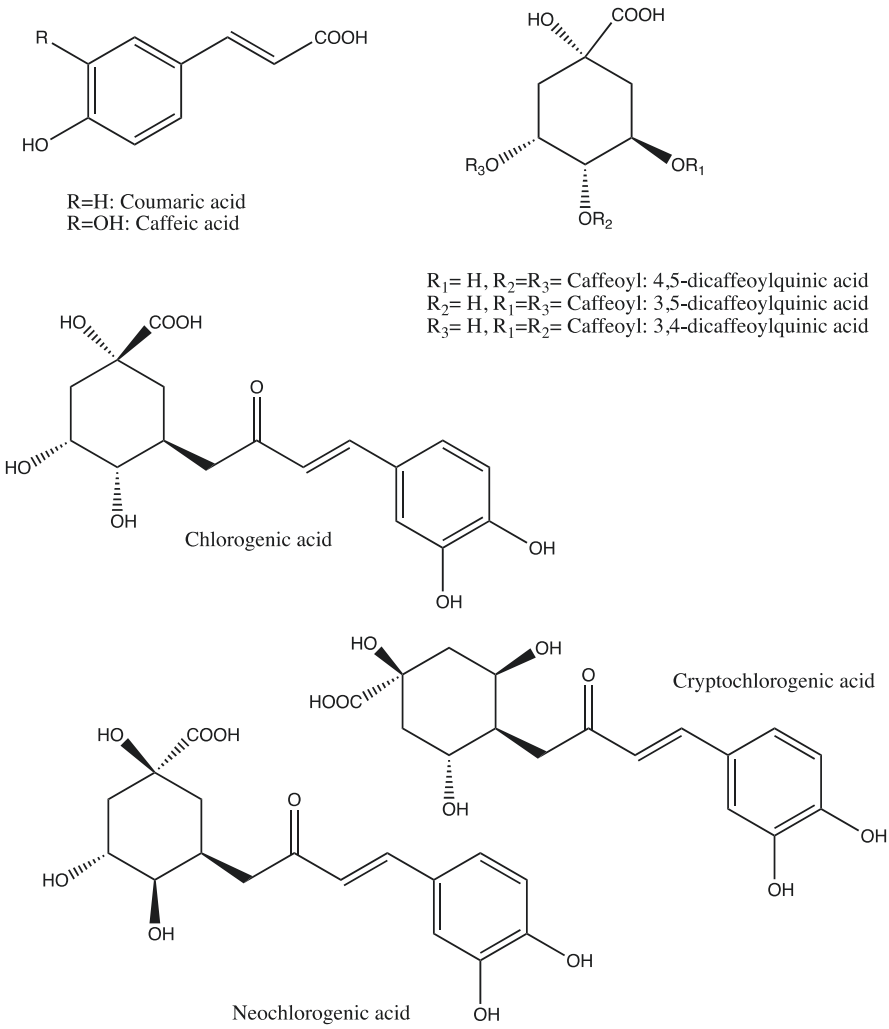
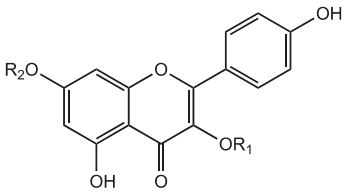
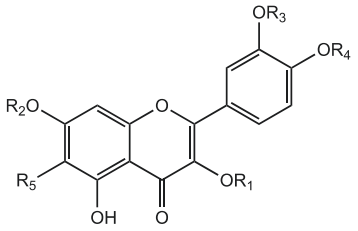


Fig. 4 Structures of some phenolic acids of *Cotula cinerea*

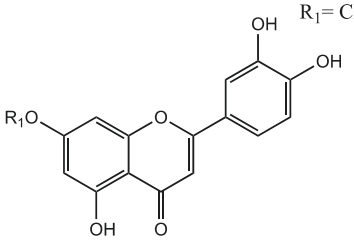
have been reported while glycosides' adducts of quercetin have been identified within the flavonols subgroup. In another sample from Algeria seventeen flavonoids have been isolated and structurally elucidated (Dendougui et al. 2012). Among the identified compounds in this study, free apigenin and luteolin have been evidenced. Additionally, several methoxylated, mono-, and diglycosylated derivatives of luteolin, apigenin, and quercetin have also been reported. Glycosides of luteolin and quercetin with hexoses and pentoses moieties have also been found in another sample from Algeria (Ghouti et al. 2018a, b). Some phytochemicals of this sample were acylated with malonyl derivatives of luteolin and quercetin hexosides.



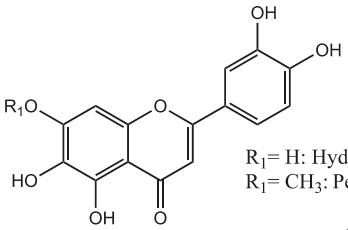
R_1=R_2= CH_3: Kaempferol 3,7-*O*-Me
R_1=R_2= H: Kaempferol



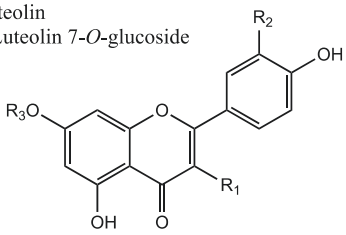
R_1=R_2= CH_3, R_3=R_4= H, R_5= OCH_3: Chrysospenol D
R_1=R_2=R_3= CH_3, R_4= H, R_5= OCH_3: Chrysosplenetin
R_1=R_2=R_4= CH_3, R_3= H, R_5= OH: Oxyayanin B
R_1= CH_3, R_2=R_3=R_4= H, R_5= OCH_3: Axillarin
R_1= CH_3, R_2=R_3=R_4=R_5= H: 3-Methyl quercetin
R_1= Glu, R_2=R_3=R_4=R_5= H: Quercetin 3-*O*-Glucoside
R_1= CH_3, R_2= Glu, R_3=R_4= H, R_5= OCH_3: Axillarin 7-*O*-glucoside



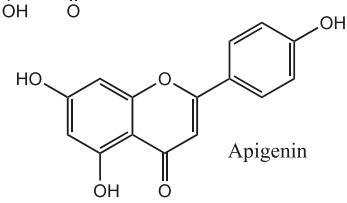
R_1= H: Luteolin
R_1= Glu: Luteolin 7-*O*-glucoside



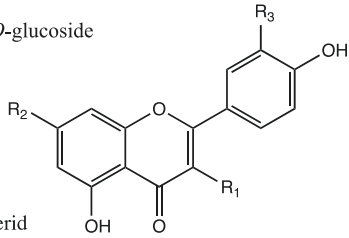
R_1= H: Hydroxyluteolin
R_1= CH_3: Pedalitin



R_1= OSO_3H, R_2= H, R_3= Glu: Kaempferol 3-sulfate 7-*O*-glucoside
R_1= OSO_3H, R_2=R_3= H: Kaempferol 3-*O*-sulfate
R_1= H, R_2=OH, R_3= SO_3H: Luteolin 7-*O*-sulfate
R_1=R_2=H, R_3= SO_3H: Apigenin 7-*O*-sulfate



Apigenin



R_1= OCH_3, R_2= OH, R_3= H: Isokaempferid
R_1= OGlu, R_2= OH, R_3= OCH_3: 3-glucosylisorhamnetin
R_1= OCH_3, R_2= OGlu, R_3= OH: 3-methyl-7-glucosylquercetin
R_1= H, R_2= OGlu, R_3= H: 7-O-beta-D-glucosylapigenin
R_1= OH, R_2= OGlu, R_3= OH: 7-O-beta-D-glucosylapigenin
R_1= H, R_2= OdiGlu, R_3= OH: 7-O-beta-D-diglucosylluteolin

Fig. 5 Structures of some flavonoids of *Cotula cinerea*

In addition to *Cotula cinerea* from Algeria and Egypt which were found to contain flavonoids, such phytochemicals have also been reported to occur in populations from Morocco. The flavone derivative luteolin 4'-O-glucoside was thus

reported in a sample from Errachidia region (Khallouki et al. 2015). Another more thorough investigation on a sample from Dakhla region (Morocco) has been recently reported (Guaouguaou et al. 2020b). In this study, free apigenin, luteolin and kaempferol have been found in the hydro ethanolic extract in addition to several methoxylated and glycosylated derivatives of flavones (apigenin, luteolin) and flavonols (kaempferol, quercetin).

3.4.4 Sulfated Flavonoids

In addition to the flavonoids discussed above, *Cotula cinerea* from Morocco was also found to contain the sulfated flavonoids indicated in Table 2 and Fig. 5. Thus, kaempferol 3-sulfate 7-O-glucoside, kaempferol 3-O-sulfate, luteolin 7-O-sulfate in addition to apigenin 7-O-sulfate have been evidenced in the hydroethanolic extracts of the plant. The presence of such sulfated flavonoids has been previously identified in species other than *Cotula cinerea* (Teles et al. 2018). Their occurrence in this plant agree with the fact that sulfated flavonoids are reported to occur in some specific plant families such as Asteraceae to which *Cotula cinerea* belong (Teles et al. 2018). This is also in agreement with the fact that such compounds were also reported to occur in species occurring in arid habitats such as Moroccan Sahara region from which the studied *Cotula cinerea* sample has been harvested.

3.4.5 Other Phytochemical Compounds

In addition to essential oil terpenoids and phenolic compounds, several other metabolites have been identified in *Cotula cinerea* plant from different geographical regions as indicated in Table 2 and Figs. 6 and 7. The phytochemical study of *Cotula cinerea* extracts from Egypt has been conducted in 1985 and afforded to the characterization of compounds pertaining to spiroketal enol-ether, sesquiterpene lactones, germacranolides, and guaianolide (Metwally et al. 1985) in addition to Isofraxidin and scopoletin derivatives (Greger and Hofer 1985). Among the latter are farnochrol, drimartol A, acetyldrimartol A, acetyldrimartol B, pectachol, pectachol B, acetylpectachol B in addition to scopofarnol and farnesylscopoletin (Fig. 6 and Table 2). Another sample from the Egyptian eastern desert was also found to contain several spiroketal enolethers, lactones, germacranolides, eudesmanolides, guaianolides and glaucolides (Jakupovic et al. 1988). A germacranolide compound (1α,6α-dihydroxygermacra-4E,9Z,11(13)-trien-12,8 α-olide) which structure is indicated in Fig. 7 was isolated and identified from *Cotula cinerea* hydroethanolic extract from Algeria (Dendougui et al. 2012). In a relatively recent investigation on *Cotula cinerea* extract from Morocco, tatridin and dehydrotatridin derivatives (Fig. 7) have been detected (Guaouguaou et al. 2020b).

In addition to these phytochemicals other derivatives have been isolated from the dichloromethane extract through bioguided isolation affording five main sesquiterpene lactones (Cimmino et al. 2021). These were shown to be three

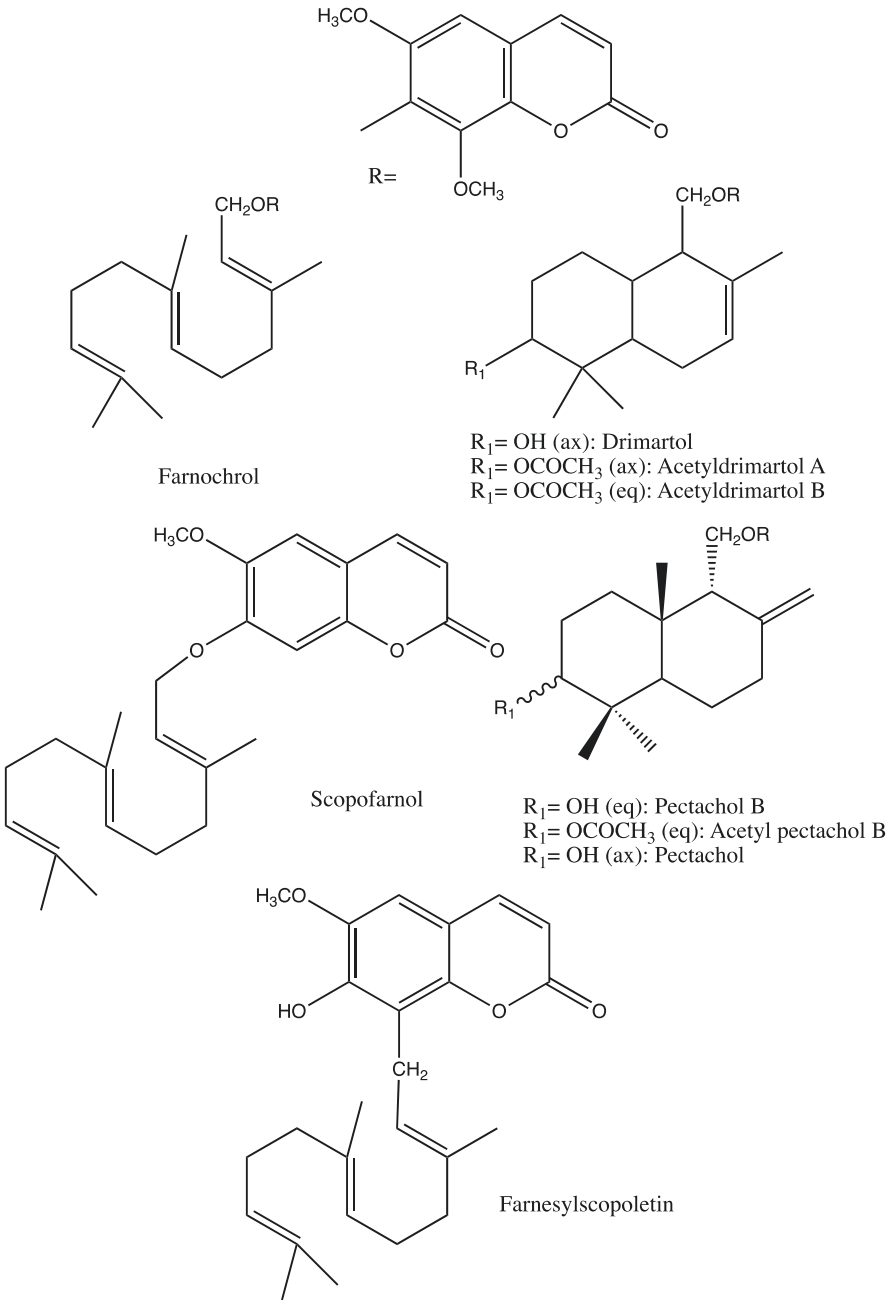
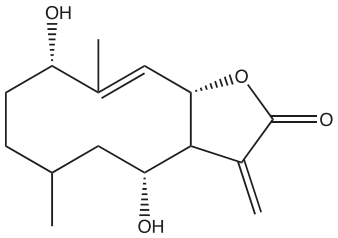
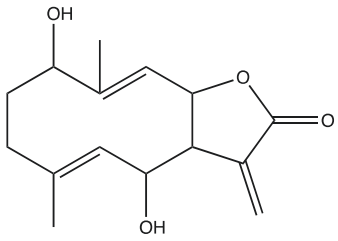


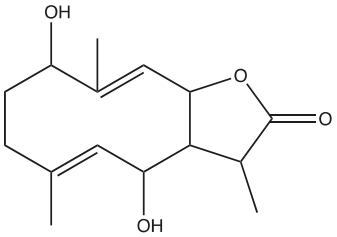
Fig. 6 Structures of some isofraxidin and scopoletin derivatives of *Cotula cinerea*



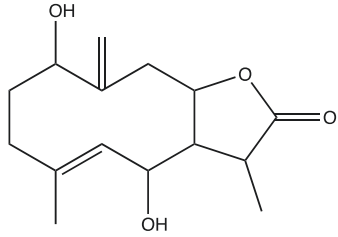
1α,6α-dihydroxygermacra-4E,9Z,11(13)-trien-12,8 α-olide



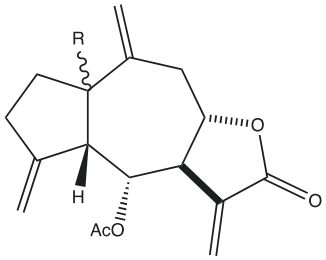
Tatridin



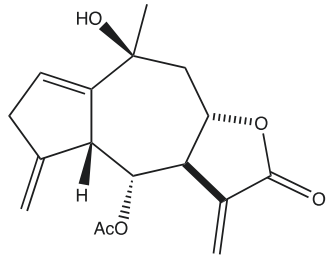
Dehydrotatridin



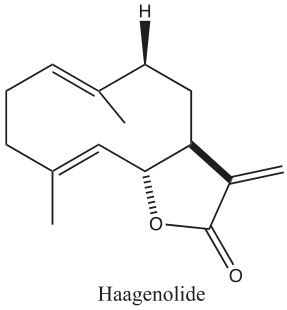
Dehydrotatridin



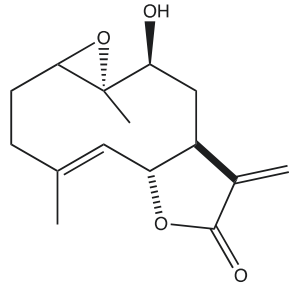
R= βOH: 6-acetoxy-1β-hydroxyguaiantrienolide
R= βOH: 6-acetoxy-1α-hydroxyguaiantrienolide



6-acetoxy-10-β-hydroxyguaiantrienolide



Haagenolide



1,10-epoxyhaagenolide

Fig. 7 Structures of some sesquiterpene lactones derivatives of *Cotula cinerea*

guaiantrianolides (6-acetoxy-1β-hydroxyguaiantrienolide, 6-acetoxy-1α-hydroxyguaiantrienolide, 6-acetoxy-10-β-hydroxyguaiantrienolide) and two germacranolides (haagenolide and 1,10-epoxyhaagenolide) (Fig. 7).

3.5 Pharmacological Investigation

3.5.1 Antimicrobial Activity

The evaluation of the antimicrobial activity (bacterial and fungal) of *Cotula cinerea* extracts and essential oil harvested in different regions of the world has been reported in numerous studies (Table 3). Several researchers have shown that *Cotula cinerea* has broad-spectrum antimicrobial activity when tested against several pathogenic bacteria and fungi. The results of the various tests of the antimicrobial activity of *Cotula cinerea* extracts and essential oil are grouped in Table 3.

The antibacterial test of *Cotula cinerea* essential oil on *Escherichia coli* and *Staphylococcus aureus* showed strong inhibition with a diameter ranging from (16.70 to 14.64 mm) (Mekhadmi et al. 2023).

Hamdouch et al. (2022) showed that the evaluation of the bacterial activity by the essential oil of *Cotula cinerea* against three bacterial strains (*Staphylococcus aureus, Listeria innocua, Pseudomonas aeruginosa*) showed that the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) are important (Table 3).

The *Staphylococcus aureus* is the bacterial strain that was inhibited with a low concentration (MIC = 0.5 µL/mL and MBC = 4 µL/mL), followed by *Pseudomonas aeruginosa* (MIC = 0.6 µL/mL and MBC = 1.5 µL/mL) and in third position is *Listeria innocua aeruginosa* (MIC = 0.8 µL/mL and MCB = 3.5 µL/mL) (Hamdouch et al. 2022).

On the other hand, the antibacterial activity of *Cotula cinerea* essential oil was evaluated on two bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) (Table 3).

The results obtained show that the two bacteria tested have a low sensitivity vis-à-vis the different prepared concentrations of this essential oil compared to the negative control (the inhibitory zones of the two strains vary between 6 and 9 mm and those of Amoxyclav between 13 and 15 mm) (Mahboub et al. 2021).

The study of the antimicrobial activity of the methanolic extract of *Cotula cinerea* revealed the effectiveness of this extract on the strains: *Escherichia coli, Escherichia coli ATCC 25922* and *Pseudomonas aeruginosa* and their inhibition diameter varies between 7.1 ± 0.6 mm and 23.2 ± 0.3 mm (Table 3).

Also, *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus aureus ATCC 25923,* and *Proteus mirabilis* showed high sensitivity toward the *Cotula cinerea* methanolic extract with a large inhibition diameter (28.7 ± 0.5 mm, 23.7 ± 0.6 mm, 21.7 ± 0.2 mm, and 36.7 ± 0.1 mm, respectively). Thus, the zones

Table 3 Antibacterial and antifungal effects of *Cotula cinerea* essential oils and extracts

Part used	Extracts	Tested strains	Key results	References
Aerial parts	Essential oil (0.45%)	*Staphylococcus aureus*	(16.70–14.64 mm)	Mekhadmi et al. (2023)
		Escherichia coli	(16.70–14.64 mm)	
Aerial parts	Essential oil (0.66%)	*Staphylococcus aureus*	MIC = 0.5 µL/mL MCB = 4 µL/mL	Hamdouch et al. (2022)
		Listeria innocua	MIC = 0.8 µL/mL MCB = 3.5 µL/mL	
		Pseudomonas aeruginosa	MIC = 0.6 µL/mL MCB = 1.5 µL/mL	
Aerial parts	Essential oil (1.07%)	*Escherichia coli*	ϕ = 6 to 9 mm	Mahboub et al. (2021)
		Pseudomonas aeruginosa	ϕ = 6 to 9 mm	
Leaves	Methanol extract (11.2%)	*Staphylococcus aureus*	ϕ = 28.7 ± 0.5 mm	Djahra et al. (2020)
		Staphylocoque epidermidis	ϕ = 23.7 ± 0.6 mm	
		Escherichia coli	ϕ = 7.3 ± 0.3 mm	
		Proteus mirabilis	ϕ = 36.7 ± 0.1 mm	
		Escherichia coli ATCC 25922	ϕ = 23.2 ± 0.3 mm	
		Pseudomonas aeruginosa	ϕ = 7.2 ± 0.3 mm	
		Staphylococcus aureus ATCC 25923	ϕ = 21.1 ± 0.2 mm	
		Trichophyton verrucosum	ϕ = 8.3 ± 0.2 mm	
Aerial parts	Essential oil (0.21%)	*Staphylococcus aureus*	ϕ = 6 mm	Mehani et al. (2019)
		Escherichia coli	ϕ = 13 mm	
		Pseudomonas aeruginosa	ϕ = 15.23 mm	
		Enterobacter cloacae	ϕ = 14 mm	
		Fusarium sporotrichioides	ϕ = 0 mm	
Aerial parts	Essential oil (0.54%)	*Bacillus cereus*	+	Ghouti et al. (2018a)
		Bacillus subtilis	MIC = 0.303 mg/mL	
		Micrococcus luteus	+	
		Pseudomonas aeruginosa	+	
		Candida albicans	+	

(continued)

Table 3 (continued)

Part used	Extracts	Tested strains	Key results	References
Aerial parts	Hydroethanolic extract	*Escherichia coli*	MIC = 10 mg/mL	Ghouti et al. (2018b)
		Pseudomonas aeruginosa	MBC and MIC >20 mg/mL	
		Klebsiella pneumoniae	MBC and MIC = 20 mg/mL	
		Proteus mirabilis	MBC and MIC >20 mg/mL	
		Morganella morganii	MBC and MIC >20 mg/mL	
		Enterococcus faecalis	MBC and MIC >20 mg/mL	
		Listeria monocytogenes	MIC = 20 mg/mL	
		MRSA	MIC = 10 mg/mL	
		MSSA	MIC = 5 mg/mL	
		Candida albicans	MFC and MIC >20 mg/mL	
	Infusion extract	*Escherichia coli*	MIC = 10 mg/mL	
		Pseudomonas aeruginosa	MBC and MIC >20 mg/mL	
		Klebsiella pneumoniae	MBC and MIC = 20 mg/mL	
		Proteus mirabilis	MBC and MIC >20 mg/mL	
		Morganella morganii	MBC and MIC >20 mg/mL	
		Enterococcus faecalis	MBC and MIC >20 mg/mL	
		Listeria monocytogenes	MIC = 20 mg/mL	
		MRSA	MIC = 10 mg/mL	
		MSSA	MIC = 5 mg/mL	
		Candida albicans	MFC and MIC >20 mg/mL	
Aerial parts	Aqueous Extracts	*Fusarium graminearum*	$\phi = 39 \pm 0.57$ mm	Salhi et al. (2017)
		Fusarium sporotrichioides	$\phi = 50 \pm 0.57$ mm	

(continued)

Table 3 (continued)

Part used	Extracts	Tested strains	Key results	References
Aerial parts	Essential oil (0.64%)	*Bacillus subtilis*	C = 1/500 v/v	Boussoula et al. (2016)
		Escherichia coli	C = 1/500 v/v	
		Staphylococcus aureus	C = 1/500 v/v	
		Micrococcus luteus	C = 1/500 v/v	
		Asper gillusniger	C = 1/250 v/v	
		Penicillium digitatum	C = 1/250 v/v	
		Penicillium expansum	C = 1/250 v/v	
		Gloeophyllum trabeum	C = 1/500 v/v	
		Coniophora puteana	C = 1/1000 v/v	
		Poria placenta	C = 1/2000 v/v	
		Coriolus versicolor	C = 1/500 v/v	
Aerial parts	Essential oil (0.39%)	*Staphylococcus aureus*	ϕ = 50 mm to 21 mm	Atef et al. (2015)
		Enterococcus faecium	ϕ = 50 mm	
		Escherichia coli	ϕ = 50 mm to 21 mm	
		Morganella morganii	ϕ = 50 mm to 21 mm	
		Citrobacter freundii	ϕ = 16 mm to 11 mm	
		Pseudomonas aeruginosa	ϕ = 21 mm	
		Proteus vulgaris	ϕ = 50 mm to 21 mm	
		Acinetobacter baumannii	ϕ = 50 mm to 21 mm	
		Klebsiella pneumoniae	ϕ = 16 mm to 11 mm	

(continued)

Table 3 (continued)

Part used	Extracts	Tested strains	Key results	References
Aerial parts	Petroleum ether (1.0%)	*Staphylococcus aureus*	+	Bensizerara et al. (2013)
		Escherichia coli	+	
		Pseudomonas aeruginosa	+	
		Klebsiella pneumoniae	ϕ = 17 ± 1.73 mm	
		Candida albicans	+	
	Ethyl acetate (1.2%)	*Staphylococcus aureus*	ϕ = 11.67 ± 3.79 mm	
		Escherichia coli	+	
		Pseudomonas aeruginosa	+	
		Klebsiella pneumoniae	+	
		Candida albicans	+	
	n-butanol (6.0%)	*Staphylococcus aureus*	ϕ = 12 ± 5.20 mm	
		Escherichia coli	+	
		Pseudomonas aeruginosa	+	
		Klebsiella pneumoniae	ϕ = 16.67 ± 5.77 mm	
		Candida albicans	+	
Aerial parts	Essential oil (0.87%)	*Candida albicans CCMM L4*	ϕ = 25.3 ± 0.6 mm	Bouzidi et al. (2011)
		Candida albicans CCMM L5	ϕ = 20.3 ± 0.6 mm	
		Candida krusei	ϕ = 19.3 ± 0.6 mm	
		Candida glabrata	ϕ = 21.3 ± 1.5 mm	
		Candida parapsilosis	ϕ = 24.3 ± 0.6 mm	

(continued)

Table 3 (continued)

Part used	Extracts	Tested strains	Key results	References
Aerial parts	Ethyl acetate extract (0.64%)	*Pseudomonas fluorescens 456–2*	MIC = 200 µg/mL	Markouk et al. (1999a, b)
		Pseudomonas savastanoui T12–10	MIC = 200 µg/mL	
		Pseudomonas savastanoui 73–29/88	MIC = 200 µg/mL	
		Bacillus sp. *VP5*	MIC = 200 µg/mL	
		Bacillus brevis VP7	MIC = 200 µg/mL	
		Bacillus sp. *326*	MIC = 200 µg/mL	
		Bacillus sphaericus 324	MIC = 200 µg/mL	
		Bacillus sp. *459–1*	MIC = 200 µg/mL	
	n-Butanol extract (2.02%)	*Pseudomonas fluorescens 456–2*	MIC = 12 µg/mL	
		Pseudomonas savastanoui T12–10	MIC = 100 µg/mL	
		Pseudomonas savastanoui 73–29/88	MIC = 50 µg/mL	
		Bacillus sp. *VP5*	MIC = 25 µg/mL	
		Bacillus brevis VP7	MIC = 25 µg/mL	
		Bacillus sp. *326*	MIC = 25 µg/mL	
		Bacillus sphaericus 324	MIC = 200 µg/mL	
		Bacillus sp. *459–1*	MIC = 12 µg/mL	

of inhibition measured exceed that of the antibiotic tested as reference (Amoxicillin) (Djahra et al. 2020).

The study of the antimicrobial activity of *Cotula cinerea* essential oil on bacterial strains showed that *Enterobacter cloacae* and *Escherichia coli* are moderately sensitive ($\phi = 14$ mm and $\phi = 13$ mm, respectively) (Table 3). In addition, *Pseudomonas aeruginosa* is the most sensitive strain to this essential oil with a zone of inhibition of 15.23 mm.

On the other hand, the *Staphylococcus aureus* strain is more resistant with an inhibition zone of 6 mm. However, *Fusarium sporotrichioides* showed strong resistance to different concentrations of *Cotula cinerea* essential oil, and no mycelial growth was observed (Mehani et al. 2019).

The evaluation of the antibacterial activity of *Cotula cinerea* essential oil by the disk diffusion method on four strains of bacteria (*Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa*) and on a yeast (*Candida albicans*) has revealed the effectiveness of this essential oil with moderate minimum inhibitory concentration (MIC). The *Bacillus subtilis* strain gave the best inhibition with MIC = 0.303 mg/mL (Table 3) (Ghouti et al. 2018a).

The study of the antimicrobial activity of *Cotula cinerea* hydroethanolic extract and the infusion extract tested on ten microbial strains (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Morganella morganii, Enterococcus faecalis, Listeria monocytogenes, MRSA, MSSA,* and *Candida albicans*) showed inhibitory effects that ranged from moderate to weak and which are expressed as minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) (Table 3). The MIC values varied between 5 and 20 mg/mL and the inhibitory effect of these extracts tested against all bacterial strains was more bacteriostatic than bactericidal (Ghouti et al. 2018b).

The results of the study of *Cotula cinerea* aqueous extract on *Fusarium graminearum* and on *Fusarium sporotrichioides* (Table 3) revealed the effectiveness of this extract in inhibiting the growth of mycelia with the two concentrations of 10% and 20%. The growth inhibition zones of these two fungi are $\phi = 39 \pm 0.57$ mm and $\phi = 50 \pm 0.57$ mm, respectively (Salhi et al. 2017).

The antibacterial and antifungal activity of *Cotula cinerea* essential oil showed significant inhibitory effects (Table 3). For the four bacterial strains (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Micrococcus luteus*), they were all inhibited at a concentration of 1/500 v/v. However, molds (*Asper gillusniger, Penicillium digitatum,* and *Penicillium expansum*) were less inhibited than bacteria, and their growth was stopped at a concentration of 1/250 v/v (Boussoula et al. 2016). Concerning the four strains of fungi (*Gloeophyllum trabeum, Coniophora puteana, Poria placenta,* and *Coriolus versicolor*), only *Poria placenta* which presented the greatest vulnerability compared with *Cotula cinerea* essential oil with a low concentration of 1/2000 v /v. For *Coniophora puteana*, it was inhibited with a concentration of 1/1000 v/v. However, *Coriolus versicolor* and *Gloeophyllum trabeum* showed resistance to the *Cotula cinerea* essential oil, and they were inhibited only with the concentration 1/500 v/v (Boussoula et al. 2016).

The antibacterial activity of *Cotula cinerea* essential oil tested on *Enterococcus faecium* showed a very high inhibitory effect (50 mm) by comparing with the diameter of antibiotic inhibition (Lincomycin: 32 mm) (Atef et al. 2015). Also, *Escherichia coli, Morganella morganii, Proteus vulgaris, Staphylococcus aureus,* and *Acinetobacter baumannii* showed great sensitivity to this essential oil with the concentrations (1/1, 1/2, 1/4, 1/8) where the diameter of inhibition varied between 50 mm and 21 mm (Table 3). However, *Citrobacter freundii* and *Klebsiella pneumoniae* showed great sensitivity just at the three concentrations (1/1, 1/2, 1/4) with an inhibition diameter which reached 50 mm. *Pseudomonas aeruginosa* showed strong resistance with all tested concentrations of *Cotula cinerea* essential oil (Atef et al. 2015).

The antimicrobial activity of *Cotula cinerea* n-butanol and petroleum ether extracts tested on *Klebsiella pneumoniae* showed a major inhibitory effect (16.67 ± 5.77 mm and 17 ± 1.73 mm respectively) (Table 3). Also, a strong activity against *Staphylococcus aureus* was revealed with the n-butanol and ethyl acetate extracts where the zones of inhibition were 12 ± 5.20 mm and 11.67 ± 3.79 mm, respectively. However, weak antimicrobial activity was observed against *Escherichia*

coli, Pseudomonas aeruginosa, Staphylococcus aureus, and *Candida albicans* with the concentration 0.25 mg/mL (Bensizerara et al. 2013).

Analysis of the antimicrobial activity of *Cotula cinerea* essential oil showed strong activity against all Candida genus yeasts studied (*Candida albicans CCMM L4, Candida albicans CCMM L5, Candida krusei, Candida glabrata,* and *Candida parapsilosis*) (Table 3), with inhibition zones ranging from 19.3 to 25.3 mm (Bouzidi et al. 2011).

Markouk et al. (1999a, b), tested the antibacterial activity of two extracts (n-butanol and ethyl acetate) of *Cotula cinerea* on eight bacterial strains (*Pseudomonas fluorescens 456-2, Pseudomonas savastanoui T12-10, Pseudomonas savastanoui 73-29/88, Bacillus* sp. *VP5, Bacillus brevis VP7, Bacillus* sp. *326, Bacillus sphaericus 324,* and *Bacillus* sp. *459-1*) (Table 3). They found the n-butanol extract to be highly effective against the bacterial strains tested with minimum inhibitory concentrations ranging from 12 to 200 µg/mL. Furthermore, *Pseudomonas fluorescens 456-2* and *Bacillus* sp. *459-1* were inhibited at a low concentration of 12 µg/mL. However, the ethyl acetate extract inhibited the growth of all the bacteria studied at a concentration of 200 µg/mL (Markouk et al. 1999a, b).

3.5.2 Antioxidant Activity

The antioxidant effect of *Cotula cinerea* extracts and essential oil obtained by extracting different parts of the plant has been proven by several studies. The antioxidant activity of this plant was carried out by the DPPH, ABTS, reducing power, β-carotene bleaching inhibition, TBARS inhibition, FRAP, and ORAC tests. Table 4 brings together all the work on the antioxidant activity of *Cotula cinerea.*

Hamdouch et al. (2022) showed that *Cotula cinerea* essential oil has a low antioxidant activity compared to the selected positive controls (butylhydroxytoluene (BHT) and Cov-iox T_{50}) with an IC_{50} of 0.080 ± 0.014 mg/mL (Table 4).

On the other hand, Mahboub et al. (2021) showed that the *Cotula cinerea* essential oil evaluated for its antioxidant power using the DPPH test showed a moderate antioxidant effect compared to ascorbic acid (IC_{50} = 79.28 mg/mL) (Table 4).

The study of the antioxidant activity of *Cotula cinerea* essential oil and extracts was carried out by two methods: DPPH and ABTS and several concentrations were tested (Guaouguaou et al. 2020a).

The DPPH test showed a higher antioxidant effect than that of ABTS and this for three extracts of *Cotula cinerea* (hexane, ethyl acetate, and n-butanol) with IC_{50} values of 0.0602, 0.0644 and 0.0641 mg/mL respectively (Table 4). For the essential oil of the same plant, it showed a moderate effect (IC_{50} = 0.1832 mg/mL). However, the ABTS test showed that *Cotula cinerea* essential oil has powerful antioxidant activity compared to the other extracts tested (IC_{50} = 0.0093 mg/mL). Also, the n-butanol extract shows strong antioxidant activity with the ABTS test (0.0698 mg/mL) (Guaouguaou et al. 2020a).

Table 4 Antioxidant activity of *Cotula cinerea* essential oils and extracts

Part used	Extracts	Used methods	Key results	References
Aerial parts	Essential oil (0.66%)	DPPH assay	IC_{50} = 0.080 ± 0.014 mg/ mL	Hamdouch et al. (2022)
Aerial parts	Essential oil (1.07%)	DPPH assay	IC_{50} = 79.28 mg/ mL	Mahboub et al. (2021)
Aerial parts	Essential oil (0.92%)	DPPH assay	IC_{50} = 0.183 mg/mL	Guaouguaou et al. (2020a, b)
		ABTS assay	IC_{50} = 0.009 mg/mL	
	Hexane extract (1%)	DPPH assay	IC_{50} = 0.060 mg/mL	
		ABTS assay	IC_{50} = 0.073 mg/mL	
	Ethyl acetate extract (3%)	DPPH assay	IC_{50} = 0.064 mg/mL	
		ABTS assay	IC_{50} = 0.082 mg/mL	
	n-butanol extract (4.5%)	DPPH assay	IC_{50} = 0.064 mg/mL	
		ABTS assay	IC_{50} = 0.069 mg/mL	
Aerial parts	Essential oil (0.54%)	DPPH assay	IC_{50} = 28 mg/mL	Ghouti et al. (2018a)
Aerial parts	Hydroethanolic extract	DPPH assay	EC_{50} = 26.0 ± 0.1 µg/mL	Ghouti et al. (2018b)
		Reducing power	EC_{50} = 31.9 ± 0.2 µg/mL	
		β-carotene bleaching inhibition	EC_{50} = 14.7 ± 0.2 µg/mL	
		TBARS inhibition	EC_{50} = 7.4 ± 0. 3 µg/mL	
	Infusion extract	DPPH assay	EC_{50} = 24.8 ± 0.2 µg/mL	
		Reducing power	EC_{50} = 38 ± 1 µg/mL	
		β-carotene bleaching inhibition	EC_{50} = 20.2 ± 0.8 µg/mL	
		TBARS inhibition	EC_{50} = 7.5 ± 0.2 µg/mL	
Aerial parts	Chlorogenic acid compound	DPPH assay	IC_{50} = 10.5 µM	Khallouki et al. (2015)
		FRAP assay	EC_1 = 478 µM	
		ORAC assay	3.07 units	
	Neochlorogenic acid compound	DPPH assay	IC_{50} = 11.0 µM	
		FRAP assay	EC_1 = 527 µM	
		ORAC assay	2.42 units	
	3,4-Dicaffeoylquinic acid compound	DPPH assay	IC_{50} = 30.25 µM	
		FRAP assay	EC_1 = 329 µM	
		ORAC assay	3.33 units	
	3,5-Dicaffeoylquinic acid compound	DPPH assay	IC_{50} = 23.84 µM	
		FRAP assay	EC_1 = 407 µM	
		ORAC assay	3.62 units	
	4,5-Dicaffeoylquinic acid compound	DPPH assay	IC_{50} = 31.49 µM	
		FRAP assay	EC_1 = 337 µM	
		ORAC assay	3.76 units	
	Luteolin-4´-O-glucoside compound	DPPH assay	IC_{50} = 30.25 µM	
		FRAP assay	EC_1 = 422 µM	
		ORAC assay	3.46 units	

The evaluation of the antioxidant activity of *Cotula cinerea* essential oil using the DPPH method (Table 4) showed moderate inhibition (IC_{50} = 28 mg/mL) (Ghouti et al. 2018a).

On the other hand, the evaluation of the antioxidant activity of *Cotula cinerea* hydroethanolic and infusion extracts was carried out by four tests (DPPH, Reducing power, β-carotene bleaching inhibition and TBARS inhibition) in order to be able to compare their antioxidant effects (Ghouti et al. 2018b) (Table 4). For the TBARS inhibition test, the two hydroethanolic and infusion extracts of *Cotula cinerea* showed that they are three times more effective than the positive control (TROLOX) with high values: EC_{50} = 7.4 ± 0. 3 μg/mL and EC_{50} = 7.5 ± 0.2 μg/mL respectively. Also, for the DPPH test, Ghouti et al. (2018b) showed that *Cotula cinerea* hydroethanolic and infusion extracts have significant antioxidant activity with EC_{50} = 26.0 ± 0.1 μg/mL and EC_{50} = 24.8 ± 0.2 μg/mL respectively (Ghouti et al. 2018b).

The phytochemical identification and fractionation of *Cotula cinerea* methanol extract, revealed the presence of six compounds (Chlorogenic acid, Neochlorogenic acid, 3,4-Dicaffeoylquinic acid, 3,5-Dicaffeoylquinic acid, 4,5-Dicaffeoylquinic acid, Luteolin-4´-O-glucoside) (Table 4) (Khallouki et al. 2015). These compounds were evaluated for their antioxidant effects using three tests namely DPPH, ABTS and ORAC. Furthermore, Chlorogenic acid showed strong antioxidant activity by the DPPH test with IC_{50} = 10.5 μM. For the FRAP test, 3,4-Dicaffeoylquinic acid gave an antioxidant effect with the lowest concentration (EC_1 = 329 μM). However, neochlorogenic acid was able to inhibit fluorescence with 2.42 units (Khallouki et al. 2015).

3.5.3 Anticancer Activity

As part of promoting medicinal plants, the *Cotula cinerea* essential oil and extracts have also been targeted to assess their anticancer activity on several cancer cell lines. The methods and results of the antiproliferative activity of this plant have been compiled in Table 5.

The evaluation of the cytotoxic activity of *Cotula cinerea* extracts harvested in Algeria showed that the hydroethanolic extract has significant and important cytotoxic properties against the four cancer cell lines tested (Ghouti et al. 2018b). The HepG2 line (hepatocellular carcinoma) was inhibited by the lowest concentration and this by the two extracts (hydroethanolic GI_{50} = 31 ± 2 μg/mL and the infusion extract GI_{50} = 42 ± 4 μg/mL) (Ghouti et al. 2018b). Furthermore, the *Cotula cinerea* extracts also exhibited a moderate cytotoxic effect against the MCF-7, NCI-H460, HeLa, and PLP2 lines (Table 5).

The study of the antiproliferative activity of essential oil and three extracts (hexane, ethyl acetate, and n-butanol) of *Cotula cinerea* carried out by the MTT test against two cell lines RD and VERO (Table 5) showed that for the RD cell line, the

Table 5 Anticancer activity of *Cotula cinerea* essential oils and extracts

Part used	Extracts	Cell lines	Key results	References
Aerial parts	Hydroethanolic extract	MCF-7 (breast carcinoma)	$GI_{50} = 53 \pm 4$ µg/mL	Ghouti et al. (2018a, b)
		NCI-H460 (non-small-cell lung cancer)	$GI_{50} = 50 \pm 3$ µg/mL	
		HeLa (cervical carcinoma)	$GI_{50} = 47 \pm 5$ µg/mL	
		HepG2 (hepatocellular carcinoma)	$GI_{50} = 31 \pm 2$ µg/mL	
		PLP2 (porcine liver primary cells)	$GI_{50} = 120 \pm 8$ µg/mL	
	Infusion extract	MCF-7 (breast carcinoma)	$GI_{50} = 77 \pm 6$ µg/mL	
		NCI-H460 (non-small-cell lung cancer)	$GI_{50} = 101 \pm 10$ µg/mL	
		HeLa (cervical carcinoma)	$GI_{50} = 51 \pm 4$ µg/mL	
		HepG2 (hepatocellular carcinoma)	$GI_{50} = 42 \pm 4$ µg/mL	
		PLP2 (porcine liver primary cells)	$GI_{50} = 198 \pm 5$ µg/mL	
Aerial parts	Essential oil (0.92%)	RD (human embryonal rhabdomyosarcoma)	$IC_{50} = 173.05 \pm 4.46$ µg/mL	Guaouguaou et al. (2018)
		Vero (monkey kidney cancerous cell lines)	$IC_{50} = 72.72 \pm 2.18$ µg/mL	
	Hexane extract (1%)	RD (human embryonal rhabdomyosarcoma)	$IC_{50} = 57.21 \pm 3.43$ µg/mL	
		Vero (monkey kidney cancerous cell lines)	$IC_{50} = 142.27 \pm 11.33$ µg/mL	
	Ethyl acetate extract (3%)	RD (human embryonal rhabdomyosarcoma)	$IC_{50} = 187.52 \pm 6.27$ µg/mL	
		Vero (monkey kidney cancerous cell lines)	$IC_{50} = 212.83 \pm 9.02$ µg/mL	
	n-butanol extract (4.5%)	RD (human embryonal rhabdomyosarcoma)	$IC_{50} > 500$ µg/mL	
		Vero (monkey kidney cancerous cell lines)	$IC_{50} = 447.38 \pm 6.52$ µg/mL	

hexane extract presents the highest cytotoxic effect with $IC_{50} = 57.21 \pm 3.43$ µg/mL, followed by the ethyl acetate extract ($IC_{50} = 187.52 \pm 6.27$ µg/mL), essential oil ($IC_{50} = 173.05 \pm 4.46$ µg/mL), and lastly, we find the n-butanol extract (IC50 > 500 µg/mL). However, the Vero cell line was better inhibited by the essential oil with $IC_{50} = 72.72 \pm 2.18$ µg/mL (Guaouguaou et al. 2018).

3.5.4 Other Pharmacological Activities of *Cotula cinerea*

The evaluation of other pharmacological activities of *Cotula cinerea* essential oil and extracts has been carried out by several research teams. The results of this work are grouped in Table 6.

Bettayeb et al. (2022) showed that the essential oil of the leaves and flowers of *Cotula cinerea* administered orally to mice exhibited low toxicity (LD_{50} = 1131.37 mg/kg, LD_{50} = 1264.91 mg/kg respectively).

Under the same conditions, Bettayeb et al. (2022) also showed that the essential oil of the leaves and flowers of *Cotula cinerea* possesses a significant anti-inflammatory effect at concentrations that do not exceed 300 mg/kg with percentages of 86, 16% for the leaves and 80.87% for the flowers (Table 6).

The experimental study of the acute oral toxicity of the aqueous extract of the dry and fresh aerial parts of *Cotula cinerea* at several doses (200, 400, 600 and 800 mg/kg) showed that these extracts did not cause any mortality or signs of toxicity in Wistar rats (Chlif et al. 2022).

On the other hand, the oral administration of the aqueous extract of the fresh and dry aerial parts at a dose of 200 mg/kg reduced the edema 3 h after the injection of carrageenan, with a percentage inhibition of 36.84% and 39.47%, respectively (Chlif et al. 2022). Furthermore, the study of the analgesic activity of the aqueous extract of fresh and dry aerial parts at a dose of 200 mg/kg and 400 mg/kg on rats by injecting 0.6% acetic acid showed a significant analgesic effect (Table 6). The aqueous extract of the dry aerial parts presents a higher percentage of inhibition (43.15% at the dose of 200 mg and 50.71% at the dose of 400 mg/kg) than that of the fresh parts (32.14% at the 200 mg dose and 45.51% at the 400 mg/kg dose) (Chlif et al. 2022).

By the same team and under the same conditions, Chlif et al. (2022) evaluated the antipyretic activity of the aqueous extract of the fresh and dry aerial parts of *Cotula cinerea* by the method Brewer's yeast-induced pyrexia model in rats using two different concentrations (200 and 400 mg/kg). The results showed that these extracts possess a significant antipyretic activity after 4 h of administration. The aqueous extract of the fresh parts reduced the rectal temperature for the two concentrations tested (36.62 ± 0.24°C at the dose of 200 mg and 37.74 ± 0.25 °C at the dose of 400 mg/kg). However, the aqueous extract of the dry parts was more effective in reducing the rectal temperature at the dose of 400 mg/kg (37.73 ± 0.26°C and 36.86 ± 0.41 °C, respectively) (Chlif et al. 2022).

The evaluation of the anti-inflammatory activity of *Cotula cinerea* extracts (hydroethanolic and infusion extracts) was evaluated by the Murine macrophage-like RAW method 264.7 cells and quantified through the nitric oxide (NO) production (Table 6).

The results obtained showed a lower inhibition of the production of NO compared to the positive control and this by the two extracts (the hydroethanolic extract (EC_{50} = 105 ± 9 µg/mL) and the infusion extract (EC_{50} = 122 ± 6 µg/mL)) (Ghouti et al. 2018a, b).

Table 6 Other pharmacological activities of *Cotula cinerea* essential oils and extracts

Activities	Part used	Extracts	Experimental approach	Key results	References
Acute toxicity	Leaves	Essential oil	Lorke's method	LD_{50} = 1131.37 mg/kg	Bettayeb et al. (2022)
	Flowers	Essential oil	Lorke's method	LD_{50} = 1264.91 mg/kg	
Anti-inflammatory effect	Leaves	Essential oil	Carrageenan-induced paw edema in mice	% Inhibition of Edema = 86.16%	Bettayeb et al. (2022)
	Flowers	Essential oil	Carrageenan-induced paw edema in mice	% Inhibition of Edema = 80.87%	
Acute toxicity	Fresh aerial parts	Aqueous extract	Oral administration (200, 400, 600, and 800 mg/kg)	Any acute oral toxicity effects and mortality in the Wistar rats	Chlif et al. (2022)
	Dry aerial parts	Aqueous extract	Oral administration (200, 400, 600, and 800 mg/kg)	Any acute oral toxicity effects and mortality in the Wistar rats	
Anti-inflammatory effect	Fresh aerial parts	Aqueous extract	Carrageenan-induced paw edema in rats	% Inhibition of Edema = 36.84%	Chlif et al. (2022)
	Dry aerial parts	Aqueous extract	Carrageenan-induced paw edema in rats	% Inhibition of Edema = 39.47%	
Analgesic effect	Fresh aerial parts	Aqueous extract	Acetic acid-induced writhing response	% Inhibition (200 mg/kg) = 32.14% % Inhibition (400 mg/kg) = 45.51%	Chlif et al. (2022)
	Dry aerial parts	Aqueous extract	Acetic acid-induced writhing response	% Inhibition (200 mg/kg) = 43.15% % Inhibition (400 mg/kg) = 50.71%	
Antipyretic effect	Fresh aerial parts	Aqueous extract	Brewer's yeast-induced pyrexia model in rats	Rectal temperature (200 mg/kg) = 36.62 ± 0.24 (°C) Rectal temperature (400 mg/kg) = 37.74 ± 0.25 (°C)	Chlif et al. (2022)
	Dry aerial parts	Aqueous extract	Brewer's yeast- induced pyrexia model in rats	Rectal temperature (200 mg/kg) = 37.73 ± 0.26 (°C) Rectal temperature (400 mg/kg) = 36.86 ± 0.41 (°C)	

(continued)

Table 6 (continued)

Activities	Part used	Extracts	Experimental approach	Key results	References
Acute toxicity	Aerial parts	Essential oil	Oral administration (2000 mg/kg)	Any acute oral toxicity effects and mortality in the mice	Guaouguaou et al. (2020a, b)
		Hexane extract	Oral administration (2000 mg/kg)	Any acute oral toxicity effects and mortality in the mice	
		Ethyl acetate extract	Oral administration (2000 mg/kg)	Any acute oral toxicity effects and mortality in the mice	
		n-butanol extract	Oral administration (2000 mg/kg)	Any acute oral toxicity effects and mortality in the mice	
Analgesic effect	Aerial parts	Essential oil	Tail-flick method Hot plate method	Mean Response = 10.84 ± 0.19 s Mean Response = 22.16 ± 0.60 s	Guaouguaou et al. (2020a, b)
		Hexane extract	Tail-flick method Hot plate method	Mean Response = 10.22 ± 0.07 s Mean Response = 22.25 ± 0.57 s	
		Ethyl acetate extract	Tail-flick method Hot plate method	Mean Response = 14.46 ± 0.20 s Mean Response = 25.16 ± 0.29 s	
		n-butanol extract	Tail-flick method Hot plate method	Mean Response = 14.69 ± 0.61 s Mean Response = 25.56 ± 0.59 s	
Anti-inflammatory effect	Aerial parts	Hydroethanolic extract	Murine macrophage-like RAW 264.7 cells and quantified through the nitric oxide (NO) production	EC_{50} = 105 ± 9 µg/mL	Ghouti et al. (2018a, b)
		Infusion extract	Murine macrophage-like RAW 264.7 cells and quantified through the nitric oxide (NO) production	EC_{50} = 122 ± 6 µg/mL	
Antipyretic effect	Aerial parts	Ethyl ether extract	Brewer's yeast-induced pyrexia model in rats	Reduction of fever = 89.43%	Larhsini et al. (2002)
		Ethyl acetate extract	Brewer's yeast-induced pyrexia model in rats	Reduction of fever = 90.12%	
		n-butanol extract	Brewer's yeast-induced pyrexia model in rats	Reduction of fever = 2.85%	

Acute toxicity	Aerial parts	Ethyl ether extract	Oral administration (1, 2, 3, 4, 5, and 6 g/kg)	Any acute oral toxicity effects and mortality in the mice	Markouk et al. (1999a, b)
		Ethyl acetate extract	Oral administration (1, 2, 3, 4, 5, and 6 g/kg)	Any acute oral toxicity effects and mortality in the mice	
		n-Butanol extract	Oral administration (1, 2, 3, 4, 5, and 6 g/kg)	Any acute oral toxicity effects and mortality in the mice	
Analgesic effect	Aerial parts	Ethyl ether extract	Acetic acid-induced writhing response	% Inhibition (100 mg/kg) = 62.49%	
		Ethyl acetate extract	Acetic acid-induced writhing response	% Inhibition (100 mg/kg) = 50%	
		n-Butanol extract	Acetic acid-induced writhing response	% Inhibition (100 mg/kg) = 40.21%	

The experimental study of acute oral toxicity of the *Cotula cinerea* essential oil and extracts administered orally at a dose of 2000 mg/kg showed that this plant showed no particular signs of toxicity, no lethality or no mortality was observed in treated mice (Guaouguaou et al. 2020a).

On the other hand, Guaouguaou et al. (2020a) used two methods (*Tail flick and Hot plate*) to evaluate the central analgesic activity of the *Cotula cinerea* essential oil and three extracts (hexane, ethyl acetate, and n-butanol) at a dose of 500 mg/kg. The results obtained showed that from the 45th minute, the reaction of the animals increased with the two methods used (*Tail flick and Hot plate*) and this for the four extracts tested and also for the positive control (Table 6). For analgesic effect observed by *Tail flick* method of ethyl acetate extract was 14.46 s, then n-butanol extract with 14.69 s, followed by essential oil (10.84 s) and of the hexane extract (10.22 s). However, the analgesic effect evaluated by the *Hot plate* method was higher than the first method (*Tail flick*). The n-butanol extract prolonged the reaction time to the thermal stimulus with a reaction time of 25.56 s, followed by the ethyl acetate extract (25.16 s). For the essential oil and the hexane extract, they showed moderate analgesic activity with a reaction time of 22.16 s and 22.25 s respectively (Guaouguaou et al. 2020a).

The evaluation of the antipyretic activity of *Cotula cinerea* of three extracts (ethyl ether, ethyl acetate, and n-butanol) by the method Brewer's yeast-induced pyrexia model in rats (Table 6) showed that ether ethyl and ethyl acetate extract reduced fever with a percentage of 89.43% and 90.12%, respectively (Larhsini et al. 2002). In the same direction and always on the same extracts cited in the work of Larhsini et al. (2002), Markouk et al. (1999a, b) showed that the oral administration of three extracts (ethyl ether, ethyl acetate, and n-butanol) of *Cotula cinerea* caused no mortality at doses of 1, 2, 3, 4, 5, and 6 g/kg and also the animals remained without physiological abnormality. On the other hand, the ethyl acetate and n-butanol extracts showed a moderate analgesic effect with inhibition percentages of 50% and 40.21%, respectively (Table 6). However, the ethyl ether extract gave a percentage inhibition of (62.49%) which is close to that of the positive control (acetylsalicylic acid) with a percentage inhibition of 73.9% (Markouk et al. 1999a, b).

4 Conclusion and Future Perspectives

This review was conducted to report all studies containing *Cotula cinerea* that describe its botanical description, medicinal use, chemical composition, toxicity, and pharmacological properties. Ethnopharmacological studies indicate that *Cotula cinerea* is widely used in traditional medicine to treat colic, cough, diarrhea, migraine and digestive disorders.

On the other hand, the pharmacological and toxicological activities carried out in vivo and in vitro on the various extracts and the essential oil of *Cotula cinerea* revealed numerous effects, namely the antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, analgesic, and antipyretic activities.

The *Cotula cinerea* extracts and essential oil have shown remarkable antibacterial and antifungal effects against several bacteria and fungi. The antioxidant activity of *Cotula cinerea* extracts and essential oil was evaluated in vitro by several tests. The results obtained showed significant antioxidant effects. The results of the antiproliferative activity of *Cotula cinerea* extracts and essential oil show significant and encouraging cytotoxic effects against the cancer cell lines tested, and could then be considered as a source of new antitumor agents. The toxicity study reveals that the *Cotula cinerea* extracts and essential oil do not cause any signs of mortality or signs of toxicity when administered to animals orally. With regard to the analgesic effect of *Cotula cinerea* extracts and essential oil, the results obtained showed a significant analgesic effect and this for all the methods used.

This review is an opportunity by which we invite the authors to further pursue their research in order to understand the physiological mechanism behind the biological activities and pharmacological effects of *Cotula cinerea* extracts. Other pharmacological and toxicological tests seem more than necessary and other therapeutic virtues remain to be revealed in the hope of finding a place for this plant in modern pharmacy.

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Essential Oil as a Source of Bioactive Compounds for the Pharmaceutical Industry

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Abstract Essential oils have been known as therapeutic agents since ancient times. Due to their properties, many biological effects have been investigated and demonstrated in the literature so far, reinforcing the understanding that these can be used with the most diverse applications. In order to provide an overview of the subject, this chapter gathers and presents information regarding some of the main essential oils activities, addressing pharmacological effects in agriculture, food and cosmetics as a viable alternative without toxicity.

Keywords Pharmacological · Activity · Antiparasitic · Antioxidant · Anxiolytic · Cosmetics · Foods · Agriculture

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1 Introduction

Natural products are important tools in therapeutic procedures since they search for relief and cure of diseases through the use of medicinal plants, which was possibly one of the first ways of employing such materials (Mukherjee et al. 2010).

Essential oils are volatile secondary metabolites extracted from plants. They are characterized by complex mixtures of odoriferous, organic compounds that can be applied in several areas, with a world market estimated to be worth US$ 1.8 billion. Throughout history, these oils have been traditionally used due to their various properties. In Egypt, India, and China, people would use aromatic extracts for different purposes, such as physical well-being, beauty care, cooking, and spiritual applications (Kar et al. 2018).

Over the last decades, there has been a significant increase in natural plant-based therapies, both in developing countries and in regions where conventional medicine is still predominant (Fernandes Da Silveira et al. 2008). Currently, the search for natural products, as well as the reduction of degradation products generated by industries, and the increasing resistance to common pathogens have allowed the application of essential oils to avoid lipid deterioration, oxidation, and contamination by microorganisms, in pharmaceutical, cosmetics, food, beverage, and cosmetic industries (Miranda et al. 2016).

As in the past, essential oils maintain their importance nowadays, being applied in the prevention and treatment of human diseases, besides their cosmetic, sanitary, agricultural, and food applications. In this context, considering the properties of volatile oils, many investigations have been conducted to characterize their potential biological uses since effects as anti-inflammatory and antioxidant have been demonstrated so far (Paola Angelini 2012; Muzammil et al. 2023).

In addition, some species have also shown activity against microorganisms (bacteria, fungi, and viruses), parasites, and insects, proving to be potential antimicrobial, antiparasitic, and insecticidal agents, respectively. Due to their complex nature, effects on the central nervous system have also been demonstrated, as well as their sedative, anxiolytic, and antidepressant properties (Dougnon and Ito 2020; Alves et al. 2023). Also, other applications of essential oils are reported, such as in agriculture, veterinary medicine, cosmetics, and aromatherapy, which will be discussed in different sections of this chapter (de Sousa et al. 2015; Mossa 2016; Bedini et al. 2019; Batista De Oliveira et al. 2020).

Thus, considering their historical context, costs, and scientific importance, the present chapter aimed at presenting an updated literature review based on evidence about the benefits and applicability of oils for therapeutic purposes.

2 Biological Activities

2.1 In Vitro

2.1.1 Antimicrobial Activity

The therapeutic potential of natural products and some of their constituents has been the object of several studies. It is noted that many of these compounds have contributed to the obtaining of several drugs of ample clinical use. Thus, more substances might be used in the future as medicinal agents (Bertin Carnevalli and Paula Serra de Araújo Resumo 2013).

Infections caused by microorganisms are widespread worldwide, especially in underdeveloped countries, and have been one of the leading causes of human morbidity and mortality (Górniak et al. 2019). Therefore, studies related to the biological activity of plants represent a great challenge for discovering and identifying new drug prototypes. It was not until 1928 that penicillin, the first proper antibiotic, was discovered by Alexander Fleming. Later, in the 1930s, sulfa drugs and arsenic were also identified (Bashir et al. 2016; Lima et al. 2022).

Antimicrobials have played an essential role over the past 60 years in treating diseases caused by microorganisms. However, a frequent increase in resistant bacteria has been observed (Saleem et al. 2010).

On the other hand, plants have been widely applied in the treatment of various diseases, and in 2007 it was observed that about 25% of available drugs were derived from plants used in folk medicine (Cushnie et al. 2008).

Essential oils, which are secondary metabolites of plants with differentiated biological properties, have also drawn attention for being an alternative against resistant strains of microorganisms.

Studies have shown that several plant families have essential oils with antimicrobial activity determined by chemical characterization. Data are show in Table 1 (Amorim et al. 2011; Aparecida Andrade et al. 2012; Sarrazin et al. 2012; Bedoya-Serna et al. 2018; Krishnamoorthy et al. 2021; Wintola et al. 2021; Badekova et al. 2021; Santos et al. 2021; Zhao et al. 2021).

2.1.2 Antioxidant Activity

Secondary metabolites with antioxidant properties are compounds that have retarding effect on oxidation rates, which occur by excessive production of oxygen-free radicals from pathophysiological processes or environmental causes. These substances have been increasingly exploited in food products, cosmetics, and pharmaceuticals, due to their antioxidant protection against cellular aging (Djeridane et al. 2006).

Several scientific studies have demonstrated the importance of essential oils as valuable sources of antioxidant compounds. Some species were tested by FRAP

Table 1 Antimicrobial activity of essential oils

Title	Authors	Study objectives	Methods	Main results
Enhanced antibacterial effect of antibiotics by the essential oil of *Aloysia gratissima* (Gillies & Hook.) Tronc, and its major constituent beta-caryophyllene	Santos et al. (2021)	Characterize the chemical profile and evaluate the antibacterial capacity and antibiotic activity of essential oil obtained from *Aloysia gratissima* (EOAG) and β-caryophyllene	Analysis of antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* by determining the MIC	A reduction in the MIC of the antibiotics against strains treated simultaneously with the essential oil or β-caryophyllene was observed
Chemical composition, antioxidant activities and antibacterial activities of essential oil from *Erythrina caffra* Thunb. growing in South Africa	Wintola et al. (2021)	Antibacterial analysis of the essential oil of *Erythrina caffra* Thunb	In vitro antibacterial susceptibility assay by agar diffusion method	The susceptibility study showed that all bacterial isolates were susceptible to essential oil. with the exception of *Salmonella typhimurium* and *Pseudomonas aeruginosa*
Composition and screening of *Origanum vulgare* essential oil for antimicrobial activity	Badekova et al. (2021)	This study aims to formulate a new *Origanum vulgare* anticaries dental gel with high antimicrobial activity	The effectiveness of *O. vulgare* essential oil was tested in vitro for *Streptococcus mutans* biofilm using colorimetric analysis	*O. vulgare* essential oil inhibited the growth of S. mutans biofilm by 98% compared with unexposed control bacteria ($p < 0.05$)
Chemical composition and antifungal activity of essential oil from *Origanum vulgare* against *Botrytis cinerea*	Zao et al. (2021)	Characterization of the chemical composition and the antifungal activity of the *Origanum vulgare* essential oil	*B. cinerea* in vitro mycelial growth and spore germination	*O. vulgare* EO exhibited high antifungal activity against *B. cinerea* in vitro and in vivo

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Table 1 (continued)

Title	Authors	Study objectives	Methods	Main results
Antifungal activity of nanoemulsion from *Cleome viscosa* essential oil against food-borne pathogenic *Candida albicans*	Krishnamoorthy et al. (2021)	Pathogenic and spoilage fungi cause enormous challenges to food related fatal infections. Plant essential oil based classical emulsions can functions as antifungal agents	The minimum inhibitory and fungicidal concentration of essential oil nanoemulsion (EONE) was tested against food borne pathogenic C. albicans	The MIC and MFC values ranged from 16.5 to 33 ml/ml with significant reduction on biofilm of C. albicans isolates
Antifungal activity of nanoemulsions encapsulating oregano (*Origanum vulgare*) essential oil: in vitro study and application in Minas Padrão cheese	Bedoya-Serna et al. (2018)	The objective of this study was to evaluate the antifungal activity of nanoemulsions encapsulating essential oil of oregano (*Origanum vulgare*), both in vitro and after application on Minas Padrão cheese	Minimal inhibitory concentrations of nonencapsulated and encapsulated oregano essential oil were determined	Nanoencapsulated oregano essential oil presented an inhibitory effect against the three genera of fungi evaluated. I
Chemical characterization and antibacterial activity of essential oils from medicinal and condiment plants against *Staphylococcus aureus* and *Escherichia coli*	Millezi et al. (2014)	Analyze the activity against microorganisms, for use in the food industry through the minimum inhibitory concentration (MIC)	Determination of MIC on *Staphylococcus aureus* ATCC 2592 and *Escherichia coli* ATCC 25922	The MIC of the oils tested against *E. coli* and *S. aureus* was 1.5%, except for the essential oil from *S. montana* on *S. aureus*, which was sensitive to this oil from the concentration of 5.0%
Chemical composition and antimicrobial activity of the essential oil of *Lippia grandis* Schauer (Verbenaceae) from the western Amazon	Sarrazin et al. (2012)	Analyze the antimicrobial potential of extracts and essential oils from several species of Lippia against several different microorganisms	In vitro antimicrobial susceptibility assay by agar diffusion method	The essential oil was effective against 75% of the micro-organisms analyzed, in particular, *S. aureus, E. faecalis,* and *E. coli*

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Table 1 (continued)

Title	Authors	Study objectives	Methods	Main results
Essential oils of *Cinnamomum zeylanicum, Cymbopogon nardus and Zingiber officinale:* composition, antioxidant and antibacterial activities	Aparecida Andrade et al. (2012)	The aims of this study were to chemically characterize and to evaluate the antioxidant and antibacterial activities of the citronella, cinnamon and ginger essential oils	Evaluation of antibacterial activity was performed by using agar well diffusion method, with *S. aureus*, *L. monocytogenes*, *E. coli*, *S. cholerasuis*, and *P. aeruginosa*	The essential oils showed antibacterial activity for both Gram-negative and Gram-positive microorganisms, and the most efficient was C. zeylanicum essential oil
Antibacterial activity of essential oils and extracts on the development of Ralstonia Solanacearum in banana seedlings	Amorim et al. (2011)	This study aimed to evaluate the activity of different concentrations of essential oils and plant extracts to the control of *Ralstonia solanacearum*	In vitro antimicrobial susceptibility assay by agar diffusion method against phytopathogen *Ralstonia solanacearum*	The ginger extract, citronella, clove and ginger oils were able to inhibit the growth of R. *solanacearum* at all concentrations tested, with emphasis on clove oil, followed by ginger extract

(ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods, and satisfactory results were found (Baschieri et al. 2017).

From the kinetic evaluation of antioxidant behavior in nonphenolic compounds, it was found that limonene, linalool, and citral acted as enhancers of the antioxidant effect. Although the tests were performed in a narrow concentration range with some oxidizable substrates, these compounds enhanced the antioxidant potential of essential oils (Bączek et al. 2017).

The profile of some essential oils was evaluated, and the following species presented antioxidant activity: *Tanacetum parthenium*, *Cymbopogon nardus*, *Origanum vulgare, Foeniculum vulgare, Thymus serpyllum, Xylopia aromatica, Piper nigrum, Syzygium aromaticum, Cymbopogon citratus, Lippia alba,* and *Piper marginatum,* as shown in Table 2 (Hurtado et al. 2016; Bączek et al. 2017; Kačániová et al. 2017; Morshedloo et al. 2018; Farias et al. 2019; Costa et al. 2021).

Studies on free radicals and the development of new methods to evaluate antioxidant activity have increased considerably over the years, as evidenced in published papers. The knowledge of the effects of free radicals on cells and their relationship with some diseases, acting as catalysts in cellular aging, stimulated the search for new organic substances, mainly obtained from plant products capable of preventing or minimizing oxidative damage to living cells 46.

Table 2 Antioxidant activity of essential oils

Title	Authors	Study objectives	Methods	Main results
Antibacterial and antioxidant activity of essential oils and extracts from costmary (Tanacetum balsamita L.) and tansy (Tanacetum vulgare L.)	Baczek et al. (2017)	Comparison of *Tanacetum balsamita* L. (costmary) and *Tanacetum vulgare* L. (tansy) in terms of the antibacterial and antioxidant activity of essential oils and hydroethanolic extracts in relation with their chemical profile	DPPH scavenging reaction and ferric reducing antioxidant power (FRAP) assay	The results obtained in the present study do not indicate on the relationship between the presence of identified flavonoids and the antioxidant activity of the investigated Tanacetum extracts
The antioxidant and antimicrobial activity of essential oils against Pseudomonas spp. isolated from fish	Kačániová et al. (2017)	Determination the antibacterial and antioxidant activity of 21 EO against 10 Pseudomonas species isolated from freshwater fish	Free radical scavenging activity of samples was measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH)	The EOs of Cymbopogon nardus, *Origanum vulgare*, *Foeniculum vulgare*, and *Thymus serpyllum* showed the highest antioxidant activity
Antioxidant activity of ethanolic extracts and essential oils from *Xylopia aromatica* and *Piper nigrum*	Costa et al. (2021)	Compare the content, activity antioxidant and chemical composition of essential oils from monkey and black pepper fruits (white and black)	DPPH scavenging reaction and ferric reducing antioxidant power (FRAP) assay	The ability to reduce DPPH was 21.13% for PM, and 12.68% and 5.48% for PP and PB, respectively
Antioxidant activity and characterization of the essential oil from the roots of *Piper marginatum* Jacq.	Bay-Hurtado et al. (2016)	This study aimed to extract, identify and quantify the essential oil of fresh roots, as well as its antioxidant activity	DPPH scavenging reaction and ferric reducing antioxidant power (FRAP) assay	The following CE50 and %AA values were found: Ginkgo biloba (used as reference) 46.96 mg/L and 75.26 mg/L for the essential oil from the roots of *P. marginatum*

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Table 2 (continued)

Title	Authors	Study objectives	Methods	Main results
Antioxidant activity of essential oils from condiment plants and their effect on lactic cultures and pathogenic bacteria	Farias et al. (2019)	Evaluation the antioxidant properties and antimicrobial activity of essential oils deriving from *Syzygium aromaticum*, *Cymbopogon citratus*, and *Lippia alba* against lactic and pathogenic bacteria responsible for food-borne diseases	The Free Radical Scavenging Capacity (RSC) was found by measuring the scavenging activity of essential oils assessed in 2.2-diphenyl-1-picrylhydrazyl (DPPH) and OH radicals	The essential oil of S. aromaticum presented better antioxidant activity, with IC_{50} equal to 5.76 μg/mL and antioxidant activity index of 6.94, and it was considered strong (AAI > 2.0) in comparison to the other evaluated oils
Chemical composition and antioxidant activity of essential oils in *Origanum vulgare* subsp. gracile at different phenological stages and plant parts	Morshedloo et al. (2018)	Determination the chemical composition and antioxidant activity of the essential oils of *Origanum vulgare* subsp. gracile in different plant parts and at different phenological stages	Free radical scavenging activity of samples was measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH)	All the essential oils exhibited high radical-scavenging properties as shown in the DPPH* assay

2.2 Antiparasitic Activity

Over the years, many essential oils have been characterized for their antiparasitic effect against pathogens capable of causing diseases in humans and animals (Setzer 2012; Bero et al. 2014; Dawood et al. 2021). Studies published between 1988 and 2012 concerning the evaluation of the antiprotozoal effect of essential oils revealed that until that moment, more than 60 plant species had shown activity on at least one protozoan. Essential oils were investigated against *Plasmodium ssp*., *Trypanosoma ssp*., *Leishmania ssp*., and other intestinal parasites, and it was noted that the main compounds were probably related to antiparasitic activity were monoterpenes, sesquiterpenes, and phenylpropanoids (Setzer 2012).

In addition, several essential oils from different species were tested for antiparasitic activity against *Plasmodium falciparum*, *Trypanosoma cruzi*, *Leishmania toxoplasma*, *Giardia lamblia*, *Entamoeba histolytica*, and *Schistosoma mansoni*. Data are described in Table 3 (Mota et al. 2012; Borges et al. 2012; Gonçalves et al. 2019; Ghadimi et al. 2020; Islam et al. 2020; Dawood et al. 2021).

Table 3 Antiparasitic activity of essential oils

Title	Authors	Study objectives	Methods	Main results
Systematic review on medicinal plants used for the treatment of Giardia infection	Alnomasy et al. (2021)	This study was aimed at systematically reviewing the existing literature in herbal medicines to treat giardiasis	Preclinical Systematic Review and Meta-Analysis Facility (SyRF) database	The plant-based anti-Giardia agents are very promising as alternative and complementary resource for treating giardiasis since had low significant toxicity
Antiparasitic and Antibacterial Functionality of Essential Oils: An Alternative Approach for Sustainable Aquaculture	Dawood et al. (2021)	To explore the effectiveness of EOs against fish parasites and pathogenic bacteria as an environment-friendly phytotherapeutic in the aquaculture industry	Preclinical Systematic Review	Essential oils (EOs) show beneficial effects on growth, immunity, antibacterial and antiparasitic activities in fish culture and are used as anesthetic compounds during fish handling and transportation
Trypanocidal and cytotoxic activities of essential oils from medicinal plants of Northeast of Brazil	Borges et al. (2012)	Determination of the antiparasitic activity of essential oils extracted from traditional medicinal plants in the search for alternatives for the treatment of Chagas disease	In vitro assay of trypanocidal activity	All essential oils tested demonstrated an inhibitory effect on the parasite growth and survival. L. sidoides and L. origanoides essential oils were the most effective against trypomastigote and amastigote forms respectively
The leishmanicidal activity of essential oils: A systematic Review	Ghadimi et al. (2020)	To explore the effectiveness of EOs against *Leishmania amazonensis*, *Leishmania infantum*, and *Leishmania major*	Systematic Review	Frequently, substantial differences were found between the observed IC_{50}s of one EO against promastigotes of different species of Leishmania

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Table 3 (continued)

Title	Authors	Study objectives	Methods	Main results
In vitro Anti-parasitic Activity of Pelargonium X. asperum Essential Oil Against *Toxoplasma gondii*	Huang et al. (2021)	In this study, five essential oils (EO) were screened for their antiparasitic activity against *T. gondii*	The cytotoxicity of essential oils was evaluated using the MTT assay on human foreskin fibroblast cells	Only PaEO exhibited antiparasitic activity, and inhibited the growth of *T. gondii* in a dose-dependent manner
Anti-Schistosoma mansoni effects of essential oils and their Components	Islam et al. (2020)	This review aimed at summarizing available in vitro, in vivo, and clinical trials showing evidence and mechanisms of actions of essential oils and their derivatives acting against S. mansoni	Systematic Review	The findings suggest that a number of essential oils and/or their components act against S. mansoni
In Vitro and In Vivo Antimalarial Activity of Essential Oils and Chemical Components from Three Medicinal Plants Found in Northeastern Brazil	Mota et al. (2012)	Determination of the antiparasitic activity against the human malaria parasite, *P. falciparum* (K1 strain) and the in vivo activity of EOs in mice infected with *P. berghei*	The acute toxicity of these oils was assessed in healthy mice and in vitro cytotoxicity was determined at different concentrations against HeLa cells and mice macrophages	This is the first study showing evidence for the antimalarial activity of these species from northeastern Brazil and the low toxicity of their EOs

2.3 Anti-Inflammatory Activity

The human body has defense mechanisms against various external agents given by the immune system. Such a network of cells and molecules is characterized by recognizing and developing responses of destruction or inactivation in the presence of these external agents (Abbas and Janeway 2000).

Inflammation is a defense response of the immune system that occurs after cellular damage caused by external agents, such as bacteria, fungi, viruses, and protozoa, as well as by physical agents, chemicals, tissue necrosis, and immune reactions (Miguel 2010).

Fundamentally, inflammation is a protective response to fight foreign body invasion (Zuzarte et al. 2013). The increase in infection-stimulating agents associated with diseases that have no effective treatment due to resistance and adverse

reactions to drugs, has stimulated the development of less toxic, more effective, and cheaper medicines for the control of such diseases (da Silveira E Sá et al. 2014).

Essential oils are beautiful regarding the production of new interfering agents for the organism's homeostasis. The secondary metabolites in essential oils, especially terpenes, present several bioactivities, including anti-inflammatory effects with low incidence of adverse effects. The profiles of some essential oils with anti-inflammatory properties were evaluated. They belong to the botanical families Myrtaceae, Apiaceae, Lamiaceae, Cyperaceae, and Verbenaceae, as can see in Table 4 (Mendes et al. 2010; de Bupleurum et al. 2013; Lv et al. 2015; da Silva et al. 2019; Ma et al. 2021).

Table 4 Anti-inflammatory activity of essential oils

Species	Authors	Majority compounds	Methods	Main results
Eugenia dysenterica DC.	Silva et al. (2019)	β-cariofileno, α-humuleno	Inhibition of lipopolysaccharide (LPS) induced nitric oxide (NO) production in the macrophage cell line (RAW 264.7)	The inhibition of nitric oxide by oEd and α-humulene suggested an anti-inflammatory effect.
Bluperium rigidum	Zuzarte et al. (2021)	α-pineno, β-pineno, limoneno	Inhibition of NO production	The essential oil of B. rigidum subsp. the anti-inflammatory exerts inhibitory activity and effects on the production of N.O., with no toxicity
Origanum Vulgare	Mir et al. (2021)	Timol, carvacrol, p-cimeno, borneol, Linalol acetato de linalilo, α-pineno, α-terpineno, β-bisabolol, β-cariofileno	Inhibition of proinflammatory cytokine	Inhibition of the production of inflammatory mediators such as TNF-α, IL-1β, NO, and PGE2 in RAW 264.7 macrophages
Cyperus articulares	Mir et al. (2021)	Monoterpenos, Sesquiterpenes, Cetonas, Sesquiterpênicas	Inhibition of the production of inflammatory mediators such as TNF-α, IL-1β, NO, and PGE2 in RAW 264.7 macrophages	OECA exerts potent anti-inflammatory activity and inhibitory effects on the production of important inflammatory mediators such as TNF-α, IL-1β, NO, and PGE2 in macrophages of the RAW 264.7 lineage
Lippia gracilis	Mendes et al. (2010)	Limoneno, β-cariofileno, p-cimeno cânfora, linalool, α-pineno, timol	Anti-inflammatory activity of the EO was evaluated using paw edema and peritonitis methods	The EO of Lippia gracilis leaves shows antinociceptive and anti-inflammatory activities

2.4 Antiviral Activity

Viral infections are a significant threat to human health. Many diseases have no effective treatments, and drug resistance threatens standard therapies' effectiveness. Drug resistance can arise in the presence of antivirals or due to the pre-existence of reduced susceptibility to such medications (Munir et al. 2017). Viral resistance and recurrent infections in immunocompromised patients require the development of new compounds with antiviral properties, which lead to the utilization of medicinal plants with significant pharmacological activities (Choi 2018).

The antiviral properties of essential oils are predominantly due to the presence of secondary metabolites such as cineole, linalool, β-pinene, linalyl acetate, thymol, and carvacrol (Feriotto et al. 2018; Panikar et al. 2021).

The antiviral activity of plant essential oils has been widely studied for severe acute respiratory syndrome (SARS) caused by coronavirus 2, acting synergistically with other drugs (da Silva et al. 2020; Panikar et al. 2021). Fourteen compounds derived from essential oils from Brazilian plants showed virucidal properties against herpes simplex virus 1 and 2 (HSV-1 and -2), dengue, Zika, and yellow fever. Also, they presented low toxicity as an anti-coronavirus agent (Carson et al. 2001).

Herpes simplex virus (HSV) is one of the most common causes of human viral infections, being responsible for encephalitis, dermatitis, genitourinary infections, and cervical cancer (Brezáni et al. 2018; Almeida et al. 2022). There are reports of the antiviral activity of *Eucalyptus globulus* essential oil against HSV-1. It demonstrated excellent efficacy when compared with acyclovir, the standard drug in clinical use for treating such diseases (Santoyo et al. 2014).

The essential oils of the species *Thymus vulgaris*, *Thymus hyemalis,* and *Thymus zygis* demonstrated, in vitro assays, antiviral action on HSV-1 by intracellular inhibition of viral replication, as well as prevented viral adhesion to host cells (Vanti et al. 2020). *Melissa officinalis* essential oil showed antiviral activity against HSV-1 when used in gallbladder infections (Tseliou et al. 2019). Also, essential oils extracted from *Melaleuca alternifolia* topically applied in a clinical trial showed antiviral efficacy against herpes labialis (Brezáni et al. 2018).

Upper respiratory tract infections caused by influenza viruses of types A, B and C are characterized by their high mutation rates. An in vitro trial demonstrated that the combination of essential oils of *Thymbra capitata* (L) Cav., *Origanum dictamnus* L., *Salvia pomifera* L., and *Salvia dendata* showed inhibitory effect on influenza A virus subtype H1N1, influenza B, as well as antiviral effect on human rhinovirus 14 (HRV-14) (Dorra et al. 2019).

Essential oils extracted from various plants demonstrated antiviral activity against the influenza A virus, with lower cytotoxicity compared to oseltamivir, an antiviral drug used to prevent and treat this disease. The species with the highest antiviral activity were *Thymus mastichina* L., *Salvia sclarea* L., and *Pimpinella anisum* L., with linalool being the common component to all (Feriotto et al. 2018).

Human immunodeficiency virus (HIV), a lentivirus of the retrovirus subgroup, causes the destruction of immune cells, and the most advanced stage of this

infection is acquired immunodeficiency syndrome (AIDS). Species *Rosmarinus officinalis*, *Thymus vulgaris*, and *Cymbopogon citratus* demonstrated antiviral action by inhibiting HIV-1 transcription, and may be promising sources of antiviral drugs for patients refractory to standard antiretroviral therapies (Panikar et al. 2021). Antiviral activity of essential oils against HIV has also been reported.

For many viral infections, there is no effective treatment available. This occurs due to the narrow spectrum of antivirals and in response to resistance and mutations of viruses (Ma and Yao 2020). Essential oils are considered solid therapeutic agents for viral diseases and prototypes of new antiviral drugs (de Sousa et al. 2015).

2.5 Anxiolytic and Antidepressant Activities

Souza et al. (Zhang and Yao 2019), who systematically reviewed essential oils with anxiolytic-like effects in animal models, listed more than 30 plant species whose oils presented such action until 2014. *Lavandula angustifolia* showed the best anxiolytic profile, and *Citrus aurantium* showed significant effects in various animal models when administered by different routes. Other essential oils considered promising were those of *Achillea wilhelmsii*, *Alpinia zerumbet*, *Citrus sinensis*, *Citrus aurantium*, *Spiranthera odoratissima*, and *Citrus bergamia*.

Zhang and Yao (2019) obtained essential oils from plants of the Lamiaceae and Rutaceae families, and in addition to volatile oils with characterized anxiolytic effects, the authors presented a variety of plant species investigated in clinical tests. Among the essential oils clinically evaluated, that obtained from *Lavandula angustifolia* was the most investigated, capable of relieving anxiety by inhalation, topical application, or ingestion.

Some essential oils have been shown to promote more than one effect on the central nervous system. This is the case of *Boswellia* sp., *Cananga odorata, Cinnamomum verum, Citrus aurantium, Citrus bergamia, Citrus sinensis, Cymbopogon citratus, Lavandula angustifolia, Citrus paradisi, Rosa damascena, Rosmarinus officinalis,* and *Salvia sclarea*, which have been characterized in preclinical and/or clinical trials as having both anxiolytic and antidepressant activities (Irie et al. 2004).

Regarding their antidepressant effect, essential oils of several species have been investigated in vivo, including those obtained from *Asarum heterotropoides, Citrus limon, Eugenia uniflora, Perilla frutescens, Salvia sclarea, Syzygium aromaticum, Toona ciliata* var. *yunnanensis,* and *Valeriana wallichii* were the most promising. Many compounds with antidepressant action have been detected in volatile oils, with eugenol and linalool being the most studied (Tao et al. 2005; Guzmán-Gutiérrez et al. 2012, 2015; de Sousa et al. 2017; Chandharakool et al. 2020).

The ability that essential oils have to act on different neural pathways without having the side effects of synthetic drugs makes them potential alternatives for treating mental disorders, including depression, anxiety, and dementia (Pharm n.d.).

2.6 *Sedative Activity*

In various species, the main components of essential oils responsible for their sedative effect are linalool, limonene, γ-terpinene, borneol, isovaleric acid, α-pinene, 1,8-cineole, sabinene, and β-caryophyllene, as can be seen in Fig. 1 (Can and Sümer 2019; Hirai and Ito 2019; Zhong et al. 2019; Das et al. 2021; Koriem 2021; Pharm n.d.). And due to their structure, they can cross the blood–brain barrier. For instance, *Lantana camara* and *Ocimum basilicum* essential oils were demonstrated to have strong sedative activity when administered in vivo by inhalation (Guzmán-Gutiérrez et al. 2012; Dougnon and Ito 2020).

In tests with volunteers, an electroencephalogram showed that the diluted essential oil of Citrus tangerine presented sedative activity by effectively decreasing alpha and beta wave power and increasing theta brain waves (Pharm n.d.). When evaluating neuronal waves, inhalation of *Michelia alba* essential oil resulted in sedative activity in humans (Sattayakhom et al. 2021). Similarly, inhalation of *Litsea cubeba* essential oil showed a sedative effect in a human model by a reduction in alpha and beta wave power involving frontal, temporal, parietal, and occipital lobes of the brain (Shan et al. 2021).

Regarding studies on animals, species *Valeriana officinalis* and *V. jatamansi* demonstrated sedative effects, possibly due to interaction with the GABAA receptor (Can and Sümer 2019). In rats, intraperitoneal administration of the essential oil extracted from *Citrus aurantium* flowers was shown to promote sedative-hypnotic activity by potentiating the chloride ion-mediated GABAA receptor (Viana et al. 2020). Similarly, *Citrus limon* essential oil presented a sedative-hypnotic effect without causing motor coordination deficit in rats (Diniz et al. 2019). *Annona vepretorum* essential oil also demonstrated a sedative effect in vivo (Abbasi-Maleki et al. 2020); low concentrations of the essential oils of *Mentha piperita* and *Lavandula angustifolia* also showed a sedative effect in fish (Maïga et al. 2019).

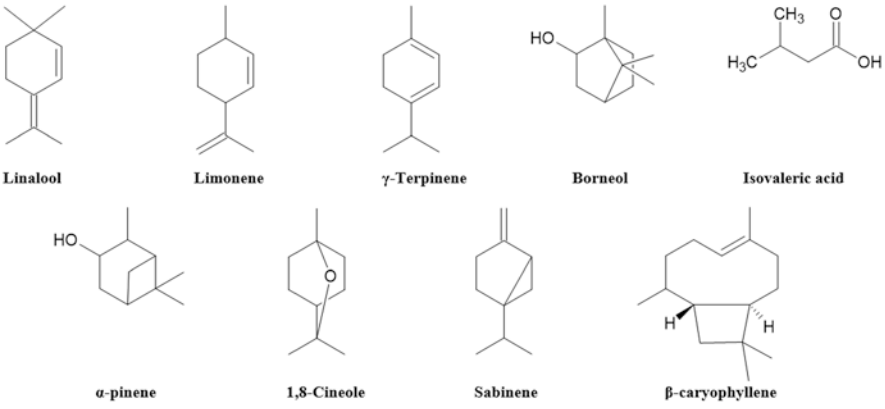

Fig. 1 Majority components of essential oils responsible for their sedative activity

Patients who take conventional antidepressants need these drugs to have the least possible adverse effects. Thus, medicinal plants may be a viable alternative with reduced side effects and greater treatment efficacy (Zhong et al. 2019).

2.7 Repellent and Insecticidal Activities

It is common knowledge that many insects can act as vectors in the transmission cycles of disease-promoting agents in humans, posing severe risks to public health worldwide. *Aedes aegypti*, for instance, is responsible for carrying pathogens that cause dengue, urban yellow fever, chikungunya, and Zika, while *Culex quinquefasciatus* and mosquitoes of the genus Anopheles are related to the transmission of filariasis and malaria, respectively (Enan 2001; Samy et al. 2016; Najafi-Sharjabad et al. 2022).

The mechanism of action involved in the insecticidal activity of essential oils is not completely clarified, but their toxicity indicates a neurotoxic mode of action. The most prominent symptoms are hyperactivity followed by hyperexcitation, leading to rapid knockdown, immobilization, and death of the insect (Prajapati et al. 2005; Pavela 2016).

Considering the importance of repellents and insecticides to prevent mosquito-borne diseases, many studies have focused on the search for essential oils that present such properties (Nerio et al. 2010; Said- et al. 2017; Castillo et al. 2017; Stevenson et al. 2017). And among the oils tested, several species proved to have insecticidal and repellent actions, as seen in Table 5.

With population growth and changes in global eating habits, the need to improve crop productivity intensifies the interest in insect pest control. In addition to losses caused during the crop-growing process, storage pests can subsequently cause damage to agricultural products stored in barns and warehouses. Thus, essential oils have been investigated to obtain insecticides that can be used in the farming sector (Prajapati et al. 2005; Demeter et al. 2021).

2.8 Essential Oils in Veterinary Medicine

Essential oils can effectively act as insecticides and repellents. Because they generally have low mammalian toxicity and high biodegradability, essential oils are considered promising agents for developing low-toxicity and eco-friendly products for pest control (Zhai et al. 2018; Nehme et al. 2021).

The larvae of flies that belong to the families Oestridae and Calliphoridae impair the welfare of domestic and wild animals due to the incidence of myiasis. The essential oils of *Clinopodium nubigenum* and *Lavandula angustifolia* Mill. demonstrated harmful activity by contact and/or fumigation against eggs and adults of the blowfly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), which is the agent

Table 5 Repellent and insecticidal activities of essential oils

Species	Indication	Vector
Cymbopogon citratus and *Cymbopogon nardus*	Repellent activity	*Aedes aegypti, Anopheles dirus,* and *Culex quinquefasciatus*
Cymbopogon winterianus	Repellent activity	*Aedes aegypti and Culex quinquefasciatus*
Eucalyptus tereticornis and *Eucalyptus deglupta*	Repellent activity	*Culex quinquefasciatus*
Eucalyptus nitens and Eucalyptus citriodora	Repellent activity	*Aedes aegypti*
Ocimum basilicum	Repellent activity	*Anopheles*
Ocimum gratissimum	Repellent activity	*Aedes aegypti*
Ocimum americanum	Repellent activity	*Aedes aegypti, Aedes dirus and Culex quinquefasciatus*
Lippia origanoides	Insecticidal activity	*Aedes aegypti*
Citrus sinensis	Insecticidal activity	*Aedes aegypti*
Cananga odorata,	Insecticidal activity	*Aedes aegypti*
Cymbopogon flexuosus	Insecticidal activity	*Aedes aegypti*
Lippia alba	Insecticidal activity	*Aedes aegypti*
Eucalyptus citriodora	Insecticidal activity	*Aedes aegypti*
Cananga odorata	Insecticidal activity	*Aedes aegypti*

responsible for the parasitic infestation in living mammals (myiasis) (Zhai et al. 2018). In a test with 25 essential oils, 16 showed insecticidal activity against wheat weevil, *Sitophilus granarius*, being considered the main pest of stored grains. The essential oils with the highest toxicity to *Sitophilus granaries,* when applied to grains, were those from *Allium sativum*, *Gaultheria procumbens* L., *Ocimum sanctum* L., *Mentha arvensis*, *Thymus vulgaris,* and *Eucalyptus dives* (Zhai et al. 2018).

Essential oils have multiple promising effects on rumen microbiota and can cause changes in rumen fermentation of cattle, pigs, goats, and poultry (Nocera et al. 2020; Nehme et al. 2021).

In vitro tests, essential oils from *Cinnamomum zeylanicum, Melissa offcinalis,* and *Leptospermum scoparium* demonstrated antibacterial and bactericidal activity against resistant strains of *Staphylococcus pseudintermedius* isolated from canines with pyodermitis (Rust 2020). And essential oils from *Schinus mole* L., *Cinnamomum osmophloeum, Taiwania cryptomerioides, Plectranthus amboinicus, Ocimum gratissimum,* and *Cinnamomum* spp. are toxic to cat fleas (*Ctenocephalides felis*) 106.

2.9 Aromatherapy

The Food and Drug Administration (FDA) guidelines of the United States classify essential oils for aromatherapy as cosmetics because they are not drugs for the treatment and/or prevention of diseases (Farrar and Farrar 2020). These oils are used daily for their aromas, with different applications, such as perfumes, shampoos, air fresheners, fabric softeners, food flavorings, and health care. They promote comfort and balance, thus reducing symptoms of depression and stress. The oils are obtained by steam distillation or cold press from different plant parts: seeds, flowers, bark, fruits, roots, and rhizomes (Cristina De Souza et al. 2019).

It was observed in most articles that *Lavandula angustifolia* essential oil was the most used in pain relief during labor, justified by its major compounds, linalool, and linalyl acetate, which seemed to contribute to the action of female hormones, increasing concentration, and tranquility (Lima et al. 2021). Recent studies evidence the activity of this oil on the nervous system, decreasing stress and anxiety (Montibeler et al. 2018).

The hospital environment presents high levels of occupational stress due to the constant contact with suffering and pain. Thus, creating strategies to minimize stress becomes increasingly relevant, which may improve healthcare workers' quality of life. Aromatherapy associated with massage in surgical centers has been used to reduce stress levels through the relaxation resulting from exposure to the aroma of essential oils. From an experimental study, it was observed that this practice has contributed to the decrease in heart rate and blood pressure of these professionals. The essential oils used were those from *Lavandula angustifolia* and *Pelargonium graveolens* (Buckle 2019).

When used correctly and with pure essential oils, aromatherapy will always be a vital resource in the search for self-care and improvement of quality of life. This therapy has a multiprofessional character, presenting several possibilities of use, depending on the types of treatment and administration. The simultaneous reach of physical, psychic, and spiritual dimensions is also considered (Kadunc et al. 2012).

3 Final Considerations

Essential oils can be used in a broad spectrum of possibilities related to several areas. They also present specific actions, such as antimicrobial and antiparasitic activities against *Plasmodium Falciparum, Trypanosoma Cruzi, Leishmania Toxoplasma, Giardia lamblia, Entamoeba histolytica,* and *Schistosoma mansoni.*

In addition, essential oils may be potent therapeutic agents for many viral infections for which no effective drug treatment exists. Thus, prototypes of new antiviral drugs may be developed since they are less toxic and residue-free.

Essential oils also have shown importance in formulating repellents and insecticides as a type of prevention against diseases transmitted by mosquitoes since many

insects can act as vectors in transmission cycles, representing severe risks to public health worldwide.

Another possibility of application was demonstrated with a sedative-hypnotic effect without causing a deficit in animal motor coordination. Also, the potential use of essential oils for aesthetic purposes and rejuvenation has been verified, increasing their demand.

Finally, it was found that essential oils do not present toxic characteristics and are a viable alternative to synthetic antioxidants with anticarcinogenic effects. They also enable a decrease in degradation products caused by harmful compounds in industries, which allows a new perspective for possible applications of these oils, increasing their range of use in pharmaceutical, food, agriculture, beverage, aromatherapy, and cosmetic industries.

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Natural Products from the Amazon Used by the Cosmetic Industry

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Abstract The Amazon rainforest is known for its incredible biodiversity, which has made it a rich source of natural products used in the cosmetics industry. Over the years, a number of cosmetic companies have turned to the Amazon in search of unique and effective ingredients for their products. One of the most popular ingredients used in cosmetics derived from the Amazon is the açai berry. Açai is a powerful antioxidant that is rich in vitamins and minerals and is believed to have antiaging properties. While the use of natural products from the Amazon has been embraced by the cosmetic industry, it is not without its challenges. One of the biggest challenges is ensuring that the products are harvested in a sustainable and ethical manner. Many of the plants used in cosmetics are native to the Amazon and are often harvested by local communities who rely on these resources for their livelihoods. Here, we examine the use of natural products from the Amazon by the cosmetic industry and the potential benefits and challenges associated with their use.

Keywords Amazon · Compound · Cosmetic industry · Natural product

1 Introduction

The Amazon is the region with the most incredible biodiversity on the planet and the largest biome in Brazil. Sources of plant species are of great importance in the world because they have extractive processes from their native species (Antunes et al. 2021; Li et al. 2023). The fruits from these plants have diversified chemical compositions with biological properties, such as significant economic, nutritional,

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and therapeutic ones, so that these fruits have awakened great interest in both scientific and industrial circles for uses such as renewable fuels, foods, pharmaceutical products, and cosmetics, among others (Bruno and Almeida 2021; Alves et al. 2023). The Amazonian territory has undergone several stages of development, generating potential new resources. Therefore, studies in the Amazon aim to discover new nutritional and functional sources that are beneficial for human health. The vast diversity of bioactive components inspires ongoing investigations into gathering information on their physicochemical, chemical, biological, and sensory properties (da Silva and Pierre 2021; da Silva et al. 2023).

Biotechnology is still characterized as a new area of research. Still, it has shown great promise for regional development, combined with bioeconomy, thus showing new resources from the Amazonian fauna and flora for developing new pharmaceutical products from this region (Santos et al. 2021). The current focus of the bioindustry is on the high demand for investments in plant raw materials for formulations that have beneficial effects on human skin, leading to positive changes in its structure and functions (Funasaki et al. 2016). In this context, developing pharmaceutical products from plant ingredients is crucial, resulting in higher-quality, safer, more-effective, and more-innovative products. There is broad interest in products derived from Amazonian biodiversity, both nationally and internationally, primarily when the scientific research on raw materials produces satisfactory results (Lima et al. 2022).

The phytocosmetics market is one of the most promising. Currently, there is a strong demand for products that do not harm the skin and do not contain toxic ingredients in their composition. This strong growth in demand for natural and vegan products, free of synthetic chemicals and not tested on animals, comes from public awareness of artificial products (Liu and Hong 2016). The production chains of cosmetics and herbal medicines are expanding, and companies and institutions are modernizing in response to productive communities, whether scientific or business (Martins et al. 2016). With this, phytocosmetics and the use of natural resources in the development of new bioproducts have been expanding in the current market, bringing new perspectives on the production of cosmetics. This theme is based mainly on the study aimed at applying knowledge of the action of active substances from species of the plant kingdom to personal hygiene, aesthetics, and the maintenance of the whole healthy state of the human body. The widespread interest in developing phytocosmetics with innovative characteristics has propelled expanding scientific knowledge on the therapeutic, pharmacological, and cosmetic properties of the Brazilian flora (Homma 2012).

The use of resources sustainably extracted from nature has increased significantly in today's industrial world, to fight diseases and keep the human body and its structures healthy. These resources are mainly due to the Amazonian plants and fruits with aromatic properties, research on which has already been an important worldwide trend in the field of phytocosmetics. The importance of this study is related mainly to the provision of new references and studies on the potential of these fruits in cosmetology, as well as the impulses of the Brazilian market for the cosmetic industry, which presents great economic potential and resources for the

commercialization of these products while taking social and environmental responsibility (Nobre et al. 2016).

The characteristics, particular properties, and various sources of the Amazon's plant derivatives, such as saponins, flavonoids, triterpene acids, mucilages, essential oils, and fixed oils, are discussed in this section. The occurrence of more than 8000 phenolic compounds in plants has already been detected. Flavonoids, for example, promote natural pigmentation and are widely distributed in the plant kingdom. Phenolic acids, flavonoids, and other plant polyphenols have been characterized as phytochemicals. In cosmetology, products containing them are used as functional cosmetics because they have therapeutic properties for the body. The functions of flavonoids in plant biology have been explored for their therapeutic activities, such as antioxidant, antifungal, bactericidal, and ultraviolet protection (Nicaretta et al. 2022).

In the last decades of the twentieth century, the environment has gained prominence among consumers' concerns, natural products are gaining more and more demand in the world market, and the pharmaceutical and cosmetic industries are obliged to respond to this natural and sustainable trend. The Amazon has always been a great source of raw materials thanks to its community that uses oils and extracts. The growing interest in phytotherapy and phytocosmetics has led to the search for information on the origin of plants, their chemical compositions, their levels of compatibility, and their actions in the human body because the various types of phytoderivatives can provide us with pharmaceutical ingredients such as astringents, emollients, humectants, tonics, stimulants, dyes, antiseptics, anti-inflammatories, and antioxidants (Maia and Andrade 2009).

The Amazon itself has numerous fruits with unusual characteristics, showing the bioavailability of the species in this region; fruit species cover 220 species of edible plants, with a percentage close to 44% of the diversity of native fruits in Brazil. This bioavailability of plants, especially native ones, has been promoting the heavy development of new bioproducts, mainly in the production of cosmetics (Giannino 2020).

The *Euterpe oleracea* Mart (açai), for example, is a complete food because it contains qualities such as dietary and functional fibers, relevant proteins, and antioxidant compounds. Its fruit shows a high concentration of anthocyanins and phenolic compounds (Gouvea and Kassicieh 2005). As described in the literature, the main constituents of açai are palmitic acid, oleic acid, and volatile acids; regarding its cosmetic use, studies discovered that it has febrifuge, purgative, repellent, healing, emollient, antiseptic, moisturizing, and softening effects (Morsello 2006).

The development of cosmetics from the fruit of cupuaçu (*Theobroma grandiflorum*) is also based on its various properties, particularly polyphenols, described as phytonutrients with antioxidant properties, but also amino acids, fatty acids, phosphorus, fiber, vitamin C, and vitamins B1, B2, and B3 (Garcia et al. 2014). These attributes offer excellent opportunities for the aesthetic and cosmetic industries because they can be used in products to delay the premature aging of the skin; they also improve elasticity and combat harmful actions of ultraviolet A (UVA) and

ultraviolet (UVB) rays, thus reducing wrinkles and reducing certain skin irritations and hydrating the skin (Peixoto Araujo et al. 2021).

One of the most powerful antioxidants, beta-carotene, is recognized for its broad cell-renewal capacity and ability to absorb radicals from visible and ultraviolet (UV) light, so Amazon ian fruit oils end up becoming extremely efficient in the pharmaceutical and cosmetic industries because they could be used to produce a natural sunscreen that reduces dryness and protects the skin (da Filho et al. 2010). Buriti oil (*Mauritia flexuosa* L.), for example, also contains carotenoids and tocopherols, which are often used in the field of cosmetology; according to the literature, the benefits of the oil include lubrication and the regeneration of the hydrolipidic barrier of the skin, which suffers from injuries from inadequate lighting or UV rays (Cândido and Silva 2017).

In cosmetic formulations, these carotenoids confer antiaging activities thanks to their high antioxidant action, with the potential to absorb UV radiation and capture reactive oxygen species and free radicals. They also have depigmentation and anti-acne activities. Other bioactive substances, such as fatty acids and phenolic compounds, are present in the chemical composition of several plant species in the Amazon (Koolen et al. 2018). Thus, carotenoids play important roles in cosmetic formulations because their antioxidant activities stimulate the synthesis of elastin and collagen, inhibit elastase, and reduce melanocyte and melanin levels. The depigmentation and the reduction of oiliness occur thanks to the functionality of the sebaceous glands, thus protecting the skin and preventing premature aging (Gouvea and Kassicieh 2005).

2 Cosmetic Industry

Industries have proven to be highly relevant in the income of countries after the pandemic crisis; the greatest effort is needed to achieve and maintain national sovereignty (Ansorge-Schumacher and Thum 2013). The cosmetic consumption index has been increasing since 2019, reaching about USD 95 million, and an increase of 2% to 5% is expected within the next five years. Such expansion is explained by the high level of pharmaceutical consumption, mainly in the United States but also in China and Brazil (Eshun and He 2004).

Sustainable development requires a balance between human development and the detrimental effects that human activities have on the diversity and functions of ecological systems (Rodrigues et al. 2015). According to the literature, sustainable development is an economic development that guarantees the production of services and products that can meet the needs of human beings but in a way that protects the environment (Kumar et al. 2006).

The growing movement of companies toward prioritizing environmental perspectives is closely linked to public demands and the controls and taxes from government regulations. Another factor to be highlighted is the possibility of building competitive advantages, resulting in developing and incorporating products in a

way that cares for the environment so that companies' images and reputations reflect sustainability values (Bom et al. 2019).

3 Amazonian Plant Species in the Production of Cosmetics

The plant species of the Brazilian biome have become known worldwide for having compounds with certain biological activities, so the Amazon is considered one of the greatest sources of ethnopharmacological knowledge (Merry et al. 2009). Studies on bioproducts, biopharmaceuticals, bioinputs, and sustainable technologies have been gaining some attention in industry, bringing raw materials that have strong potential for the development of natural medicines and natural cosmetics (Nepstad et al. 2006).

Given the ongoing search for bioactive molecules, we show how these new bioproducts and sustainable technologies work in a circular economy so that the byproducts of a given product are used to generate income. The most important of these bioproducts come from Amazonian fruits such as açai (*Euterpe oleracea*), andiroba (*Carapa guianensis* Aublet), buriti (*Mauritia flexuosa*), and cupuaçu (*Theobroma grandiflorum*), which need to be further investigated to find new bioactive molecules (Rodrigues Da Silva Enríquez and Drummond 2007; Lessmann et al. 2016).

In the evolution of the pharmaceutical and cosmetics industries, these fruits have been gaining economic prominence from the dissemination among and increased use by people. Technological and phytochemical advances have consolidated their high efficiency, biocompatibility, and low toxicity thanks to the use of natural products made from plant sources; these species have a variety of valuable properties for industrialization, including several useful chemical characteristics (Greve and Song 2017; Baugh et al. 2018).

*3.1 Açai (*Euterpe oleracea*)*

The açai tree from *Euterpe oleracea* (Fig. 1) is a palm tree native to the Amazon, derived from the Arecaceae family, measuring approximately 3 to 20 meters in height, with a smooth stem of 7 to 18 meters in diameter. With fruitful characteristics for the entire annual period, it shows greater growth in flooded areas because they contain moist soils and enable natural regeneration. The fruits, called açai berries, are smooth and globose, measure 1.2 to 1.3 meters in diameter, and are violet in color during the mature phase (Laurindo et al. 2023).

The açai trees generate small spherical dark-purple fruits found in clusters on the tops their palms. Its fruiting is abundant from July to December. A palm tree has a production capacity of about 120 kg each harvest; the essential extraction of this fruit occurs through its pulp, which occurs through maceration processes to remove

Fig. 1 Açai tree

undesirable residues and reduce particle sizes. The seeds constitute 85% of the total weight, 15% of which corresponds to the epicarp and mesocarp pulp, which can be pressed to extract its oil (Al Nasser and Mellor 2022).

The main consumer, producer, and exporter of açai berries is Brazil, specifically the north and northeast areas, comprising 42% of the current diet of the Amazonian community, where Pará is the largest state producer of the fruit, accounting for 95% of national production. The fruit, usually in its frozen pulp form, is widely exported to several countries, mainly China, Japan, the United States, Canada, and several European countries, usually sold and consumed in energy drinks (Yamaguchi et al. 2015).

The fruit of the açai tree demonstrates high socioeconomic relevance for the Amazon region given its enormous potential for the necessary improvement of this fruit, particularly the production and commercialization of the açai pulp, which is obtained from mechanical or manual extraction processes. The fruit is also used in popular foods and in industrial and artisanal products such as juices, ice creams, popsicles, jellies, and liqueurs, among others (Yamaguchi et al. 2015).

Its leaves are used as coverings for houses in areas where açai is planted and used in the treatment of snake bites, muscle aches, and pains in the chest. Açai seeds are widely used for handicrafts and mainly as organic fertilizer; from their stem, they are extracted from the hearts of palms. Another relevant application of açai pulp is the production of natural dyes; its ripe fruits end up providing a certain pigment derived from anthocyanins, which can produce colors varying from dark purple to dark bluish green, depending on the environment and the extraction method. Lastly,

oils can be extracted from the pulp of the fruit, which contains a dark green viscous fluid that has an aroma reminiscent of açai (Cavalcante et al. 2018).

*3.2 Andiroba (*Carapa guianensis*)*

Carapa guianensis (Fig. 2), popularly known as andiroba, is part of the Meliaceae family, which is widely used in traditional Brazilian medicine and activities in the Amazon region. The name andiroba comes from Tupi and means "bitter oil"; the tree of this species is the most important medicinal plants used in the production of herbal medicines by the Indigenous peoples and traditional inhabitants of the Amazon rainforest (Martinez et al. 2018).

It is found naturally in the northeast, specifically in the states of Acre, Amazonas, Amapá, and Pará, and in the northeast, it is only in the region of Maranhão and the Amazon in general. This species is adaptable in that it grows on forest canopies and develops best in clayey, muddy soils rich in organic matter. The parts that are generally used from this fruit in the extraction and production processes are the seeds, from which oils are extracted (Peixoto Araujo et al. 2021).

This species contains various bioactive compounds, such as coumarins, terpenes, and flavonoids. Andiroba fruit oil contains fatty acids such as linoleic, oleic, and palmitic acids. The so-called unsaponifiable fractions of this oil contain mainly limonoids and meliacins, bitter substances that exert a strong influence on the biological activities of this fruit (Figueiredo et al. 2022).

Fig. 2 Andiroba (*Carapa guianensis*)

*3.3 Buriti (*Mauritia flexuosa*)*

Buriti (*Mauritia flexuosa*) (Fig. 3) is derived from a palm that belongs to the Arecaceae family. When mature, it can reach 30 meters in height. It flowers in the months between April and August and is found mainly in the wetlands and marshes of Brazilian regions with tropical or subtropical climates. It has spread primarily to the states of Amapá, Amazonas, Pará, Rondônia, Goiás, Bahia, Minas Gerais, Mato Grosso, Ceará Maranhão, Piauí, and Tocantins (Delgado et al. 2007; Virapongse et al. 2017).

Buriti, popularly known as miriti, has wide territorial distribution throughout the Amazon region. It has a high population density and great genetic diversity. This species is considered the most important among the 11 in Brazil that have been called "trees of life," because every part of them is useful. This tree produces fruits between late December and June, ranging from 2000 to 6000 fruits per palm tree (Pereira Freire et al. 2016; van der Hoek et al. 2019). The fruit is described as an elliptical fruit, in which the peel, pulp, fiber, and seed have an average mass and concentration of 11.08 g and 22.07%, 12.80 g and 24.25%, 10.49 g and 21.03%, and 16.86 g and 32.65%, respectively (Koolen et al. 2013).

Mauritia flexuosa is important to its environment because it is part of the diets of several wild animals and acts as shelter for other species that choose to build nests at the top of these palm trees. This fruit can keep its soil moist and acts as a natural water resource. Buriti is also used for its core, which is rich in carbon. It is used in the production of filters for water purification (Albuquerque et al. 2003; Manzi and Coomes 2009). The pulp of buriti is a source of income for riverside and Indigenous communities, mainly in the Amazonian territory; in the food sector, the part of the

Fig. 3 Buriti (*Mauritia flexuosa*) fruit

mesocarp is widely used in products such as jellies, wines, sweets/candies, cakes, ice creams, and liqueurs (de Oliveira et al. 2013; Endress et al. 2013).

*3.4 Cupuaçu (*Theobroma grandiflorum*)*

The cupuaçu (*Theobroma grandiflorum*) comes from a tree in the Malvaceae family that is native to the Amazon. Each produces an amount of fruit that has an average weight of 4 kg. Approximately 20% of this fruit is composed of seeds; 35% of its interior is pulp; and 45% of its exterior is peel. The pulp of this species is the greatest source of economic exploitation because it can be used in the food industry and in cosmetic production. This species is usually found in forested areas in the northern parts of the Amazon and in the northeast of Maranhão (Rogez et al. 2004; Pereira et al. 2018).

In its natural habitat, the cupuaçu tree (Fig. 4) is medium size, reaching about 20 meters in height. Its foliage has elongated physiological characteristics: They are approximately 30 cm long and 15 cm wide, with a leaf color ranging from pink when young to dark green when mature, and their flowers are large and have a reddish color (Pugliese et al. 2013).

The cupuaçu grows mainly in the Amazon region, where more than 30,000 seedlings are planted. The largest plantation of cupuaçu trees is in the state of Pará, followed by the state of Amazonas, together making Brazil the largest producer (Costa et al. 2015; Abdullah et al. 2020). The economic activity of the Amazon region is mostly composed of family farming, where the cultivation of the fruit requires heavy labor and generates income for the local communities. Normally, production centers are formed by two or three farm properties, which support these families in

Fig. 4 Cupuaçu (*Theobroma grandiflorum*) fruit

a way that producers are included in the entire process, from soil preparation, management, cultivation, and harvesting to the commercialization of the final product (Vriesmann and de Oliveira Petkowicz 2009; de Oliveira and Genovese 2013).

Cupuaçu oils are extracted from cupuaçu seeds, which have a high fat content and are similar to cocoa butter, showing their great potential in industrial production, which can be as food, pharmaceuticals, and even cosmetics. This part of the fruit, previously characterized as a byproduct, has been featured in studies because it can be used to obtain products similar to those made from cocoa seeds and because its stages of development are identical to those of cocoa seeds in industrialization (Rogez et al. 2004; Alves et al. 2007).

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Correction to: Natural Biopolymers as Scaffold

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Correction to:
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In Chapter 2 of the original version of this book, the term "Biopolymers" was misspelt as "Bioplymers" in the chapter title. It was incorrectly titled as "Natural Bioplymers as Scaffold", whereas it should be "Natural Biopolymers as Scaffold". This has now been rectified.

The updated original version of this chapter can be found at
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