An-Najah National University

Faculty of Graduate Studies

VARIATIONS OF THE CHEMICAL CONSTITUENTS AND PHARMACOLOGICAL ACTIVITIES OF *ARTEMISIA*, *CHILIADENUS IPHIONOIDES*, *TEUCRIUM POLIUM*. ESSENTIAL OILS FROM JERICHO-PALESTINE

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Dedication

This thesis is wholeheartedly dedicated to my mother, Father, Sisters and my brother Mohammad who gave me moral, spiritual, and emotional support.

To my close friends Haya Janem, Rafeef Odwan, and Mohammad Jamal who have been my source of strength and passion.

I dedicate this work

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First and foremost, I want to express my gratitude to God Almighty for providing me with the chance and direction I needed to accomplish my goal and succeed in this area of my life.

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Last but not least, I want to thank my family, especially my mother.Without her wonderful support and understanding throughout the previous few years, I would not have been able to finish my education.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

VARIATIONS OF THE CHEMICAL CONSTITUENTS AND PHARMACOLOGICAL ACTIVITIES OF *ARTEMISIA*, *CHILIADENUS IPHIONOIDES*, *TEUCRIUM POLIUM*. ESSENTIAL OILS FROM JERICHO-PALESTINE

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: إيمان عزت ساعد

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Date: 8-3-2025

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Abstract

The growing fascination with medicinal plants is primarily due to the perception that certain plants can treat various conditions without causing negative side effects. In our region, three common herbs, *Artemisia, Chiliadenus iphionoides, and Teucrium polium,* have been employed in several traditional treatments for a considerable duration. This study aims to examine the chemical compositions of the essential oils (EO) from *Artemisia, C. iphionoides, and T.polium* gathered in Jericho, as well as their antioxidant, antibacterial, antilipase, and anti-amylase properties. Essential oils were extracted from *Artemisia, C. phionoides, and T.polium* using hydrodistillation, with their chemical contents characterized subjectively and quantitatively via GC-MS analysis. The antioxidant efficacy of the essential oils was evaluated by inhibiting 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radicals. The micro-dilution technique was employed to assess antibacterial efficacy. The anti-lipase activity was assessed utilizing p-nitrophenyl butyrate (PNPB). The activity of anti-amylase was evaluated utilizing 3,5-dinitrosalicylic acid (DNSA). Seventeen chemicals were found in *T. Polium,* of which E-nerolidol (27.11%), geranyl acetone (23.26%), germacrene D (19.08%), β-caryophyllene (17.78%), α-caryophyllene (3.35%), and bicyclo germacrene (3.08%) as the major constituents. In *C.iphionoides,* 47 chemicals were found, comprising 98.81% of the total oil, with cresol methyl ether (52.93%), ethyl oct-2-note (14.36%), epi-cadinol (6.56%), 1,8-cineole (4.25%), and epi-α-eudesmol (3.66%) being the major constituents. Fifty-one chemicals were discovered in *Artemisia*, with 1,8-cineole (28.67%) as the predominant component, followed by trans-thujone (24.0%), cis-thujone (17.69%), camphor (12.76%), and terpinen-4-ol (8.34%).

The essential oils of *T. Polium, C. iphionoides, and Artemisia* leaves exhibited notable antioxidant activity, with IC50 values of 19.18± 0.34 µg/mL, 17.03± 0.59 µg/mL, and

35.00± 0.47 µg/mL, respectively. The 1:1 mixtures of *Artemisia* essential oil (EO) *with C. iphionoides* EO, *Artemisia* EO with *T. Polium EO, and C.iphionoides* EO with *T. Polium* EO showed comparable antioxidant activity, with IC50 values of 17.13 ± 0.70 µg/mL, 28.96 ± 0.16 µg/mL, and 18.47 ± 0.86 µg/mL, respectively, all of which are less effective than Trolox (IC50 = 4.3 ± 0.58 µg/mL).*Artemisia* essential oil and *C. iphionoides* EO exhibited moderate antibacterial activity, with MIC values of 3.906 and 31.3 µL/mL and MBC values of 250.0 µL/mL. *T.Polium* had negligible antibacterial activity. The extracted essential oil demonstrated inhibitory effects on swine pancreatic lipase, with IC50 values of 534±0.19 µg/mL for *Artemisia*, 368.13±0.62 µg/mL for *T.polium*, and 931.58±0.91 µg/mL for *C. iphionoides*. The isolated essential oil demonstrated α-amylase inhibitory action with IC50 values of 569 ± 0.20 for *Artemisia*, 569 ± 0.20 µg/mL for *T. Polium* and 1550 ± 0.25 µg/mL for *C. iphionoides*.

Keywords: *Artemisia, Chiliadenus iphionoides, Teucrium polium,* α-amylase inhibitory, pancreatic lipase, antibacterial activity, antioxidant activity.

Chapter One

Introduction

1.1 Medicinal plant

Therapeutic plants (restorative herbs) have been discovered for centuries and have been employed in traditional medicine since antiquity. Plants produce numerous chemical and biological components for functions including protection against insects, herbivorous mammals, parasites, and diseases. Numerous phytochemicals with established or prospective natural efficacy have been identified in plants [1]. Given the increasing prevalence of infections, promoting and maintaining health and well-being has become a priority for all individuals. Given this health risk, the pursuit of alternative treatments and pharmaceuticals that are both effective and safe is essential [2,3].

Medications are classified into two categories: mainstream contemporary medicine and herbal or alternative medicine. Herbal remedies were extensively employed for the treatment of various infections throughout many countries in the 18th century. Over time and with enhancements in pharmaceuticals, synthetic solutions began to progressively supplant traditional remedies. While it is a beneficial practice, it may result in several adverse effects, ranging from back pain and headaches to more severe complications such as heavy bleeding, hemorrhage, and respiratory difficulties [4].

Despite the swift progress of modern pharmaceuticals, their technological advancements have encountered a stalemate[5]. In the United States, deaths due to adverse reactions or side effects of drugs surpass those caused by intoxicated drivers by a factor of at least three. The fatalities linked to herbal substances are comparatively infrequent. As a result, many turn to herbal medicines, believing them to be free of detrimental side effects [6].

Current estimates indicate that more than 10% of all recognized plant species, over 50,000 types, are employed in the development of pharmaceuticals and cosmetic items. These plants are esteemed for both their bioactive chemicals and their cultural importance, as well as the historical knowledge associated with their utilization. Traditional medicine systems, including Ayurveda and Traditional Chinese Medicine, have utilized these botanical medicines for ages, highlighting the significance of plants in global healthcare practices [7].

Ongoing research reveals the therapeutic potential of various plant species, resulting in the creation of novel drugs and health products sourced from nature. This tendency highlights the persistent connection between people and the plant kingdom, demonstrating a profound comprehension of nature's therapeutic attributes [8].

Patients seek herbal medicines for several practical and psychological reasons. A notable factor is cost; herbal alternatives are frequently more economical than traditional treatments, rendering them attractive, particularly for individuals with constrained financial means. Furthermore, numerous herbs are readily obtainable, either via local markets or online, hence increasing their attractiveness for those pursuing prompt treatment. An additional significant component is the psychological reassurance that herbal treatments can offer. A multitude of individuals assert that utilizing these natural alternatives provides them with a sensation of agency regarding their wellness. This sense of empowerment can be especially advantageous for individuals who may feel powerless due to chronic sickness or when conventional treatments fail to produce the expected outcomes. Opting for herbal alternatives enables patients to engage actively in their healthcare, potentially reducing anxiety and promoting a sense of well-being [9].

Furthermore, societal factors may augment the implementation of the "man-soil relationship." It is believed that when illness occurs in specific areas, the plants in those regions will react to this anomaly and have the ability to provide a treatment. The World Health Organization reports that over 80% of the global population uses traditional medicine [10,11].

Taking all of the above into account, there is a significant and growing demand for plant-based medicines, health products, pharmaceuticals, nutritional supplements, and cosmetics in both developing and developed countries. This trend has prompted pharmacists and pharmaceutical companies to shift their focus from synthetic compounds to natural products in order to meet consumer demands and enhance their understanding of botanical sources [11].

Research has demonstrated that compounds derived from medicinal plants exhibit a range of beneficial properties, including antioxidant effects and inhibitory activities against cancer cells, bacteria, and various viruses, such as hepatitis B, HIV, herpes, and influenza. For instance, phytochemicals like flavonoids and alkaloids have been shown to exert

anticancer effects by inducing apoptosis in tumor cells and inhibiting metastasis. Notably, approximately 60% of commonly used anticancer drugs are derived from natural products, underscoring the importance of phytochemicals in modern pharmacotherapy [12].

The fundamental difference between conventional and herbal medicine is that the former contains a single active ingredient that is chemically produced or extracted from creatures, microorganisms, or plants, while the latter involves several substances that work synergistically. They may originate from the flowers, leaves, stems, bark, or roots of plants [12].

All plants generate chemical compounds as byproducts of their normal metabolic activities. This includes primary metabolites, such as carbohydrates and lipids, found in all plants, as well as secondary metabolites, which are exclusive to certain plant species. These auxiliary metabolites are essential for the growth of medicinal plants and may have advantageous effects on humans [13].

1.2 Secondary metabolites

Secondary metabolites (SMs) are natural substances formed by plants, typically after growth, and are not directly implicated in the plant's growth, development, or reproduction. Although they are not critical for the immediate survival of plants, their absence may influence reproductive capabilities, environmental perception, or long-term resilience. At times, they may lack any recognizable alternative. In addition to their use in solutions, they function as distinct sources of tastes, colorants, and aromatics [14].

SMs are classified into three major classes: terpenes, phenols, and alkaloids. Their classification is based on their composition, chemical make-up, dissolvability in numerous solvents, or strategy of union. Basic essential oils are lipophilic and highly volatile secondary plant metabolites [15,16].

1.2.1 Terpenes

They are also referred to as terpenoids or isoprenoids. They are made from C5 isoprene units that are joined together from the head to the tail by rearranging and cyclizing the carbon skeleton. Hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes

(C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}), and tetraterpenes (C_{40}), which are referred to as carotenoids, are among the various types of terpenes [17].

They serve an assortment of purposes in plants, counting thermo protection, signaling, taste, and solvents [18]. They too have a few therapeutic applications. It has been found that the cannabis plant, which is the foremost common source of terpene, has anticancer, antimicrobial, antifungal, antiviral, anti-hyperglycemic, pain-relieving, anti-inflammatory, and antiparasitic action [18].

1.2.1.1 Hemiterpenes (C_5)

Hemiterpenes, classified as C_5 compounds, are the simplest form of terpenes with a general chemical formula of C_5H_8. This basic structure signifies their classification within the broader terpene family, which is known for its diverse range of biological activities and applications. Hemiterpenes are typically sourced from the resins of various plants and herbs, serving as natural reservoirs for these compounds. Their production is often associated with the metabolic pathways of plants, where they play a role in defense mechanisms, pollinator attraction, and overall plant ecology. Hemiterpenes are important not only for their structural simplicity but also as essential precursors for more complex terpenes, affecting the aroma, taste, and therapeutic properties of their source plants [19].

Isovaleric acid, tiglic acid, and prenol are all important compounds found in various plants, each contributing unique properties and applications in the fields of perfumery, food, and medicine[20,21]. The differences and similarities between these three compounds shown in Appendix A shows (Table S1).

1.2.1.2 Monoterpenes (C_{10})

The conventional formula for monoterpenes is $C_{10}H_{16}$. They are made up of two isoprene units that can be cyclized or oxidized to form a range of compounds. These compounds have a particular fragrance. They are the major terpenes in essential oils that give plants their scent. This unusual fragrance is due to the distinct functional clusters connected with them, as well as their participation in ecological processes. They are divided into non-cyclic, monocyclic, and bicyclic monoterpenes [17]. Here are a few instances of hemiterpenes:

Naturally occurring organic compounds such as limonene, pinene, menthol, myrcene, and geraniol have a diverse variety of applications due to their distinct qualities. Limonene, a monoterpene found in citrus peels such as lemons, oranges, and grapefruits, is known for its strong citrus aroma. It is widely used as a cleaning agent, air freshener, and flavoring component. Research reveals that limonene has antioxidant and anti-inflammatory properties, making it useful in health and wellness products [22,23].

Pinene, sourced from pine trees and a principal constituent of turpentine, manifests in two isomeric forms: α-pinene and β-pinene. Both isomers are employed in the synthesis of various organic compounds, rendering pinene indispensable in chemical manufacture. Additionally, pinene has healing properties like reducing inflammation and widening airways, which suggests that it could be used in respiratory treatments [24,25].

Menthol, extracted from peppermint and other mint oils, is acknowledged for its cooling sensation, which arises from its engagement with cold-sensitive receptors in the dermis. It is employed in pharmaceutical formulations, encompassing topical analgesics, decongestants, and oral hygiene products like toothpaste and mouthwash. Menthol is a common flavoring in confections, imparting a minty flavor and a chilling sensation[26,27].

Myrcene is a musky, earthy scent that comes from plants like ylang-ylang, wild thyme, parsley, and hops. This terpene is used a lot in the fragrance business because it has a unique smell. It is also used to add flavor to food. Also, myrcene can help you sleep and relax your muscles, which makes it useful in aromatherapy and health settings [28].

Geraniol is known for its sweet, flowery scent and is found in geraniums, lemons, and roses. It is widely used as a flavoring and in cosmetics and perfumes. Furthermore, geraniol is frequently used as an ingredient in natural pesticides due to its insect-repelling qualities. Geraniol's uses in health and personal care products are growing as research shows that it also has antibacterial, anti-inflammatory, and antioxidant properties [29,30].

1.2.1.3 Sesquiterpenes ($C_{15}H_{24}$)

Sesquiterpenes, with the molecular formula $C_{15}H_{24}$, are highly versatile compounds composed of three isoprene units. These compounds display considerable structural diversity, ranging from cyclic and monocyclic to bicyclic and tricyclic forms. Their

properties are similar to those of monoterpenes, but the additional isoprene unit allows for more complex structures and greater functional diversity. The arrangement of the carbon skeletons, functional groups, and substituents contributes significantly to the various chemical and biological activities exhibited by sesquiterpenes. Their wide-ranging applications span industries such as fragrance, cosmetics, pharmaceuticals, and even agriculture due to their natural origin and bioactive properties [31].

Farnesol is a notable sesquiterpene. It is found in essential oils derived from numerous plants, such as citronella, lemongrass, tuberose, cyclamen, rose, neroli, balsam, and musk. Farnesol is frequently utilized in perfumes due to its agreeable flowery aroma. Nonetheless, it has been investigated for its potential anti-tumor, antibacterial, and antifungal activities, rendering it a viable option for pharmaceutical applications [32].

Humulene, or α-caryophyllene, is a monocyclic sesquiterpene found naturally in plants such as hops. It is an isomer of β-caryophyllene; however, it does not possess the significant CB_2 receptor activity that β-caryophyllene demonstrates, which is crucial for anti-inflammatory and analgesic actions. Humulene is utilized in brewing, enhancing the scent of hops. It has demonstrated potential as an appetite suppressant and anti-inflammatory drug. Furthermore, studies suggest that humulene may possess anti-cancer capabilities, which are presently being investigation in the medical domain [33].

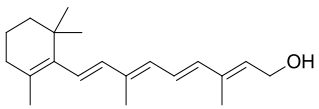
Bisabolol, a monocyclic sesquiterpene alcohol, was first isolated from chamomile flowers in 1951. It exists in four stereo isomeric configurations. The α-(-)-bisabolol isomer is mostly used in commercial applications. It is a key ingredient in many dermatological and cosmetic formulations due to its relaxing and anti-inflammatory properties, making it suited for use in aftershave creams, lotions, sunscreens, and newborn care products. Bisabolol is known for its ability to improve wound healing and protect the skin from injury, making it an essential element in many skincare products [34,35].

Chamazulene, while not formally categorized as a sesquiterpene, is intimately linked to them due to their derivation from sesquiterpenes during steam distillation. It is produced during the distillation of chamomile and other botanicals from the breakdown of matricin, a sesquiterpene lactone. Chamazulene possesses anti-inflammatory and antioxidant characteristics, rendering it beneficial in essential oils and aromatherapy [36,37].

1.2.1.4 Diterpenes (C_{20}) and Sesterterpenes (C_{25})

Diterpenes (C_{20}) and sesterterpenes (C_{25}) are two fascinating and structurally unique types of terpenes. They vary in the quantity of carbon atoms, and isoprene units are found naturally in a diverse array of creatures, encompassing terrestrial and marine plants, fungus, bacteria, and mammals. Diterpenes exhibit a variety of chemical structures and functions, including applications in pharmaceuticals, cosmetics, and the food industry. C_{20} molecules consist of four isoprene (C_5H_8) units and are present in both terrestrial and marine ecosystems, including plants, organisms, bacteria, and animals [38,39].

Diterpenes are essential for the production of numerous physiologically significant chemicals. Retinol **1** (vitamin A), retinal **2** (associated with vision), and phytol **3** (a constituent of chlorophyll) are all synthesized from diterpenes. Retinoids, encompassing retinol and retinal, are crucial for vision, immunological function, and dermatological health. Phytol is an alcohol formed from chlorophyll that is essential in photosynthesis. These molecules underscore the biological significance of diterpenes in human health and plant physiology [38,39].



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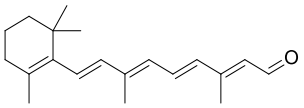

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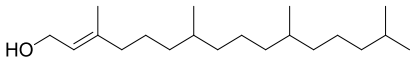

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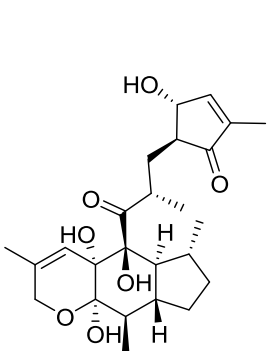
Sesterterpenes (C_{25}):

Sesterterpenes represent a tiny subclass of terpenoids primarily found in marine environments; however, they are also produced by terrestrial species such as fungi, and to a lesser extent, by higher plants, lichens, and insects. Sesterterpenes exhibit notable natural qualities, such as anti-inflammatory, cytotoxic, anticancer, antibacterial, antitubercular, and anti-biofilm activity. Leucosesterpenone 4 and leucosterlactone 5 are tetracyclic sesterterpenoids extractable from plants [40].



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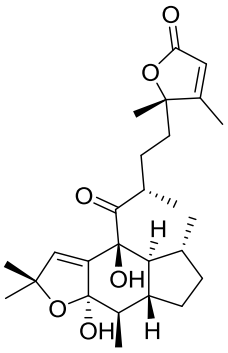

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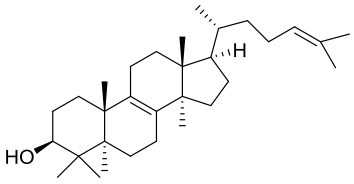
1.2.1.5Triterpenes (C_{30}) and Tetraterpenes (C_{40})

Triterpenes are substances consisting of six isoprene molecules. They may be either acyclic or cyclic. Lanosterol **6** and Hopane**7** are present in wheat germ and olives, while cholesterol **8** exemplifies triterpenes. The chemical structures of many essential triterpenes are detailed below [41].



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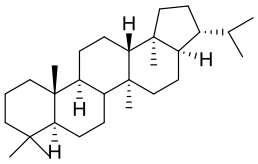

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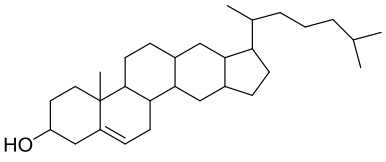

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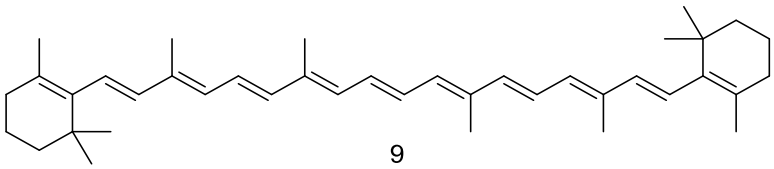
Tetraterpenes:

Tetraterpenes, the most branched category within this family, are composed of 40 carbon atoms and 8 isoprene units, with carotenoids serving as the predominant colored compounds abundant in plant pigmentation. In addition to carotenoids, several plant elements are expelled, similar to the green microalga [42].

Botryococcusbraunii has also been identified as a source of tetraterpenoids; however, information regarding their biological characteristics is lacking [42].

Carotenoids occur in nature in various geometric configurations due to the ability to rotate around double bonds. Nonetheless, the predominant configuration is the all-trans arrangement [42]. The two main structures in the carotenoid group are the oxygen-free carotenes, such as ß-carotene **9** and lycopene and the oxygenated **10** carotenoids such as lutein **11**, and cryptoxanthin **12** [42].



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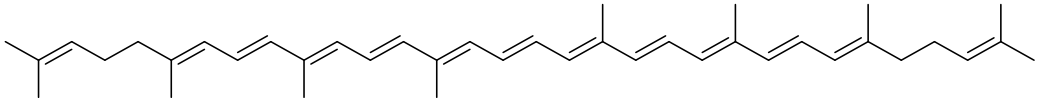

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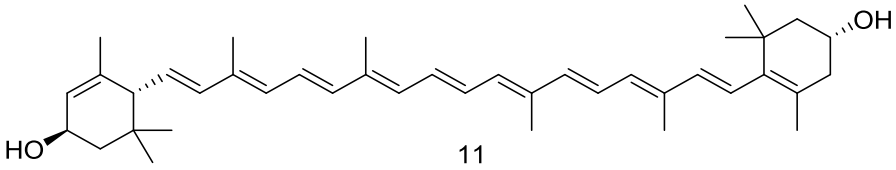

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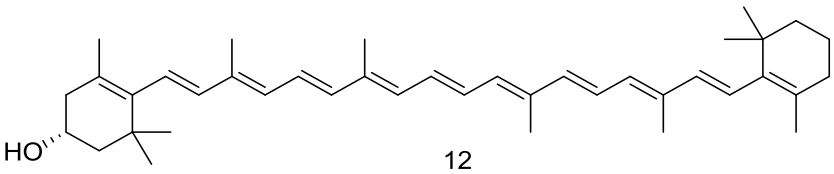

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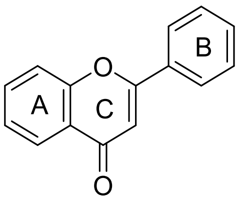
1.2.2 Poly Phenols

Polyphenols are characterized by the presence of aromatic rings bearing hydroxyl groups. They are present in several plant species and constitute the primary secondary metabolites of plants. Over 8,000 phenolic compounds have been identified, encompassing fundamental molecules like phenolic acids and extensively polymerized entities such as

tannins. Plant phenolics primarily serve as protection against ultraviolet radiation and threats from viruses, parasites, and predators, while also adding to the coloration of plants. They are ubiquitous in all plant organs and hence constitute a vital component of the human diet [43].Polyphenols are broad constituents of plant nourishments (fruits, vegetable cereals, olives, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.). Polyphenols play a significant role in the overall organoleptic properties of plant-based foods and beverages. For example, phenolics contribute to the sharpness and astringency of natural products and fruit juices. This is due to the interaction between phenolics, specifically procyanidin, and the glycoprotein in spit [43].

Anthocyanins, a subclass of the extensive class of plant polyphenols known as flavonoids, are responsible for the orange, red, blue, and purple hues found in several fruits and vegetables, including apples, berries, beets, and onions. Phenolics are the primary components that significantly affect the flavor and color differentiation among white, pink, and red wines; they interact with oxygen and are essential for the preservation, development, and aging of wine [43].

They are categorized into six categories based on the oxidation state of the C ring: flavones **13**, flavanols **14**, flavanones **15**, isoflavones **16**, and anthocyanins **17**. Phenolic acids **18** and tannins **19** constitute the two primary groups of polyphenols, but the less explored categories include stilbenes **20** and lignans. Polyphenols possess robust antioxidant effects [43].



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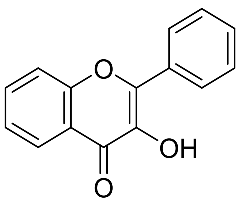

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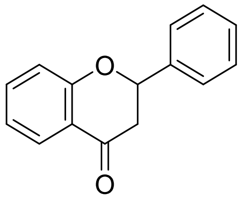

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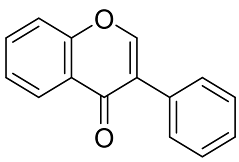

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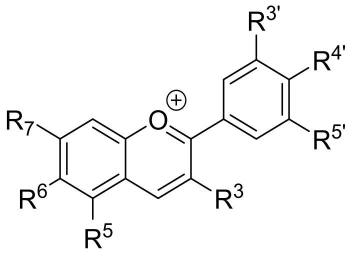

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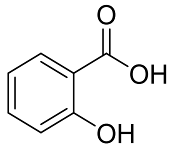

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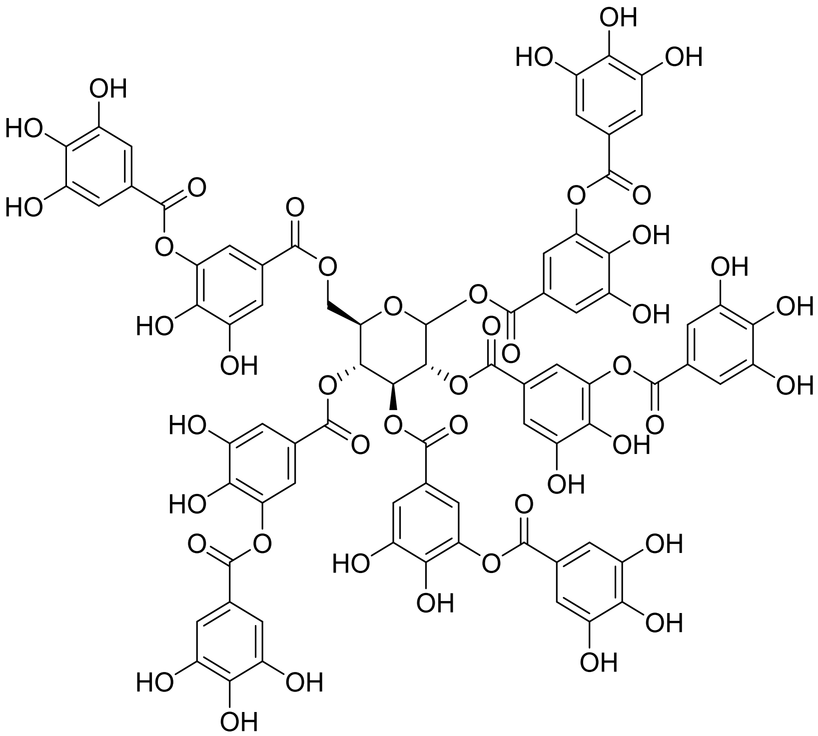

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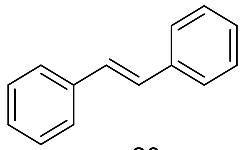

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1.2.3 Alkaloids

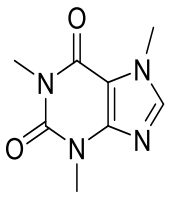
Alkaloids are a diverse group of naturally occurring organic compounds that contain a basic nitrogen atom, typically with a heterocyclic structure, which is essential for their classification. The nitrogen atom can be located at various positions within the molecule, but it is not part of amide or peptide bonds. Most alkaloids are alkaline, which is reflected in their ability to react with acids to form salts. These compounds are predominantly derived from plants, though some alkaloids are also found in fungi, bacteria, and animals.

Due to their potent physiological effects on humans and animals, alkaloids have long been used in medicine, pharmacology, and even as poisons [44].

Opium, derived from the poppy plant (*Papaver somniferum*), was the first alkaloid to be isolated in history. It has been used for centuries as a powerful analgesic and narcotic, particularly in treating pain. Following the isolation of opium, morphine was extracted from it in the early 19th century, marking a major advancement in medical treatments for severe pain. Morphine remains in use today for pain management, particularly in cases of chronic or post-surgical pain, due to its strong pain-relieving properties [45,46].

In addition to morphine, several other alkaloids play crucial roles in medicine and everyday life. Caffeine **21**, found in coffee, tea, and cacao plants, is a well-known stimulant of the central nervous system and is widely consumed globally for its energizing effects[47]. Strychnine **22**, derived from the seeds of the Strychnosnux-vomica tree, is a highly toxic alkaloid historically used as a pesticide, but in small doses, it has been used in some cultures as a performance-enhancing substance. Quinine **23**, an alkaloid obtained from the bark of the cinchona tree, has been instrumental in the treatment of malaria for centuries. It works by interfering with the parasite that causes the disease and is still used in some forms for this purpose[48]. Coniine **24**, an alkaloid found in hemlock (Conium maculatum), is infamous for its toxic properties and was historically used in executions, most notably in the death of the philosopher Socrates[49].

The broad spectrum of biological activities exhibited by alkaloids, from stimulating and sedative effects to toxicity and therapeutic properties, makes them an invaluable subject of study in pharmacology and medicine. Their complex chemistry and diverse range of effects continue to inspire both scientific research and practical applications in drug development [50].



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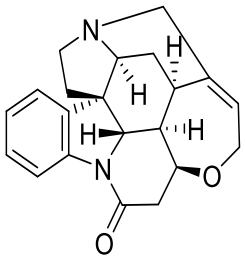

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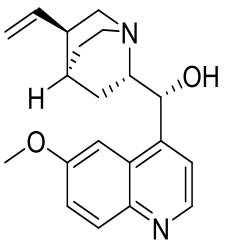

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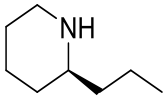

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1.3 Essential oils

Essential oils are volatile, natural, complex compounds characterized by a solid scent and are shaped by fragrant plants as auxiliary metabolites [51].Essential oils are typically obtained through processes such as steam or hydro-distillation, techniques that date back to the Middle Ages and were pioneered by scholars in the Middle Eastern region. Renowned for their purifying properties, essential oils possess bactericidal, veridical, and fungicidal characteristics, which make them valuable in various applications [52]. They are widely used in embalming, food preservation, and as natural remedies with a range of therapeutic effects. These effects include antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic, and local anesthetic properties [53].

Despite the long history of their use, the fundamental characteristics of essential oils have remained relatively unchanged over the centuries. However, contemporary research has significantly enhanced our understanding of their mechanisms of action, particularly regarding their antimicrobial efficacy. Studies have revealed insights into how these compounds interact with microbial pathogens, leading to potential applications in modern medicine and natural health solutions [54].

In nature, essential oils play a critical partin the assurance of the plants as antibacterial, antiviral, antifungal, and bug sprays additionally against herbivores by decreasing their craving for such plants. They moreover may pull in a few creepy crawlies to support the scattering of dusts and seeds or repulse undesirable others [55].

Essential oils are concentrated extracts obtained from a wide range of aromatic plants, predominantly located in temperate to warm climates, including Mediterranean and tropical regions. These oils are typically derived from plant parts such as leaves, flowers, stems, bark, and roots, and are known for their distinctive fragrances and therapeutic properties. In these regions, essential oils have become integral components of traditional pharmacopeias, often utilized for their medicinal benefits, aromatic qualities, and various applications in herbal remedies. Their use spans centuries, and they are valued for their roles in holistic healing practices, aromatherapy, and as natural alternatives in various health and wellness treatments. The rich biodiversity of these climates contributes to a diverse array of essential oils, each with unique compositions and potential health benefits. They are fluid, unstable, limpid, and seldom colored, lipid dissolvable, and

dissolvable in natural solvents with a for the most part lower thickness than that of water. They can be synthesized by all plant organs, i.e. buds, blooms, takes off, stems, twigs, seeds, natural products, roots, wood or bark, and are put away in secretory cells, cavities, canals, epidermis cells, or glandular trichomes [55].

1.3.1 Extraction Methods of EO

A few strategies were created to extricate EOs, the reason for all these distinctive procedures is to produce with high quality, in expansion, to get oils with worthy yields. It is especially imperative to select a strategy that does not crush the helpful properties, where a few solvents may influence them. Besides, the ultimate product's aim utilization decides which strategy will be utilized [56].

In common, extraction procedures are isolated into two fundamental categories, the conventional extraction methods, and the green extraction strategies

1.3.1.1 Conventional extraction

Conventional extraction uses water or organic solvents under atmospheric pressure. These strategies are recognized for their straightforwardness, wide openness, and low cost. The solvents enter the strong plant matter and solubilize the polar comparable chemicals interior, so the choice of the appropriate dissolvable is basic sort incorporates steam distillation, hydro diffusion, hydro distillation, cold pressing, and solvent extraction[57].

1.3.1.2 Steam Distillation

The plant in this strategy is infused into the alembic without water maceration, at that point uncovered to the steam that streams from the foot to the beat of the alembic. At that point, the basic oil-filled vapor passes through a "swan-neck" column followed by condensation. At last, it'll be collected in a Florentine carafe. The thought behind this method is to induce the whole vapor weight to rise to surrounding weight at around 100 °C so that the unstable substances, which are bubbling at temperatures running from 150 to 300 °C, can be evaporated at a temperature comparable to that of water [58].

1.3.1.3 Hydro Diffusion

This strategy is considered the simplest, most effortless, and most seasoned strategy-changing oil. A Clevenger device which comprises of warming source, a vessel, a condenser, and a decanter utilized in this strategy, where the plant is drenched in the water interior of the vessel, and warmed for 3-6 hours, at that point vapor from the vessel is changed over to fluid by the condenser, that collected within the decanter where the EO is isolated with small sum of water, and dried with anhydrous sodium sulfate (Na_2SO_4)[59,60].

1.3.1.4 Cold pressing

The cold squeezing strategy happens without warming, EO is extricated by mechanical strategies, particularly from the skins of natural products having a place in the citrus family, such as lemons, oranges, and grapefruits. The result of this strategy is within the shape of juicy oil, so a partition preparation is utilized to isolate oils from water. A drawback of this method is that these oils tend to deteriorate more quickly than other types of oils. Its effortlessness, commonality, security, eco-friendly, conventional, and less costly fetch are considered preferences for this strategy. Other points of interesting utilizing this strategy incorporate the conservation of certain temperature-sensitive phenolic compounds and lipophilic phytochemicals like antioxidants, which serve to keep us healthy and prevent disease [59,61].

1.3.1.5 Solvent Extraction

This method has been utilized for delicate or touch flower materials that cannot withstand warm or steam distillation. The plant is dissolved after being submerged in a solvent bath and extricated by utilizing direct warming. The solvents may be alcohol, hexane, ethanol, petroleum ether, and methanol. The choice of extraction solvents could be a pivotal figure in this strategy, and the specialists avoid solvents that can disable the extraction strategy or respond with the extrication. The filtered extract is concentrated at that point. The resulting concentrate comprises EO and other compounds, so it is blended with alcohol to extricate the oil taken after by refining at low temperature. This strategy is utilized within the perfume industry, whereas it isn't secure to be utilized for nourishment applications where it may contain a buildup from the solvent that has been utilized [60,62,63].

1.3.1.6 Green extraction

Green extraction of natural products can be one of the arrangements from the past to long-standing times of humankind as ecologic and economic chemistry, and turning to "Green Chemistry of Natural Products". Green extraction is based on the disclosure and plan of extraction forms which will reduce energy consumption, permit the utilization of elective solvents and renewable natural products, and guarantee a secure and high-quality extract/product". In this definition, the most thought of escalated is distinguished. The posting of green extraction standards was built up to direct analysts from the scholarly community and industry in their demarche towards green advancement, not as it were with respect to the method, but in all perspectives of solid-liquid extraction [64].

1.3.1.7 Ultrasound-assisted extraction (UAE)

UAE offers a naturally inviting, clean extraction with a few benefits advertised by ultrasonic helped extraction. In expansion, compared to other inventive extraction strategies, ultrasound is comparatively reasonable, flexible, and versatile. Acoustic cavitations, which happen because of the arrangement of cavitation bubbles are the essential calculation that drives the extraction impacts of sonication. Two diverse shapes of cavitation bubbles exist. When a medium is subjected to ultrasound, two sorts of cavitations are made: transitory (inertial) cavitations and steady (no inertial) cavitations. Various passing cavitations severely collapse, changing the temperature and weight. Within the case of EO, these changes result in the burst of EOs organs, which energizes the discharge of EO from the plant [58,65].

1.3.1.8 Supercritical fluid extraction (SFE)

Supercritical liquid extraction is the foremost viable and productive way to extricate important constituent botanicals. Supercritical Fluid Extraction (SFE) is the method of isolating one component (the extractant) from another (the matrix) utilizing supercritical fluids like carbon dioxide as the extracting solvent. carbon dioxide is the ruler of extraction solvents for botanicals. Extraction conditions for supercritical CO_2 are over the basic temperature of 31°C and critical pressure of 74 bar. Supercritical fluids are exceedingly compressed gases, which have combined properties of gases and fluids in an interesting way. Supercritical liquids can lead to responses, which are troublesome or indeed inconceivable to attain in conventional solvents. It could be a quick handle

completed in 10 to 60 minutes. A supercritical fluid can be isolated from the analyte by basically releasing pressure, taking off nearly no follow, and yielding an unadulterated buildup [66].

1.3.1.9 Microwave-assisted extraction (MAE)

Microwaves are non-ionizing electromagnetic (EM) waves found between the radio-frequency extend at the lower recurrence and infrared at the higher recurrence within the electromagnetic range, inside the recurrence band of 300 MHz to 300 GHz; 915 MHz is considered most valuable for mechanical applications with its more noteworthy infiltration profundity, whereas 2,450 MHz recurrence is for the most part utilized in residential microwave stoves and for extraction applications with a wide extend of commercial units planned for explanation chemistry purposes [67].

1.4 Biological activities of essential oils

Biological activity is a substance`s impact on living things. The different composition of EOs gives rise to a wide range of bioactivities, subsequently, they can take part in the treatment of a number of ailments, since their natural exercises have been detailed in many investigations such as anticancer, anti-microbial, and antioxidant exercises [68,69].

1.4.1 Antioxidant activity

Measuring the antioxidant activity/capacity of nourishments and natural tests is in this manner basic not as it were in guaranteeing the quality of useful nourishments, but more vitally in examining the proficiency of nourishment cancer prevention agents in avoiding and treating the illnesses related to oxidative stretch. Antioxidants are compounds which, when displayed in nourishments or the human body in exceptionally low concentrations, delay, control, or avoid oxidative forms driving nourishment quality disintegration or the event and proliferation of degenerative illnesses within the life form. A number of strategies and activities are included within the handle of hindering the oxidation by these antioxidant compounds. Particles with antioxidant properties may be delivered endogenously or ingested exogenously by counting calories or nourishment supplements. The most endogenous antioxidant chemicals are grass, catalase (CAT), and glutathione peroxidase (GSH-Px). Turf changes over the superoxide anion, which could be a substrate for CAT and GSH-Px. Catalase metabolizes H_2O_2in water and oxygen, and GSH-Px

diminishes both H_2O_2 and natural hydro peroxides when responding with glutathione (GSH) [70].

Exogenous antioxidants, like vitamins E and C, may exist within the living being within the cell film and the intracellular and extracellular fluid. They respond with ROS to dispose of or to restrain them. The hydrophobic lipid insides of the layers require a distinct spectrum of cancer prevention agents. Fat-soluble vitamin E is the foremost vital antioxidant in this environment, securing against the misfortune of film judgment [70].

1.4.2 Classification of Antioxidants

Antioxidants are substances that prevent or slow damage to cells caused by free radicals, which are unstable molecules that can harm cellular structures. They play a crucial role in protecting the body from oxidative stress, a condition that can lead to various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. Antioxidants can be classified based on several criteria, including their origin, solubility, mechanism of action, and chemical structure [71].

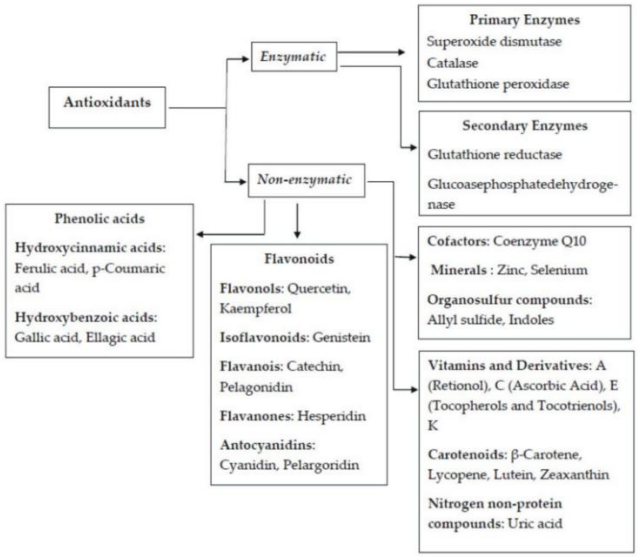
Antioxidants can be categorized into two main groups: enzymatic and non-enzymatic antioxidants, each playing a vital role in neutralizing free radicals and reactive oxygen species (ROS) that contribute to oxidative stress in the body [72].

Enzymatic antioxidants are naturally occurring enzymes that promote metabolic processes to reduce oxidative damage. The crucial antioxidant enzyme known as superoxide dismutase (SOD) converts superoxide radicals into hydrogen peroxide. This reduces oxidative stress in cells. Catalase is a vital antioxidant enzyme that breaks down hydrogen peroxide into harmless water and oxygen, mitigating its potentially harmful effects. Furthermore, glutathione peroxidase is essential for reducing hydrogen peroxide and lipid peroxides, thereby safeguarding cellular components from oxidative damage. These enzymatic antioxidants are essential for maintaining cellular health and function[73].In contrast, non-enzymatic antioxidants operate independently of enzymatic action to neutralize free radicals. They directly neutralize free radicals through several mechanisms. Vitamins C and E are acknowledged non-enzymatic antioxidants; vitamin C, a hydrophilic antioxidant, neutralizes free radicals in aqueous conditions, while vitamin E, a lipophilic antioxidant, protects cell membranes from oxidative damage. Additionally, flavonoids and other phytochemicals are naturally occurring compounds

that also exhibit significant antioxidant activity. These compounds, found in fruits, vegetables, and other plant sources, help combat oxidative stress by donating electrons to free radicals, thus stabilizing them. Together, both enzymatic and non-enzymatic antioxidants work synergistically to protect the body from oxidative damage and contribute to overall health [74].

Scheme 1.1

Classification of Antioxidants



1.4.3 Measuring the Antioxidant Activity

The strategies and disobedience utilized to degree the action of the cancer prevention agents have made surprising advances within the past few decades. Early strategies degree the productivity of the cancer prevention agents against the arrangement of specific species of oxidation items and in this way, are based on measuring lipid oxidation In this way distant, different chemical tests coupled with exceedingly touchy and mechanized location technologies have been utilized to assess antioxidant movement by uncommon strategies, like for occasion rummaging movement against diverse sorts of free radicals or ROS, decreasing control and metal chelation, among others. Oxidation substrates have to be amplified from nourishment show frameworks to chemical compounds, natural materials, cellular lines and indeed living tissues [70].

Scheme 1.2

Different Techniques Used to Measure Antioxidant Activity [51]

Techniques	Antioxidant Capacity Assay	Principle of the Method	End-Product Determination
Spectrometry	ORAC	Antioxidant reaction with peroxyl radicals, induced by 2,2′-azobis-2-amidino-propane (AAPH)	Loss of fluorescence of fluorescein
	HORAC	Antioxidant capacity to quench OH radicals generated by a Co(II) based Fenton-like system	Loss of fluorescence of fluorescein
	TRAP	Antioxidant capacity to scavenge luminol-derived radicals, generated from AAPH decomposition	Chemiluminescence quenching
	CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
	FRAP	Antioxidant reaction with a Fe(III) complex	Colorimetry
	PFRAP	Potassium ferricyanide reduction by antioxidants and subsequent reaction of potassium ferrocyanide with Fe^{3+}	Colorimetry
	ABTS	Antioxidant reaction with an organic cation radical	Colorimetry
	DPPH	Antioxidant reaction with an organic radical	Colorimetry
	FluorimetricAnalysis	Emission of light by a compound, which has absorbed light or other electromagnetic radiation of a different wavelength	Recording of fluorescence excitation/emission spectra

1.4.4 Anti-microbial activity (bacteria)

A substantial number of studies have been conducted to evaluate the antimicrobial properties of various plant extracts utilized in traditional medicine. These studies often focus on essential oils or isolated compounds, which include a diverse range of phytochemicals such as alkaloids, flavonoids, sesquiterpene lactones, diterpenes, triterpenes, and naphthoquinones, among others [75].

These bioactive compounds have garnered attention due to their potential efficacy in combating microbial infections. For example, alkaloids are known for their diverse pharmacological activities, while flavonoids exhibit strong antioxidant and antimicrobial properties. Sesquiterpene lactones and diterpenes have also been shown to possess significant antimicrobial effects. Triterpenes are recognized for their ability to disrupt microbial membranes, and naphthoquinones have demonstrated activity against a range of pathogens [76].

By examining these plant-derived extracts and compounds, researchers aim to uncover their mechanisms of action, effectiveness against various microbes, and potential applications in modern medicine. This body of research highlights the importance of traditional plant use in developing new antimicrobial agents and understanding the

therapeutic potential of natural products. A few of these compounds were disconnected or gotten by bio-guided segregation after already identifying antimicrobial action on the portion of the plant [75]. Antibacterial drugs are presently commonly alluded to as anti-microbial. Microbes may be actually safe to a few anti-microbial, but they can moreover create resistance to them through chromosomal quality changes and level quality exchange. Anti-microbial resistance is one of the greatest dangers to human well-being. Concurring to the foremost later World Financial Gathering Worldwide Dangers appraisals [76]. *Escherichia coli* and Klebsiella are sorts of microbes commonly found in animals, which are the foremost commonplace causes of circulatory system contamination in patients as well as the foremost ordinary causes of urinary tract diseases. *Staphylococcus aureus* is the foremost visited cause of skin diseases and the second-most visited cause of circulation system diseases in patients [77]. There are exceptionally few treatment alternatives for contaminations caused by anti-microbial safe microscopic organisms. Besides, it is very exceptional to discover unused and moved forward anti-microbial as a result. It's imperative to explore other alternatives that might work to treat these serious bacterial diseases. Plant extricates have been found to be promising medicines for sicknesses and antimicrobial treatment, where there's a bounty of plans for creating restorative plant phytochemicals as bio-enhancers of anti-microbial [78,79].

Numerous studies have demonstrated that essential oils possess antibacterial properties, which can be attributed to their hydrophobic nature. This characteristic allows essential oils to penetrate the lipid membranes of bacterial cells, leading to increased membrane permeability and subsequent structural disruption. Such alterations in the membrane can result in the loss of essential cellular components and critical molecules, ultimately leading to cell death. Research indicates that essential oils with significant antibacterial activity are typically high in phenolic compounds, such as carvacrol, eugenol, and thymol. These compounds have antibacterial activity against a variety of bacteria, including Escherichia coli, Bacillus cereus, Listeria monocytogenes, Salmonella enterica, Clostridium jejuni, Lactobacillus sakei, Staphylococcus aureus, and Helicobacter pylori. The efficacy of essential oils against various diseases demonstrates their promise as natural antibiotics [80].

1.5 *Artemisia*

Artemisia is a genus including small plants and shrubs primarily located in northern temperate zones. This genus is part of the vast family Compositae, or Asteraceae, noted for its diversity, comprising around 1,000 genera and 20,000 species, with around 500 species predominantly found in Asia, Europe, and North America [81].

Furthermore, the essential oils extracted from *Artemisia* species find applications in the cosmetics and pharmaceutical industries, highlighting their versatility and importance in various sectors. The rich diversity of *Artemisia* species not only contributes to ecological balance but also provides numerous benefits in terms of health and aesthetic appeal [81].

Essential oils for the most part have a wider range of bioactivity, owing to the nearness of a few dynamic fixing auxiliary metabolites, which work through different modes of activity. The auxiliary digestion system in a plant not as it were plays a part in its survival by creating attractants for pollinators, but it also acts as a chemical resistance against predators and illness. Concurring to the mode of extraction utilized, generally refining from fragrant plants, basic oils contain an assortment of unstable atoms such as terpenes, phenolic-derived fragrant, and aliphatic components. The expansive class *Artemisia* from the Anhimidae comprises imperative restorative plants which are as of now the subject of phytochemical consideration because of their organic and chemical differences, and basic oil generation [81].

Figure 1.1

Artemisia plant

1.6 Chiliadenus iphionoide

Chiliadenus class has a place in the Asteraceae family, which may be a little sort that incorporates ten species primarily conveyed all through the southern edge of the Mediterranean Ocean. Most of its species develop in rough places and semidry arrives recognized by pappus with double rows of hairs, the external push is exceptionally brief setae whereas the inward is equaling the corolla [82].

C.iphionoides develops wild in a rough environment, deserts and extraordinary deserts of the Iran-Turanian, Sahara-Arabian, and Mediterranean locale. It is conveyed all through Palestine, Jordan, Syria and Lebanon.*C.iphionoides*is commonly utilized in conventional medication as a decoction or implantation for the treatment of distinctive sicknesses [82].

C. iphionoides has been reported to show numerous pharmacological helpful properties such as anticancer, antidiabetic, antimicrobial, antioxidant, antispasmodic, and antiplatelet exercises [82].

Figure 1.2

Chiliadenusiphionoide plant

1.7 *Teucrium polium*

Teucrium polium (Fig S2) is one of the wild-growing blossoming species of this sort and is found inexhaustibly in Iran. This plant is utilized for home-grown tea and as a conventional medication. The tea of *T. Polium* is utilized as an appetizer particularly in children additionally as a flavor. An implantation of the takes off and blooms of the plant is devoured as a reviving refreshment. The organic exercises of *T. Polium* are broadly detailed and it has appeared to have anti-inflammatory, anti-nociceptive, anti-bacterial, anti-hypertensive, hypolipidemic, anti-rheumatoid, and hypoglycemic impacts [83].

1.8 Objectives of the Study

This study involves extracting the essential oils of *Artemisia*, *Chiliadenus iphionoides*, and *Teucrium polium* from their dried leaves, analyzing their components, evaluating their bioactivities, and determining the synergistic effects of particular biological activities. The specific objectives of this study are:

1. Extract the essential oil of *Artemisia*,*Chiliadenus iphionoides*, and *Teucrium polium*.
2. Utilize the GC-MS technique to examine the chemical makeup of the EOs from the leaves of *Artemisia*, *Chiliadenus iphionoides*, and *Teucrium polium*.

3. Investigating the antioxidant properties of the Eos *Artemisia*, *Chiliadenus iphionoides*, *Teucrium polium* using DPPH assay.
4. Assessing the antibacterial activities of, *Chiliadenus iphionoides,* and *Teucrium polium* by evaluating MIC using broth-micro dilution method.
5. Assessing the enzymatic properties of *Artemisia*, *Chiliadenus iphionoides*, and *Teucrium polium* including antilipase, anti-amylase activities.

Chapter Two

Experimental Part

2.1 Materials and Methods

2.1.1 Chemicals and reagents

The materials utilized in this study were of analytical grade and required no additional purification. DPPH (1,1-Diphenyl-2-picrylhydrazyl) (Sigma-Aldrich, Germany), Methanol (Lobachemie, India), Trolox ((s)-(-)-6-hydroxy-2,5,7,8- tetramethychroman-2-carboxylic acid) (Sigma-Aldrich, Denmark), drying agent $CaCl_2$, Dimethyl sulfoxide (DMSO) 10% (Riedeldehaen, Germany), RPMI-1640 medium (Roswell Park Memorial Institute-1640 medium) (Sigma-Aldrich, R0883, UK) and Trypsin EDTA which was purchased from Sigma-Aldrich.

2.1.2 Instrumentation

Gas Chromatography-Mass Spectrometry (GC-MS) (QP-5000 GC-MS Shimadzu, Japan), Electronic balance (Wagl, AS 220/C/2, Radwag,Poland), hydro distillation apparatus, 96-well plates (Greiner bio-one, North America), UV-Vis (Ultraviolet-Visible) spectrophotometer (Jenway 7315, England), micropipettes (Finnpipette, Finland), and Inverted biological microscope (MRC, 2017-170529, China), Microplate Absorbance (Bio Tek, 1903217-2019, USA).

2.1.3 plants collection and preparation

Artemisia, *Chiliadenus iphionoides*, and *Teucrium polium* leaves were collected from Jericho in Palestine in June 2023: Jericho, the world's deepest place. The plants were washed with distilled water and then dried in the shade for a week at room temperature (25±3 °C) and humidity (55±4 RH). After being dried the leaves were separated from the stems and coarsely ground, then stored in paper bags for further isolation process.

2.1.4 Extraction of Artemisia, *Chiliadenus iphionoides*, Teucrium polium essential oils

The EOs were extracted by hydro-distillation; 100g of the dried, ground leaves were added to a 500 mL volumetric flask along with distilled water. For three hours, the flask was exposed to the Clevenger apparatus (Figure 2.1). The extracted EO was dried with calcium chloride and then placed in small, clean glass vials sealed with a tight cover. The

plant name, date, and sample code were written on the vials. After that, the vials were stored in a refrigerator at 4 °C.

2.2 Identification of the chemical composition of *Artemisia, Chiliadenus iphionoides,* and *Teucrium polium* EOs by gas chromatography-mass spectrometry (GC–MS)

The components of *Artemisia, Chiliadenus iphionoides*, and *Teucrium polium* were separated and identified using the Perkin Elmer Elite-5-MS fused silica capillary column (30 m 0.25 mm, film thickness of 0.25 m). The carrier gas used in the experiment was helium, with a typical flow rate of 1.1 mL/min. The temperature of the injector was adjusted to 250 °C, with a 4.0 °C/min ramp to 280 °C, a 5-minute initial hold, and a temperature of 50 °C. The entire running time was 62.5 minutes, with a solvent delay ranging from 0 to 4.0 minutes. An MS scan with a mass range of 50.00 to 300.00 m/z took 4 to 62.5 minutes.

The compounds were identified using both computed retention indices (RI) and mass spectral data. The equation used to calculate the linear-temperature-programmed RI was: $RIx = 100n + 100\ (x) - (n)\ (n+1) + (n)$, where $t(x)$ denotes the retention time of the compound under consideration (x) and $tR(n)$ and $tR(n+1)$ are the retention times of n-alkanes (leaving the chromatographic column before and after the compound under consideration).

The molecular components of both species were identified by comparing the mass spectra of *Artemisia, Chiliadenus iphionoides*, *Teucrium polium* to reference spectra from the NIST's MS Data Centre and by relating their Kovats and retention indices to those reported in the literature [84–86]. Quantitative calculations were used to determine the percentage areas for each component from the EOs of *Artemisia, Chiliadenusiphionoides*, and *Teucrium polium*.

2.3 DPPH radical method for evaluation of antioxidant activity

Using a literature-based technique, the free 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was used to assess the antioxidant activity of *Artemisia, Chiliadenus iphionoides, Teucrium polium*, and 1:1 combination of *Artemisia* and *C. iphionoides* essential oils, *Artemisia* and *T. Polium* essential oils, and *C. iphionoides* and *T. Polium* [87]. The antioxidant reduces the strength of the violet DPPH radicals and converts them

into stable pale-yellow DPPH molecules by attaching hydrogen atoms to them. To evaluate the antioxidant activity, the residual violet DPPH radicals are measured at a wavelength of around 515–520 nm using a UV-Vis spectrophotometer [88].

For every EO, a stock solution containing 100 µg/mL was created by dissolving 10 mg of the oil in 100 mL of methanol. Subsequently, a trolox solution, serving as the reference product, was made using the same methodology. Using a 10 mL volumetric flask, six dilutions were made by taking amounts of the stock solution (EO and Trolox) that were 0.5, 1.0, 2.0, 5.0, 8.0, and 10.0 mL and raising them to 10 mL with methanol.

(VF), correspondingly, working solutions containing 5, 10, 20, 50, 80, and 100 µg/ml were prepared. The freshly synthesized DPPH solution had a concentration of 0.002% w/v and was made by dissolving 2 mg of DPPH in 100 mL of methanol using 100 mL VF. One milliliter of the methanolic DPPH solution was added to each EO dilution. After adding 1 mL of methanol, the final working capacity was 3 mL. The series concentrations' blank control was created by dissolving DPPH in methanol at 1:2 ratios without the addition of EO. After that, all of those solutions were let to stand at room temperature in complete darkness for thirty minutes. Using a UV-Vis spectrophotometer set at 517 nm, the absorbance of those solutions was measured after the incubation period. The spectrophotometer was reset to zero using methanol.

Using the following equation, the antioxidant activity of the EOs was calculated in terms of inhibition percentage:

$$\boldsymbol{In}\% = \frac{\boldsymbol{A\ blank - A\ sample}}{\boldsymbol{A\ blank}} \times \mathbf{100} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.1)$$

Equation (2.1): Inhibition% of antioxidant activity [80]

Where A blank represents the absorbance of the control reaction (which contains all reagents excluding the test compound) and A sample represents the absorbance of the test compound.

2.4 Antibacterial activity tests

The American Type Culture Collection (ATCC) provided six reference bacterial strains for this study: *Enterococcus faecalis* "ATCC; 29212", *Pseudomonas aeruginosa* "ATCC; 27853", *Staphylococcus aureus* "ATCC; 25923", *Staphylococcus aureus* "ATCC; 6538", *Escherichia coli* "ATCC; 25922"

2.4.1 Preparation of bacterial suspensions

A stock solution of each essential oil was prepared at a concentration of 500 mg/mL by dissolving the essential oil in 88% dimethyl sulfoxide (DMSO). These stock solutions were then serially diluted two-fold in sterile Muller-Hinton broth to obtain final concentrations of 250.0, 125.0, 62.5, 31.25 mg/mL, and so on.

The dilution series was performed aseptically in 96-well microtiter plates. For each bacterial strain, wells 1 to 11 were inoculated with the bacterial suspension, and wells 1 to 10 received the diluted essential oil solutions. Well 12, containing neither essential oil nor bacteria, served as the negative control, ensuring no microbial contamination. Well 11, containing only the bacterial suspension without essential oil, served as the positive control for bacterial growth.

The microtiter plates were incubated at 37 °C for 24 hours. The Minimum Inhibitory Concentration (MIC) of each essential oil was determined as the lowest concentration at which no visible bacterial growth was observed and the minimum bactericidal concentration (MBC) at all wells until 1 below the MIC well to which no bacterial growth on Petri dish after 24 hours. Ciprofloxacin was used as a positive control to validate the antibacterial activity of the essential oils. All experiments were performed in duplicate to ensure reproducibility and accuracy.

2.5 Porcine pancreatic lipase inhibition assay

The porcine pancreatic lipase inhibition was assessed employing a previously reported procedure [89].

2.5.1 Preparation of stock and working solutions

A stock solution of *Artemisia, Chiliadenus iphionoides,* and *Teucrium polium* essential oil (EO) was prepared at a concentration of 500 μg/mL in 10% dimethyl sulfoxide (DMSO). From this stock, a dilution series was made, producing five different

concentrations of 50, 100, 200, and 500 µg/mL. Additionally, a fresh stock solution of porcine pancreatic lipase (1 mg/mL) was prepared in Tris-HCl buffer, along with a stock solution of 20.9 mg of p-nitrophenyl butyrate (PNPB) dissolved in 2 mL of acetonitrile.

2.5.2 Assay of pancreatic lipase PL inhibition

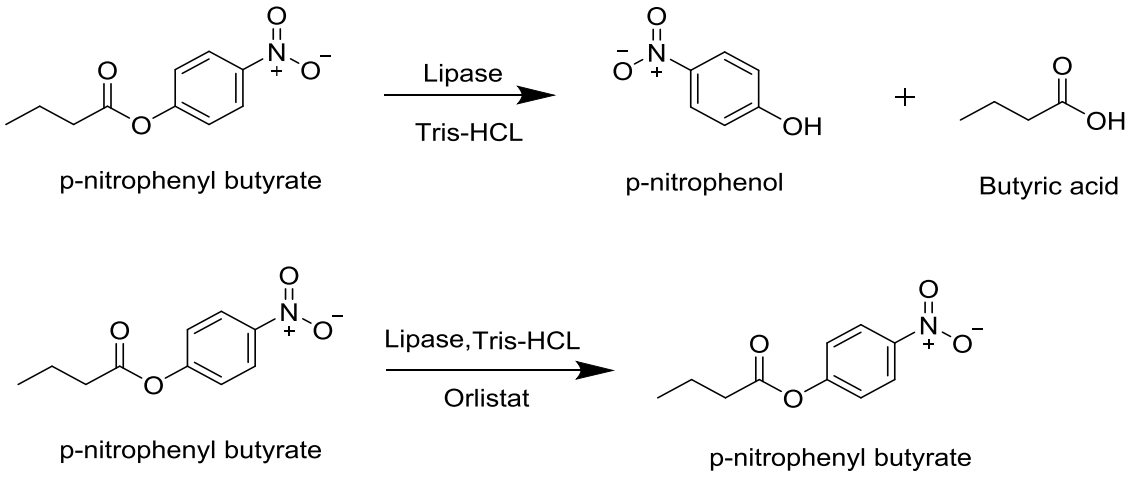
For each working solution, 0.1 mL of porcine pancreatic lipase (1 mg/mL) was combined in a separate test tube with 0.2 mL of *Artemisia*, *Chiliadenus iphionoides*, and *Teucrium polium* essential oil (EO) from each dilution in the series. Tris-HCl buffer was then added to adjust the final volume to 1 mL. The mixtures were incubated at 37 °C for 15 minutes, followed by the addition of 0.1 mL of p-nitrophenyl butyrate (PNPB) in acetonitrile to each test tube. The resulting mixtures were further incubated at 37 °C for 30 minutes. The pancreatic lipase activity was determined by measuring the hydrolysis of PNPB into p-nitro phenolate using a UV spectrophotometer at 410 nm. The same procedure was conducted using orlistat as a positive control. The percentage inhibition of lipase activity by the plant EO was calculated using the following equation:

$$Lipase\ inhibition\% = \frac{AB - Ats}{AB} \times 100\% \quad \text{..... (2.2)}$$

Equation (2.2): Pancreatic lipase inhibition Where *AB* represents the recorded absorbance of the blank solution, and *Ats* represents the recorded absorbance of the tested sample solution.

Scheme 2.1

Different Hydrolysis of p-nitrophenyl butyrate (PNPB) with and without Orlistat [90]



p-nitrophenyl butyrate | p-nitrophenol | Butyric acid

p-nitrophenyl butyrate | p-nitrophenyl butyrate

2.6 α-Amylase Inhibition Assay

A modified standard method was used to evaluate the α-amylase inhibitory activity of *Artemisia*, *Chiliadenusiphionoides*, and *Teucrium polium* essential oils (EO) [91].

The essential oil (100 mg) was first dissolved in a small amount of 10% DMSO, followed by the addition of 0.02 M Na_2 HPO_4 /NaH_2 PO_4 and 0.006 M NaCl buffer solution (pH 6.9) to a final volume of 100 mL, creating a stock solution with a concentration of 1 mg/mL. From this stock, dilutions of 50, 100, 300, and 500 µg/mL were prepared using 10% buffer as the diluent.

A 0.2 mL aliquot of each essential oil solution was mixed with 0.2 mL of porcine pancreatic α-amylase (2 units/mL). After a 10-minute incubation at 30 °C, 0.2 mL of freshly prepared 1% starch solution was added, and the mixture was incubated for an additional 3 minutes. The reaction was terminated by adding 3,5-dinitrosalicylic acid (DNSA), followed by dilution with 5 mL of distilled water. The mixture was then heated at 90 °C in a water bath for 10 minutes. After cooling to room temperature, the absorbance was measured at 540 nm.

The blank control was prepared using the same quantities as described above, but with 0.2 mL of buffer in place of the essential oil. The α-amylase inhibitory activity was calculated using the following equation:

$$\alpha - Amylase\ inhibition\% = \frac{AB - AT}{AB} \times 100\% \quad \text{..... (2.3)}$$

Equation (2.3): α-Amylase inhibition

Where *AB* represents the absorbance of the blank sample and *AT* denotes the absorbance of the test sample.

Chapter Three

Results

3.1 GC–MS characterization of *Artemisia, Chiliadenus iphionoides, Teucrium polium* essential oils

Hydro distillation of the dried leaves of *Artemisia*, *Childenus iphionoides*, and *Teucrium polium* yielded pale yellow oils, producing 0.25%, 0.07%, and 0.32%, respectively. The phytochemical composition of the three oils was examined using GC-MS, as depicted in Figure 1. Table 1 displays the names, retention times (RT), retention indices (RI), and percentages of identified components in the essential oils (EOs). 17 compounds representing 99.58% of the total EO of *T. Polium* were discovered, with E-nerolidol (27.11%), geranyl acetone (23.26%), germacrene D (19.08%), β-caryophellen (17.78%), α-caryophellen (3.35%), and bicyclogermacrene (3.08%), being the predominant components.

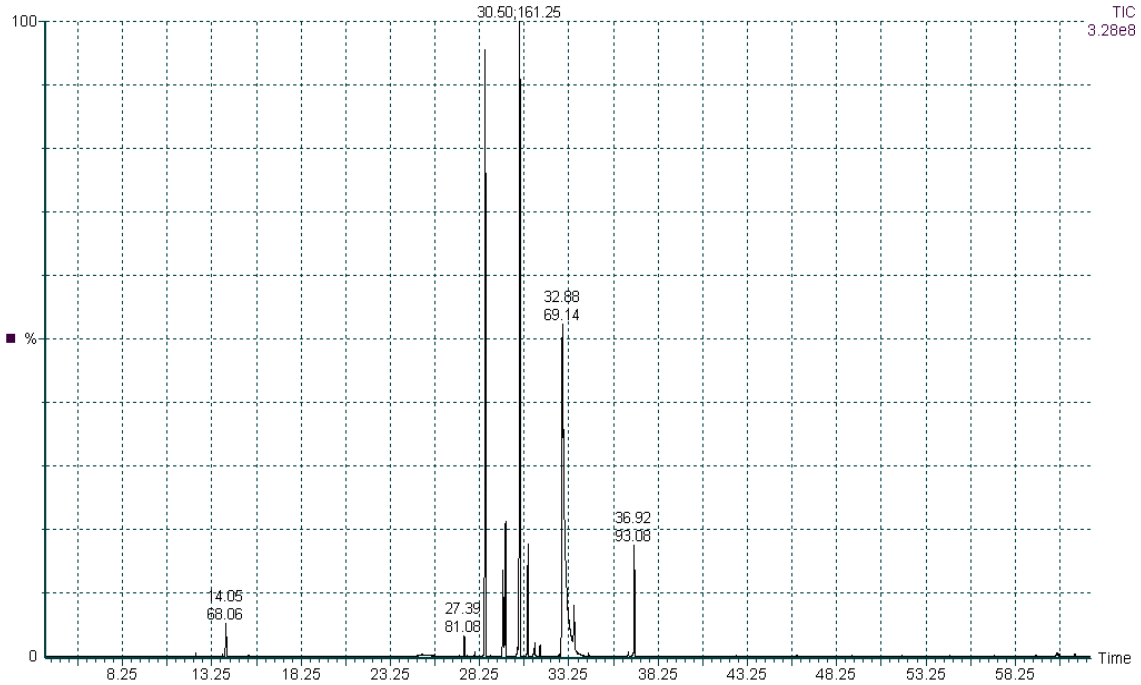
Table 3.1

GC-MS chromatogram of Teucrium polium

	Compounds	R.T	RI	Area%
1	Myrcene	12.35	987	0.05
2	p-Cymene	13.84	1022	0.03
3	Sylvestrene	14.05	1027	0.96
4	γ-Terpinene	15.3	1056	0.02
5	2-Methoxy-4-vinylphenol	25.07	1312	0.45
6	2.3,5-Trimethylphenol	25.75	1333	0.03
7	α –Copaene	27.12	1374	0.03
8	β-Bourbonene	27.39	1382	0.54
9	Sesquithujene	27.98	1399	0.09
10	β-Caryophellene	28.57	1418	17.78
11	Nd	28.87	1426	0.03
12	Geranyl acetone	29.56	1450	23.26
13	α-caryophellene	29.7	1454	3.35
14	Germacrene D	30.5	1480	19.08
15	Bicyclogermacrene	30.95	1495	3.08
16	E-Nerolidol	32.88	1560	27.11
17	Caryophellene oxide	33.55	1582	1.12
18	Nd	34.36	1610	0.05
19	Nd	36.59	1690	0.12
20	β-Sinensal	36.92	1702	2.56
21	Nd	54.58		0.01
22	Nd	57.07		0.02
23	Nd	59.43		0.02
24	Nd	60.59		0.15
25	Nd	60.73		0.07
	Total identified%			99.58
	Monoterpene hydrocarbons			1.06
	Oxygenated monoterpenes			23.26
	Sesquiterpene hydrocarbons			43.95
	Oxygenated sesquiterpenes			30.79
	Others			0.48

Figure 3.1

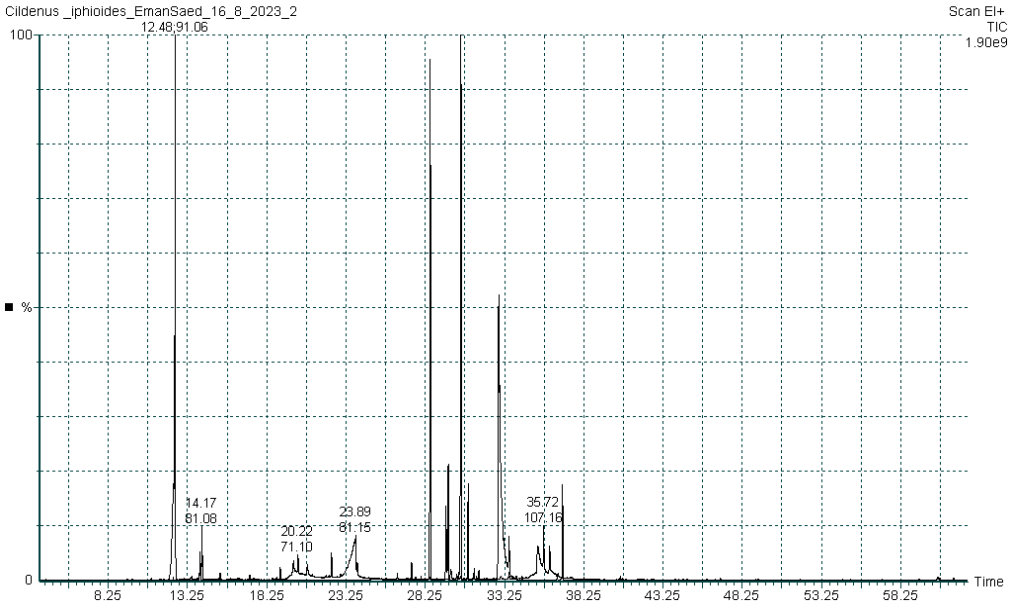
GC-MS chromatogram of Teucrium polium



GC-MS analyses of the *Chiliadenus iphionoide* EO resulted in the identification of 47 compounds accounting for 98.81% of the total oil, with cresol methyl ether (52.93%), ethyl oct-2-ynoate (14.36%), epi cadinol (6.56%), 1,8-cineole (4.25%), and epi-**α**-eudesmol (3.66%).

Figure 3.2

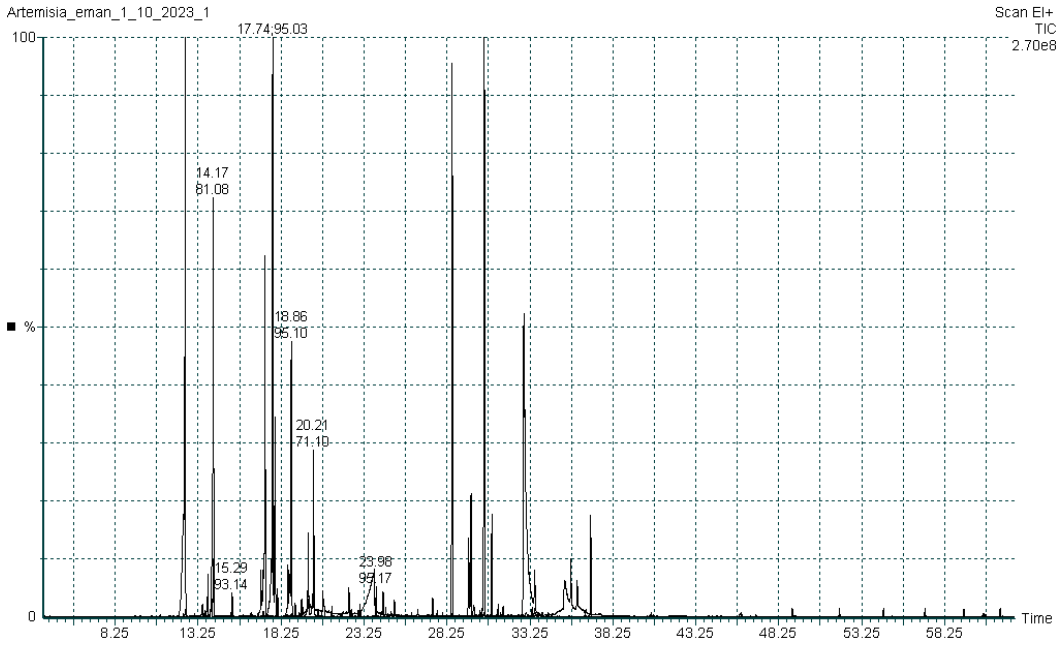
GC-MS chromatogram of Chiliadenusiphionoide



GC-MS analysis of the *Artemisia* essential oil identified 51 components, constituting 98.96% of the total oil composition. Linalool acetate, a monoterpene ester, constituted 49.1% of the identified chemical. The sequence included oxygenated monoterpene bicyclic ether 1,8-cineole (28.67%), followed by trans-thujone (24.0%), cis-thujone (17.69%), camphor (12.76%), and concluding with terpinen-4-ol (8.34%). The five principal components are classified as oxygenated monoterpenes, accounting for 90% of the total contribution.

Figure 3.3

GC-MS chromatogram of Artemisia



3.2 Antioxidant Activity of *Artemisia, Chiliadenus iphionoides, Teucrium polium,* mix of *Chiliadenus iphionoides* and *Teucrium polium,* mix of *Artemisia* and *Teucrium polium,* mix of *Artemisia* and *Chiliadenus iphionoides*

The antioxidant capabilities of *Artemisia*, *Chiliadenus iphionoides*, *Teucrium polium*, mix of *Chiliadenus iphionoides* and *Teucrium polium*, mix of *Artemisia* and *Teucrium polium*, mix of *Artemisia* and *Chiliadenus iphionoides*.EO's were assessed utilizing DPPH radical scavenging and reduction power activities. For each of the EOs, the antioxidant half-maximal inhibitory doses (IC_{50}) (giving 50% inhibition) were calculated. Both oils were found to exhibit effective in scavenging the DPPH radicalswithIC_{50} values

Table 3.2

Artemisia EO, Chiliadenus iphionoides EO, Teucrium polium EO activity versus DPPH

Concentrations (μg/mL)	Trolox	*Artemisia* EO	*C.iphionoides* EO	*T. Polium* EO
5	58.71 ± 0.26	23.86 ± 0.53	19.31 ± 1.07	17.23 ±0.26
20	93.01 ± 0.33	42.23 ± 0.27	57.58 ± 0.54	51.89± 0.54
50	93.34 ± 0.33	57.77 ± 0.27	65.72 ± 0.27	62.69± 0.27
80	93.67 ± 0.33	72.54 ± 0.80	68.94 ± 0.54	72.16± 0.80
100	93.67 ± 0.68	87.12± 0.54	75.76 ± 0.54	90.34± 0.27
IC_{50}	4.3 ± 0.58	35.00± 0.47	17.03± 0.59	19.18± 0.34

Values are the mean ± SD (n = 3/group). DPPH is 1,1-diphenyl-2-picrylhydrazyl, and IC_{50} is the half-maximum inhibitory concentration.

Table 3.3

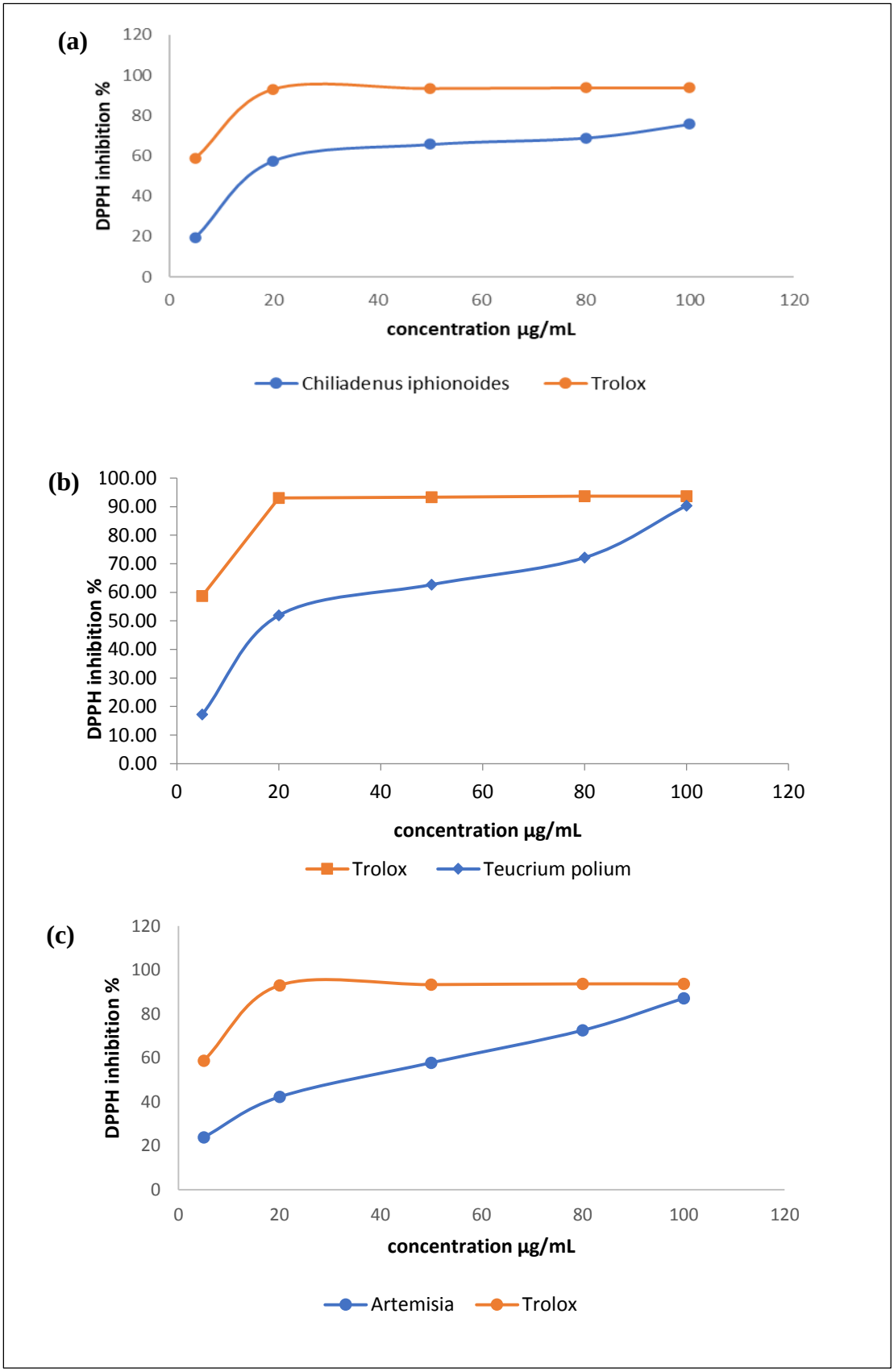
Antioxidant Activity of mix of Chiliadenus iphionoides and Teucrium polium, mix of Artemisia and Teucrium polium, mix of Artemisia and Chiliadenus iphionoides

Concentrations (μg/mL)	Trolox	Mix of *C.iphionoides* and *T.polium*	mix of *Artemisia* and *T. Polium*	mix of *Artemisia* and *C. iphionoides*
5	58.71 ± 0.26	18.18 ± 3.21	24.43± 0.26	11.55 ± 0.26
20	93.01 ± 0.33	53.60± 0.27	46.21± 0.00	59.09± 0.54
50	93.34 ± 0.33	66.48± 0.27	58.90± 0.27	60.98± 0.54
80	93.67 ± 0.33	74.81± 0.27	74.81± 0.27	69.51± 0.80
100	93.67 ± 0.68	80.87± 0.27	77.27± 0.00	75.95± 1.34
IC_{50}	4.3 ± 0.58	18.47± 0.86	28.96± 0.16	17.13±0.70

Values are the mean ± SD (n = 3/group). DPPH is 1,1-diphenyl-2-picrylhydrazyl, and IC_{50} is the half maximum inhibitory concentration.

Figure 3.4

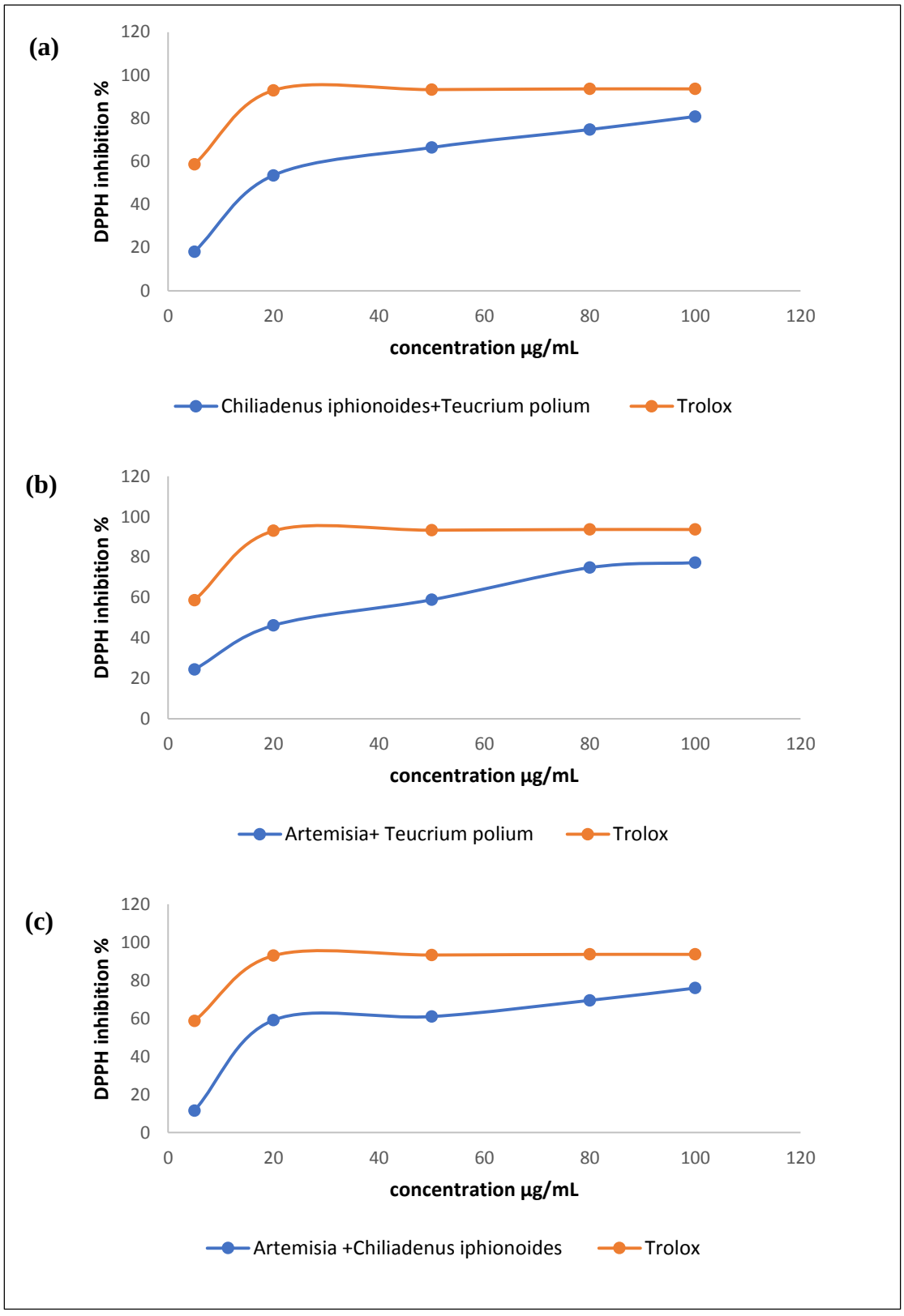
DPPH Inhibition % by Trolox and Chiliadenus iphionoides, Teucrium polium, Artemisia EO



(a) Chiliadenus iphionoides (b) Teucrium polium(c) Artemisia EO.

Figure 3.5

DPPH Inhibition% by Trolox and mix of (Chiliadenus iphionoides, Teucrium polium), (Artemisia, Teucrium polium), and (Artemisia,Chiliadenus iphionoides) EO



(a) (Chiliadenus iphionoides, Teucrium polium) (b) (Artemisia, Teucrium polium) (c) (Artemisia,Chiliadenus iphionoides) EO

3.3 Anti-microbial activity

Table 3.4

Antimicrobial effects MIC (mg/mL) of AEO and CEO and positive control

	Bacteria				
ATCC Number/strain	29212	27853	25923	6538	25922
Bacteria	*E.faecalis*	*P.aeruginosa*	*S.aureus*	*S.aureus*	*E.coli*
AEO	R	R	3.906	R	R
CEO	R	R	R	31.3	R
Ciprofloxacin	0.894	0.351	0.288	0.945	0.190

Where R = Resistance

Table 3.5

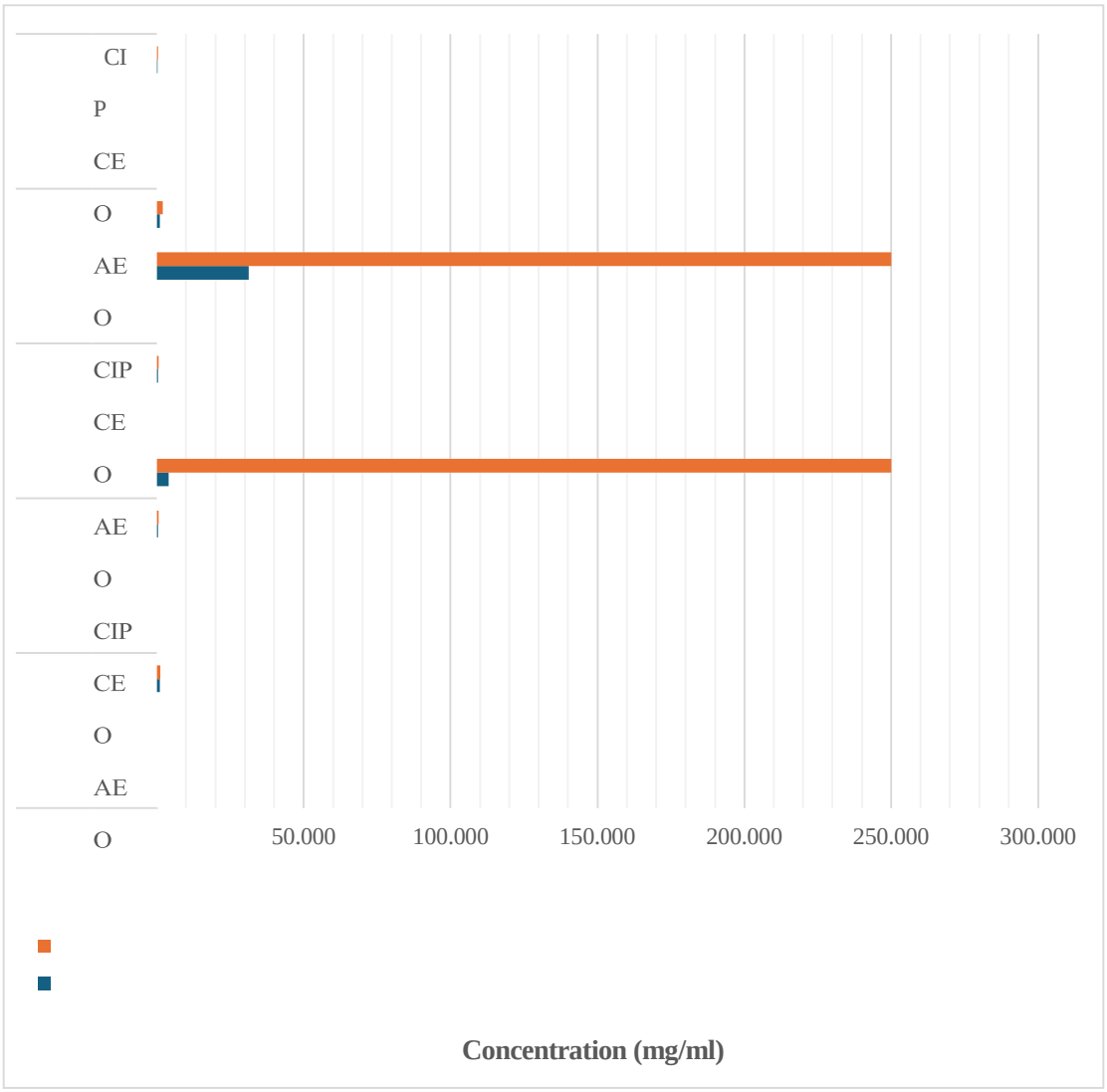
Antimicrobial effects MBC (mg/mL) of AEO and CEO and positive control

	Bacteria				
ATCC Number/strain	29212	27853	25923	6538	25922
Microbe	*E.faecalis*	*P.aeruginosa*	*S.aureus*	*S.aureus*	*E.coli*
AEO	R	R	250.0	R	R
CEO	R	R	R	250.0	R
Ciprofloxacin	1.26	0.667	0.478	1.87	0.39

Where R = Resistance

Figure 3.6

MIC and MBC for AEO and CEO.



	E.faecalis			*P.aeruginosa*			*S.aureus*(25923)			*S.aureus*(6538)			*E.coli*		
	AEO	CEO	CIP	AEO	AEO	CEO	CIP	CEO	CIP	AEO	CEO	CIP	AEO	CEO	CIP
MBC	0.000	0.000	1.260	0.000	0.000	0.000	0.390	0.000	0.667	250.0	0.000	0.478	0.000	250.0	1.870
MIC	0.000	0.000	0.894	0.000	0.000	0.000	0.190	0.000	0.351	3.906	0.000	0.288	0.000	31.30	0.945

The antimicrobial efficacy of AEO against various bacteria was found to be negligible. However, when tested against *Staphylococcus aureus* strain ATCC 25923, AEO exhibited MIC of 3.906 mg/ml and an MBC of 250.0 mg/ml, from start solution 500.0 mg/ml.

Notably, the deference between MICs and MBCs was 16-fold, indicating a bacteriostatic effect. Similarly, the CEO showed limited activity against all bacteria except for *Staphylococcus aureus* strain ATCC 6538. For this strain, CEOs demonstrated a MIC of 31.3 mg/ml and an MBC of 250.0 mg/ml, also from the start solution of 500.0 mg/ml.

The difference between MICs and MBCs was 8-fold, suggesting a bacterio static effect. These findings suggest that both AEOs and CEOs could be explored as topical skin ointments for treating infections caused by *Staphylococcus aureus*. However, oral consumption of such high concentrations may have adverse effects on human health. Further research should focus on isolating and studying the specific antimicrobial compounds present in these essential oils.

3.4 Porcine pancreatic lipase inhibitory activity

In this assay, the anti-obesity activity of *Artemisia*, *Chiliadenus iphionoides*, *Teucrium polium* was compared with that of orlistat, a potent lipase inhibitory agent which is responsible for the digestion of dietary fat.

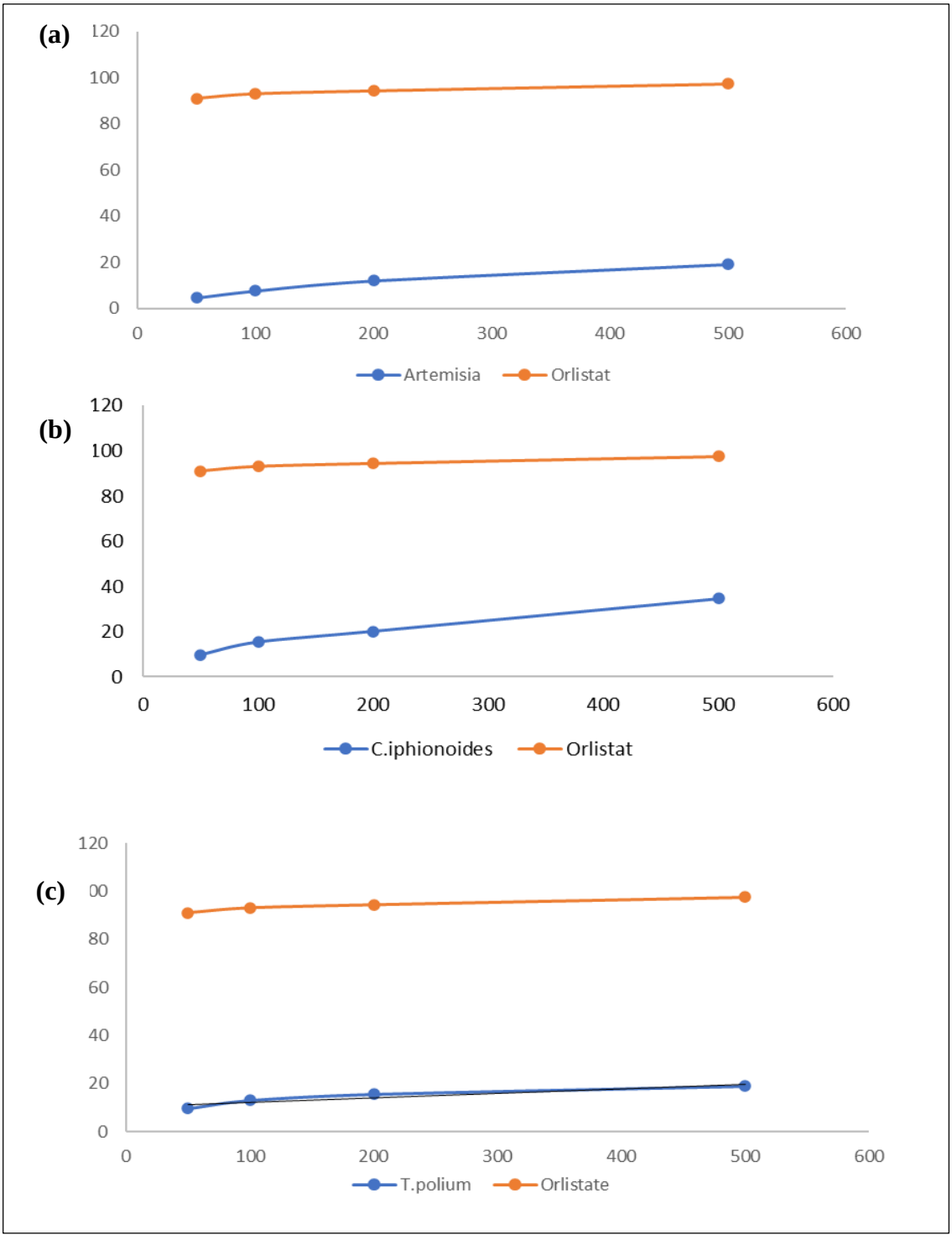
Table 3.6

The inhibitory activity of Artemisia, Chiliadenus iphionoides, Teucrium polium essential oil and orlistat against porcine pancreatic lipase.

Concentrations (μg/mL)	Orlistat	*Artemisia EO*	*C.iphionoides EO*	*T.polium EO*
50	91.05 ± 0.77	4.74±0.19	9.75±0.38	7.31±0.38
100	93.1 ± 0.42	7.72± 0.19	15.45±0.77	17.98± 0.38
200	94.3 ± 0.42	12.06± 0.19	20.19± 2.11	30.76± 1.34
500	97.5±0.00	19.11± 0.19	34.69± 0.38	45.26± 0.38
IC_{50}	93.87 ±0.40	534± 0.19	931.58± 0.91	368.13± 0.62

Figure 3.7

Inhibition percentage of lipase by Artemisia, Chiliadenus iphionoides, Teucrium polium EO essential oil and orlistat



(a) Artemisia, (b) Chiliadenus iphionoides, (c) Teucrium polium EO essential oil and orlistat

3.5 α-Amylase activity

The lipase-catalyzed hydrolysis of p-nitrophenyl butyrate to the chromophore (p-nitrophenol) assay was used to determine the inhibitory activity of *Artemisia*, *Chiliadenus iphionoides, Teucrium polium* EO on the porcine pancreatic α-amylase enzyme. The assay was compared with acarbose (positive control), a potentα-amylase inhibitory agent, and the IC50 values were calculated for *Artemisia*, *Chiliadenus iphionoides*, *Teucrium polium* EO, and acarbose (Table 3.7 and Fig. 3.6)

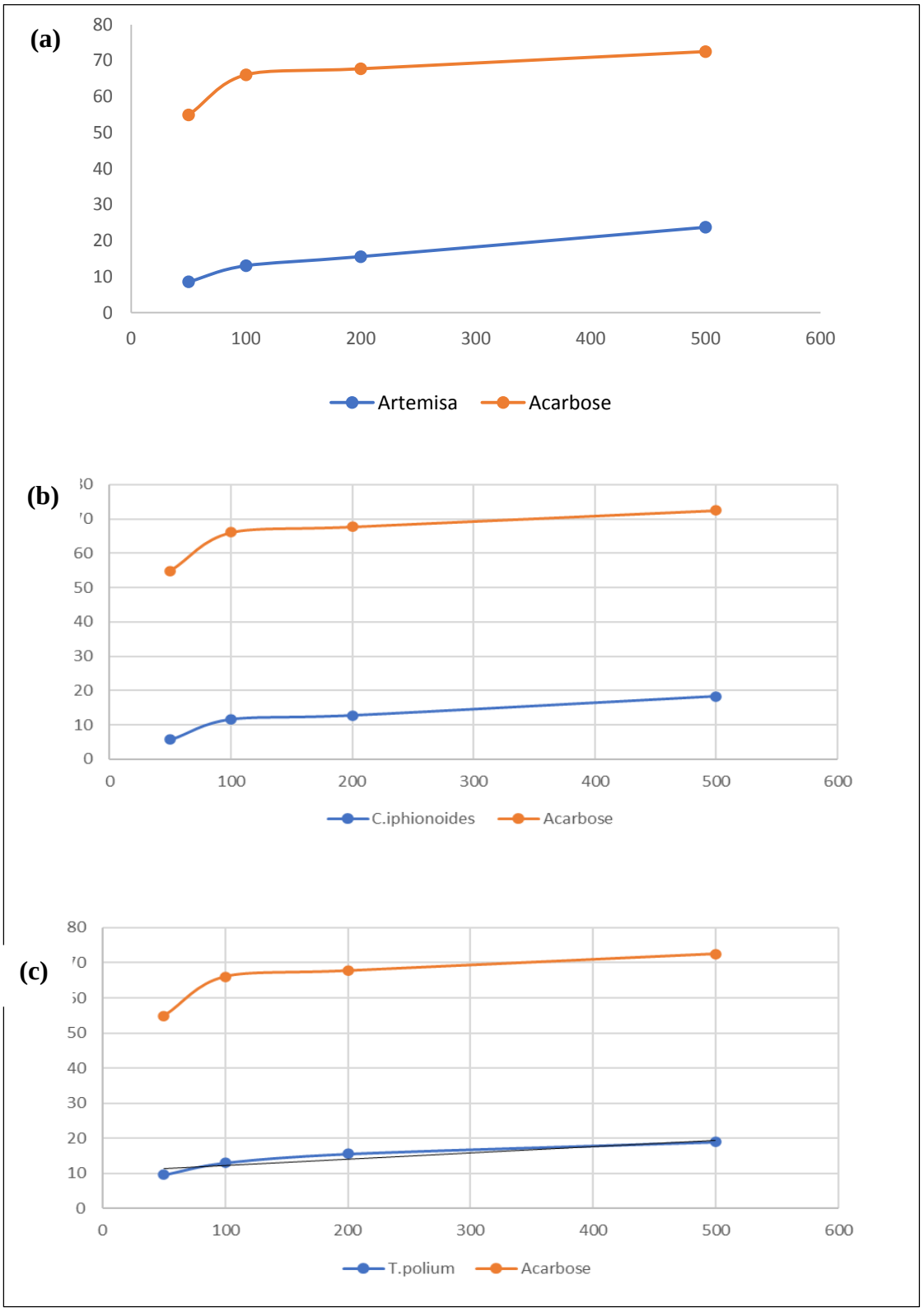
Table 3.7

α-Amylase inhibition of ***Artemisia, Chiliadenus iphionoides, Teucrium polium*** *essential oil compared with acarbose.*

Concentrations (μg/mL)	Acarbose	*Artemisia* EO	*C.iphionoides* EO	*T.polium* EO
50	54.91 ± 0.58	8.56±0.19	5.75±0.19	09.69±0.19
100	66.1 ± 1.62	13.06± 0.20	11.66±0.20	13.06± 0.20
200	69.3±1.53	15.59± 0.20	12.78± 0.20	15.59± 0.20
500	72.54 ± 1.37	23.74± 0.20	18.26± 0.40	18.96± 0.20
IC_{50}	56.42± 1.275	569± 0.20	1550± 0.25	569± 0.20

Figure 3.8

α-Amylase inhibition of Artemisia, Chiliadenus iphionoides, and Teucrium polium essential oils compared with acarbose



(a)Artemisia(b) Chiliadenus iphionoides (c) Teucrium polium essential oils compared with acarbose.

Chapter Four

Discussion

4.1 Phytochemistry of *Teucrium polium*, *C. iphionoides*, and *Artemisia* essential oils

4.1.1 Phytochemistry of *Teucrium polium* essential oils

The chemical composition of EOs recovered by hydrodistillation in **0.32%** yield from dried *Teucrium polium* leaves harvested in Jericho in early April 2023 was investigated. The phytochemical composition of three oils was analyzed using GC-MS (Figure 1). Table 1 presents the names, retention times (RT), retention indices (RI), and percentages of the identified constituents for the essential oil (EO). GC-MS analysis of the extracted *Teucrium polium* EO revealed 17 compounds, which together made up 99.58% of the whole essential oil. E-nerolidol (27.11%), geranyl acetone (23.26%), germacrene D (19.08%), β-caryophyllene (17.78%), α-caryophyllene (3.35%), and bicyclo germacrene (3.08%) were the main components. The 17 discovered components of the essential oil can be categorized into four groups, with sesquiterpene hydrocarbons constituting the predominant category at 43.95%, followed by oxygenated sesquiterpenes at 30.79% and oxygenated monoterpenes at 23.26%.

Prior research conducted by (Emre Sevindik et al.) detailed the analysis of essential oil extracted from *Teucrium polium* obtained from ArdahanCıldır Lake (elevation 1970 m) and its surrounding area in Turkey, indicating that 14 to 20 constituents comprised 77.22% of *T. Polium*.The compounds present in the highest concentrations were (Z)-b-farnesene (15.49%), b-phellandrene (10.77%), and a-farnesene (10.71%), whilst b-pinene displayed the lowest concentration at 0.74% [92]. A distinct study carried out in the eastern region of Turkey, specifically in Ağrı, was published by (Toplan et al.).The analysis of *Teucrium polium* revealed a complex chemical composition characterized by a predominance of sesquiterpenes and monoterpenes. The primary compounds identified were β-caryophyllene (8.8%), α-cadinol (5.4%), (E)-nerolidol (5%), and α-pinene (4.7%). These findings confirm the essential oil's significant chemical diversity and its potential for therapeutic applications[93]. Farahbakhsh documented the identification of 64 compounds in the essential oil of *T. Polium*'s aerial parts, collected from Iran, of which hvalerianol (21.44%), β-pinene (12.97%), epi-α-bisabolol (9.86%), α-pinene (6.7%), caryophyllene (4.71%), limonene (3.45%), and carvone (3.85%)were the main

components [94]. El Atki et al. reported the identification of twenty-two constituents accounting for 94.49% of the total essential oil extracted via hydro distillation from the aerial parts of *T. Polium* subs *T. Polium* collected in Morocco, with 3-carene (16.49%), c-muurolene (14.03%), a-pinene (9.94%), a-phellandrene (6.93%), and caryophyllene (7.51%) as the primary constituents.Sesquiterpene hydrocarbons are the predominant category, comprising 54.3% of total oil, whereas monoterpene hydrocarbons account for 38.23% [95].The GC/MS study of the essential oil extracted from the aerial portions of *T. Polium*, collected during the flowering stage in June from Tunisia, identified 71 compounds, which constituted 89.66% of the total oil. The principal components of essential oils comprised α-pinene (17.04%), β-pinene (12.68%), and limonene (6.65%), followed by β-myrcene (6.07%) and germacrene D (5.89%). Unlike prominent literature studies, the essential oil had a significant proportion of monoterpene hydrocarbons (48.73%), followed by sesquiterpenes (20.14%), oxygenated monoterpenes (14.00%), and a minimal 5.54% of oxygenated sesquiterpenes [96].

A study conducted by the (Kabouche et al. Group) in Algeria revealed that the hydro distillation of the aerial parts of *Teucrium polium*.*T.polium* yielded twenty-one compounds, accounting for 91.5% of the essential oil. The predominant components included α-cadinol (46.8%), 3-β-hydroxy-α-muurolene (22.5%), α-pinene (9.5%), and β-pinene (8.3%) [97].

4.1.2 Phytochemistry of *C.iphionoid* essential oils

The chemical makeup of essential oil extracted using hydrodistillation, yielding 0.07% from dried *C. iphionoides* leaves collected in Jericho in early April 2023, was examined. GC-MS analysis of the extracted *C. iphionoides* essential oil identified 47 components, constituting 98.81% of the total oil, with cresol methyl ether (52.93%), ethyl oct-2-ynoate (14.36%), epi-cadinol (6.56%), 1,8-cineole (4.25%), and epi-α-eudesmol (3.66%) were the major components. The 47 identified constituents of the essential oil can be classified into four categories: others at 68.22%, oxygenated monoterpenes at 19.95%, oxygenated sesquiterpenes at 7.39%, and sesquiterpene hydrocarbons at 2.38%. Previous investigations have extensively studied the essential oil composition of *C. iphionoides* using gas chromatography-mass spectrometry (GC-MS), as described by (Hilla Tamir). The principal component is Eucalyptol (1,8-cineole), comprising up to 36.08% of the oil, making it the predominant oxygenated monoterpene.Borneol (49.3%), camphor (3.7%),

α-terpineol (3.8%), and bornyl acetate (2.9%) have been detected as well. Research conducted on samples gathered throughout Israel revealed considerable intraspecific heterogeneity in chemical composition attributable to environmental and geographic influences. Northern populations exhibited elevated amounts of 1,8-cineole and t-cadino.Southern populations exhibit elevated concentrations of camphor, α-pinene, and fokienol[98].(AlNaimat et al.) reported the identification of twenty-three compounds in the essential oil of *C.iphionoides* leaves collected in As-Subayhi, Al Balqa, Jordan, via GC-MS analysis. The examination of the crucial oil disclosed significant amounts of eucalyptol (42.6%) and trans-chrysanthemum (20.65%). Additional chemicals identified were Yomogialcohol (10.04%), γ-terpinene (4.09%), and o-cymene (3.40%)[99].

4.1.3 Phytochemistry of *Artemisia* essential oils

The chemical makeup of essential oils obtained through hydro distillation, yielding 0.25% from dried *Artemisia* leaves collected in Jericho in early April 2023, was examined. The GC-MS analysis of the extracted *Artemisia* essential oil identified 51 components, which comprised 98.96% of the overall oil content. Linalool acetate, a monoterpene ester, comprised 49.1% of the discovered compound. The sequence comprised oxygenated monoterpene bicyclic ether 1,8-cineole (28.67%), followed by trans-thujone (24.0%), cis-thujone (17.69%), camphor (12.76%), and culminating with terpinen-4-ol (8.34%).The 51 identified constituents of the essential oil can be grouped into four categories, with the five primary components designated as oxygenated monoterpenes, comprising 90% of the overall contribution.

(Kordalietal) investigated the EO of *Artemisia* herba-alba leaves taken from Turkey, identifying 19 main chemicals that constituted 98.93% of the total oil. The primary components comprised camphor (51.14%), 1,8-cineole (19.30%), chrysanthenone (5.68%), and camphene (6.90%). Camphor and 1,8-cineole are extensively recognized for their antibacterial and antioxidant characteristics. The prevalence of oxygenated monoterpenes (86.23%) indicates significant potential for industrial and medicinal applications[100].(Nidal et al.) reported the identification of nineteen components in the essential oil of *Artemisia* jordani causing gas chromatography-mass spectrometry (GC-MS). The compounds were both subjectively and quantitatively described within the essential oil of the leaves, constituting 100% of the total EO mass, with bornyl acetate (63.40%) and endo-borneol (17.75%) identified as the predominant constituents.

Furthermore, the predominant phytochemical classes were oxygenated monoterpenoids (85.98%) and oxygenated sesquiterpenoids (8.01%) [101].

4.2 Evaluation of the Antioxidant of *Teucrium polium, C.iphionoides,* and *Artemisia* Activities

The free radical scavenging activity of the studied essential oils was assessed utilizing the established DPPH assay, as previously delineated.DPPH• is a stable free radical that can take an electron or hydrogen from an antioxidant, thereby converting it into a stable molecule [102].

4.2.1 Evaluation of the Antioxidant of *Teucrium polium* Activities

Figure 3.4 demonstrates that the essential oil isolated from the desiccated leaves of *Teucrium polium* possesses significant antioxidant ability, as evidenced by an IC50 value of 20.08 ± 1.004 µg/mL. The elevated antioxidant capacity of the essential oil from *Teucrium polium* can be attributed to its chemical composition, which includes a high content of E-nerolidol and the presence of phenolic substances such as 2-methoxy-4-vinylphenol and 2,3,5-trimethylphenol. It was reported that cis-nerolidol displayed significant scavenger capacity against DPPH radical with an IC50 value of 1.48 mM. Our findings align with those reported by Bakari et al. regarding the DPPH radical scavenging capacity of *T. Polium* essential oil from Tunisia, which exhibited an IC50 value of 20 ± 1.004 µg/mL [95]. The notable antioxidant activity was attributed to the presence of α-pinene, β-pinene, p-cymene, and borneol, all of which had antioxidant properties.In a Moroccan study, El Atki et al. [96].Discovered that the essential oil of *T. Polium*, characterized by major components t-cadinol (18.3%), germacrene D (15.3%), and β-pinene (10.5%), exhibits DPPH radical scavenging activity with an IC50 value of 7.2 ± 0.55 mg/ml It.

4.2.2 Evaluation of the Antioxidant of *C.iphionoides* Activities

Figure 3.4 demonstrates that the essential oil isolated from desiccated leaves of *C.iphionoides* had significant antioxidant ability, as evidenced by anIC50 value of 17.03± 0.59 µg/mL The high antioxidant for the EO could be referred to because the chemical composition of this essential oilcontains phenolic compounds like Cresol methyl ether. According to previous investigations,the EO was extracted from dry leaves of *C.iphionoides* from Jenin-Palestine. The antioxidant activity of *C. iphionoides* has been

measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay reportedby (Reem Sbiehet al.,). The plant extracts demonstrated significant radical scavenging ability:IC50 value of Hexane extract: 218 μg/mL.The antioxidant activity of *C. iphionoides* is attributed to its ability to scavenge free radicals and inhibit oxidative stress, which plays a key role in preventing diseases such as cancer, diabetes, and cardiovascular disorders.[103].

4.2.3 Evaluation of the Antioxidant of *Artemisia* Activities

Figure 3.4 demonstrates that the essential oil isolated from desiccated leaves of *Artemisia* had significant antioxidant ability, as evidenced by an IC 50 value of 22.17 ± 1.11 μg/mL.The elevated antioxidant capacity of *Artemisia* essential oil can be attributed to its substantial terpenoid composition, including 1,8-cineole and camphor, both of which exhibit significant antioxidant properties.

Previous experiments by Jaradat et al. indicated that the essential oil of *Artemisia* jordanica leaves exhibited a dose-dependent inhibitory effect on DPPH free radical activity, demonstrating 74.88% of the antioxidant capacity relative to the standard antioxidant chemical Trolox. The IC50 was 2.18 ± 0.24 μg/mL [101].Taherkhani et al. showed that the essential oil of *Artemisia* diffusa, sourced from Iran, demonstrated a dose-dependent scavenging effect on DPPH, with an IC50 value of 13.01 mg/ml [104]

Khan et al. demonstrated antioxidant activity in two local wild *Artemisia* species. The EO of *Artemisia* scoparia had an IC 50 of 285 ± 0.82 μg/mL in DPPH, whereas the EO of *Artemisia* absinthium had an IC 50 of 416 ± 0.45 μg/mL. They reasoned that the remarkable antioxidant activity of A. scoparia EO was due to the presence of large amounts of tocopherol and sesquiterpene derivatives[105].

4.3 Evaluation of the Antilipase, and Anti-α-Amylase of *Teucrium polium, C. iphionoides,*and *Artemisia* Activities

4.3.1 Evaluation of the Antilipase, and Anti-α-Amylase of *Teucrium polium* Activities

The inhibitory effects of EO on lipase and α-amylase were assessed. The EO extracted from *Teucrium polium* as in Figure 3.7 demonstrates low lipase inhibition activity, with

IC50=368.15±0.62µg/mL andFigure 3.8 demonstrates lowα-amylase inhibition activity, with an IC50 of 715±0.20µg/mL.

Best of our knowledge this is the first reported lipase inhibition activity for the EO extracted from *Teucrium polium*.

Other groups have been reported the IC50 for α-amylase inhibition for EO derived of form *Teucrium polium*.(Benchikha, et al.) reported that the EO derived from *Teucrium polium* showed a pronounced anti-hyperglycemic activity using α-amylase inhibitory assay (IC50 = 111.68 µg/mL) [106].

4.3.2 Evaluation of the Antilipase, and Anti-α-Amylase of *C. iphionoides* Activities

The inhibitory effects of *C. iphionoides* EO on lipase and α-amylase were assessed.The EO extracted from *C. iphionoides*, as shown in Figure 3.7, demonstrates low lipase inhibition activity, with IC50=931.58± 0.91µg/mL and Figure 3.8 demonstrates lowα-amylase inhibition activity, with an IC_{50} of1550± 0.25µg/mL.

To the best of our knowledge, this is the first reported lipase & α-amylase inhibition activity for the EO extracted from *C. iphionoides.*

4.3.3 Evaluation of the Antilipase, and Anti-α-Amylase of *Artemisia* Activities

The inhibitory effects of *Artemisia* EO on lipase and α-amylase were assessed.The EO extracted from *Artemisia* as in Figure 3.7demonstrates low lipase inhibition activity, with IC50=534± 0.19µg/mL, and Figure 3.8 demonstrates lowα-amylase inhibition activity, with an IC_{50} of569± 0.20 µg/mL.

(Jaradat, et al.) reported the EO of the *Artemisia* jordanica plant showed dose-dependent inhibitory activity against porcine the results showed that the *Artemisia* jordanicaexhibited weak lipase inhibitory potential with an IC50 value of 51.41 ± 0.91 µg/mL [101].

(Jaradat, et al.) reported The results showed that *Artemisia* jordanica exhibited α-amylase with IC50 values of 14.17 ± 0.39µg/mL and 8.53 ± 0.72 µg/mL, respectively[101].

4.4 Antimicrobial activity of *C. iphionoides* and *Artemisia*

The antimicrobial activity of *C. iphionoides* and and *Artemisia* essential oils was tested in a broth microdilution assay against three gram-positive bacteria *Enterococcus faecalis*, Staphylococcus aureus, *Staphylococcus aureus* the *C.iphionoides* EO showed and two gram-negative bacteria, *Pseudomonas aeruginosa*, and Escherichia coli. The *C. iphionoides* essential oil showed limited activity against all bacteria except for *Staphylococcus aureus* strain ATCC 6538. The antimicrobial efficacy of *Artemisia* essential oil against various bacteria was found to be negligible. For this strain, *C. iphionoides* essential oil demonstrated a MIC of 31.3 mg/ml and MBC of 250.0 mg/ml and *Artemisia* essential oil exhibited a MIC of 3.906 mg/ml and an MBC of 250.0 mg/ml.

According to previous investigations, The antimicrobial activity of *C. iphionoides* extracts has been evaluated against Gram-positive and Gram-negative bacteria as well as fungi reported by (Reem Sbiehet al.,).Results demonstrated of Hexane extract: Displayed variable activity against *S. aureus, Pseudomonas aeruginosa,* and *C. albicans* [103].

(Jaradat, N) reported that the antimicrobial activity of *Artemisia jordanica* essential oil was established using the broth microdilution strategy. The *Artemisia jordanica* essential oil inhibited the growth of most of the tested microbial strains. Depicts that *Artemisia jordanica* essential oil has remarkable antimicrobial effects against *MRSA, S. aureus, P. vulgaris, and C. albicans* compared with the positive antimicrobial controls, the commercial antibiotics ciprofloxacin and ampicillin, and commercial antifungal drug fluconazole, while the *P. aeruginosa* and *E. coli strains* were resistant to *Artemisia jordanica* essential oil [103].

4.5 Conclusion

This research investigated the chemical composition of *C. iphionoides*, *Artemisia*, and *Teucrium polium*. The samples were sourced from Jericho. Seventeen compounds were identified in *T. Polium*, with E-nerolidol, geranyl acetone, germacrene D, β-caryophyllene, α-caryophyllene, and bicyclo germacrene as the principal ingredients. Forty-seven compounds were identified in *Chiliadenusiphionoides*, constituting 98.81% of the total oil, including cresol methyl ether, ethyl oct-2-ynoate, epi-cadinol, 1,8-cineole, and epi-α-eudesmol. Fifty-one compounds were identified in *Artemisia*, including 1,8-cineole, trans-thujone, cis-thujone, camphor, and terpinen-4-ol. The analyzed essential

oils demonstrate significant antioxidant properties. A 1:1 combination of *Artemisia* and *C. iphionoides* essential oils, *Artemisia* and *T. Polium* essential oils, and *C. iphionoides* and *T. Polium* did not exhibit a significant enhancement in their antioxidant properties.

The antibacterial efficacy of *Artemisia* essential oil against several microorganisms was found to be negligible.Similarly, the CEO demonstrated little effectiveness against all germs, save for the *Staphylococcus aureus* strain ATCC 6538.The essential oils of *C. iphionoides*, *Artemisia*, and *Teucrium polium* demonstrate potential antioxidant, antilipase, and α-amylase enzyme inhibitory effects in comparison to the positive controls (orlistat and acarbose, respectively). *Teucrium polium* displayed modest lipase inhibitory action and showed minimal inhibition of α-amylase compared to lipase inhibition.*C. iphionoides* demonstrated reduced lipase inhibition activity and limited α-amylase inhibition relative to lipase inhibition. *Artemisia* displayed negligible lipase inhibitory activity and showed minimal inhibition of α-amylase compared to lipase inhibition. The antimicrobial efficacy of *Artemisia* EO against various bacteria was found to be negligible. Similarly, the *C. iphionoides* EO showed limited activity against all bacteria except for *Staphylococcus aureus* strain ATCC 6538.

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Appendices

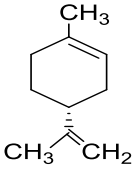
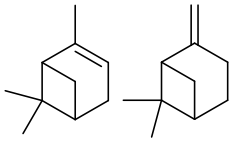
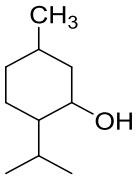
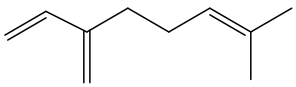
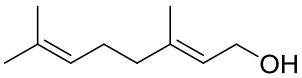
Appendix A

Supporting Information

The following table summarizes the differences between these compounds:

Table A.1

Types of Monoterpenes

Compound	Sources	Scent	Common uses	Properties	Structures
Limonene	Citrus fruit rinds (lemons, oranges)	Strong citrus fragrance	Cleaning products, air fresheners, flavoring	Antioxidant, anti-inflammatory	
Pinene	Pine trees (turpentine)	Pine-like	Organic synthesis, anti-inflammatory treatments	Bronchodilator, α- and β-pinene isomers	 α-Pinene β-Pinene
Menthol	Peppermint, other mint oils	Cooling, minty	Medicinal products, decongestants, toothpaste, chewing gum	Cooling effect, interaction with cold-sensitive receptors	
Myrcene	Ylang-ylang, hops, wild thyme	Musky, earthy	Fragrance industry, food flavoring	Sedative, muscle relaxant potential	
Geraniol	Geraniums, lemons, roses	Sweet, floral	Perfumes, cosmetics, flavorings, insect repellents	Antioxidant, anti-inflammatory, antimicrobial properties	

The following table provides a detailed comparison of these sesquiterpenes:

Table A.2

Types of Sesquiterpenes

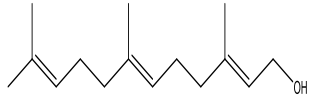
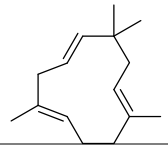
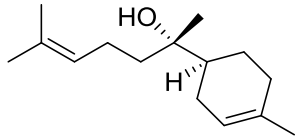
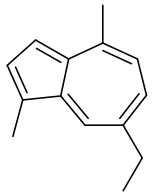
Compound	Structure type	Source	Molecular Formula	Common Uses	Additional properties	Structure
Farnesol	Alcohol-based sesquiterpene	Essential oils from citronella, lemongrass, rose	$C_{15}H_{26}O$	Fragrance industry, cholesterol metabolism	Endogenously produced, non-sterol isoprenoid	
Humulene	Monocyclic sesquiterpene	Found in hops and various plants	$C_{15}H_{24}$	Used in brewing (hops), lacks CB2 receptor activity	Isomer of β-caryophyllene, structural role in plant aromas	
Bisabolol	Monocyclic sesquiterpene alcohol	Chamomile flowers	$C_{15}H_{26}O$	Cosmetic formulations, skin care, sun care, infant care	Anti-inflammatory, soothing for skin conditions	
Chamazulene	Bicyclic unsaturated hydrocarbon	Formed during steam distillation of chamomile	$C_{14}H_{16}$	Used in essential oils, aromatherapy	Used in essential oils, aromatherapy	

Table A.3

Types of Hemiterpenes

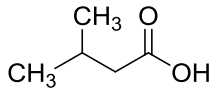
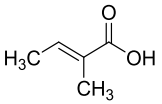
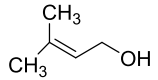
Compound	Type	Sources	Key Characteristics	Potential Applications	Figures
Isovaleric Acid	Hemiterpene carboxylic acid	Valerian root, fermented foods	Strong pungent odor	Flavoring Aroma enhancement	
Tiglic Acid	Geometric isomer of Angelic acid	Chamomile, Roman Chamomile	Antimicrobial properties	Natural preservatives, health products	
Preno	Hemiterpene Alcohol	Hops, Citrus fruits, grapes	Contributes to plant aroma; precursor to terpenes	Fragrance industry, flavoring agents	

Table A.4

GC-MS chromatogram of Chiliadenus iphionoide

	R.T	RI	%
2-Methyl-3(E)-octen-5-yne	12.357	988	
Cresol methyl ether	12.48	990	52.92
Anisole	13.53	1014	0.35
p-cymene	13.85	1022	0.07
1,8-Cineole	14.08	1027	4.25
Benzene acetaldehyde	14.67	1041	0.05
γ –Terpinene	15.31	1056	0.59
cis-Vertocitral C	15.98	1072	0.11
p-Mentha-2,4(8)-diene	16.45	1083	0.06
Linalool	16.53	1085	
Pinene oxide	17.18	1100	0.29
1,3,8-p-Menthatriene	17.43	1107	0.08
1,5,7-Octatrien-3-ol, 3,7-dimethyl-	17.54	1109	0.02
trans-a-Necrodol	18.61	1137	0.12
Camphor	18.87	1144	0.14
Nerol oxide	19.1	1149	0.75
Borneol	19.93	1171	1.71
Terpinen-4-ol	20.23	1193	1.48
Methyl 2-octynoate	21.12	1201	0.13
trans-Dihydrocarvone	21.22	1204	1.25
Pulegone	22.34	1235	1.66
Ethyl 2-octynoate	23.73	1274	14.36
lavandulyl acetate	23.96	1281	0.75
Neryl acetate	26.49	1355	0.41
Ethyl cis-cinnamate	27.03	1371	0.03
Carvyl acetate	27.13	1374	0.05
Carvyl acetate	27.33	1380	0.13
Z-Jasmone	27.66	1390	0.19
Nd	28.22	1407	0.04
β-Caryohyllene	28.56	1418	0.26
α-Trans-Bergamotene	28.96	1431	0.09
Aromadendrene	29.38	1441	0.02
Nd	29.45	1447	0.02
α–Humulene	29.69	1455	0.02
allo-aromadendrene	29.85	1457	1.23

Ethyl E-cinnamate	30	1464	0.04
Nd	30.09		0.02
Isomethyl- α-ionone	30.25	1472	0.47
Germacrene D	30.49	1480	0.28
10,11-Epoxycalamenene	30.68	1486	0.13
β-Selinene	30.95	1495	0.05
α –Muurolene	31.04	1498	0.02
Lavandulyl isovalerate	31.28	1505	0.03
γ –Cadinene	31.48	1512	0.19
d-Cadinene	31.64	1518	0.18
Nd	32.14	1535	0.11
4-[(2E)-2-Butenyl]-1,2-dimethylbenzene	32.55	1548	0.31
Nd	33.14	1568	0.05
Nd	33.8	1591	0.21
Nd	33.99		0.24
Epi-α-Cadinol	35.37	1647	6.55
α –Eudesmol	35.72	1659	2.73
7-epi-a-Eudesmol	36.11	1672	3.65
5-neo-Cedranol	36.63	1691	0.35
Nd	40.56	1842	0.29
Nd	40.72	1848	0.09
	Total identified%		99.90
	Monoterpene hydrocarbons		0.81
	Oxygenated monoterpenes		19.95
	Sesquiterpene hydrocarbons		2.38
	Oxygenated sesquiterpenes		7.39
	Others		68.22

Table A.5

GC-MS chromatogram of Artemisia

	RT	RI	%
Sabinen	11.57	970	0.02
Octen-3-ol	12.01	980	0.02
Mesitylene	12.48	990	0.35
α-phelandrene	13.04	1003	0.11
α-Terpinene	13.51	1014	0.42
1,2,4-Trimethyl benzene	13.65	1017	0.18
p-cymene	13.84	1022	1.54
1,8-Cineole	14.17	1029	23.12
Nd	14.42	1035	0.01
3-Methylcyclohex-2-en-1-one	14.9	1047	0.01
γ-Terpinene	15.29	1056	1.00
(3Z)-Hexenyl oxy-acetaldehyde	15.7	1065	0.01
p-Mentha-3,8-diene	15.83	1068	0.03
Artemisia alcohol	16.26	1079	0.03
Isoterpinolene	16.44	1083	0.10
Nd	16.5	1084	0.02
p-Cymenene	16.62	1087	0.03
cis-4-Thujanol	16.86	1093	0.03
Nd	16.96	1095	0.03
Dimethylphenol	17.04	1097	1.41
cis-Thujone	17.28	1103	14.27
trans-Thujone	17.74	1114	19.36
Chrysanthenone	17.89	1118	7.19
trans-Pinene hydrate	18.03	1122	0.91
E-Epoxyocimene	18.59	1136	0.08
4(10)-Thujen-3-ol	18.67	1138	1.56
Camphor	18.86	1143	10.29
Nd	19.22	1153	0.02
Sabina ketone	19.35	1156	0.12
Pinocarvone	19.48	1159	0.59
Borneol	19.88	1169	3.76
Terpinen-4-ol	20.21	1178	6.73
Nd	20.47	1185	0.25
α-Terpineol	20.77	1192	1.85
trans-Piperitol	21.31	1207	0.36

Nd	21.63	1216	0.03
m-Cuminol	21.94	1224	0.07
Ascaridole	22.39	1237	0.06
Cumin aldehyde	22.48	1239	0.23
Carvone	22.55	1241	0.04
Carvotanacetone	22.73	1246	0.04
Piperitone	22.92	1252	0.16
Nd	23.46	1267	0.05
2-Methyl isoborneol	23.77	1275	0.04
Isobornyl acetate	23.98	1281	0.98
Thymol	24.3	1290	0.13
Carvacrol	24.56	1297	0.32
Nd	24.88	1307	0.19
Patchenol	25.09	1313	0.56
Nd	25.85	1335	0.05
α-Terpinyl acetate	26.12	1344	0.13
E-Jasmone	27.66	1390	0.21
Nd	30.24	1471	0.06
Germacrene D	30.49	1480	0.10
Artedouglasiaoxide C	31.52	1513	0.04
Artedouglasiaoxide A	31.89	1526	0.05
Geranyl butanoate	32.84	1558	0.05
γ-Undecalactone	33.14	1568	0.04
Spathulenol	33.39	1578	0.30
Nd	33.59	1583	0.14
Salvial-4(14)-en-1-one	33.84	1592	0.12
	Total identified%		100.00
	Monoterpene hydrocarbons		1.66
	Oxygenated monoterpenes		92.48
	Sesquiterpene hydrocarbons		0.13
	Oxygenated sesquiterpenes		1.27
	Others		3.62

Appendix B

Figures

Figure B.1

Teucriumpolium plant

جامعة النجاح الوطنية
كلية الدراسات العليا

نباتات المكونات الكيميائية والأنشطة الدوائية لزيوت العطرية لنباتات الشيح، والجعدة والكتيلة من منقطة اريحا في فلسطين

إعداد
إيمان عزمي قعدان ساعد

إشراف
أ. د. نواف المحاريق

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء،
من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس – فلسطين.

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الملخص

يرجع الاهتمام المتزايد بالنباتات الطبية إلى الاعتقاد بقدرتها على علاج الأمراض دون آثار جانبية. في منطقتنا، تستخدم نباتات الشيح (Artemisia)، الكتيلة (Chiliadenus iphionoides)، والجعدة (Teucrium polium) تقليديا في العلاج. تهدف هذه الدراسة إلى تحليل التركيب الكيميائي لزيوتها العطرية المستخلصة من منطقة أريحا، وتقييم نشاطها المضاد للأكسدة، والبكتيريا، وإنزيمي الليباز والأميليز.

تم استخلاص الزيوت بتقنية التقطير المائي، وتحليل مكوناتها باستخدام GC-MS. جرى تقييم النشاط المضاد للأكسدة باختبار DPPH، والفعالية المضادة للبكتيريا بطريقة التخفيف الدقيق، والنشاط المثبط لإنزيم الليباز باستخدام PNPB، ولإنزيم الأميليز بطريقة DNSA.

أظهر زيت الجعدة احتواءه على 17 مركبًا رئيسيًا، أبرزها E-nerolidol (27.11%)، وجيرانييل أسيتون (23.26%). أما زيت الكتيلة فضم 47 مركباً شكلت 98.81% من مكوناته، أهمها كريسول ميثيل إيثر (52.93%). وزيت الشيح احتوى على 51 مركباً، منها 1,8-سينول (28.67%) وترانس-ثوجون (24.0%).

أظهرت الزيوت خصائص مضادة للأكسدة، حيث بلغت قيم IC50 للجعدة، الكتيلة، والشيح: 19.18، 17.03، و35.00 ميكروغرام/مل على التوالي. الخلطات بين الزيوت أعطت نتائج متقاربة (17.13-28.96)، لكنها أقل من Trolox (4.3).

في الذ شاط المـ ضاد للبكتيريا، أظهر زيتا الـ شيح والكتيلة فعالية متو سطة (MIC = 3.906 و31.3 ميكرولتر/مل)، بينما كان زيت الجعدة ضعيف التأثير.

أما في تثبيط الليباز، فكانت القيم: الشيح (534)، الجعدة (368.13)، الكتيلة (931.58) ميكروغرام/مل. وبالنسبة لإنزيم الأميليز، بلغت القيم: الشيح والجعدة (569)، والكتيلة (1550).

تؤكد النتائج أن الزيوت المدرو سة تمتلك خ صائص مـ ضادة للأكـ سدة واعدة، مع إمكانيات محدودة في مكافحة البكتيريا وتثبيط إنزيمات الهضم.

الكلمات المفتاحية: الشـــيح، Chiliadenus iphionoides، Teucrium polium، مثبط ألفا أميليز، الليباز البنكرياسي، النشاط المضاد للبكتيريا، النشاط المضاد للأكسدة