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PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SOME ASTERACEAE SPECIES GROWING IN OSMANİYE PROVINCE

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IN

BIOCHEMISTRY SCIENCE AND TECHNOLOGY

BY

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Supervisor

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ABSTRACT

PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SOME ASTERACEAE SPECIES GROWING IN OSMANİYE PROVINCE

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In this study, the biological activities and chemical compositions of ethanol and methanol extracts of seven plant species (*Anthemis cotula*, *Achillea falcata*, *Scorzonera kotschyi*, *Artemisia absinthium*, *Tanacetum parthenium*, *Senecio othonnae*, and *Tussilago farfara*) collected from Osmaniye province were investigated. GC-MS analyses revealed that compounds such as caryophyllene, α-terpinenyl acetate, anethole, d-limonene, and quercetin were associated with biological activities. Phenolic analyses showed that chlorogenic, fumaric, hydroxycinnamic, and ellagic acids, as well as flavonoids such as kaempferol, quercetin, and myricetin, play an important role in the pharmacological effects of the plants. Antimicrobial tests showed that extracts of *A. absinthium*, *A. cotula*, and *T. parthenium* were particularly effective against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida* species. *A. absinthium* extracts showed significant inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), while ethanol extracts of *A. cotula*, *A. falcata*, *S. kotschyi*, and *T. farfara* dose-dependently reduced cell viability in A549 lung cancer cells. In conclusion, it was determined that the plants used in our study can be considered as natural sources of antioxidants, antimicrobials, and enzyme inhibitors.

Key Words: Biological activity, Astereceae, Antioxidant, Antimicrobial, Antiproliferative, Anticholinesterase

ÖZET

OSMANİYE İLİNDE YETİŞEN BAZI ASTERACEAE TÜRLERİNİN FİTOKIMYASAL KOMPOZİSYONU VE BİYOLOJİK AKTİVİTELERİ

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132 sayfa

Bu çalışmada, Osmaniye ilinden toplanan yedi bitki türünün (*Anthemis cotula*, *Achillea falcata*, *Scorzonera kotschyi*, *Artemisia absinthium*, *Tanacetum parthenium*, *Senecio othonnae* ve *Tussilago farfara)* etanol ve metanol ekstraktlarının biyolojik aktiviteleri ve kimyasal bileşimleri incelenmiştir. GC-MS analizleri, karyofillen, α-terpinenil asetat, anetol, d-limonen ve kuersetin gibi bileşiklerin biyolojik aktivitelerle ilişkili olduğunu ortaya koymuştur. Fenolik analizler, klorojenik, fumarik, hidroksisinamik ve ellagik asitler ile kaempferol, kuersetin ve mirisetin gibi flavonoidlerin bitkilerin farmakolojik etkilerinde önemli rol oynadığını göstermiştir. Antimikrobiyal testler, özellikle *A. absinthium*, *A. cotula* ve *T. parthenium* ekstraktlarının *Staphylococcus aureus*, *Enterococcus faecalis* ve *Candida* türlerine karşı etkili olduğunu göstermiştir. *A. absinthium* ekstraktları, asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BChE) enzimleri üzerinde belirgin inhibitör etki gösterirken, *A. cotula*, *A. falcata*, *S. kotschyi* ve *T. farfara*'nın etanol ekstraktları A549 akciğer kanseri hücrelerinde hücre canlılığını doza bağlı olarak azaltmıştır. Sonuç olarak, çalışmamızda kullanılan bitkilerin doğal antioksidan, antimikrobiyal ve enzim inhibitörü kaynakları olarak değerlendirilebileceği belirlenmiştir.

Anahtar Kelimeler: Biyolojik aktivite, Astereceae, Antioksidan, Antimikrobiyal, Antiproliferatif, Anticholinesterase

“Canım aileme”

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CHAPTER I
INTRODUCTION

In antiquity, the basis of knowledge regarding the use of flora and fauna was established through experimentation. As this experimentation advanced, the uses of plants likewise expanded. During this time, folks acknowledged the therapeutic potential of plants and began employing them to enhance well-being. With the progression of iatrochemistry after the 16th century, medicinal and aromatic herbs gained heightened importance. Recently, medicinal and aromatic plants have played a crucial role in providing active chemicals for the pharmaceutical sector, employed in disease prevention and treatment. The increasing importance of medicinal and aromatic plants is due to the rising detrimental effects of synthetic drugs on living organisms over time. The World Health Organization (WHO) contends that a thorough comprehension of natural plants requires medical and pharmacological data, as well as the reliability of these plant groups throughout their whole life cycle. Historical evidence indicates that herbal remedies were employed in ancient times. The oldest of these is the Nagpur Sumerian clay tablet. This plate comprises approximately 12 phytotherapeutic compounds originating from botanical sources. Subsequently, notable examples include the Chinese text "Pen T'Sao" on roots and herbs by Emperor Shen Nung; spices mentioned in sacred Indian texts; the use of St. John's wort, coriander, and juniper in the Eber papyrus; the application of aromatic plants, including incense, in the Bible and the Jewish Talmud; and Dioscorides's "De Materia Medica" regarding medicinal plants (Thorwald, 1991; Stojanoski, 1999; Schippmann et al., 2006; Wiart, 2006; Kelly, 2009; Anonymous, 2015).

Medicinal and fragrant plants are employed in diverse areas, including cosmetics, personal care, incense, sanitation, food, spices, and the pharmaceutical business. The availability of aromatic plants for many purposes is also crucial. These plants are either cultivated or obtained directly from their native environments. These procurement situations are generally sourced straight from their respective surroundings, both domestically and abroad. Some therapeutic and aromatic plants are used fresh, while

others are dried and applied in specific contexts. The aerial components, including as stems, leaves, fruits, seeds, flowers, and rarely roots, are generally preferred for the specified purposes. The acknowledged characteristics of medicinal and aromatic plants highlight the economic importance of these products. Furthermore, evaluations by the World Health Organization suggest that flora historically acknowledged for their therapeutic properties in the healthcare frameworks of developing countries continue to be beneficial. The export of medicinal and aromatic plants, once negligible to the economy, now generates significant financial advantages for countries such as China, Hong Kong, the USA, Italy, Spain, and France (Faydaoğlu and Sürücüoğlu, 2011; Dzoyem et al., 2013; Vasisht et al., 2016; Tripathi et al., 2017).

With more than 12,000 different vascular plant species, Turkey has an exceptionally diverse flora. Of these species, around one-third are native to the nation. The Aegean, Marmara, Mediterranean, Eastern Black Sea, and Southeastern Anatolia areas are the main geographic locations for medicinal and aromatic plants. This is mostly because Turkey has a diverse range of climates. The Turkish indigenous community uses 1,546 types of medicinal plants, according to ethnobotanical studies. Of these species, around 350 are harvested directly from their native distribution regions and utilized locally, while about 100 have the potential to be exported. Currently, around 20 kinds of aromatic and therapeutic plants are grown on about 1,3 million decares of land nationwide, however this has fluctuated over time. When it comes to output volume, species like mint, thyme, poppy, black tea, red pepper, and anise stand prominently. Upon examining current data on the cultivation areas and yields of some high-value plants in Turkey, it is reported that 132,793 tons of black tea were produced from 76,207 hectares, 20,413 tons from 11,289 hectares, 3,073 tons from 61,591 hectares, cumin 1,690 tons from 27,024 hectares, mint 1,494 tons from 1,058 hectares, thyme 1,300 tons from 10,486 hectares, rose oil produced 948 tons from 2,824 hectares, anise 905 tons from 13,812 hectares, and fennel produced 146 tons from 1,551 hectares (Bayram et al., 2010; Akalın et al., 2020).

Despite its favorable geographical location and rich biodiversity, Türkiye's yearly export earnings for medicinal and aromatic plants is limited to approximately $300 million. In Türkiye, most medicinal and aromatic plants are obtained by natural harvesting. To leverage this position for the national economy's advantage, it is

essential to protect the environment and prioritize investment in research and development of high-value patented products. (Máthé et al., 2023).

1.1 Asteraceae Family and Systematics

The Asteraceae family is among the largest plant families globally, with 1,600 genera and over 25,000 species worldwide. Notable taxa include chamomile, artichoke, lettuce, chicory, and dandelion. Employed for therapeutic purposes over 3,000 years, some species within the Asteraceae family has a profound history in traditional medicine. Analysis of their chemical compositions reveals that several species within this diverse family have remarkably similar chemical structures. Many plants within the Asteraceae family demonstrate notable antioxidant, anti-inflammatory, and antibacterial properties, as well as diuretic and wound-healing functions. Furthermore, they demonstrate a broad spectrum of pharmacological effects attributable to their diverse phytochemicals, such as polyphenols, phenolic acids, flavonoids, and acetylenes (Bessada et al., 2015; Nikolić and Stevović, 2015; Rolnik et al., 2021). Members of the Asteraceae family are widely distributed across polar to tropical latitudes and occupy many ecosystems, including deserts, swamps, plains, rainforests, and alpine tundra. This characteristic makes them ideal for worldwide study (Palazzesi et al., 2022).

Kingdom: Plantae
Subkingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Asteridae
Order: Asterales
Family: Asteraceae

1.1.1 *Anthemis cotula* L.

It is a member of the Asteraceae family. It is designated in the literature as "mayweed chamomile". The geographical distribution in Europe encompasses Germany, Georgia, Greece, Albania, Austria, Luxembourg, Malta, Moldova, Montenegro, Belarus, Belgium, Bosnia-Herzegovina, Romania, Russia, Serbia, Slovakia, Spain, Sweden, Switzerland, Tunisia, Ukraine, Bulgaria, Croatia, Cyprus, the Czech Republic,

Denmark, France, Great Britain, Hungary, Italy, Kosovo, Netherlands, Norway, and Portugal. In Türkiye, it is distributed over provinces such as Osmaniye, Istanbul, Kars, Şırnak, Karabük, Artvin, Edirne, Elazığ, Mersin, İzmir, Konya, Sakarya, Samsun, and Trabzon. It is a perennial, herbaceous plant. It blooms in the sixth and seventh months. It thrives in pastures, beside roadways, in unoccupied spaces, and on sandy soils. It reaches a height of 15 to 60 cm. The leaves of the plant are between 2,5 and 6,5 cm in length and are around 2 cm in width. The leaves are vibrant green, petiolate, alternate, fleshy, and may be either glabrous or pubescent, as well as complex. Each branch tip has 10–16 tridentate white ray florets around a capitulum comprised of involucral bracts and yellow disc florets. The disc florets are hermaphroditic, with 70-200 slender, yellow corolla tubes per capitulum. The fruits are little, approximately 1,6 mm in length, brown, and resemble achene-like cypselae with an almost cylindrical shape. They can produce roughly 5,000 to 27,000 seeds (Adhikari et al., 2020; Lo'ay et al., 2023; TUBİVES).

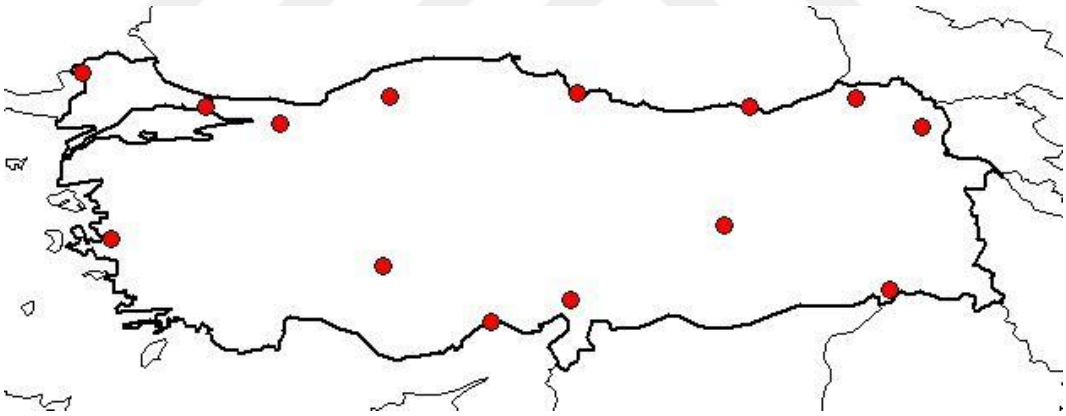

Figure 1.1 Distribution of *A. cotula* in Türkiye (TUBİVES)

1.1.2 *Achillea falcata* L.

It is a member of the Asteraceae family. In literature, it is designated as "Sarçane". It is naturally distributed in Europe, Asia, North Africa, Australia, and New Zealand. In Türkiye, it is situated in the provinces of Osmaniye, Antalya, Mersin, Muğla, Niğde, and Karaman. *Achillea*, an important medicinal genus, consists of around 130 perennial flowering species. The plants are clustered and primarily rhizomatous. The flowers are grouped in clusters of three to eight, positioned on little discs, and typically

display colors from white to pink. The leaves develop down the stem and are frequently larger than the lower leaves. The leaves reach lengths of up to 20 cm and are covered in hairs. *Achillea* is mostly pollinated by insects. The sarçane is a perennial herbaceous plant. In Türkiye, sarçane often blooms during the sixth and eighth months. It flourishes in rocky slopes, *Pinus* forests, and *Quercus* shrublands as its habitats (Saeidnia et al., 2011; Salehi et al., 2020; TUBİVES).

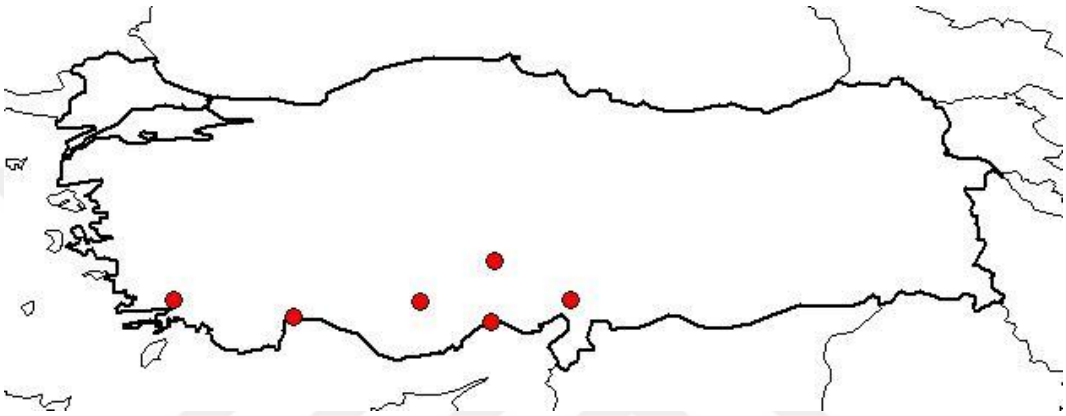

Figure 1.2 Distribution of *A. falcata* in Türkiye (TUBİVES)

1.1.3 *Scorzonera kotschyi* BOISS.

It is a member of the Asteraceae family. In literary studies, it is termed "Nurtekesakalı". The genus *Scorzonera*, originating in the ancient Mediterranean, includes over 180 species and is common in the arid regions of Eurasia and North Africa. In Türkiye, it is distributed over provinces such as Adana, Osmaniye, Gaziantep, Mardin, Adıyaman, Diyarbakır, Hatay, Kahramanmaraş, and Şanlıurfa. Perennial plants and shrubs of the genus *Scorzonera* exhibit simple, whole, and rarely pinnate leaves. They primarily exhibit hollow-stemmed or sessile seeds. Nurtekesakalı is a perennial herbaceous plant species. Its flowers appear in the fourth and fifth months. Its habitats include Quercus shrubland, limestone, and serpentine (Martin et al., 2012; Makbul et al., 2016; TUBİVES).

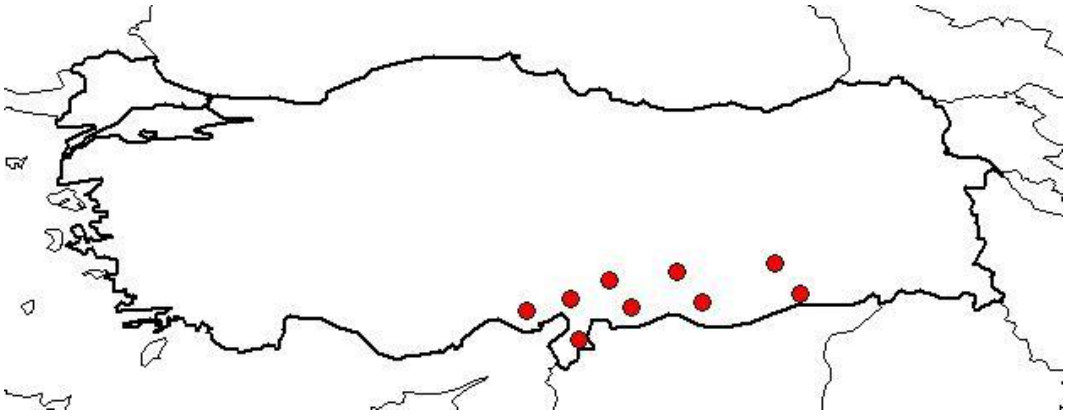

Figure 1.3 Distribution of *S. kotschyi* in Türkiye (TUBİVES)

1.1.4 *Artemisia absinthium* L.

It is a member of the Asteraceae family. In literary contexts, it is designated as "wormwood". It is geographically dispersed throughout Europe, Western Asia, and North Africa. It is situated in Poland, Scotland, and England. The species has been brought to North and South America and has adapted to the climate. In Türkiye, it is distributed over provinces such as Osmaniye, Bolu, Istanbul, Kars, Hakkari, Kastamonu, Ağrı, Ankara, Antalya, Gümüşhane, Mersin, Kahramanmaraş, Muş, Sivas, Tunceli, Bayburt, and Karaman. It is a perennial shrub that reaches a height of up to 80 cm. In specific environments, it reaches a height of 1,5 meters. The leaves include trichomes and glands that secrete protective oils and are equipped with T-hairs, which shield the plant from high temperatures and prolonged drought. The stem is gray-green, highly pubescent, and ribbed, usually displaying five flattened, longitudinal furrows. The flowering stem reaches a maximum diameter of 2,5 mm. The basal leaves have lengthy petioles and contain a triangular or oval morphology, characterized by two or three lobes; the lower leaves are less finely divided, whilst the upper leaves are lanceolate. Capitular inflorescences are formed in loose racemes that arise from the leaf axils. These semicircular or circular heterogamous capitula include pale yellow ligulate female flowers and tubular hermaphroditic blossoms. The involucral bracts surrounding the capitulum are elongated and gray, comprising ensiform outer leaves and oval inner leaves. Habitats include riparian zones, meadows, slopes, and steppes (Batiha et al., 2020; Szopa et al., 2020; TUBİVES).

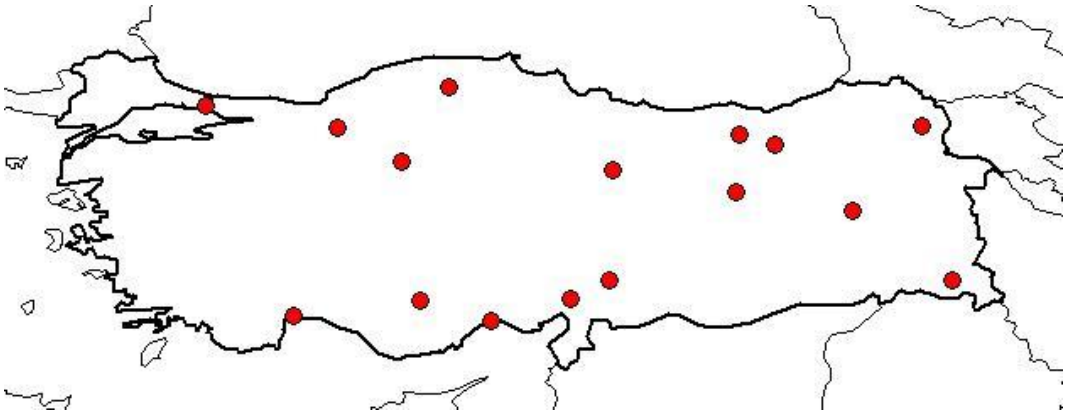

Figure 1.4 Distribution of *A. absinthium* in Türkiye (TUBİVES)

1.1.5 *Tanacetum parthenium* (L.) SCHULTZ BIP.

It is a member of the Asteraceae family. It is designated as "feverfew" in the literature. It is geographically distributed in Australia, Europe, China, Canada, Japan, and North Africa. In Türkiye, it is situated in the provinces of Osmaniye, Bolu, Istanbul, Hakkari, Kastamonu, Amasya, Artvin, Giresun, Mersin, Izmir, Kırklareli, Konya, Samsun, Trabzon, and Tunceli. It is termed "feather grass" because to its pubescent foliage. It is a small, compact, aromatic perennial plant that reaches a height of 0,3-1 m. The yellow-green leaves often measure less than 8 cm in length, are nearly glabrous, and display a pinnate-bipinnate arrangement. The yellow flowers flourish from July to October and measure around 2 cm in diameter. They resemble the flowers of the daisy (*Matricaria chamomilla*), with which they are sometimes confused, and feature uniseriate white outer ray florets. Habitats include walls, derelict lots, stream banks, shady forests, and rock outcrops (Ernst and Pittler, 2000; Pareek et al., 2011; TUBİVES).

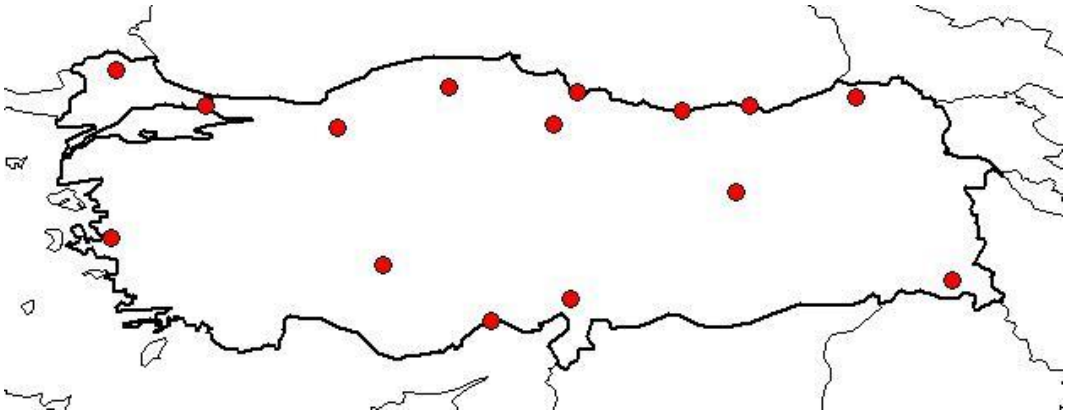

Figure 1.5 Distribution of *T. parthenium* in Türkiye (TUBİVES)

1.1.6 *Senecio othonnae* BIEB.

It belongs to the Asteraceae family. *Senecio* L. is the most extensive genus within Asteraceae, with over 1500 species documented. Thirty-nine of these species are naturalized in Türkiye, whereas fourteen are indigenous. It is geographically located in the Central and Eastern Balkan Peninsula, the Caucasus, and Iran. In Türkiye, it disseminates naturally in provinces like Osmaniye, Istanbul, Hakkari, Bitlis, Artvin, Balıkesir, Isparta, Kırklareli, Muş, and Tunceli. It is a perennial herb. The blossoming period occurs between the sixth and eighth months. It disseminates at an elevation of roughly 700-3050 meters. Habitats comprise rocky forest slopes, shrublands, and humid regions (Üçüncü et al., 2010; TUBİVES).

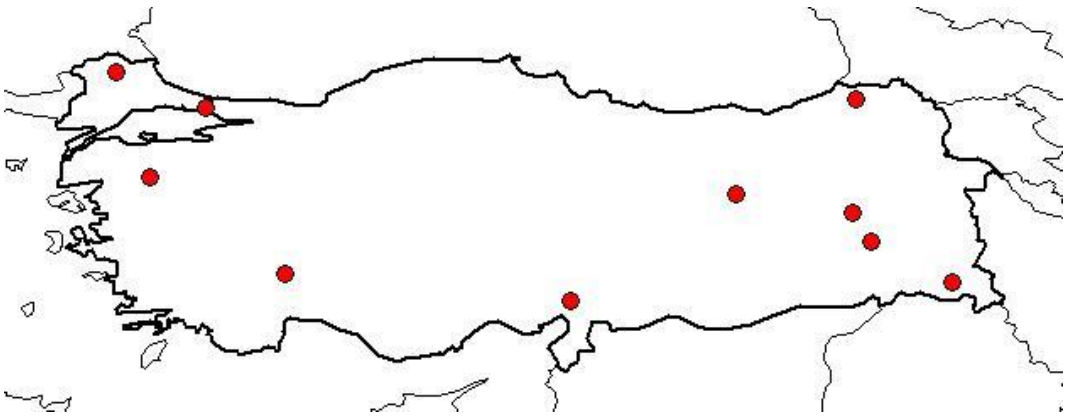

Figure 1.6 Distribution of *S. othonnae* in Türkiye (TUBİVES)

1.1.7 *Tussilago farfara* L.

It belongs to the family Asteraceae. It is known in literary settings as "coltsfoot". Originally from Nepal, North Africa, and temperate Eurasia, it is now found in 46 countries worldwide. Osmaniye, Ankara, Elazığ, Antalya, Bolu, İzmir, Istanbul, Kastamonu, Erzurum, Gümüşhane, Isparta, Samsun, Konya, Niğde, Sivas, Çanakkale, and Tekirdağ are among the provinces in Turkey where it naturally spreads. Although they may expand up to 30 cm during fruit dispersion, plants typically reach a height of 5 to 15 cm above the ground. The flower buds are 12,5 cm in length and 0,5-1 cm in diameter. They are elongated and spherical, with one to three consecutive bases. Numerous fish-scale leaves adorn their robust top stem, which taper to a short stalk. They have a pleasant scent. They have bright yellow blossoms that resemble dandelions. Its leaves are long, sturdy, rounded, and heart-shaped. They are 3 to 12 cm long and 4 to 14 cm wide, with radial veins and wrinkled, somewhat serrated edges. Both dry, sandy settings and wet regions are among the habitats (Chen et al., 2021; Norani et al., 2023; TUBİVES).

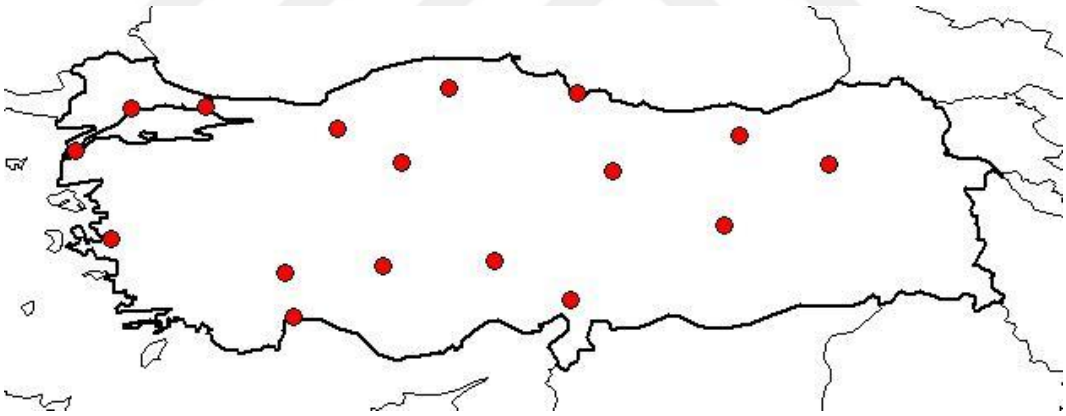

Figure 1.7 Distribution of *T. farfara* in Türkiye (TUBİVES)

1.2 Antioxidants

Species having unpaired electrons in their outer orbitals can link with other species because of these electrons. These extremely reactive species are known as free radicals. Factors such as the intake of processed and packaged foods, tobacco use, prolonged exposure to harmful UV radiation, and chronic stress increase the likelihood of a free radical chain reaction. These variables undermine the integrity and functionality of healthy cells during metabolism, leading to the oxidation of lipids,

amino acids, and nucleic acids (Riley, 1994; Harman, 2002; Inoue et al., 2003; Bansal and Bilaspuri, 2011). Free radicals may exist as cations (positively charged), anions (negatively charged), or in a neutral form (uncharged). The O2 (oxygen) molecule is categorized as a biradical (·O-O·) due to the presence of unpaired electrons at both termini of the molecule. Due to its high reactivity, biradical oxygen can engage with both free radicals and non-radical species. Reactions involving free radicals occur with significantly greater ease than those involving non-radical substances. Singlet oxygen (O-O:) is produced when an electron in biradical oxygen is excited and moves to an orbital with an opposite spin. Singlet oxygen is a non-radical reactive oxygen species distinguished by the lack of an unpaired electron. Oxidation reactions in the body occur by the transfer of single electrons to molecular oxygen via enzymes that contain transition metals, such as Fe2+ and Cu+ (Kochi, 1970; Inze and Montagu, 2002).

The imbalance between the body's antioxidant defense system and the production of reactive oxygen species (ROS), also known as free radicals, is known as oxidative stress (Persson et al., 2014). During metabolic processes, this disturbance may result in tissue and cellular damage. High concentrations of free radicals, which are very chemically reactive substances, are an unavoidable consequence of several metabolic processes. Furthermore, the generation of free radicals may also be triggered by exposure to oxidizing contaminants including nitrogen dioxide, ozone, and electromagnetic radiation. These radicals destroy tissue irreversibly when antioxidant mechanisms are weak. Chemical entities with an unpaired electron are known as free radicals. The molecule in issue is more chemically reactive as a result of this unpaired electron. The hydroxyl radical, superoxide anion, transition metals including iron and copper, nitric oxide, and peroxynitrite radicals are examples of major free radicals. The imbalance brought on by a lack of antioxidants, which counteract the damaging effects of free radicals, is known as oxidative stress. Cell death may result from this because it may compromise the structural integrity of biomolecules including DNA, proteins, and lipids in cell membranes. The rise in free radicals leads to the development of the cancer phenotype by speeding up the metabolic alterations that encourage the production of cancer cells (Halliwell et al., 1994; Tandon et al., 2005; Yousri et al., 2011; Rahman et al., 2012).

In order to lessen the harm that reactive oxygen species, in particular, do to cellular structures, biological systems have developed defensive mechanisms. These systems' antioxidant chemicals shield cells from harm by either lowering the damage already done by free radicals or preventing their creation. Foods naturally contain antioxidants, which are essential for the maintenance of physiological systems. Thus, optimizing the advantages of antioxidants requires a sufficient and well-balanced diet. Antioxidants are compounds that either stop or lessen the body's oxidative stress. Numerous biological and environmental factors, such as ionizing radiation, air pollution, heavy metal accumulation, bacterial and fungal infections, excessive physical exertion, addictive behaviors like smoking and drinking, and inadequate antioxidant intake, are linked to the development or aggravation of oxidative stress in the body. Elevated oxidative stress may cause cellular malfunction, DNA damage, and eventually cell death. The development of chronic illnesses is also significantly influenced by this cellular damage, which is brought on by an increase in free radicals. The two primary categories of antioxidants are endogenous (produced by the body) and exogenous (derived from outside sources). There are two types of endogenous antioxidants: enzymatic and non-enzymatic. Superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, catalase, and mitochondrial cytochrome oxidase are the primary enzymatic antioxidants. Melatonin, transferrin, ceruloplasmin, lactoferrin, glutathione, cysteine, uric acid, glucose, albumin, and bilirubin are examples of endogenous antioxidants that are not enzymatic. Exogenous antioxidants are substances that are often found in food or prescription drugs. These include pharmacological antioxidants like ebselen, acetylcysteine, mannitol, and iron chelators, as well as vitamins like vitamin E, vitamin C, β-carotene, and coenzyme Q (Dröge et al., 2002; Willcox et al., 2004; Valko et al., 2007; Pham et al., 2008; Sen et al., 2010; Sen et al., 2011; Shinde et al., 2012).

1.3 Antimicrobial

The germ theory of disease is the dominant scientific explanation for various ailments. Microorganisms, known as pathogens or "microbes" can cause disease. These microbes, invisible without magnification, invade humans, other animals, and numerous biological hosts. Their proliferation and replication inside hosts can cause disease. Environmental factors, such as humidity, nutritional composition of food,

temperature, and pH, influence microbial processes. "Microbe" refers to bacteria and any microorganisms, including protists and fungus, as well as non-living pathogens such as viruses, prions, or viroids that can cause disease (John and Rose, 2005; Susser and Stein, 2009; Ding and He, 2010). Antimicrobial agents are substances that eradicate or inhibit the growth of bacteria. Antimicrobial drugs employed for illness treatment are classified according to the specific pathogen they target. The effectiveness of antimicrobials may diminish over time. This phenomenon, defined by microbes developing a defense mechanism against antimicrobials, is referred to as antimicrobial resistance. Bacteria can develop new defensive mechanisms against drugs via structural alterations. The identification of new antimicrobials and the prudent use of current medications have become essential for disease management. Agents that combat bacteria are designated as antibiotics, whereas those that target fungi are classified as antifungals (Leekha et al., 2011). Prior to the identification of germs by humans, the belief in the medicinal properties of certain plants established the basis for modern antimicrobial theory. Since antiquity, humanity has employed plants to combat common infectious diseases. Secondary metabolites, such as phenolic compounds, alkaloids, terpenoids, and flavonoids, are considered the source of plants' antibacterial properties. A variety of herbs is essential to the traditional treatment of many diseases. The use of bearberry and cranberry juices is recognized as advantageous for urinary tract infections in various phytotherapy guidelines, while several other medicinal and aromatic plants, such as lemon balm, garlic, tea tree, turmeric, lavender, and thyme, are identified as broad-spectrum antimicrobial agents (Bennett and Wallsgrove, 1994; Heinrich et al., 2004; Crozier et al., 2006; Demain and Sanchez, 2009).

1.4 Phytochemicals

Medicinal and aromatic plants are important as bionutrients or bioactive phytochemicals. This plant community is a significant source of bionutrients, or bioactive phytochemicals. Medicinal and aromatic plants include a diverse array of bioactive substances, such as carbohydrates, tannins, terpenoids, flavonoids, alkaloids, and steroids. Phytochemicals serve numerous roles in pharmacology, including anticancer, detoxifying, antioxidant, antibacterial, antiviral, anti-inflammatory, pathological enhancement, and neuropharmacological effects. Furthermore, many

phytochemicals from distinct chemical families have been reported to demonstrate inhibitory effects on different bacteria in vitro (Yadav and Agarwala, 2011; Saxena et al., 2013).

1.4.1 Essential oils

Essential oils are prevalent in therapeutic and aromatic flora. They are often derived from various components of medicinal and aromatic plants, including fruits, flowers, leaves, bark, roots, buds, and seeds, through a range of extraction techniques. The techniques encompass distillation (water distillation, steam distillation, vacuum distillation), extraction (solvent extraction, supercritical fluid extraction, microwave extraction, compressed solvent extraction, solid-phase microextraction), multi-method extraction, and mechanical approaches. They are especially prevalent in flora cultivated in tropical and subtropical regions. They are a volatile, aromatic natural chemical that remains liquid at ambient temperature and readily crystallizes. They frequently lack vibrancy or display a faint yellow hue. They are referred to as essential oils because of their agreeable fragrance. Despite being classified as oils due to their immiscibility with water, they are distinct from fixed oils (Guenther et al., 1959; Kılıç, 2008; Turek and Stintzing, 2013; Lukas et al., 2015). Terpenes form the most complete category in terms of their chemical structure. Nonetheless, trace quantities of alcohols, aldehydes, esters, phenols, and molecules containing nitrogen and sulfur are also present. Oxidized terpenes produce oxygenated compounds that possess aroma, taste, and medicinal attributes. Essential oils, possessing a lower density than water, typically exhibit elevated refractive indices and show optical activity. Due to their propensity to resinify when exposed to light and oxygen, they must be stored in opaque, airtight containers for prolonged preservation. Essential oils were widely utilized in Roman, Greek, and particularly Egyptian civilizations. In recent years, the prevalence of aromatherapy, regarded as a facet of alternative medicine, has augmented the utilization of essential oils. Essential oils are utilized in therapeutic massages or soothing baths. Essential oils are extensively utilized in the fragrance, cosmetics, food and beverage sectors, as well as in domestic cleaning goods. The concentration of essential oils may fluctuate based on geographic distribution and plant growth. Recently, essential oils have been extensively utilized in studies concerning their antioxidant, anti-inflammatory, antiviral, antibacterial, and antifungal properties

(Hajhashemi et al., 2003; Perry et al., 2003; Silva et al., 2003; Pichersky et al., 2006; Bakkali et al., 2008).

1.4.2 Phenolic compounds

The presence of one or more acidic phenolic hydroxyl groups distinguishes plant phenolics from other aromatic compounds. They are widely distributed across the kingdom of plants. Fruits, legumes, vegetables, tea, wine, and coffee are the main sources of phenolics, which improve the sensory aspects of plant-based diets. In a similar manner, phenolic compounds increase fruit bitterness by interacting with salivary glycoproteins. Benzoic acid derivatives, which include gallic acid, and cinnamic acid derivatives, which include coumaric and ferulic acids, are the two groups of phenols. Fruits and vegetables contain caffeine, which is often esterified with quinic acid. One common phenolic acid found in grains is ferulic acid, which is often esterified with hemicelluloses. Phenolics found in plants improve pigmentation and provide defense against UV rays, infections, parasites, and herbivores. They are found in almost every part of the plant. Basic phenols like salicylic acid and complex polymer molecules like lignin and suberin are examples of the signaling molecule. Hydroxycinnamic acids, flavonoids, flavones, isoflavones, flavonols, anthocyanins, and tannins are the main types of phenolics. Condensed tannins and hydrolyzable tannins are the two common categories into which tannins fall. The main polyphenols in human diet are flavonoids. With over 10,000 different compounds, plant phenolics are a remarkably varied category (Rice-Evans et al., 1997; Taiz and Zeiger, 2008; de la Rosa et al., 2019; Alara et al., 2021). Because they may be synthesized in a variety of ways, plant phenolics show a great deal of metabolic variability. The malonic acid and shikimic acid pathways are the main metabolic activities. Many plant phenolics are synthesized by the shikimic acid route. In bacteria and fungi, the malonic acid pathway is a vital source of phenolic secondary metabolites; in higher plants, however, its significance is lessened. Phenylalanine is the source of the main secondary phenolic compounds found in plants. Phenylalanine is converted to cinnamic acid by removing one ammonium molecule. Perhaps the most researched enzyme in plant secondary metabolism is phenylalanine ammonium lyase (PAL). By enabling an essential regulatory step in the production of various phenolic compounds, PAL plays a critical role in linking primary and secondary metabolism (Yamasaki, 1997; Croteau et al.,

2000; Grace and Logan, 2000; Close and McArthur, 2002; Santos-Sánchez et al., 2019).

1.5 Anticholinesterase

Bavarian neuropsychiatrist Alois Alzheimer was the first to identify Alzheimer's disease (AD), a disorder of the central nervous system, in 1906 (Hostettmann et al., 2006). Due to increased life expectancy, the illness has become more common in the population over the 20th century. There are emotional, social, and financial obstacles in caring for those affected by this illness (Akhondzadeh and Noroozian, 2002). Inhibiting cholinesterase is crucial for the treatment of several illnesses, including Alzheimer's (de Almeida et al., 2022). In those 65 years of age and older, Alzheimer's disease (AD) affects the central nervous system (CNS). Alzheimer's disease is characterized by the degeneration of cholinergic neurons and increased activity of the enzymes butyrylcholinesterase (BChE) and acetylcholinesterase (AChE), which enable the rapid hydrolysis of acetylcholine at cholinergic synapses (Whitehouse et al., 1981; Rao et al., 2007). According to Alvarez et al. (1997) and Campos et al. (1998), AChE promotes the excessive aggregation of β-amyloid into expanding fibers, also known as β-sheet deposits. Many authors have repeatedly noted a positive correlation between Alzheimer's disease and β-amyloid fibrils (Seloke and Schenk, 2003). Since the 1980s, research has been done on cholinesterase inhibitors that reduce AChE and BChE activity in order to treat Alzheimer's disease. Numerous writers have drawn attention to the fact that patients often experience a range of negative side effects after using cholinesterase inhibitors for an extended period of time (Karczmar, 1998; Giacobini, 2004; Sabbagh et al., 2006). The investigation of new inhibitors and dietary sources rich in these inhibitors has thus gained considerable importance (Szwajgier, 2013). The history of medication discovery demonstrates that plants have active compounds that the pharmaceutical industry might explore as new resources. According to Howes and Houghton (2003), plant components may work in concert with compounds from the same species to increase their effectiveness and lessen the negative effects of chemicals from other plant species. Historically, a wide range of botanicals have been used to treat cognitive problems, such as neuropharmacological illnesses and neurodegenerative diseases (Mukherjee et al., 2007). Numerous neurological conditions have been successfully treated with yokukansan, a Chinese

herbal treatment, with no negative side effects (De Caires and Steenkamp, 2010). Additionally, the US Food and Drug Administration has authorized galanthamine, an alkaloid obtained from the snowdrop plant, to treat Alzheimer's disease (Ingkaninan et al., 2003; Heinrich and Teoh, 2004). Modern treatment techniques are focusing on phytotherapy since Alzheimer's disease has been classified as a public health concern and because of the negative effects of accessible synthetic drugs (Adewusi et al., 2010).

1.6 Purpose of the Study

Plants possess numerous biological activities thanks to the bioactive compounds they contain (Wink, 2015). This study aimed to determine the antimicrobial, anticholinesterase, antioxidant, and antiproliferative activities, as well as the phenolic content, essential oil content, total phenolic, and flavonoid contents of *Anthemis cotula* L., *Achillea falcata* L., *Scorzonera kotschyi* BOISS., *Artemisia absinthium* L., *Tanacetum parthenium* (L.) SCHULTZ BIP., *Tussilago farfara* L., and *Senecio othonnae* BIEB, all members of the Asteraceae family and distributed around Osmaniye.

CHAPTER 2

LITERATURE REVIEW

Zebarjad and Farjam (2015), investigated the chemical composition and cytotoxicity of the essential oil obtained from the flowers of *Anthemis odontostephana* var. compared the composition and antimicrobial activity of essential oils obtained from different organs (flower, leaf, bark and root) of the species Odontostephana. Chrysanthemum (9,7%), borneol (31,3%), (-)-bornyl acetate (13,9%) and myristicin (9,8%) were identified as the dominant components in the flower oil. Borneol (19,2%), (-)-bornyl acetate (6,6%), myristicin (13,3%) and trans-nerolidol (7,1%) were identified in the leaves; borneol (27,0%), (-)-bornyl acetate (7,9%), myristicin (11,2%) and trans-nerolidol (6,1%) were identified in the bark; and borneol (8,2%), myristicin (8,7%), trans-nerolidol (10,9%) and β-eudesmol (5,1%) were identified in the root. In antimicrobial tests performed on *E. coli*, *Klebsiella* spp., *S. aureus*, *S. epidermidis*, *Corynebacterium glutamicum*, *Aspergillus niger*, *Fusarium solani* and *Alternaria alternata*, minimum inhibitory concentration (MIC) values were reported to vary between 8-128 µg/mL.

Jasspi et al. (2016), investigated the cytotoxic potential of *A. mirheydari*. All parts of the plant were extracted with ethanol and dichloromethane for four days and the obtained extracts were tested on LS180 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma) and MOLT-4 (acute lymphoblastic leukemia) cell lines by colorimetric methods. The dichloromethane extract was fractionated by silica gel column chromatography and thin layer chromatography (TLC); identification of the compounds was done by NMR and EI-MS data. IC_{50} values of this extract were found to be 30.8, 25.2 and 8.6 mg/mL, respectively. In contrast, the methanol extract was reported to have no significant effect on the tested cell lines. Less polar fractions were reported to exhibit significant cytotoxicity.

The antioxidant activity and phenolic component composition of samples of *S. latifolia* gathered from various parts of Türkiye were compared by Açıkara et al. (2017). The

greatest quantities of hyperoside (652,32 µg/g) and chlorogenic acid (1246,78 µg/g) were discovered in aerial samples taken from the Kars area. While root samples from Bayburt had the lowest MDA levels (4,41 nmol/mL), samples from the Kayseri area had the greatest DPPH radical scavenging activity (IC_{50} = 1,036 mg/mL).

Çolak et al. (2017), investigated the levels of antioxidant capacity, total phenolic substance (TPC), and total flavonoid (TF) in capitulum samples of 41 taxa (Asteraceae, Anthemideae) from seven distinct genera that are native to Turkey. The average TPC value was 33,73 mg GAE/g DM, the TF amount was 16,58 mg QE/g DM, the ORAC value was 28,46 µmol TE/g DM, the DPPH value was 125,23 µmol TE/g DM, the FRAP value was 421,56 µmol TE/g DM, and the CUPRAC value was 107,44 µmol TE/g DM.

The chemical makeup and biological effects of the essential oil extracted from the aerial portions of the Libyan-origin *A. monosperma* species were examined by El Zalabani et al. (2017). In the research, 16-20 compounds were found to make up 97,63-99,00% of the oil's total chemical composition. Group A3 had a saturated hydrocarbon ratio of 88,36%, while groups A1 and A2 had ratios of 63,56 and 66,55%, respectively. The major components among the monoterpenes were found to be sabinene (13,15-22,85%), β-pinene (9,00-24,03%), and β-cis-ocimene (3,73-12,92%). Bornyl acetate was particularly high in group A1 at 8,00-31,00% in the oxygenated fractions (A3: 11,29%; A2: 31,08%; A1: 35,44%), whereas only the A2 group had β-eudesmol (8,01%). At minimal inhibitory concentrations of 0,98 and 0,24 µg/mL, respectively, the A2 fraction demonstrated strong antifungal activity against *A. fumigatus* and *Geotrichum candidum*.

The essential oil content and biological activity of *A. biebersteinii* and *A. teretifolia* were compared by Köse et al. (2017). Piperitone (44,7%), 1,8-cineole (15,9%), and camphor (4,9%) were the main constituents of *A. biebersteinii* oil, while 1,8-cineole (18,8%), camphor (17,6%), p-cymene (13,7%), and chrysanthemum (10,2%) were the main constituents of *A. teretifolia* oil. While *A. teretifolia* oil showed suppression of the tested bacteria in the range of 250>2000 µg/mL, *A. biebersteinii* essential oil shown antibacterial activity in the concentration range of 31,25>2000 µg/mL. Additionally, at 31,25 and 125 µg/mL, respectively, *A. biebersteinii* oil dramatically inhibited the development of the pathogenic yeast species *C. utilis* and *C. krusei*.

The essential oil extracted by hydrodistillation from the aerial sections of the wild *A. biebersteinii* species, which is found natively in Iran, was examined by Mirahmadi and Norouzi (2017), for its composition, phenolic content, and ability to scavenge free radicals. According to GC and GC-MS studies, a total of 11 components were found to make up 96,6% of the oil. 1,8-cineole (45,2%), p-cymene (20,8%), and cis-chrysanthemyl acetate (20,4%) were found to be the oil's primary constituents. With a phenolic concentration of 113,34 mg GAE/g DM, the extract extracted at the bud stage had the greatest total phenolic content. It also demonstrated potent antioxidant activity against the DPPH radical (IC_{50}=4,64 µg/mL).

The essential oil content of *A. wiedemanniana* (Asteraceae) cultivated in Iran at various phenological phases was compared by Nejadhabibvash (2017). The blossoming stage produced the maximum oil output. At the vegetative stage, 13 chemicals (0,77-25,03%) were found, followed by 15 compounds (0,46-44,31%) during flowering and 16 compounds (0,49-41,49%) during fruit production. The main constituents throughout the vegetative stage were β-eudesmol (15,01%), spatulenol (11,58%), 2-pentanone (25,03%), and α-farnesene (7,34%). The main compounds throughout the blooming stage were 2-pentanone (36,53%), α-farnesene (7,01%), and caryophyllene oxide (6,73%). The primary components during fruit commencement were 2-pentanone (41,49%), α-farnesene (10,88%), and caryophyllene oxide (10,88%); considerable levels of β-sesquiphellandrene (4,49%) and spatulenol (4,34%) were also found.

Using the Soxhlet microdilution technique, Toptanone et al. (2017), assessed the antibacterial activity of methanol and chloroform extracts made from the aerial portions of *A. tricolor* Boiss., which is indigenous to the Turkish Republic of Northern Cyprus. According to reports, the extracts' minimum inhibitory concentrations (MIC) against strains of *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, and *C. albicans* ranged from 78 to 1250 µg/mL.

Wu et al. (2017), examined the chemical composition and biological properties of essential oils extracted from *A. annua* plants that were gathered in China at various stages of growth. The pre-bud, early blooming, flowering, and post-flowering phases were identified by mass spectrometric studies as 21, 23, 21, and 22 chemicals, respectively. These compounds were found to make up 93,9%, 95,3%, 97,1%, and

90,6% of the total oil content. As phenological development progressed, the total hydrocarbon concentration fell and the total oxygenated compound content rose. In vitro experiments shown that the essential oils had variable degrees of growth suppressive effects on *S. aureus*, *B. subtilis*, *E. coli*, and *S. typhimurium*, and antioxidant activity was especially strong during the blooming and post-flowering periods.

S. acaulis' aboveground portions were assessed by Ashour et al. (2018), from a chemical and biological standpoint. Twenty-two components, or 81,08% of the total, made up the essential oil content. D-limonene (13,32%), β-pinene (11,54%), and sabinene (10,79%) were found to be the main constituents. Secondary metabolites were also identified from the plant, including β-amyrin, β-sitosterol, lupeol, oleanolic acid, β-amyrin-3-O-β-glucopyranoside, isorhamnetin-3-O-β-glucopyranoside, isoquercitrin, and quercitrin. Significant antibacterial and limited antileishmanial activity against *S. aureus* and *Cryptococcus neoformans*, as well as modest antimalarial activity against both chloroquine-susceptible and chloroquine-resistant strains of *Plasmodium falciparum*, were seen in the essential oil.

Hichri et al. (2018), investigated various biological properties of the essential oil obtained from *A. cretica*. Among the DPPH, ABTS, and iron reduction tests, the ABTS method reported the strongest antioxidant activity, with IC_{50} values ranging from 62-70 µg/mL. Antibacterial tests conducted using the liquid dilution method revealed that the essential oil exhibited significant inhibition against two Gram-positive and three Gram-negative bacterial species, particularly *S. aureus*.

The chemical makeup and biological activity of *S. vernalis*, a species indigenous to Türkiye's Lakes Region, were examined by Balpınar and Okmen (2019). Four distinct bacterial species were discovered to be susceptible to the antibacterial activity of ethanol-derived extracts from leaf and floral samples. Leaf and flower extracts were shown to have minimum inhibitory values of 13,000 µg/mL against strains of *Yersinia enterocolitica* and *E. coli*. The total phenolic content of the floral methanolic extract was determined to be 4,73 mg/mL gallic acid equivalent, and it demonstrated 78% DPPH radical scavenging action. In addition, the methanolic leaf extract was found to contain seven distinct chemicals.

Sarı et al. (2019), assessed the biological activities of the phenolic compounds that were extracted from the roots of *S. hieraciifolia*. Nine distinct compounds were found as a consequence of the analyses: 5-O-feruloylquinic acid methyl ester, 1,5-di-O-feruloylquinic acid, methyl ester of chlorogenic acid, 3-(4'-hydroxyphenyl)-2-propenoic acid (4"-carboxyl)-phenyl ester, 3-O-caffeoylquinic acid methyl ester, 1,3-di-O-caffeoylquinic acid methyl ester, 3,5-di-O-caffeoylquinic acid methyl ester, 4,5-di-O-caffeoylquinic acid methyl ester, and caffeic acid. In the superoxide, DPPH, and ABTS assays, the EC_{50} values of antioxidant activity were 0,54-5,88 mg/mL, 0,140-4,77 mg/mL, and 0,60-9,19 mg/mL, respectively. The measured values for TEAC and FRAP were 0,55-2,17 mM and 0,75-2,884 mM, by the way. Maximum inhibition of 79,21% for COX-1 and 67,69% for COX-2 were obtained in the COX inhibition test. Fractions derived from the roots' ethanolic extract, however, showed little antibacterial activity against other bacterial or fungal species and very modest antibacterial activity against *E. faecalis* (MIC: 1250 mg/L; standard: 8 mg/L).

Terenzhev et al. (2019), evaluated the chemical and biological properties of an ethanolic extract obtained from *T. farfara* flowers. The resulting extract was determined to exhibit antimicrobial activity against both human and plant pathogens. Minimum inhibitory, bactericidal, and fungicidal concentrations were found to be in the range of 2500-5000 µg/mL. The phytopathogen *Alternaria solani* was reported to be the most sensitive species to the extract components. Furthermore, the extract was determined to exhibit moderate antioxidant activity at concentrations of 0,001 mg/mL and above.

The biological activities of *T. farfara* and *Tragopogon dubius* were assessed by Uysal et al. (2019). High antioxidant capacity has been demonstrated for extracts from both species. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were most effectively inhibited by ethyl acetate extracts. Additionally, the α-glucosidase enzyme was significantly inhibited by the methanol extract of *T. farfara* and the ethyl acetate extract of *T. dubius*. Both plants contained phenolic chemicals, including rosmarinic acid and chlorogenic acid, according to HPLC studies. Furthermore, the *T. dubius* ethyl acetate extract demonstrated potent antifungal activity against every fungal strain that was examined.

Al-Shuneigat et al. (2020), looked into *A. biebersteinii* essential oil's antibacterial properties and its capacity to prevent the development of biofilms. The results showed that the essential oil was very active against every kind of bacterium that was tested. Minimum biofilm inhibitory concentration (MBIC) values were found to be between 0,125 and 4 mg/mL, whereas minimum inhibitory concentration (MIC) values were found to be between 0,125 and 1 mg/mL. Additionally, methicillin-resistant *S. aureus* (MRSA) strains were shown to be susceptible to the effects of *A. biebersteinii* essential oil, even at subinhibitory dosages.

The phytochemical content, antioxidant activity, enzyme inhibition capability, and antiproliferative characteristics of samples derived from the *A. filipendulina* species were examined by Asghari et al. (2020). The research contrasted the biological activity and composition of ethanolic extracts (EtOH) with essential oils (EO) extracted from flowers and leaves. The essential oils included 35 and 38 volatile compounds, which accounted for 98,88% and 97,26% of the total content, respectively. Chrysanthemyl acetate (19,11% and 16,26%), 1,8-cineole (17,18% and 12,01%), borneol (9,70% and 7,66%), and α-pinene (9,60% and 8,77%) were the chemicals that were discovered in the largest concentrations. Eleven phenolic acids and flavonoids were found in the ethanolic extracts' phenolic profile; the principal constituents were apigenin (204,89 and 228,28 mg/100 g), cinnamic acid (116,67 and 90,41 mg/100 g), and chlorogenic acid (214,95 and 154,54 mg/100 g). According to the findings, all samples exhibited strong antityrosinase activity and moderate to high levels of inhibition against important enzymes linked to diabetes and Alzheimer's disease. In cell culture assays, the floral component of the plant was shown to have the highest antiproliferative impact against MCF-7 and Hep-G2 cell lines, but the essential oils were not cytotoxic.

Using dichloromethane, ethyl acetate, hexane, water, and methanol, Dall'Acqua et al. (2020), investigated the phenolic content, antioxidant activity, and fundamental enzyme inhibitory qualities of extracts made from the roots and aerial portions of *S. tomentosa*. The results showed that the highest concentrations of total polyphenols were found in methanolic extracts and plant infusions, which had respective values of 40,33 and 39,30 mg gallic acid equivalents (GAE)/g. The methanolic extract made from the aerial portions had a total phenolic level of 22,42 mg GAE/g, whereas the root infusion had a total phenolic value of 21,84 mg GAE/g. In aerial extracts, the total

flavonoid content ranged from 2,96 to 34,93 mg rutin equivalent (RE)/g, but in root extracts, it stayed within the range of 0,40 to 2,65 mg RE/g. In DPPH and ABTS assays, methanolic and aqueous extracts exhibited the highest levels of radical scavenging activity, with values ranging from 1,05 to 45,89 mg Trolox equivalent (TE)/g. Furthermore, all extracts had their total antioxidant capacity (1,82-82,94 mg TE/g), CUPRAC (39,40-164,06 mg TE/g), and FRAP (15,54-79,78 mg TE/g) activities measured. The tyrosinase enzyme was significantly inhibited by all samples; values for the aerial portions ranged from 16,40 to 64,83 mg kojic acid equivalent (KAE)/g, while values for the roots ranged from 6,29 to 65,46 mg KAE/g. Furthermore, α-amylase (0,08-0,61 mmol acarbose equivalent (ACAE)/g) and α-glucosidase (0,09-0,83 mmol ACAE/g) showed minor inhibitory effects.

Mohammed et al. (2020), looked at *S. papposa*'s antibacterial and antioxidant properties. Ethanol was used to extract the plant's roots and aerial parts; Rel Assay Diagnostics kits were used to measure the antioxidant capacity and oxidant levels; and the agar dilution technique was used to measure the antibacterial activity against bacterial and fungal species. According to the research, *S. papposa* has a significant level of antioxidant activity. Oxidative stress index (OSI) was determined to be between 0,182 and 0,473, total antioxidant status (TAS) to be between 4,817 and 6,328 mmol/L, and total oxidant status (TOS) to be between 11,525 and 24,199 μmol/L. In the concentration range of 50-800 μg/mL, the extract was proven to be efficient against the tested strains of microorganisms.

Sarikurkcu (2020), The biological potential and phytochemical composition of *A. chia* were investigated. In the study, phytochemical contents, antioxidant activities, and enzyme inhibition potentials of ethyl acetate (EtOAc), methanol (MeOH) and aqueous extracts were compared. The highest total phenolic (65,22 mg GAE/g) and flavonoid (73,32 mg quercetin equivalent (QE)/g) contents were determined in the methanol extract. LC-ESI-MS/MS analyses revealed chlorogenic acid, protocatechuic acid, hyperoside, caffeic acid, apigenin-7-glucoside, and luteolin-7-glucoside as the main phenolic compounds. In the antioxidant capacity measurements made by CUPRAC, FRAP, DPPH and ABTS methods, 350,36, 655,18, 435,78, 265,33 and 325,25 mg Trolox equivalent (TE)/g values were recorded, respectively.

Sultan et al. (2020), *A. absinthium* evaluated the biologically active components of heat-treated methanol extract obtained from leaves for their in vitro cytotoxic and antibacterial activities. GC–MS analyses revealed that epiyangambin, flavone, octadecanoic acid 2,3-dihydroxypropyl ester, palmitic acid β-monoglyceride, β-mannofuranoside, camphor, and terpineol were the major components. The heat-treated methanol extract showed significant antiproliferative effect on human breast cancer cell line MCF-7 (ATCC) (IC_{50} = 80,96 µg/mL), whereas it did not cause any cytotoxic effect on colon cancer cell line HCT-116 (ATCC). It was also determined that the same extract exhibited moderate inhibitory activity against the tested bacterial species.

Sweidan et al. (2020), The antibacterial and antibiofilm activities of *S. macmeliana* were investigated. The results showed that Gram-negative bacterial strains were more susceptible and the minimum inhibitory concentration (MIC) values ranged from 48,98-341,85 mg/mL. The aqueous stem extract, rich in phenolic compounds, showed the highest antibacterial activity (MIC = 48,98 mg/mL). While the resin-rich varicella zoster extract exhibited MIC value of 160,85 mg/mL against *P. aeruginosa*, the ethanolic root extract with high terpenoid content was effective on *S. epidermidis* with MIC value of 284,35 mg/mL. In addition, the aqueous stem extract showed the highest antibiofilm activity with an eradication rate of 91% at a minimal biofilm eradication concentration (MBEC) of 0,1 mg/mL.

Younos et al. (2020), The chemical composition and cytotoxic properties of the essential oil obtained from the aerial parts of *A. falcata* were evaluated on HeLa and Caco-2 human cancer cell lines. The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus, and its components were determined by GC–MS profiling. Antioxidant capacity was measured by DPPH radical scavenging and total phenolic content, while cytotoxicity was evaluated by MTT assay. The essential oil showed low DPPH radical scavenging activity, and its total phenolic content was determined as 32,467 µg gallic acid equivalent/g. However, a dose-dependent cytotoxic effect was observed in both HeLa and Caco-2 cell lines, indicating the presence of potential anticancer compounds in the essential oil of *A. falcata*.

Elsharkawy et al. (2021), The antioxidant and antibacterial activities of *A. fragrantissima* samples collected from the Tabuk and Arar regions of Saudi Arabia

were compared. Samples from the Arar region were reported to have higher antioxidant capacity, with a DPPH IC_{50} value of 0,21 g/L. Furthermore, these samples exhibited significant antibacterial activity against the Gram-positive bacteria *S. epidermidis*, *S. aureus*, and *Bacillus cereus*, with inhibition zones reaching up to 14,5 mm.

Erik et al. (2021), Three new dihydroisocoumarin glycoside compounds isoscorzopigmaecoside, scorzoaucherioside I, and scorzoaucherioside II as well as five known compounds scorzopigmaecoside, scorzocreticoside II, 3,5-O-dicafeoyl-epi-quinic acid, 3,5-O-dicafeoylquinic acid, and 3,4-dihydroxyphenyl caffeate were isolated from the aerial parts of *S. aucheriana* collected from Sivas Province, Turkey. Of these compounds, isoscorzopigmaecoside, scorzoaucherioside I, and 3,4-dihydroxyphenyl caffeate exhibited significant anti-Pseudomonas activity. Additionally, isoscorzopigmaekoside and scorzoaucherioside II were reported to provide strong inhibition on *Mycobacterium smegmatis* with MIC values of 25,6 µg/mL and 21,2 µg/mL, respectively.

Kese ve Kak (2021), investigated the in vitro antibacterial, antiradical, anticancer and phytochemical properties of the endemic species *S. semicana*. It was reported that both water (94,81%) and methanol (98,87%) extracts showed high activity in ABTS radical scavenging assay and reached values above BHT (93,24%). In terms of hydroxyl radical scavenging capacity, water (84,97%), ethanol (84,29%) and methanol (84,62%) extracts exceeded the effect of BHT (75,77%). The methanolic extract had the highest flavonoid (5555,34 µg catechin equivalent (CE)/g), proanthocyanidin (1860,78 µg CE/g) and total phenolic (134,89 mg GAE/g) contents. All extracts exhibited stronger antibacterial activity than standard antibiotics against *Listeria monocytogenes* strains, and it was also determined that the plant exhibited dose-dependent high cytotoxic activity on LNCaP, MCF-7 and HCT-116 cancer cell lines.

Salomon et al. (2021), The phenolic compound profiles and antioxidant potential of *A. atrata and A. millefolium* were investigated. The aerial parts of both species were sequentially extracted with solvents of increasing polarity (dichloromethane, n-butanol, and ethyl acetate) and the antioxidant activities of the obtained fractions were evaluated by the DPPH method. The strongest activity was observed in the ethyl acetate extract of *A. atrata*, with an IC_{50} value of 12,2 µg/mL. The *A. millefolium*

extract showed slightly lower activity (IC_{50} = 17,0 µg/mL). The most polar fractions of both species contained luteolin, apigenin, centaureidin, and nevadensin, which are flavonoids with well-known free radical scavenging properties.

Stoyanova and Perifanova-Nemska (2021), The biochemical profile of *T. farfara* was characterized by examining the phenolic acid, organic acid and amino acid composition. The most dominant phenolic compound was caffeic acid (1867,34 µg/g), followed by ferulic acid (545,23 µg/g), while chlorogenic acid was not detected. HPLC analysis revealed that ascorbic acid (67,43 mg/g) and malic acid (45,25 mg/g) were the highest organic acids. In addition, 17 amino acids were identified; among these, phenylalanine (6,23 g/100 g protein) and valine (5,56 g/100 g protein) were identified as the most dominant essential amino acids. Threonine (4,90 g/100 g protein), leucine (4,64 g/100 g protein), isoleucine (3,76 g/100 g protein) and lysine (2,45 g/100 g protein) were also detected at significant levels.

Deveci (2022), *S. mollis* subsp. *szowitzii* the cytotoxic, antioxidant and enzyme inhibition potentials of the extracts were evaluated by LC–ESI–MS/MS methods. A total of 56 phytochemical compounds were identified, and chlorogenic acid was determined as the dominant compound in acetone (0,118 µg/mg) and methanol (34,105 µg/mg); gallic acid (0,153 µg/mg) was detected at the highest amount in the aqueous extract. The methanolic extract showed the strongest cytotoxic effect on the DLD-1 cancer cell line (IC_{50} = 19,46 µg/mL) and exhibited the highest antioxidant activity in β-carotene/linoleic acid, DPPH, ABTS, CUPRAC and phosphomolybdenum assays. In enzyme inhibition tests, hexane extract showed an effect above the standard control (78,93 %) with urease inhibition of 89,18 %.

Using GC and LC chromatographic techniques, Micallizzi et al. (2022), assessed the chemical composition and antioxidant capacity of both fresh and dried aerial portions of *A. parlatoreana*. The results showed that the two samples' predominant chemical classes were ketones and esters. The primary constituent of the fresh samples was β-Thujone, while the primary constituent of the dried samples was β-artemisia acetate. The dried plant material also included significant amounts of α-pinene and δ^3-carene. Fifteen hydroxycinnamic derivatives, eleven flavonoids, two sesquiterpenoids, two organic acids, and one hydroxybenzoic acid derivative were found by phenolic compound analysis. With results of 122,53 mg/g in the DPPH radical scavenging test

and 98,58 mg/g in the metal chelation study, the dried methanolic extract exhibited the greatest total phenolic content (358,03 mg/100 g) and the strongest antioxidant activity among the extracts.

The phytochemical, antioxidant, and cytotoxic qualities of *A. absinthium*, which is used medicinally every year in Central Saudi Arabia, were thoroughly assessed by Mohammed (2022). A total of 34 components, or 99,98% of the essential oil, were identified by GC–MS analysis. Cis-davanone was the most prevalent chemical (52,51%), followed by osimenone (2,03%), α-gurjunene (7,15%), camazulen (3,38%), camphene (3,27%), γ-eudesmol (2,49%), and pinocarvone (2,18%). Significant antioxidant ability was shown by the essential oil: ferric reducing power (FRAP) was 10,54 mg TE/g, DPPH radical scavenging activity was 24,00 mg TE/g, and total antioxidant capacity (TAC) was 35,59 mg Trolox equivalent/g. Additionally, 29,87 mg EDTA equivalent/g was found to be the metal chelation activity (MCA).

The phenolic and volatile components of the indigenous Turkish species *S. pisidica* and *S. sandrasica*, as well as their tyrosinase inhibitory and antibacterial properties, were assessed by Yaylı et al. 2022. Gallic acid, which was detected in the methanolic extracts of both species at 6,33 mg/g and 2,63 mg/g, respectively, was the most abundant phenolic component, according to LC-MS studies. Strong tyrosinase inhibition was shown by the methanol extracts of *S. pisidica* and *S. sandrasica*, with IC_{50} values of 0,495 µg/mL and 0,699 µg/mL, respectively. The essential oil of S. pisidica shown comparable effectiveness against *M. smegmatis* in antimicrobial testing, but the methanol extract of *S. sandrasica* generated a 12 mm inhibitory zone against the same microbe.

The phytochemical composition and biological activity of ethanolic extracts made from *T. parthenium* L. aerial and root sections at various phenological phases were thoroughly examined by Afshin et al. (2023). The highest levels of total phenols (62,3 mg GAE/g DM), flavonoids (35,7 mg QE/g DM), alkaloids (73,2 mg atropine equivalent/g DM), and total anthocyanins (4,2 mg cyanidin-3-glucoside equivalent/g DM) were found in the shoot extract made during the flowering period. The maximum antioxidant activity was 75,3 % in the extract that was collected during the same time; however, in the vegetative stage, this value dropped to 16,5 % in the root extract. *S. aureus* had the greatest antibacterial activity; the flowering shoot extract's inhibitory

zone width was measured at 21 mm. The minimum bactericidal and inhibitory values were found to be 25 mg/mL and 12,5 mg/mL, respectively.

The chemical makeup, antioxidant capacity, and antidiabetic potential of extracts derived from the *S. phaeopappa* species were examined by El Hosry et al. (2023). The maximum phenolic (2,73 mg GAE/100 mg extract) and terpene (232,42 mg LE/100 mg extract) concentrations were found in dichloromethane–ammonia extract. The best solvent for extracting flavonoids was found to be methanol; this extract demonstrated significant metal chelation capacity (IC_{50} = 0.08 mg/mL, 50% chelation) and good antioxidant activity (DPPH IC_{50} = 0,07 mg/mL). At a dosage of 6,3 mg/mL, acetone extract demonstrated the highest α-glucosidase inhibition and around two times the α-amylase inhibition of the conventional medication acarbose. According to these results, the plant could be a good source of organic substances that have antidiabetic properties.

Gamoune (2023) assessed a crude extract made from the aerial portions of *A. pedunculata* subsp. *atlantica* (Pomel) Oberpr. for its anti-inflammatory and antioxidant qualities in vitro. Compared to the standard antioxidant BHT, the extract showed significant, although reduced, free radical scavenging activity. The extract's anti-inflammatory properties were linked to its protective impact against red blood cell lysis, as shown by erythrocyte membrane stability tests. It was observed, although, that the efficacy diminished with increasing dosage, which could be connected to the extract's phytochemical makeup. According to the study's findings, the extract may be useful in lowering oxidative stress and regulating inflammation, suggesting that further research is necessary to fully explore its medicinal potential.

Güçlü et al. (2023) examined the biochemical profile and biological properties of *S. tomentosa*. According to gas chromatography results, the main compound of the essential oil was identified as 2-pentylamine, which constitutes 35,68% of the total composition. In antioxidant tests, the IC_{50} value for DPPH was 517,0 µg/mL and for ABTS was 244,8 µg/mL; the IC_{50} value of the reference antioxidant was 1,313 µg/mL. Total phenolic and flavonoid analyses revealed that flavonoids predominate among the plant's secondary metabolites.

T. parthenium samples cultivated in various Ukrainian locations were examined for their phenolic content and antioxidant capacity by Hordiei et al. (2023). All samples included 11 different phenolic chemicals, with hydroxycinnamic acids being the most common category. The concentration of hydroxycinnamic acid varied from 3,3% to 6,5%, whereas the quantity of total flavonoids ranged from 0,8% to 2,6%. These variations suggest that regional growth circumstances may affect the phytochemical composition, which in turn may affect antioxidant function.

From the indigenous Hyacinth species *S. longiana* in Türkiye, Korkmaz et al. (2023), extracted two novel dihydroisocoumarins and other terpenoid chemicals and assessed their antibacterial properties. Skorzo Longin I (1) and Skorzo Longin II (2) are the names given to the recently discovered dihydroisocoumarin compounds. Furthermore, nine known metabolites were found: cladantolide (4), dammar-24-en-3β-ol (5), taraxasterol (6), β-sitosterol (7), mangiferursanone (8), 3′,5′-dimethoxyhydrangeol (scorzo longin III, 3), and a mixture of α-amirenone (9a), β-amirenone (9b), and dammar-24-en-3-one (9c) in a ratio of roughly 1:1:2. From the dichloromethane fraction, each of these substances was separated. Scorzo longin I, II, and III were shown to create 20 mm diameter inhibition zones against strains of *E. coli*, *Y. pseudotuberculosis, C. albicans*, and *Saccharomyces cerevisiae* in antimicrobial tests conducted on eight pathogenic bacteria. The compound with the highest activity, scorzo longin II, has a MIC value of 33.8 μg/mL for *M. smegmatis*, *E. coli*, *Y. pseudotuberculosis*, *C. albicans*, and *S. cerevisiae*.

The volatile constituents of Bulgarian *T. parthenium* essential oil were investigated by Lechkova et al. (2023). 37 components, or 94,58% of the oil's overall composition, were found by GC-MS analysis. Camphor (45,47%), trans-chrysanthenyl acetate (21,6%), camphene (9,48%), and cis-isogeraniol (5,42%) were the principal constituents. This chemical profile was mostly in line with the species' previously documented chemotypes.

In order to evaluate the plant's in vitro antioxidant ability, Lo'ay et al. (2023), used GC-MS and LC-ESI–MS/MS methods to investigate the essential oil and phenolic component profile of *A. cotula*. It was discovered that the essential oils produced by hydrodistillation were mostly composed of sesquiterpene hydrocarbons. The primary constituents of the flower oil were found to be γ-muurolene and aromadendrene, whilst

the leaf oil included γ-muurolene and trans-cadinene ether. The primary phenolic components found in the alcoholic floral extracts and aqueous leaf extracts were apigenin and chlorogenic acid. Iron ion chelation (FIC), DPPH, and ABTS assays were used to assess all essential oils and extracts, and the results showed potent metal-binding and radical-scavenging properties.

Sarimahmut (2023), assessed *S. pygmaea*'s cytotoxic and antioxidant qualities. The IC_{50} values from the CUPRAC and DPPH tests were found to be 0,061 g/g and 63,53 µg/mL, respectively. With IC_{50} values of 104,63, 179,27, and 90,83 µg/mL, respectively, cytotoxicity assays demonstrated that the extract inhibited cell growth on MCF-10A (normal), MCF-7, and MDA-MB-231 (breast cancer) cell lines.

The phytochemical profiles and biological activity of *A. vulgaris* samples cultivated at various altitudes in Nepal were studied by Sharma and Adhikari (2023). According to DPPH tests, the leaf extract from Chitwan had the lowest antioxidant activity (IC_{50} = 149,62 µg/mL), whereas the root extract from Kathmandu had the greatest (IC_{50} = 67,31 µg/mL). The Gorkha region's root extract reduced α-amylase by 39,43%. The leaves from Chitwan had the lowest phenolic content (26,04 mg GAE/g), whereas the roots from Kathmandu had the greatest (96,30 mg GAE/g). The greatest flavonoid levels were found in Kathmandu leaf extracts (71,15 mg QE/g), whilst the lowest values were found in Gorkha samples (31,54 mg QE/g).

In *A. wilhelmsii* from the Kashan area of Iran, Ghavam et al. (2024), discovered a novel chemotype and examined its antibacterial qualities and essential oil composition. Oxygenated monoterpenes made up 47,87% of the composition, and the output of essential oil was 0,1071% (w/w). Fragranol (33,22%), fragranyl acetate (16,18%), and oleic acid (6,33%) were the most prevalent components. The oil shown comparable action to rifampin (≈9 mm) against Gram-negative organisms such *Acinetobacter baumannii* and *Shigella dysenteriae*, and it created inhibition zones of around 10 mm against *C. albicans* and *S. aureus*.

Güçlü (2024), investigated the methanolic and aqueous extracts of *A. kotschyana* Boiss's in vitro antioxidant properties. variation. GC-MS tests revealed that the primary constituent in the water extract was 2-propenoic acid tridecyl ester, whereas the primary constituent in the methanol extract was cyclododecane. Both the methanol

extract and the aqueous extract had greater antioxidant activity in DPPH and ABTS, respectively, according to the results of the DPPH and ABTS tests. In both extracts, it was discovered that the total phenolic content (TPC) was greater than the total flavonoid content (TFC). TFC values were 24,3 QE/g and 65,6 QE/g, respectively, whereas TPC was 83,7 mg GAE/g in the aqueous extract and 170,7 mg GAE/g in the methanol extract.

Yıldırım et al. (2024), employed a bioactivity-guided fractionation technique based on GC/MS and LC-MS/MS to assess the anti-inflammatory potential of many *Anthemis* species, which are historically used in the treatment of inflammatory illnesses. Finding the active metabolites in *A. cotula*, *A. tomentosa* (Austrian variation), and A. tinctoria that have anti-inflammatory properties was the main goal of the research. The methanolic extract of *A. tinctoria* demonstrated comparable potency to the reference medication indomethacin (IC_{50}: 23,88 and 18,05 mg/mL), according to 5-lipoxygenase (5-LOX) inhibition assays. Additionally, a subfraction known as ATiEA-II was shown to have strong inhibitory efficacy and probably includes a brand-new anti-inflammatory substance. *Anthemis* species are valuable natural resources for the creation of novel anti-inflammatory drugs, the researchers underlined.

CHAPTER 3
MATERIALS AND METHODS

3.1 Field Study

Several Asteraceae species (*Anthemis cotula, Achillea falcata, Scorzonera kotschyi, Artemisia absinthium, Tanacetum parthenium, Senecio othonnae*, and *Tussilago farfara*) collected from Osmaniye province were collected during the summer months of 2023 and 2024 to determine their phenolic compound and essential oil contents, total antioxidant and oxidant levels, DPPH activities, total phenolic and flavonoid contents, and antimicrobial properties. Species identification of the collected plant samples was conducted at the Biology Department of Osmaniye Korkut Ata University, Faculty of Arts and Sciences. The identification of the samples was conducted by Instructors Ali Erdem ŞABİK and Assoc. Prof. Dr. Mustafa SEVİNDİK.

3.2 Preparation of the Collected Plant Species

Unwanted components of the plant samples found during field investigations in Osmaniye province were eliminated, and dust and muddy residues were cleansed with distilled water. The pre-prepared plant samples were desiccated by positioning them on blotting paper in a controlled laboratory setting, shielded from humidity and direct sunlight. Thirty-gram samples were obtained from each dried plant specimen using a tared precision balance, and these samples were subjected to extraction with 250 mL of ethanol at 50°C utilizing a Soxhlet equipment. The solvent was eliminated via a rotary evaporator, resulting in the crude extract. The identical extraction procedure utilized with the ethanol solvent was similarly employed with the methanol solvent, resulting in the crude extract.

3.3 Antioxidant Determination

Commercial kits from Rel Assay Diagnostics (Turkey) were used to measure the total antioxidant capacity (TAS) of ethanol and methanol extracts made from plant sources

(Erel, 2004). As a calibration standard, trolox, a water-soluble form of vitamin E, was used. mmol Trolox equivalents/L was the unit of measurement used to represent the TAS results. Rel Assay Diagnostics (Turkey) kits were also used to measure the total oxidant capacity (TOS) values (Erel, 2005). Calibration was performed using hydrogen peroxide (H_2O_2), and the findings were expressed in μmol H_2O_2 equivalents/L. By dividing the TOS values by the TAS values, the oxidative stress index (OSI) value was determined, and the findings were shown as a percentage (Sevindik, 2019).

3.4 Free Radical Scavenging Activity (FRAP and DPPH Tests)

A solution of 0,004% DPPH in methanol was made in order to measure the DPPH radical scavenging activity. To do this, 150 μL of DPPH solution was combined with 50 μL of the extract at a concentration of 2 mg/mL. The combination was allowed to sit at room temperature in the dark for half an hour. The reference standard, Trolox (mg Trolox equivalent/g), was likewise subjected to the same process (Krishnaiah et al., 2011). At a wavelength of 517 nm, spectrophotometric measurements were made. Prior to the FRAP test, the reagent solution was made from scratch. 0.3 M pH 3.6 acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine), and 20 mM $FeCl_3$ solutions were combined in a 10:1:1 ratio to create the FRAP reagent. 200 μL of FRAP reagent was added to each tube after 25 μL of the extract at a concentration of 2 mg/mL for analysis. Before measuring absorbance at a wavelength of 593 nm, the combinations were let to remain at room temperature for half an hour. Trolox, the reference drug, was subjected to the same procedure. Trolox equivalents (mg TE/g) were used to represent the FRAP findings (Benzie and Strain, 1996).

3.5 Antimicrobial Determination

Based on the agar dilution method suggested by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI), the antimicrobial activity of ethanol and methanol extracts made from plant samples collected in the field was assessed. Methanol and ethanol extracts' minimum inhibitory concentrations (MICs) against certain bacterial and fungal species were determined. For the bactericidal testing, ethanol and methanol extracts were made by centrifuging at 15,000 rpm and +4 °C. *Candida krusei* ATCC 34135, *C. glabrata* ATCC 90030, and *C. albicans* ATCC 10231were the fungal strains

used in the investigation. Gram-negative bacteria were *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Acinetobacter baumannii* ATCC 19606, whereas Gram-positive bacteria were *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *S. aureus* MRSA ATCC 43300. The American Type Culture Collection (ATCC) was the source of all microorganism strains. Yeast strains were cultivated in RPMI 1640 Broth, whereas bacterial strains were pre-cultured in Mueller Hinton Broth. The turbidity of the yeast and bacterial cultures was adjusted to 0,5 McFarland standard in order to create the standard inoculum. The concentration range of 800-12,5 µg/mL was used to evaluate methanol and ethanol extracts. Standard antibiotics such as ampicillin, ciprofloxacin, and amikacin were used for bacterial strains, while fluconazole and amphotericin B were used for yeast infections. The medium for the studies was Müller Hinton Agar (MHA). After being frozen in a water bath, 9 mL of the MHA medium was divided among 15 mL sterile tubes and sterilized. One milliliter of extract was added to each tube to create a homogenous mixture, which was then moved to petri plates. In this manner, seven distinct dilutions were made for every component. Sterile plastic ring tips (0,01 mL) were used to inoculate the medium with bacterial and yeast inocula (10^6 CFU/mL). For 16-20 hours for bacteria and 48 hours for fungus, plates were incubated at 35°C. Each experimental series comprised control plates and cultures were assessed at the conclusion of the periods (Bauer et al., 1966; Hindler et al., 1992; Matuschek et al., 2014).

3.6 Total Phenolic and Flavonoid Determination

A modified Folin-Ciocalteu technique was used to determine the total phenolic content (Slinkard and Singleton, 1977). This was accomplished by vortexing a specific volume of the sample solution with diluted Folin-Ciocalteu reagent (1 mL, 1:9, v/v). Waiting for three minutes was followed by the addition of 0,75 mL of 1% Na_2CO_3 solution. Following two hours of dark incubation at room temperature, the mixture was analyzed spectrophotometrically at 760 nm. Gallic acid equivalents (mg GAE/g extract) were used to express the results.

The aluminum chloride colorimetric technique was used to determine the total flavonoid concentration (Uysal et al., 2024). 1,5 mL of 95% ethanol, 0,1 mL of 10% $AlCl_3$, 0,1 mL of 1 M potassium acetate, and 2,8 mL of filtered water were added to

0,5 mL of the produced plant extract. For half an hour, the mixture was incubated at room temperature. Using spectrophotometry, the absorbance of the reaction mixture was measured at 415 nm at the conclusion of this time frame. The calibration curve was prepared using quercetin as a reference, and the findings were reported as quercetin equivalents (mg QE/g extract).

3.7 Determination of AchE and BchE Inhibitory Activity

The colorimetric technique developed by Ellman et al. (1961) was used to measure the anticholinesterase activity of plant extracts. The study's reference standard was galantamine. The extracts were used to create functional solutions with concentrations ranging from 200 to 3,125 µg/mL. A 96-well microplate was filled with 130 µL of 0,1 M phosphate buffer (pH 8.0) for the analysis. The mixture was then incubated for 10 minutes at 25°C in the dark after 10 µL of extract stock solution and 20 µL of enzyme solution (AchE or BchE) were added. Next came 20 µL of DTNB (5,5′-dithiobis-(2-nitrobenzoic acid), 6,8 µmol) solution, followed by 20 µL of substrate (either butyrylcholine iodide or acetylcholine iodide). Measurements of absorbance at a wavelength of 412 nm were made at the conclusion of the process. The extracts' inhibition percentages were computed and reported as IC_{50} values (µg/mL).

3.8 Antiproliferative Activities of Plant Samples

The A549 human lung epithelial cell line was used in the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) test to assess the effects of ethanol and methanol extracts derived from plant materials on cell viability. 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) was used to separate the cells from the surface after they had achieved around 70-80% confluence. The cells were then moved to culture plates. Following injection, the cells were cultured for a full day. The extracts were added to the cell cultures and incubated for an additional twenty-four hours after being diluted at doses of 25, 50, 100, and 200 µg/mL. Fetal bovine serum (FCS)-free culture media was used to create the control group. The culture medium's supernatant was taken out after 48 hours of incubation, and 1 mg/mL of Sigma MTT solution was applied to each well. Purple formazan crystals formed when the cells were cultured at 37°C. Following incubation, the supernatant was discarded, and dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) was used to dissolve the

crystals that had formed. An Epoch spectrophotometer (BioTek Instruments, Winooski, VT, USA) was used to measure absorbance at a wavelength of 570 nm (Bal et al., 2017).

3.9 Determination of Phenolic Content in Plant Samples

To ascertain the levels of phenolic compounds in plant samples gathered in the field, an analysis service was hired. The LC–MS/MS technology was used to assess the samples' phenolic profiles. The service provider in this research conducted the validity tests and assessments of the technique created for the quantitative determination of a total of 26 phenolic chemicals. On a C18 analytical column in reverse phase, chromatographic separation was carried out. The temperature of the column was maintained at 40 °C. Mobile phases A (water, 5 mM ammonium formate, and 0.1% formic acid) and B (methanol, 5 mM ammonium formate, and 0,1% formic acid) were used to construct the elution gradient. In the gradient program, solvent B was administered at the following percentages: 0,40 percent, 20,9 percent, 23,99%, 24,4 percent, and 29,40 percent. The injection volume was set at 4 µL, and the flow rate was set at 0,5 mL/min. Lab Solutions software was used for data processing and computations. The studies were conducted using the multiple reaction monitoring (MRM) method. Every phenolic compound was subjected to three separate analyses. While the next two analyses were focused on technique validation studies, the first analysis was conducted for quantitative determination. The following were determined to be the ideal parameters for electrospray ionization (ESI): 350°C for the interface, 250°C for the DL (desolvation line), 400°C for the heat block, 3 L/min for the nebulizer gas, and 15 L/min for the dryer gas (Takım, 2020). Acetohydroxamic acid, resveratrol, syringic acid, thymoquinone, vanillic acid, gallic acid, oleuropein, caffeic acid, hydroxycinnamic acid, catechin, hydroxybenzoic acid, 2-hydroxy-1,4-naphthoquinone, fumaric acid, protocatechuic acid, salicylic acid, phloridzin, myricetin, ellagic acid, quercetin, butyrin, naringenin, silymarin, luteolin, kaempferol, alizarin, and curcumin are among the phenolic compounds that were used as standards in the analysis.

3.10 Determination of Essential Oils in Plant Samples

Following the grinding and homogenization of natural plant materials, enough amounts were weighed and put into 1-liter heat-resistant flasks. A Clevenger-type device was used to water distill the samples for four hours in order to extract the essential oils. After being extracted from the water in the device, the essential oils were put into Eppendorf tubes, dried with anhydrous sodium sulfate, and kept at +4°C until GC-MS analysis. The components of essential oils were determined both qualitatively and quantitatively using gas chromatography-mass spectrometry (GC-MS). Three separate analyses were conducted. Using an HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm), chromatographic separation was accomplished. The carrier gas, helium (He), had a flow rate of 3 milliliters per minute. The split injection technique was used, and the injector temperature was maintained at 280°C. The oven temperature program was set up as follows: after two minutes at 50°C, the temperature was raised to 120°C at a rate of 15°C per minute and maintained there for another two minutes. After then, the temperature was raised by 5°C every minute until 300°C, where it remained for 16 minutes. The temperature of the detector was set at 280°C.

3.11 Statistical Analyses

SPSS 22 package program was used for the statistical analysis of the data obtained from the study.

CHAPTER 4
RESULTS

4.1 Antioxidant Activity

4.1.1 DPPH and FRAP Tests

The antioxidant activities of the methanol and ethanol extracts of *Anthemis cotula, Achillea falcata, Scorzonera kotschyi, Artemisia absinthium, Tanacetum parthenium, Senecio othonnae*, and *Tussilago farfara* used in our study were determined using DPPH and FRAP tests. The results are shown in Figure 4.1. The ethanol extract of *A. cotula* showed significantly higher antioxidant capacity compared to the methanol extract. DPPH radical scavenging activity was determined as 127,33 mg TE/g and FRAP value was 148,03 mg TE/g in the ethanol extract. These values were determined as 105,06 mg TE/g (DPPH) and 132,74 mg TE/g (FRAP), respectively in the methanol extract. Similarly, the ethanol extract of *A. falcata* also exhibited stronger antioxidant activity than the methanol extract. While the DPPH value was 93,92 mg TE/g and the FRAP value was 103,05 mg TE/g in the ethanol extract. In the methanol extract, these values were recorded as 71,65 mg TE/g (DPPH) and 79,62 mg TE/g (FRAP), respectively. However, it was determined that *A. falcata* generally has a lower antioxidant capacity compared to other plants. The ethanol extract of S. kotschyi showed a relatively limited antioxidant effect with values of 62,0 mg TE/g (DPPH) and 89,83 mg TE/g (FRAP). In the methanol extract, these values were measured as 53,97 mg TE/g (DPPH) and 73,43 mg TE/g (FRAP). The obtained findings show that this species provides low level of protection against free radicals and has limited antioxidant capacity. The methanol extract of *A. absinthium* had the highest antioxidant activity among all the plants evaluated in the study. For this extract, DPPH value was determined as 155,63 mg TE/g and FRAP value was determined as 193,32 mg TE/g. High activity was also detected in the ethanol extract. Both solvents provided significant level of antioxidant activity for this species. The methanol extract of *T. parthenium* showed stronger antioxidant properties compared to the ethanol extract.

DPPH value was determined as 120,58 mg TE/g and FRAP value was determined as 150,12 mg TE/g in the methanol extract. These values were measured as 101,08 mg TE/g (DPPH) and 136,64 mg TE/g (FRAP), respectively. The ethanol extract of *T. farfara* was found to have a remarkable antioxidant potential with values of 91.38mg TE/g (DPPH) and 122,26 mg TE/g (FRAP). These values were determined as 78,60 mg TE/g (DPPH) and 103,40 mg TE/g (FRAP) in the methanol extract.

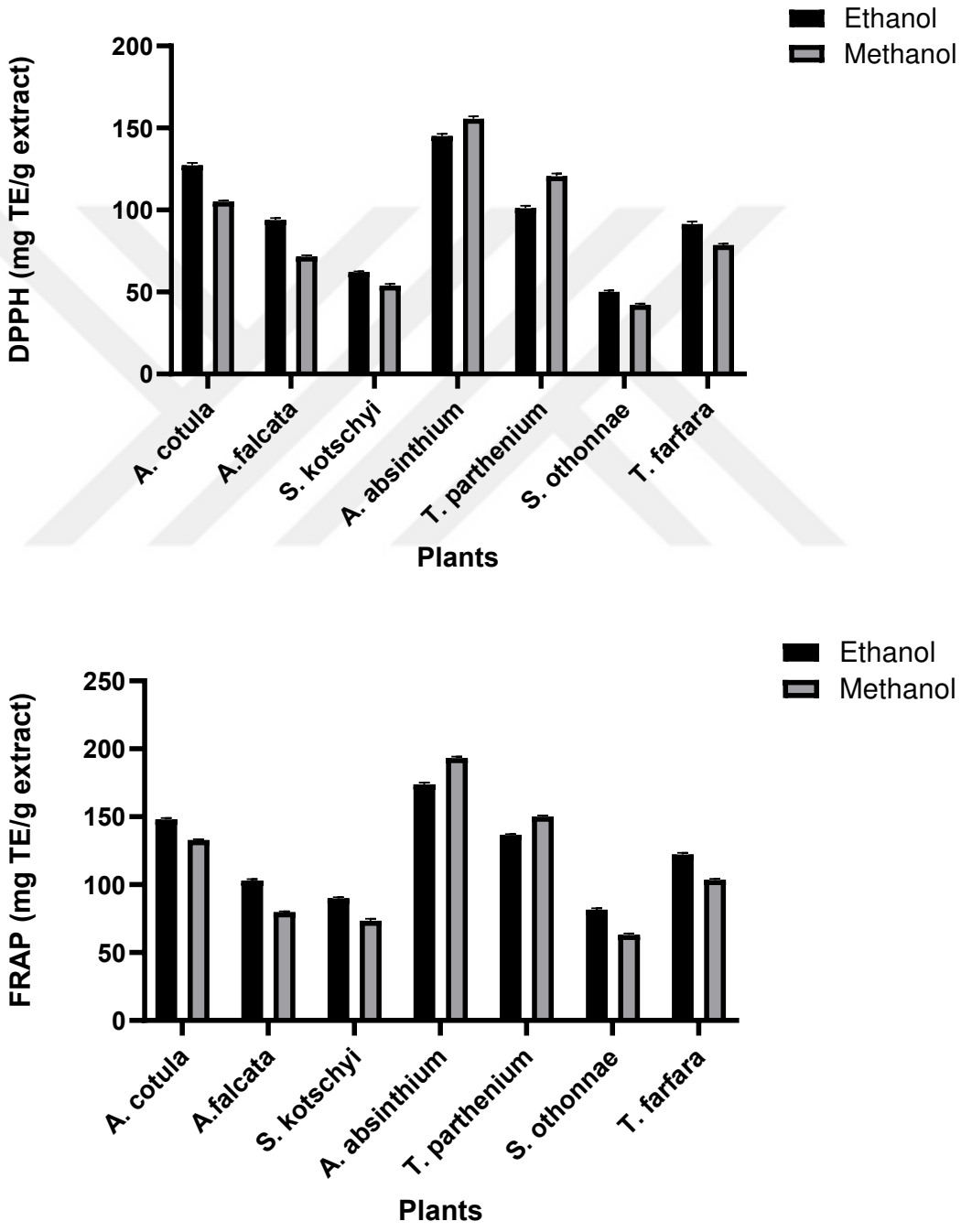

Figure 4.1 DPPH and FRAP values

The plants examined in the study exhibited significant differences in their antioxidant properties. *A. absinthium* exhibited superior antioxidant activity in both DPPH and FRAP experiments. The methanol extract exhibited the greatest values for free radical scavenging capacity and reducing power. *T. parthenium* and *A. cotula* exhibited significant antioxidant activity, but not as pronounced as that of *A. absinthium*, establishing them as noteworthy natural sources of antioxidants. *A. falcata* and *T. farfara* exhibited moderate antioxidant activity. *A. falcata* exhibited heightened DPPH and FRAP values, particularly with the ethanol extract; nevertheless, these values were lower than those of more active species such as *T. parthenium* and *A. cotula*. *T. farfara* exhibited a similar moderate antioxidant capacity. In contrast, *S. kotschyi* and *S. othonnae* demonstrated the lowest antioxidant capacity among the species analyzed. *S. othonnae* was identified as the least effective antioxidant plant in the study, demonstrating the lowest DPPH and FRAP values. *S. kotschyi* exhibited slightly higher values but was primarily defined by its reduced antioxidant capacity. *A. absinthium* and *T. parthenium* exhibited enhanced antioxidant activity in methanol extracts when evaluated by solvent type. In contrast, *A. falcata*, *T. farfara*, and *S. kotschyi* shown enhanced antioxidant capabilities when ethanol was employed as a solvent. *A. absinthium*, *T. parthenium*, and *A. cotula* displayed the highest antioxidant activity, whereas *S. kotschyi* and *S. othonnae* showed the lowest antioxidant capacity.

4.1.2 TAS, TOS, and OSI Values

The TAS, TOS, and OSI values of the methanol and ethanol extracts of the plants used in the study are shown in Figure 4.2. The TAS and TOS values for *A. cotula* were determined. It has average levels of antioxidant and oxidant compounds in both solvents. This plant's TAS value is moderate compared to other plants, while its TOS value is also at an average level. The OSI value is also quite low, indicating that the plant has a more balanced antioxidant and oxidant compound profile and a lower potential for oxidative stress. The TAS and TOS values for *A. falcata* indicates that the plant has a structure containing higher levels of oxidant compounds. Although the TAS value is lower than other plants, the TOS value is quite high, indicating that the plant has a high potential to cause oxidative stress. The OSI value is also high, indicating that the plant contains more oxidant compounds relative to its antioxidant capacity, and therefore, oxidative stress may be high.

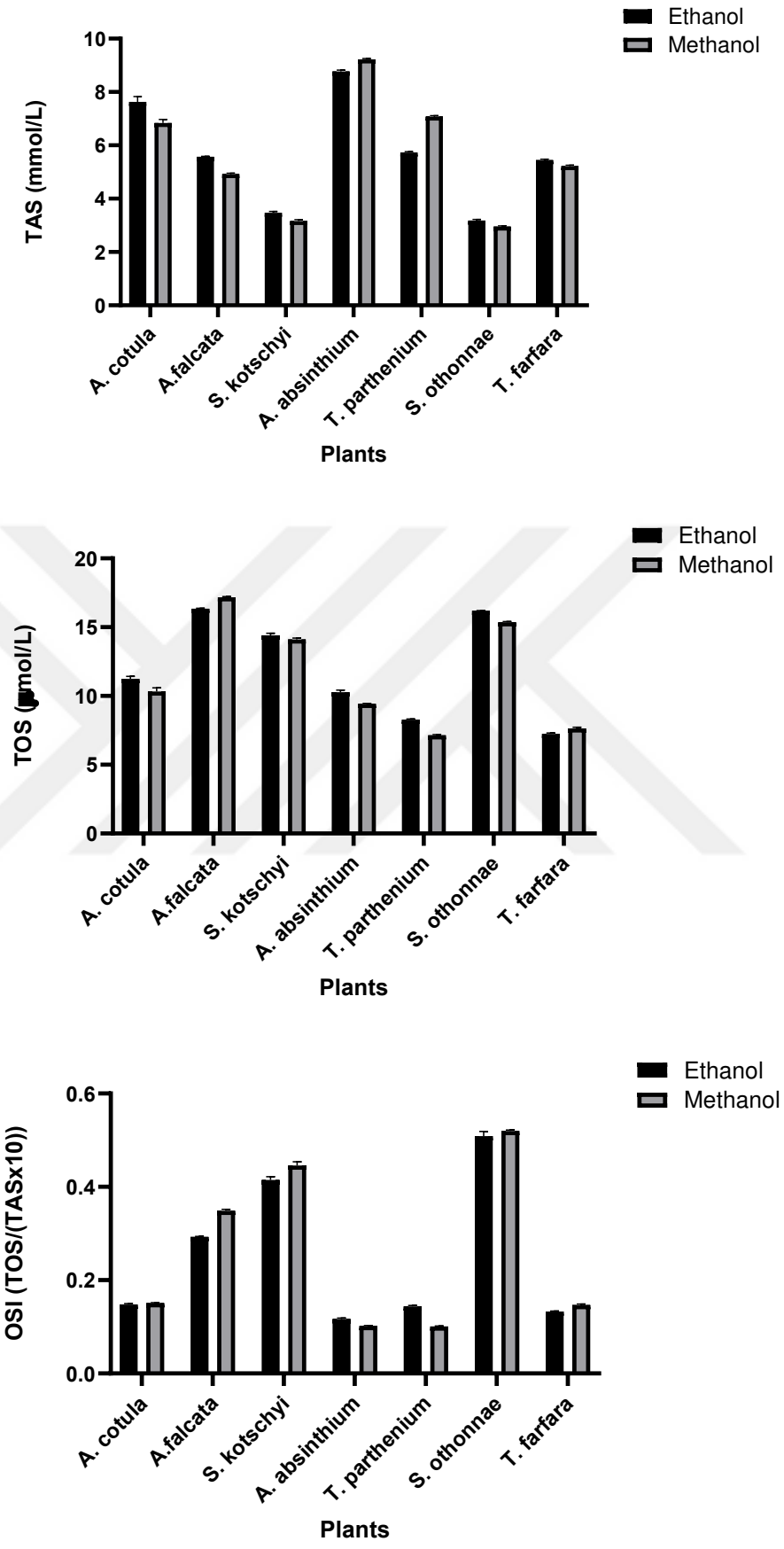

Figure 4.2 TAS, TOS, and OSI values

The TAS and TOS values for *S. kotschyi* indicate that the plant has low levels of antioxidant compounds but a relatively high level of oxidant compounds. The TAS value is quite low for this plant, while the TOS value remains high. Consequently, the OSI value is also high, indicating that the plant has a profile that creats strong oxidative stress.

The TAS and TOS values for *A. absinthium* indicate that it stands out with its high antioxidant levels in both solvents. The TAS value is quite high compared to other plants, while the TOS value is at lower levels. The OSI value is low, indicating that this plant has a high antioxidant capacity and low oxidative stress. This plant can be considered a plant with strong antioxidant properties and potential for protection against oxidative stress.

The TAS and TOS values for *T. parthenium* indicate that the plant contains moderate levels of antioxidant compounds, but oxidant compounds are relatively low. The TAS value is found at moderate levels, while the TOS value remains correspondingly low. The OSI value is also quite low, indicating that this plant has a more balanced antioxidant-oxidant profile and low levels of oxidative stress. *T. parthenium* stands out as a plant with a balanced oxidative stress/antioxidant balance.

TAS and TOS values were determined for *S. othonnae*. These values indicate that the plant has low levels of antioxidant compounds but high levels of oxidant compounds. Despite the low TAS value, the TOS value remains quite high. The OSI value is also very high, indicating that this plant is capable of inducing high oxidative stress. *S. othonnae* stands out as a plant containing potent compounds that can cause oxidative stress.

TAS and TOS values were determined for *T. farfara*. These values indicate that the plant contains moderate levels of antioxidant compounds and low levels of oxidant compounds. The TAS and TOS values remain at moderate levels. The OSI values are low, indicating that the plant's antioxidant and oxidant compounds are balanced and that oxidative stress levels are low. *T. farfara* stands out as a plant that reflects a balanced antioxidant-oxidant profile and low levels of oxidative stress.

Among the plants examined in our study, *A. absinthium* stands out with its highest TAS. The very high TAS values in both methanol and ethanol solvents indicate that

this plant contains potent antioxidant compounds. The methanol extract in particular exhibited the highest antioxidant capacity. Similarly, *A. cotula* and *T. parthenium* plants also stand out with their high TAS values.

When examined for TOS, the highest values were observed in *A. falcata* and *S. othonnae*. The methanol extract of *A. falcata* had the highest oxidant levels. *S. othonnae* also stands out with its high oxidant levels, indicating a high potential for inducing oxidative stress. Similarly, *S. kotschyi*, with its high TOS values, is among the plants with pro-oxidant properties.

When the OSI values were evaluated, the plants with the lowest OSI values were *T. parthenium* and *A. absinthium*. *T. parthenium* had the lowest OSI value in the methanol extract, indicating that its antioxidant capacity is quite high compared to its oxidant level. *T. farfara* also has a balanced structure with a low OSI value.

The highest OSI values were observed in *S. othonnae* and *S. kotschyi. S. othonnae* was determined to have the highest OSI value in the study with the methanol extract. *S. kotschyi* also stands out with its high OSI levels, while *A. falcata*, with its relatively high OSI values, is among the plants that induce oxidative stress.

When the effects of solvents were evaluated, methanol generally yielded higher TAS values, while extracts prepared with ethanol exhibited higher TOS values. *A. absinthium*, *T. parthenium*, and *A. cotula*, in particular, exhibited stronger antioxidant properties in methanol extracts. In contrast, oxidant levels were higher in ethanol extracts of *A. falcata* and *S. kotschyi* plants.

4.2 Antimicrobial Activity

The antibacterial efficacy of ethanol and methanol extracts from the plants included in this investigation was assessed with the modified agar dilution technique. The study's findings are presented in Figure 4.3. *A. cotula* extracts in ethanol and methanol shown strong antibacterial and antifungal properties against every tested bacterium. At 25-100 µg/mL, the ethanol extract was shown to be efficient against bacteria, while at 25-50 µg/mL, it was effective against fungus. At 50-100 µg/mL, the methanol extract had antibacterial activity, while at 100 µg/mL, it demonstrated antifungal activity. At 50 µg/mL, both extracts demonstrated significant suppression against *S. aureus* and

methicillin-resistant *S. aureus* (MRSA). Concentrations of 100 µg/mL of the ethanol extract and 200 µg/mL of the methanol extract were both efficient against *E. faecalis*.

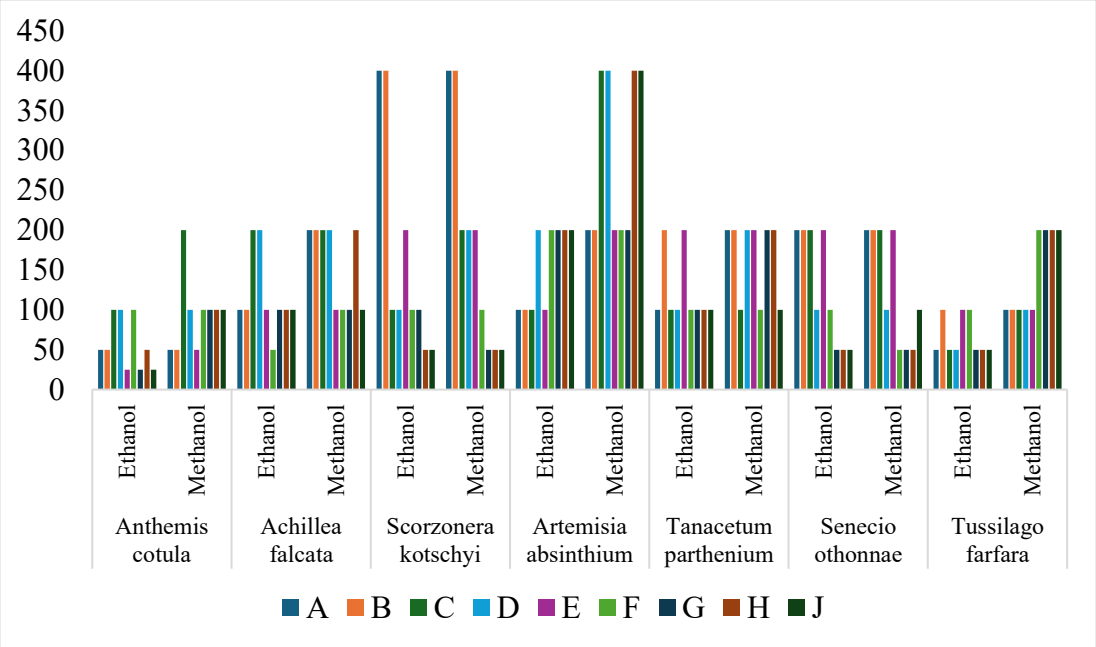

Figure 4.3 Antimicrobial activity of plants. * A: *S. aureus*, B: *S. aureus* MRSA, C: *E. faecalis*, D: *E. coli*, E: *P. aeruginosa*, F: *A. baumannii*, G: *C. glabrata*, H: *C. albicans*, J: *C. krusei.* *Extract concentrations are 400, 200, 100, 50 and 25 µg/mL.

At a dosage of 100 µg/mL, it was also shown that both extracts exhibited antibacterial activity against *A. baumannii* and *E. coli*. At 25 µg/mL, the ethanol extract and 50 µg/mL, respectively, were shown to be effective against *P. aeruginosa*. The ethanol extract significantly inhibited *C. glabrata* and *C. krusei* at 25 µg/mL and *C. albicans* at 50 µg/mL in antifungal tests. At a dose of 100 µg/mL, the methanol extract demonstrated efficacy against these species. Broad-spectrum antibacterial activity was also shown by *A. falcata*'s ethanol and methanol extracts. While the methanol extract was effective against both bacteria and fungus in the 100-200 µg/mL range, the ethanol extract was effective against bacteria in the 50-200 µg/mL range and against fungi at a dosage of 100 µg/mL. At 100 µg/mL, the ethanol extract was shown to be efficient against *S. aureus*, MRSA, and *C. albicans*; at 200 µg/mL, the methanol extract showed the same effect. At 200 µg/mL, both extracts inhibited *E. faecalis* and *E. coli* bacteria to comparable degrees. At a dose of 100 µg/mL, comparable activity was shown against *P. aeruginosa*, *C. glabrata*, and *C. krusei*. *A. baumannii* was effectively

inhibited by the methanol extract at 100 µg/mL and the ethanol extract at 50 µg/mL. According to these results, *A. falcata* has a wide range of activities and has exceptional antibacterial activity, especially against Gram-positive bacteria.

In both ethanol and methanol extracts, *S. kotschyi* demonstrated a wide range of antibacterial and antifungal properties. The ethanol extract showed antifungal effects between 50 and 100 µg/mL, and it was efficacious against bacterial strains at doses between 50 and 400 µg/mL. The methanol extract demonstrated antifungal activity at 50 µg/mL and antibacterial activity between 100 and 400 µg/mL. At 400 µg/mL, both extracts demonstrated notable efficacy against methicillin-resistant *S. aureus* (MRSA) and *S. aureus*. At 100 µg/mL, the ethanol extract inhibited *E. coli* and *E. faecalis*, but the methanol extract needed 200 µg/mL to have the same effect. At 200 µg/mL, both extracts inhibited *P. aeruginosa* growth. The minimum inhibitory concentration (MIC) of the ethanol extract against *A. baumannii* was 50 µg/mL, but the methanol extract needed 100 µg/mL. Additionally, the ethanol extract demonstrated antifungal activity against *C. glabrata* at 50 µg/mL, while both extracts shown comparable efficacy against *C. albicans* and *C. krusei* at the same concentration. These findings suggest that *S. kotschyi* has a significant antibacterial activity, especially against fungi. Additionally, *A. absinthium* showed strong antibacterial activity against every microbe that was examined. While the methanol extract shown action against both bacteria and fungi at 200-400 µg/mL, the ethanol extract inhibited bacterial strains at 100-200 µg/mL and fungal strains at 200 µg/mL. While the methanol extract needed 200 µg/mL to be effective against *S. aureus*, MRSA, and *P. aeruginosa*, the ethanol extract worked at 100 µg/mL. Ethanol extract activity for *E. faecalis* was 100 µg/mL, but methanol extract activity was 400 µg/mL. At 200 µg/mL, both extracts inhibited *A. baumannii* and *C. glabrata*, and at 200 µg/mL, *E. coli, C. albicans*, and *C. krusei*. All of these results show that *A. absinthium* has a wide range of antibacterial activity against both bacterial and fungal diseases. Both ethanol and methanol extracts of *T. parthenium* showed dose-dependent antibacterial and antifungal activity. While the methanol extract inhibited both bacterial and fungal strains at 100-200 µg/mL, the ethanol extract showed action against bacteria in the 100-200 µg/mL range and antifungal activity at 100 µg/mL. *S. aureus*, *E. coli*, *C. glabrata*, and *C. albicans* were suppressed by both extracts at 100 µg/mL for ethanol and 200 µg/mL for methanol. Likewise, both extracts shown inhibitory effects on *E. faecalis*, *A. baumannii*, and *C. krusei* at 100

µg/mL, as well as on MRSA and *P. aeruginosa* at 200 µg/mL. All things considered, *T. parthenium* showed significant antibacterial ability, successfully inhibiting the development of strains of fungi and bacteria, both Gram-positive and Gram-negative.

The antibacterial and antifungal properties of *S. othonnae* varied according to the extraction solvent. While the methanol extract showed antifungal potential at 50-100 µg/mL and antibacterial effectiveness at 50-200 µg/mL, the ethanol extract showed antibacterial effects in the 100-200 µg/mL range and antifungal activity at 50 µg/mL. At 200 µg/mL, both extracts inhibited *P. aeruginosa*, *E. faecalis*, methicillin-resistant *S. aureus* (MRSA), and *S. aureus*. In contrast, both ethanol and methanol extracts reduced *E. coli* at 100 µg/mL. The methanol extract showed greater efficacy against *A. baumannii* at 50 µg/mL, whereas the ethanol extract was effective at 100 µg/mL. At 50 µg/mL, both extracts inhibited *C. glabrata* and *C. albicans*. Additionally, at 50 µg/mL, the ethanol extract demonstrated action against *C. krusei*, but the methanol extract needed 100 µg/mL to exhibit a similar level of inhibition. All things considered, our results suggest that *S. othonnae* has more antifungal potential than antibacterial activity, especially against *Candida* species. In tests using ethanol and methanol extracts, *T. farfara* showed modest but persistent antibacterial and antifungal activity. At 50-100 µg/mL, the ethanol extract demonstrated antifungal suppression, but at 50 µg/mL, it was active against bacterial strains. The methanol extract demonstrated antifungal activity at 200 µg/mL and antibacterial activity between 100 and 200 µg/mL. With ethanol extracts active at 50 µg/mL and methanol extracts at 100 µg/mL, both extracts efficiently inhibited *S. aureus*, *E. faecalis*, and E. coli. At 100 µg/mL, both extracts demonstrated efficacy against *P. aeruginosa* and MRSA. The ethanol extract inhibited *A. baumannii* at 100 µg/mL, whereas the methanol extract inhibited it at 200 µg/mL. Furthermore, at 50 µg/mL, the ethanol extract showed antifungal activity against *C. glabrata*, *C. albicans*, and *C. krusei*, but the methanol extract needed 200 µg/mL to exhibit the same activity. All things considered, *T. farfara* showed moderate but notable antimicrobial ability, showing inhibitory action against both bacterial and fungal species. Ethanol extracts were typically more effective than methanol extracts.

4.3 Total Phenolic and Total Flavonoid Levels

The total phenolic levels (TPS) and total flavonoid levels (TFS) of the plant samples used in the study were determined and are shown in Figure 4.4.

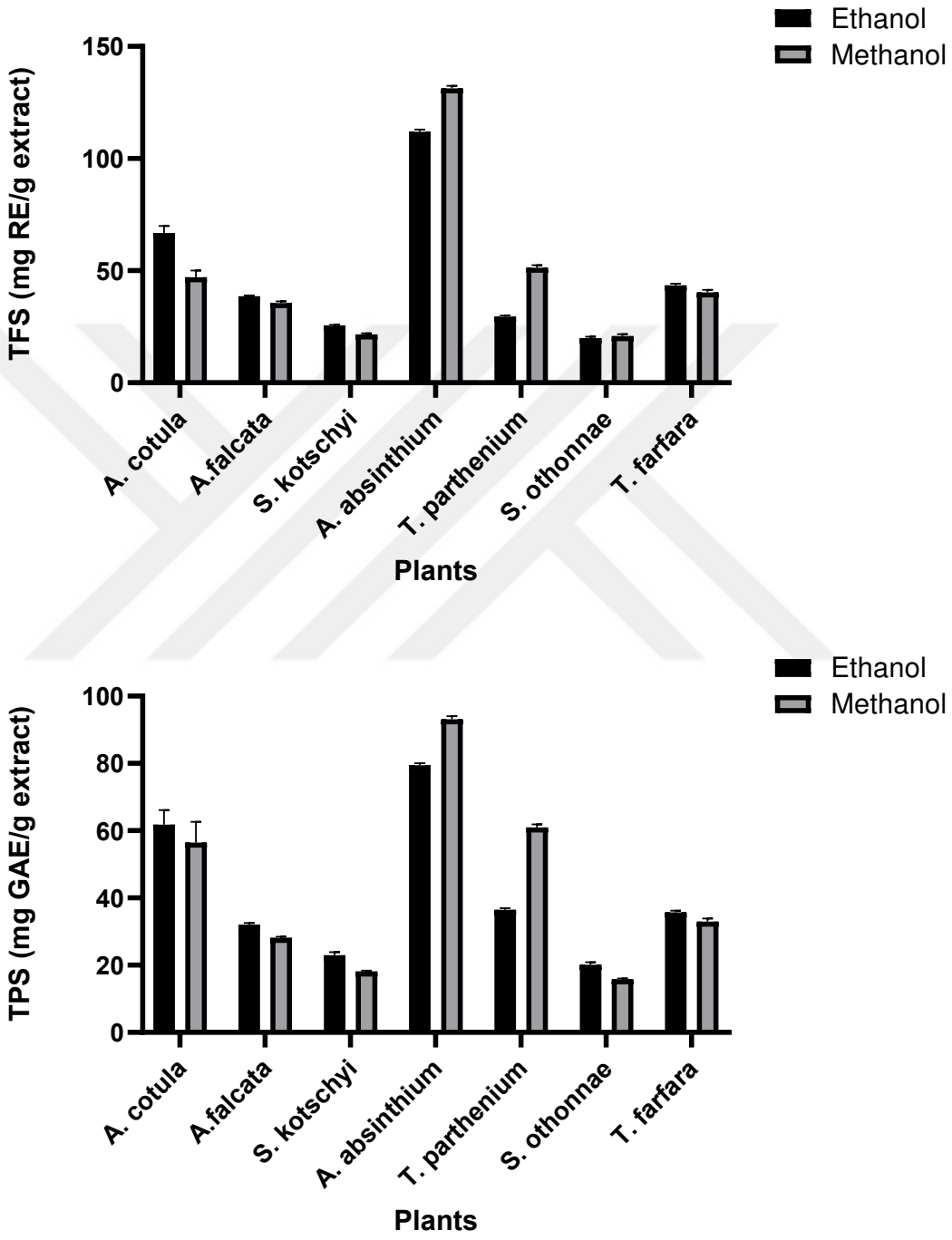

Figure 4.4 TPS and TFS values

One of the plants with higher levels of total phenolic and flavonoid contents, according to our analysis, is *A. cotula*. Compared to the methanol extract, the ethanol extract

showed higher values for total flavonoid levels (TFS) at 66,84 mg RE/g and total phenolic levels (TPS) at 61,78 mg GAE/g. Total flavonoid content (TFS) was 47,01 mg RE/g and total phenolic levels (TPS) was 56,48 mg GAE/g in the methanol extract. The results show that the best solvent for removing phenolic and flavonoid components from *A. cotula* is ethanol. Phenolic and flavonoid components were present in modest amounts in *A. falcata*. In comparison to the methanol extract, which showed TPS values of 28,14 mg GAE/g and TFS values of 35,62 mg RE/g, the ethanol extract showed improved TPS values of 32,06 mg GAE/g and TFS values of 38,61 mg RE/g. *S. kotschyi* exhibited lower amounts of flavonoids and total phenolics. TPS and TFS values for the ethanol extract were 22,97 mg GAE/g and 25,44 mg RE/g, respectively. TFS levels of 21,55 mg RE/g and TPS values of 18,09 mg GAE/g were lower in the methanol extract. The greatest total concentrations of phenolics and flavonoids in our study were found in *A. absinthium*. The highest concentrations of total phenolic substances (TPS) and total flavonoid substances (TFS) were found in the methanol extract. With a total flavonoid content (TFC) of 111,98 mg RE/g and a total phenolic content (TPC) of 79,42 mg GAE/g, the ethanol extract showed significantly higher values. The increased concentrations of flavonoid and phenolic components in both solvents suggest that this plant may have significant antioxidant qualities. Phenolic and flavonoid compounds were found in moderate to high amounts in *T. parthenium*. With a TPS of 60,5 mg GAE/g and a TFS of 51,28 mg RE/g, the methanol extract showed a greater concentration of chemicals than the ethanol extract. The lowest levels of flavonoids and total phenolics were found in *S. othonnae*. The TPS and TFS values of the ethanol extract were 20,09 mg GAE/g and 19,96 mg RE/g, respectively. The total flavonoid content (TFS) of the methanol extract was 20,84 mg RE/g, while the total phenolic content (TPS) was 15,77 mg GAE/g. According to the investigations, the content of phenolic and flavonoid chemicals in *S. othonnae* is low. *T. farfara* showed modest levels of flavonoid and phenolic compounds. Compared to the methanol extract, the ethanol extract produced somewhat higher TPS values of 35,74 mg GAE/g and TFS values of 43,45 mg RE/g.

The TPS and TFS concentrations in the plants analyzed in the research varied significantly. The plant with the greatest concentrations of flavonoid and phenolic compounds was found to be *A. absinthium*. The greatest chemical concentration among the plants was found in the methanol extract, which produced 93,14 mg GAE/g

TPS and 131,32 mg RE/g TFS. strong findings from the ethanol extract demonstrated the plant's strong phenolic and flavonoid content. With noticeably greater TPS and TFS values in the ethanol extract, *A. cotula* is classified in the high group. The phenolic content in *T. parthenium* is moderate to high. In comparison to the ethanol extract, the methanol extract showed higher concentrations of phenolic and flavonoid compounds. The values of *A. falcata* and *T. farfara* were comparable. On the other hand, the lowest levels of total phenolics and flavonoids were found in *S. kotschyi* and *S. othonnae*. Particularly in the methanol extract, *S. othonnae* showed modest quantities of phenolic compounds, with TPS measuring 15,77 mg GAE/g and TFS measuring 20,84 mg RE/g. TPS and TFS levels were also noticeably low in *S. kotschyi*. Phenolic and flavonoid concentrations were higher in *A. absinthium*, *A. cotula*, and *T. parthenium*, while they were intermediate in *A. falcata* and *T. farfara*. Methanol gave better extraction results for *A. absinthium* and T. *parthenium*, while ethanol produced better extraction results for *A. cotula*, *A. falcata*, and *T. farfara*. *S. kotschyi* and *S. othonnae* showed the lowest amounts of phenolic and flavonoid compounds.

4.4 AChE and BChE Inhibitions

The assays demonstrated the inhibition of AChE and BChE in plant samples by ethanol and methanol, as detailed in Figure 4.5. With low IC50 values, *A. absinthium* is among the most effective plants we examined in our research. With IC50 values of 5,88 µg/mL for AChE and 78,76 µg/mL for BChE, the ethanol extract demonstrated considerable inhibition, but the methanol extract demonstrated effectiveness with IC50 values of 95,40 µg/mL for AChE and 71,96 µg/mL for BChE. Compared to *A. falcata*, *T. farfara*, *A. cotula*, and *T. parthenium*, *A. absinthium* showed a more potent inhibitory profile. Suppression of AChE and BChE was less successful in species such as *S. othonnae* and *S. kotschyi*. Our study's reference, galantamine, showed an AChE value of 7,37 and a BChE value of 15,49. These numbers served as a benchmark for evaluating the plant extracts' capacity to inhibit enzymes in the research. Galantamine has noticeably more inhibitory efficacy than any of the plant extracts included in the research, according to an analysis of its IC50 values. Despite having the lowest IC50 values, *A. absinthium*'s values are noticeably higher than those associated with galantamine's activity.

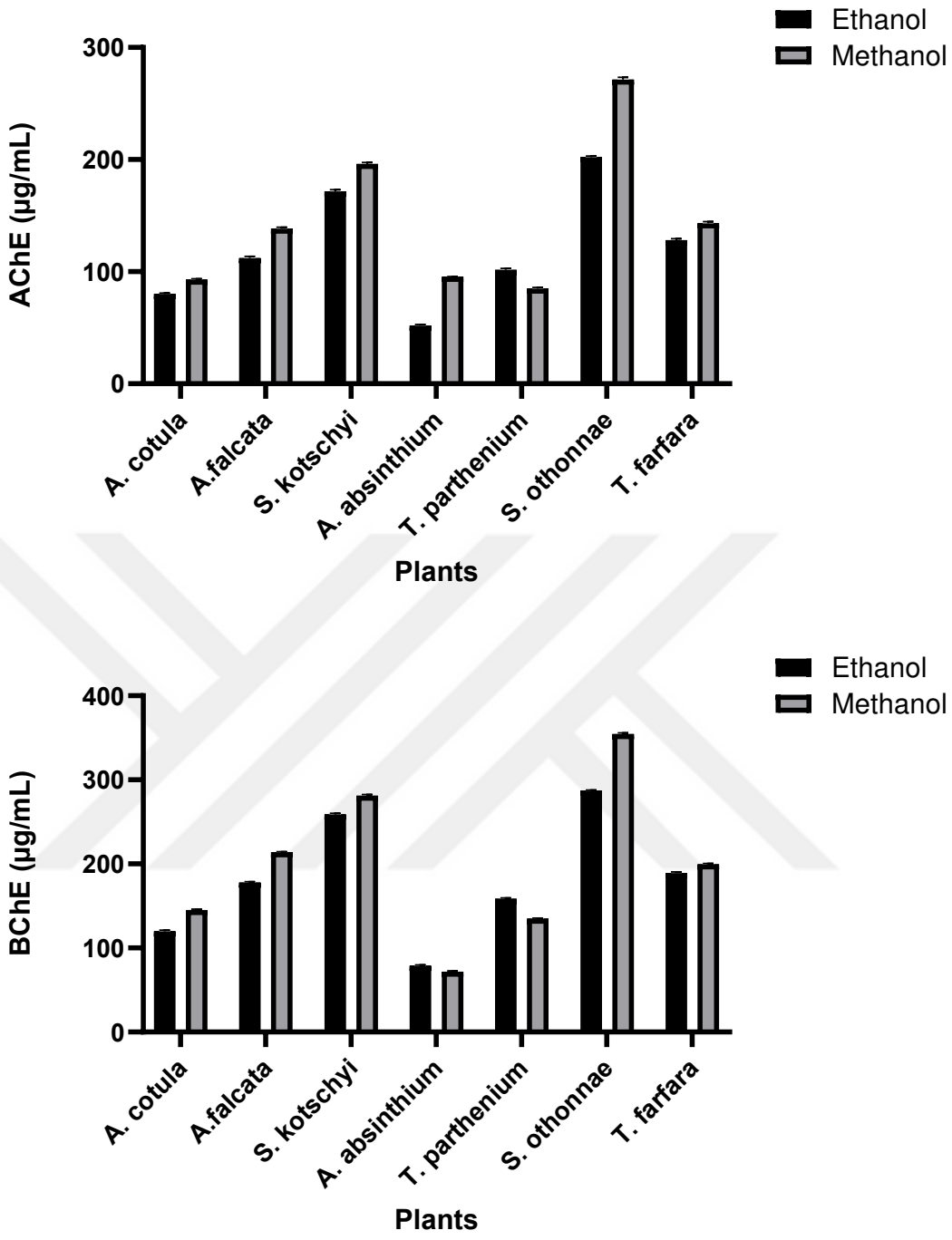

Figure 4.5 AChE and BChE inhibitions

In a similar vein, taxa with much higher IC50 values than galantamine include *A. cotula*, *T. parthenium*, *A. falcata*, and *T. farfara*. This indicates that galantamine is a synthetic standard that is more efficient in inhibiting enzymes. For the ethanol extract of *A. cotula*, the IC50 values for AChE and BChE were 80,23 µg/mL and 119,73 µg/mL, respectively; however, with the methanol extract, these values increased to 92,80 µg/mL for AChE and 145,18 µg/mL for BChE. The ethanol extract of *A.*

absinthium showed lower IC50 values than those of *A. cotula*. Compared to *A. cotula*, the ethanol extract of *T. parthenium* showed a somewhat higher level of AChE inhibition. However, compared to *A. cotula*, *S. kotschyi* and *S. othonnae* had less impact on both enzymes. With IC50 values of 101,71 µg/mL for AChE and 158,65 µg/mL for BChE, the ethanol extract of *T. parthenium* demonstrated efficient inhibition; however, the methanol extract shown higher effectiveness, with IC50 values of 84,82 µg/mL for AChE and 134,91 µg/mL for BChE. According to the results, *T. parthenium* is more effective than *A. falcata* and *A. cotula* but less effective than *A. absinthium*. In methanol extract, *T. farfara* demonstrated reduced activity with IC50 values of 143,16 µg/mL for AChE and 199,43 µg/mL for BChE, while demonstrating inhibitory effects with IC50 values of 128,22 µg/mL for AChE and 189,05 µg/mL for BChE. In comparison to *A. absinthium*, *A. cotula*, *T. parthenium*, and *A. falcata*, *T. farfara* has shown lower effectiveness. In comparison to *T. farfara*, *S. kotschyi* and *S. othonnae* shown decreased inhibitory efficacy. With IC50 values of 112,20 µg/mL for AChE and 177,99 µg/mL for BChE, the ethanol extract of *A. falcata* exhibited inhibitory effects; on the other hand, the methanol extract showed higher IC50 values of 138,27 µg/mL for AChE and 213,79 µg/mL for BChE. Compared to *A. absinthium*, *A. falcata* exhibited reduced activity. *T. parthenium* methanol extract was more effective than *A. falcata* at suppressing AChE. In ethanol solution, *A. cotula* showed a more notable impact. It was shown that *A. falcata* had a stronger impact on both enzymes than *S. kotschyi*.

With IC50 values of 171,57 µg/mL for AChE and 258,91 µg/mL for BChE in ethanol extract, *S. kotschyi* demonstrated inhibitory actions; however, in methanol extract, the IC50 values increased to 196,07 µg/mL for AChE and 281,28 µg/mL for BChE. In comparison to *S. othonnae*, *S. kotschyi* was marginally more effective. But compared to species like *A. absinthium*, *A. cotula*, and *T. parthenium*, it showed noticeably less activity. In its ethanol extract, *S. othonnae* has low activity, with IC50 values of 202,10 µg/mL for AChE and 287,24 µg/mL for BChE. For AChE and BChE, the methanol extract showed elevated IC50 values of 271,46 µg/mL and 354,45 µg/mL, respectively. This plant was less efficient and showed noticeably less suppression of both enzymes. In both solutions, *S. othonnae* had one of the highest IC50 values. This plant showed the lowest inhibitory capability and much lower effectiveness when compared to the other plants.

4.5 Antiproliferative Activity

The antiproliferative activities of ethanol and methanol extracts obtained from plant samples were determined against the lung cancer cell line A549 at concentrations of 25, 50, 100, and 150 µg/mL.

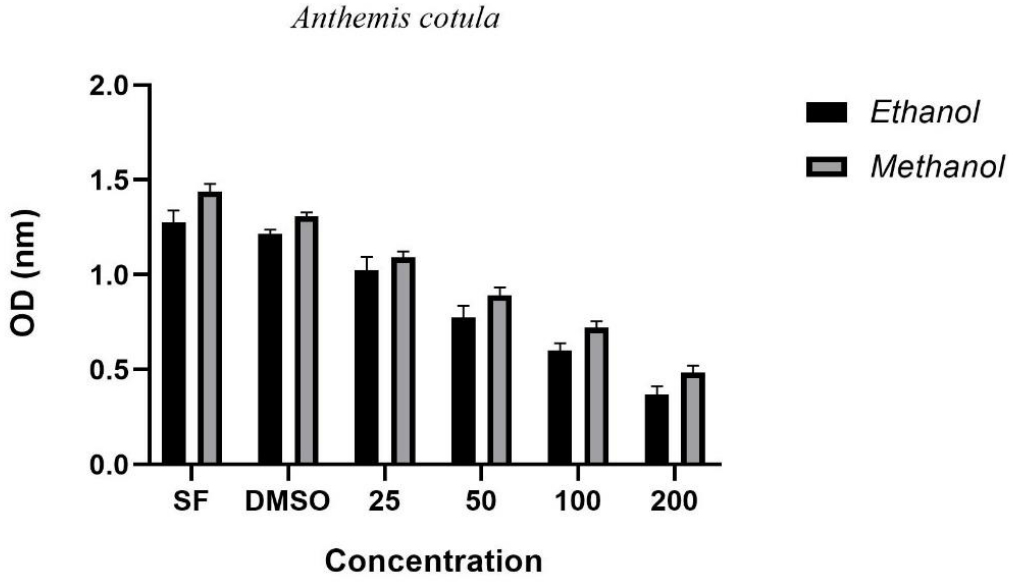

Figure 4.6 Antiproliferative activity of *A .cotula*

Figure 4.6 depicts the antiproliferative effects of ethanol and methanol extracts of *A. cotula* on the A549 lung cancer cell line. This study investigated the effects of extracts administered at varying concentrations (25, 50, 100, and 200 µg/mL) on cellular viability. Ethanol extracts exhibited a more significant antiproliferative impact than methanol extracts. Ethanol extracts, at a concentration of 200 µg/mL, markedly diminished cell viability. Methanol extracts diminished cell viability with rising concentration, albeit to a smaller degree than ethanol extracts. In both excerpts, cell viability diminished with rising concentration. This indicates a dose-dependent relationship. A greater concentration of the extracts correlates with a more pronounced effect in suppressing cancer cell proliferation.

Figure 4.7 depicts the antiproliferative effects of ethanol and methanol extracts of *A. falcata* on the A549 lung cancer cell line. We examined the effect of extracts administered at varying concentrations (25, 50, 100, and 200 µg/mL) on cellular viability. Ethanol extracts exhibited a more significant antiproliferative impact than

methanol extracts. Ethanol extracts, especially at elevated concentrations (200 µg/mL), markedly diminished cell viability. Methanol extracts diminished cell viability with increasing concentration, albeit to a smaller degree than ethanol extracts. Moreover, cell viability diminished with escalating quantities of both extracts, indicating a dose-dependent relationship. An increased concentration of the extracts leads to a more pronounced effect on suppressing cancer cell proliferation.

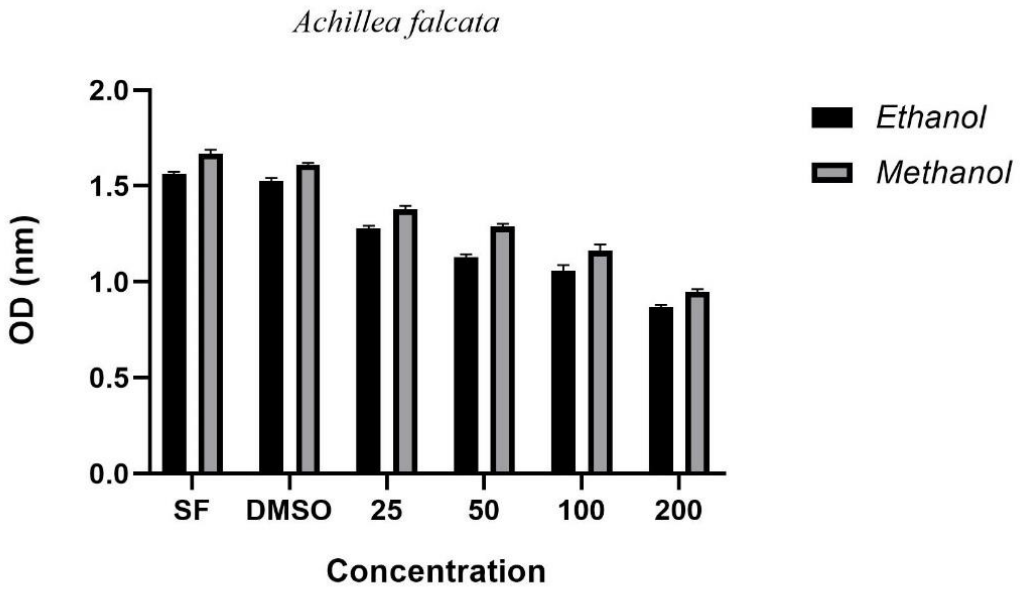

Figure 4.7 Antiproliferative activity of *A. falcata*

The antiproliferative effects of the ethanol and methanol extracts of *S. kotschyi* on the A549 lung cancer cell line are illustrated in Figure 4.8. Our study investigated the effects of extracts administered at varying concentrations (25, 50, 100, and 200 µg/mL) on cell viability. Ethanol extracts demonstrated a more significant antiproliferative impact than methanol extracts. At elevated doses (50, 100, and 200 µg/mL), ethanol extracts markedly diminished cell viability. Methanol extracts diminished cell viability with rising concentration, albeit to a smaller degree than ethanol extracts. Moreover, cell viability diminished with escalating concentrations of both extracts, indicating a dose-dependent relationship. The greater the concentration of the extracts, the more pronounced their effect in suppressing cancer cell proliferation.

Scorzonera kotschyi

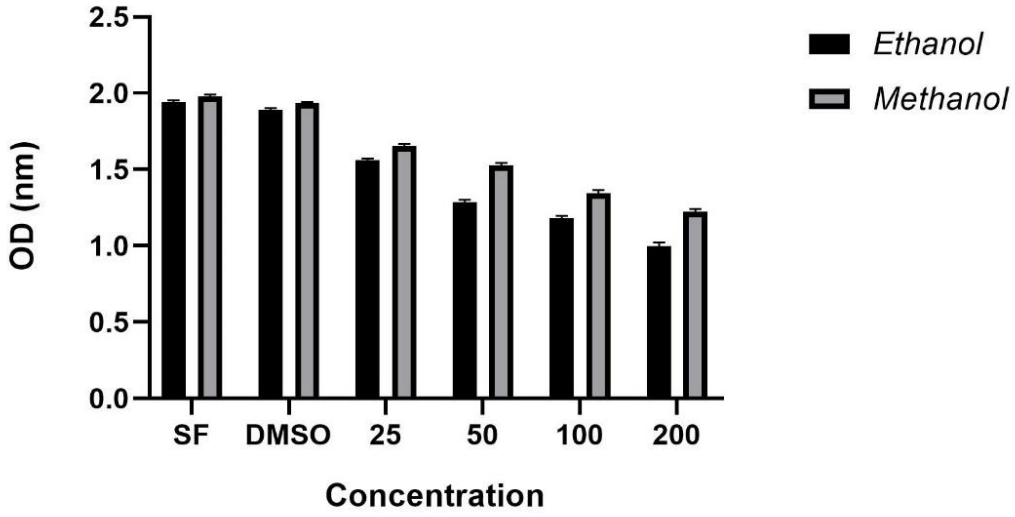

Figure 4.8 Antiproliferative activity of *S. kotschyi*

Artemisia absinthium

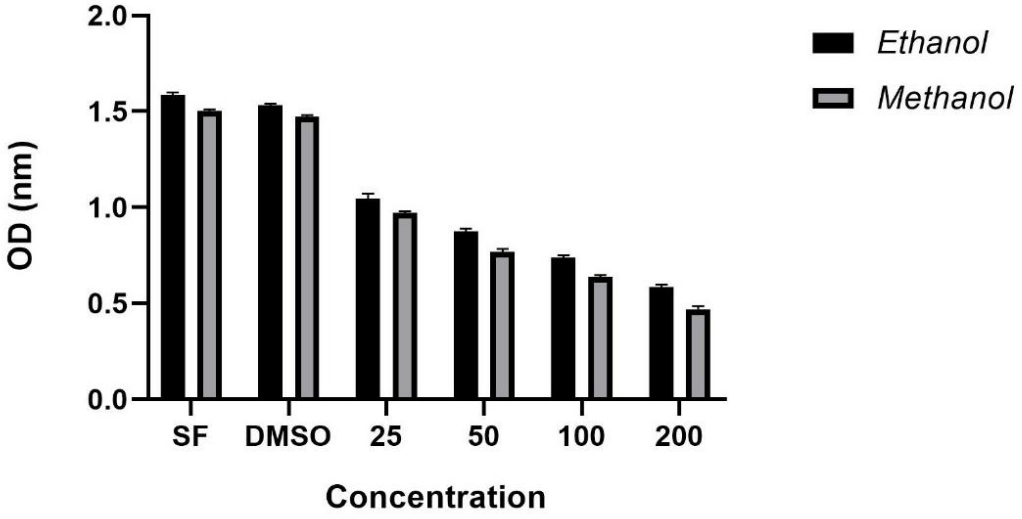

Figure 4.9 Antiproliferative activity of *A. absinthium*

The antiproliferative effects of the ethanol and methanol extracts of *A. absinthium* on the A549 lung cancer cell line are illustrated in Figure 4.9. Our study investigated the effects of extracts administered at varying concentrations (25, 50, 100, and 200 µg/mL) on cell viability. Methanol extracts demonstrated a more significant antiproliferative

impact than ethanol extracts. At elevated doses (100 and 200 µg/mL), methanol extracts markedly diminished cell viability. Ethanol extracts diminished cell viability with increasing concentration, albeit to a lesser degree than methanol extracts. Methanol extracts have efficacy at elevated doses (200 µg/mL), however this impact is more gradual or less prominent compared to that of methanol extracts. In both extracts, cell viability diminished with increasing concentration. This indicates a dose-dependent relationship. A larger concentration of the extracts correlates with an enhanced effect in inhibiting cancer cell proliferation.

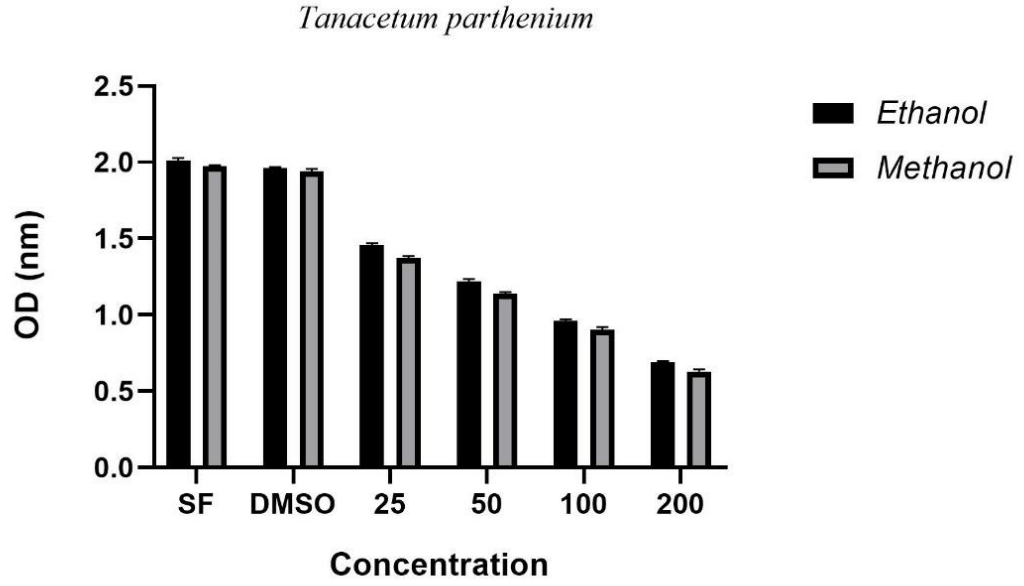

Figure 4.10 Antiproliferative activity of *T. parthenium*

The antiproliferative effects of the ethanol and methanol extracts of *T. parthenium* on the A549 lung cancer cell line are illustrated in Figure 4.10. Our study investigated the effects of extracts administered at varying concentrations (25, 50, 100, and 200 µg/mL) on cell viability. Methanol extracts demonstrated a more significant antiproliferative impact than ethanol extracts. At elevated doses (200 µg/mL), methanol extracts markedly diminished cell viability. Ethanol extracts diminished cell viability with increasing concentration, albeit to a lesser degree than methanol extracts. Moreover, cell viability diminished with escalating concentrations of both extracts, indicating a dose-dependent relationship. The greater the concentration of the extracts, the more pronounced their effect in suppressing cancer cell proliferation.

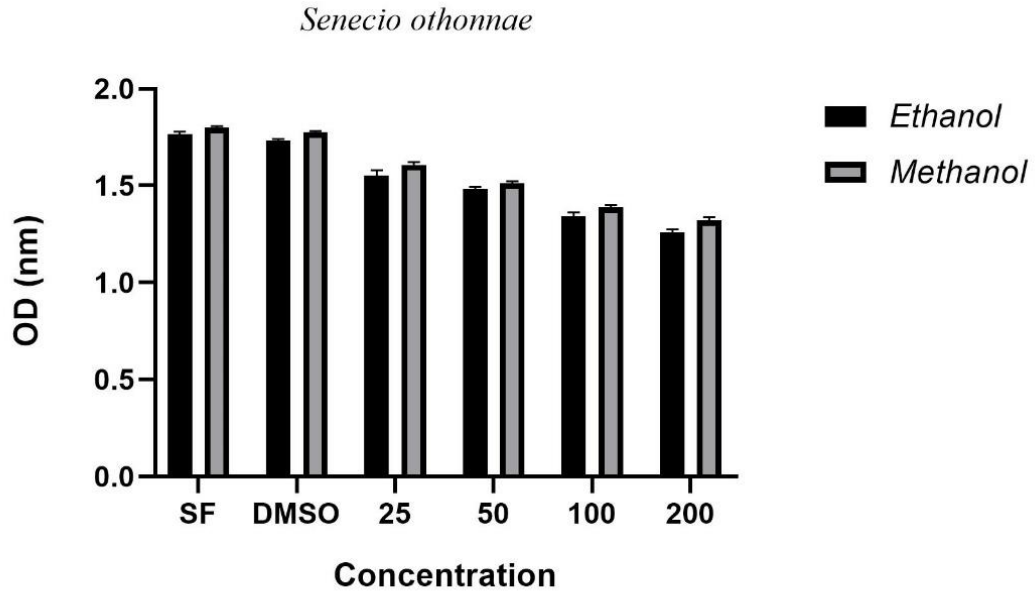

Figure 4.11 Antiproliferative activity of *S. othonnae*

The antiproliferative effects of the ethanol and methanol extracts of *S. othonnae* on the A549 lung cancer cell line are illustrated in Figure 4.11. Our study investigated the effects of extracts administered at varying concentrations (25, 50, 100, and 200 µg/mL) on cell viability. Ethanol extracts demonstrated a more significant antiproliferative impact than methanol extracts. Ethanol extracts were observed to somewhat diminish cell viability, particularly at elevated doses. Methanol extracts diminished cell viability with escalating concentration, albeit the effect was less significant compared to ethanol extracts. Moreover, cell viability diminished with escalating concentrations of both extracts. Nonetheless, both extracts demonstrated minimal efficacy.

The antiproliferative (cell proliferation-inhibiting) effects of the ethanol and methanol extracts of *T. farfara* used in our study on the A549 lung cancer cell line are shown in Figure 4.12. The effects of extracts applied at different concentrations (25, 50, 100, and 200 µg/mL) on cell viability were compared in our study. Ethanol extracts exhibited a more pronounced antiproliferative effect compared to methanol extracts. Ethanol extracts, especially at higher concentrations (200 µg/mL), were observed to significantly reduce cell viability. Methanol extracts also reduced cell viability with increasing concentration, but to a lesser extent than ethanol extracts. Furthermore, cell viability decreased with increasing concentration for both extracts, suggesting a dose-

dependent effect. That is, the higher the concentration of the extracts, the greater the effect of inhibiting the proliferation of cancer cells.

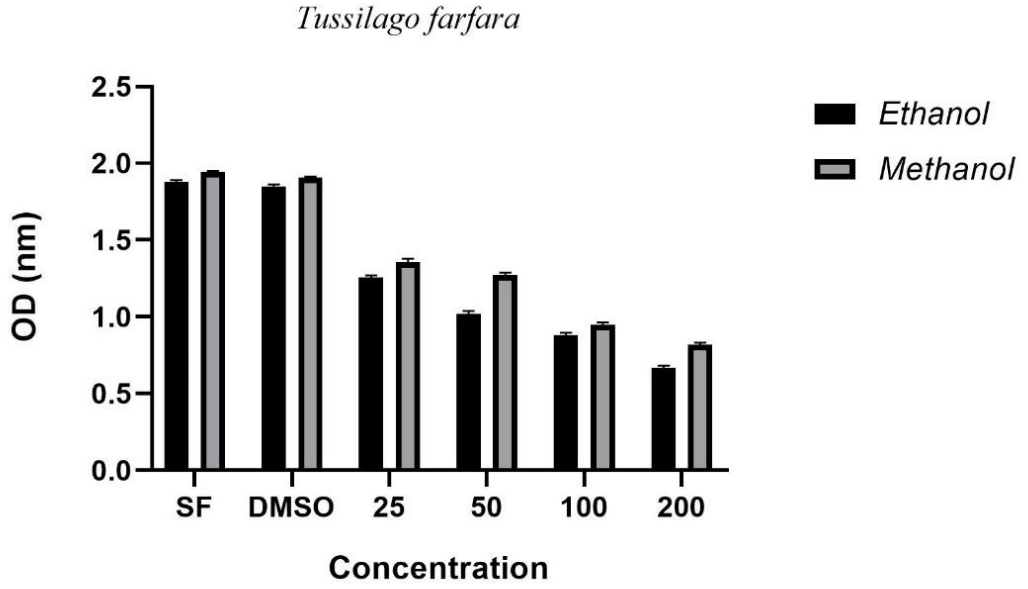

Figure 4.12 Antiproliferative activity of *T. farfara*

4.6 Chemical Compositions

The chemical compositions of the plant samples used in this study were determined using a Gas Chromatography-Mass Spectrophotometer (GC-MS) device. The mass spectra of the identified compounds were scanned against the NIST library. The findings are listed below.

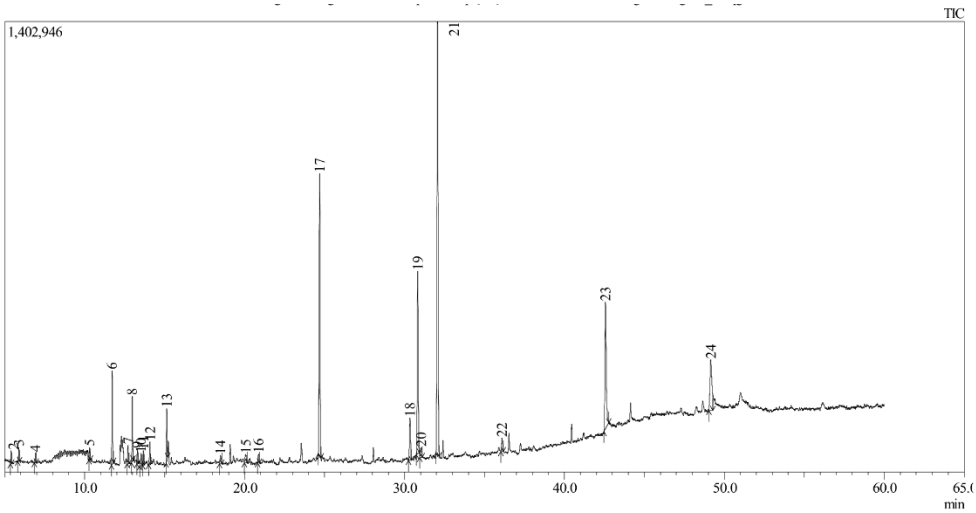

Figure 4.13 GC-MS chromatogram of *A. cotula*

Table 4.1 GC-MS results of *A. cotula*

No	Chemical compound	Retention time	Composition (%)
1	α-Pinene	3,522	0,61
2	β-Myrcene	5,391	0,51
3	D-Limonene	5,877	0,48
4	o-Cymene	6,918	0,43
5	α-Copaene	10,301	0,47
6	Caryophyllene	11,717	3,65
7	α-Humulene	12,708	0,83
8	α-Terpınenyl acetate	12,974	2,66
9	Germacrene-D	13,292	0,68
10	α-Muurolene	13,523	0,85
11	Bicyclogermacrene	13,675	0,59
12	delta-Cadinene	14,073	1,15
13	Anethole	15,135	2,27
14	Caryophyllene oxide	18,497	0,53
15	Caryophyllenyl alcohol	20,064	0,75
16	Epiglobulol	20,876	0,55
17	Palmitic acid, methyl ester	24,691	17,78
18	Methyl stearate	30,348	3,09
19	Oleic acid, methyl ester	30,828	12,35
20	Linoleic acid, methyl ester	32,067	30,36
21	1-Naphthalenepropanol, α-ethenyldecahydro-α,5,5,8a-tetramethyl-2-methylene-	36,087	1,02
22	n-Hexadecanoic acid	42,571	11,14
23	9-octadecenoic acid	49,148	6,61
Total			**100**

According to the analysis results, the compound detected at the highest rate was linoleic acid, methyl ester at 30,36%. This was followed by palmitic acid, methyl ester at 17,78%, oleic acid, methyl ester at 12,35%, and n-hexadecanoic acid at 11,14%. The total rate of these four compounds exceeded 70% and they were the dominant components in the chemical profile of the extract. Other compounds were found at lower rates, particularly those below 1%, and were considered minor compounds.

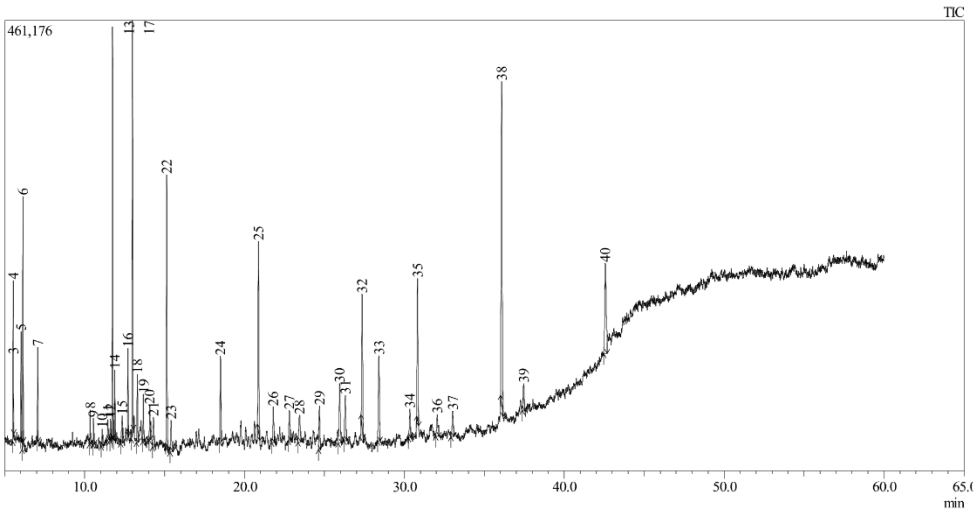

Figure 4.14 GC-MS chromatogram of *A. falcata*

Table 4.2 GC-MS results of *A. falcata*

No	Chemical compound	Retention time	Composition (%)
1	α-Pinene	3,624	8,29
2	Camphene	4,160	3,04
3	β-Pinene	4,712	1,16
4	β-Myrcene	5,526	2,01
5	D-Limonene	6.018	1,49
6	Eucalyptol (1,8-Cıneole)	6,141	3,57
7	o-Cymene	7,064	1,36
8	α-Copaene	10,351	0,57
9	Linalyl acetate	11,093	0,31

No	Chemical compound	Retention time	Composition (%)
10	Acetic acid, bornyl ester	11,450	0,45
11	β-Elemene	11,623	0,36
12	Caryophyllene	11,748	6,85
13	(+)-Aromadendrene	11,889	1,33
14	3-Cyclohexene-1-methanol, α,4-trimethyl-, 1-acetate	12,344	0,78
15	α-Humulene	12,712	1,27
16	α-Terpinyl acetate	12,992	7,33
17	Germacrene-D	13,292	1,58
18	Bicyclogermacrene	13,677	0,97
19	Δ-Cadinene	14,073	0,81
20	Propanal, 2-methyl-3-phenyl-	14,309	0,88
21	Anethole	15,136	6,44
22	Naphthalene	15,406	1,07
23	Caryophyllene oxide	18,498	2,35
24	Veridiflorol	20,872	5,30
25	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-	21,801	1,17
26	3-Allylguaiacol	22,807	0,89
27	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	23,452	1,28
28	Palmitic acid, methyl ester	24,683	1,42
29	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	26,301	1,61
30	3-Methyl-5-(2,6,6-Trımethyl-1-Cyclohexen-1-Yl)-1-Pentyn-3-O	27,357	4,30
31	Methyl stearate	30,346	0,83
32	Oleic acid, methyl ester	30,822	4,23
33	Linoleic acid, methyl ester	32,051	0,82

No	Chemical compound	Retention time	Composition (%)
34	Eugenyl isovalerate	33,029	0,96
35	Epimanool	36,085	11,67
36	1,4-Dimethyl-8-isopropylidenetricyclo[5.3.0.0(4,10)]decane	37,460	0,97
37	n-Hexadecanoic acid	42,574	4,59
Total			**100**

As a result of GC-MS analysis, the compound detected at the highest rate in the sample was epimanool with 11,67%. This was followed by α-pinene with 8,29%, α-terpinyl acetate with 7,33% and caryophyllene with 6,85%. Anethole with 6,44% and veridiflorol with 5,30% were also among the notable compounds. Other compounds detected above 4% included 3-methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1-pentyn-3-O (4,30%), oleic acid, methyl ester (4,23%) and n-Hexadecanoic acid (4,59%), while the remaining compounds were represented at lower rates (3% and below). These results show that sesquiterpene and monoterpene derivatives are particularly dominant in the chemical structure of the extract.

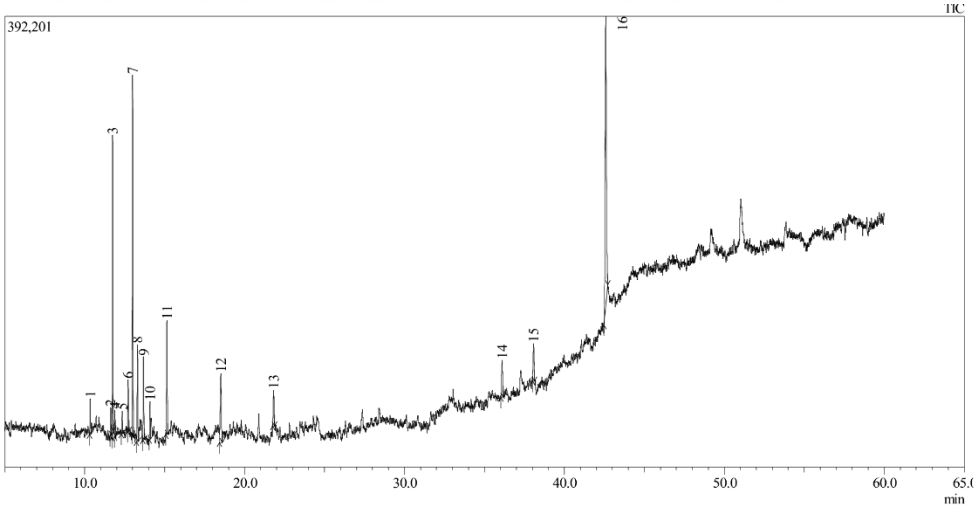

Figure 4.15 GC-MS chromatogram of *S.kotschyi*

Table 4.3 GC-MS results of *S. kotschyi*

No	Chemical compound	Retention time	Composition (%)
1	Copaene	10,349	1,79
2	β- Elemene	11,625	1,00
3	Caryophyllene	11,751	11,92
4	(+)-Aromadendrene	11,889	1,19
5	p-Menthen-8-yl acetate	12,345	1,18
6	α-Humulene	12,718	2,41
7	α-Terpinenyl acetate	12,997	16,57
8	Germacrene-D	13,299	5,46
9	Bicyclogermacrene	13,678	4,32
10	Δ-Cadinene	14,077	2,17
11	Anethole	15,144	6,56
12	Caryophyllene oxide	18,511	5,99
13	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-	21,818	2,44
14	Epimanool	36,107	3,29
15	Tetracosane	38,073	3,27
16	n-Hexadecanoic acid	42,584	30,43
Total			**100**

The compound detected at the highest rate as a result of the analysis was n-hexadecanoic acid (30,43%). This compound was followed by α-terpinenyl acetate (16,57%) and caryophyllene (11,92%). Other significant compounds such as anethole (6,56%), caryophyllene oxide (5,99%), and germacrene-d (5,46%) were also present at significant levels in the sample. Bicyclogermacrene (4,32%), epimanool (3,29%), and tetracosane (3,27%) were moderately represented at over 3%. Other compounds were in the 1-2% range and were at minor levels in the chemical profile. These data indicate that the sample is particularly rich in saturated fatty acids and sesquiterpenes.

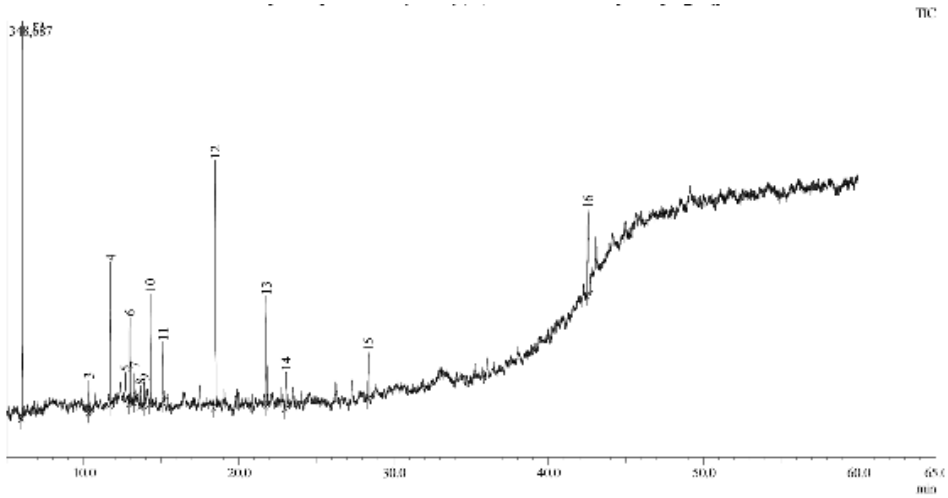

Figure 4.16 GC-MS chromatogram of *A.absinthium*

Table 4.4 GC-MS results of *A. absinthium*

No	Chemical compound	Retention time	Composition (%)
1	α-Pinene	3,620	0,85
2	D-Limonene	6,011	16,05
3	α-Copaene	10,336	1,83
4	Caryophyllene	11,738	7,18
5	α-Humulene	12,697	1,76
6	1-p-Menthen-8-yl acetate	12,983	5,02
7	Germacrene-D	13,289	1,94
8	Elixene	13,661	1,07
9	Geranyl acetate	13,898	1,15
10	α-Curcumene	14,299	6,55
11	Anethole	15,125	3,70
12	Caryophyllene oxide	18,486	20,82
13	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-	21,790	10,79
14	δ-Cadinene	23,057	3,46

No	Chemical compound	Retention time	Composition (%)
15	3-Methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-pent-1-yn-3-ol	28,380	5,22
16	L-(+)-Ascorbic acid 2,6-dihexadecanoate	42,569	12,60
Total			**100**

According to GC-MS analysis, the compound detected at the highest rate in the sample was caryophyllene oxide with 20,82%. This is followed by D-limonene with 16,05%, L-(+)-ascorbic acid 2,6-dihexadecanoate with 12,60% and 1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)] with 10,79%. Other notable compounds are caryophyllene with 7,18%, α-curcumene with 6,55%. 1-p-Menthen-8-yl acetate and 3-methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-pent-1-yn-3-ol, which are present at a moderate level, are present around 5%. Other compounds were below 4% and were included as minor components in the chemical profile. The results show that the sample is particularly rich in oxidized sesquiterpenes and monoterpene esters.

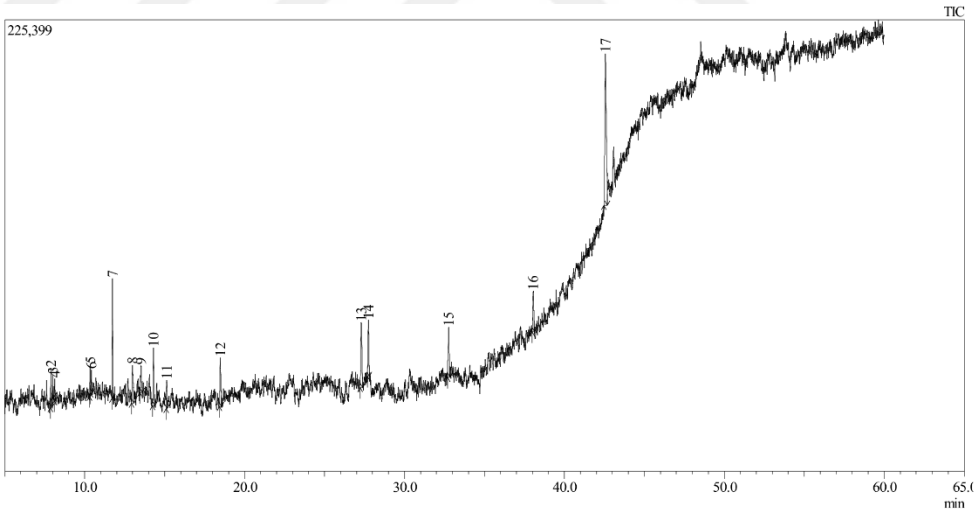

Figure 4.17 GC-MS chromatogram of *T. parthenium*

Table 4.5 GC-MS results of *T. parthenium*

No	Chemical compound	Retention time	Composition (%)
1	2,4-Dimethyl-1-heptene	2,341	2,08
2	2-Isopropyl-5-methyl-1-heptanol	7,885	2,78

No	Chemical compound	Retention time	Composition (%)
3	11-Methyldodecanol	8,006	1,00
4	α-Copaene	10,348	2,39
5	Hexadecane	10,419	1,14
6	Caryophyllene	11,739	7,60
7	3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate	12,984	5,61
8	α-Muurolene	13,509	3,68
9	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	14,300	4,98
10	Benzene, 1-methoxy-4-(1-propenyl)-	15,135	2,96
11	Caryophyllene oxide	18,479	5,72
12	Eicosane	27,298	10,04
13	9-Tricosene, (Z)-	27,740	6,65
14	Tetracosane	32,767	6,97
15	n-Hexadecanoic acid	42,566	29,75
Total			**100**

In GC-MS analysis, the compound detected at the highest rate was n-Hexadecanoic acid with 29,75%. This was followed by eicosane with 10,04%, caryophyllene with 7,60%, tetracosane with 6,97% and 9-tricosene (Z)- with 6,65%. In addition, sesquiterpene and ester derivatives such as caryophyllene oxide with 5,72% and 3-cyclohexene-1-methanol, α, α, 4-trimethyl-, acetate with 5,61% also had an important place in the sample. Compounds found at moderate levels (between 2-5%) included α-Muurolene, Benzene derivatives and 2-Isopropyl-5-methyl-1-heptanol, while other compounds were detected at lower rates (below 2%). These results indicate that the sample has a complex chemical structure rich in saturated fatty acids, long-chain alkanes and terpene derivatives.

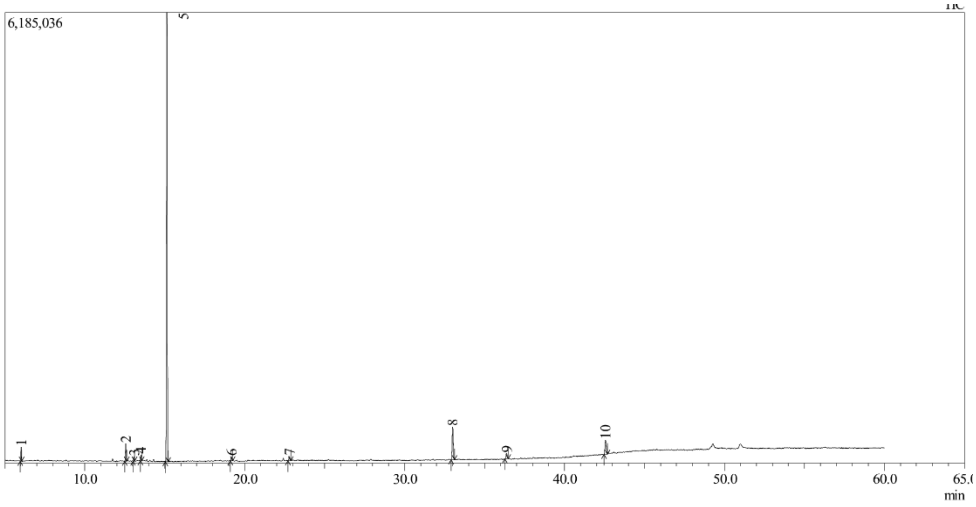

Figure 4.18 GC-MS chromatogram of *S.othonnae*

Table 4.6 GC-MS results of *S. othonnae*

No	Chemical compound	Retention time	Composition (%)
1	D-Limonene	6,016	1,42
2	Estragole	12,563	2,08
3	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	13,075	0,58
4	Esdragole	15,138	78,67
5	Benzaldehyde, 4-Methoxy-	19,177	0,83
6	Phenol, 2-methoxy-3-(2-propenyl)-	22,791	1,00
7	Butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester	33,004	8,36
8	2-(1',2'-epoxypropyl)-4-methoxyphenyl 2-methylbutanoate	36,377	1,62
9	n-Hexadecanoic acid	42,563	4,51
Total			**100**

In GC-MS analysis, the most dominant compound in the sample was determined as esdragole with 78,67%. This compound is clearly dominant in the chemical profile, accounting for the majority of the total composition. This is followed by butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester with 8,36% and n-hexadecanoic acid with 4,51%. Compounds represented at moderate levels (between 1-2%) include estragole, 2-(1',2'-epoxypropyl)-4-methoxyphenyl 2-methylbutanoate, phenol, 2-

methoxy-3-(2-propenyl)- and D-limonene. Other compounds were detected at low levels. The results show that the sample is rich in phenylpropene compounds and that an aromatic compound, especially an anethole derivative, clearly dominates the chemical structure.

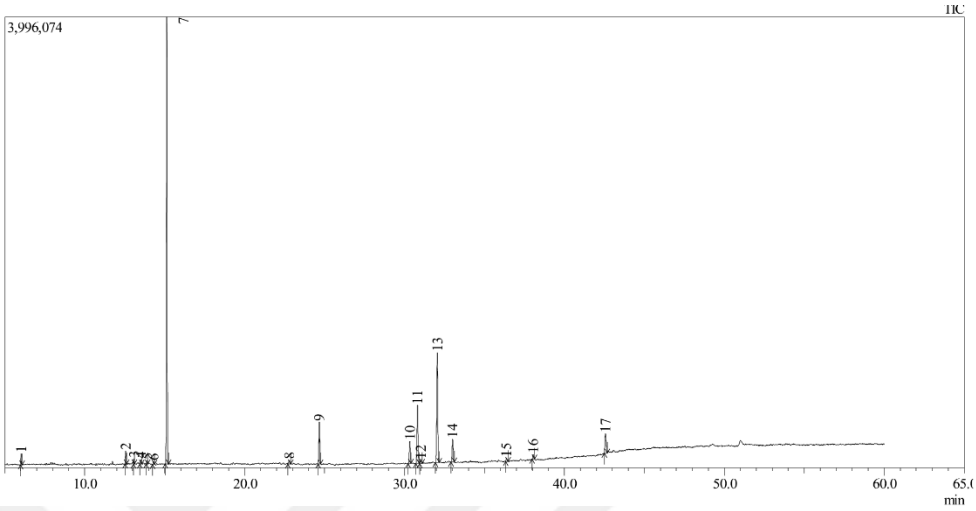

Figure 4.19 GC-MS chromatogram of *T.farfara*

Table 4.7 GC-MS results of *T. farfara*

No	Chemical compound	Retention time	Composition (%)
1	D-Limonene	6,018	0,72
2	Estragole	12,565	1,06
3	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	13,076	0,47
4	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (S)-	13,509	0,51
5	Pentafluoropropionic acid, pentadecyl ester	13,899	0,37
6	Benzaldehyde, 4-(1-methylethyl)-	14,303	0,33
7	Anethole	15,136	49,68
8	Eugenol	22,801	0,68
9	Hexadecanoic acid, methyl ester	24,673	6,19
10	Methyl stearate	30,335	3,65
11	9-Octadecenoic acid (Z)-, methyl ester	30,812	9,27

No	Chemical compound	Retention time	Composition (%)
12	9,12-Octadecadienoic acid, methyl ester, (E,E)-	32,051	17,13
13	Butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester	33,006	3,85
14	2-(1',2'-epoxypropyl)-4-methoxyphenyl 2-methylbutanoate	36,374	0,60
15	Tetracosane	38,047	0,70
16	n-Hexadecanoic acid	42,572	4,21
Total			**100**

According to the analysis results, the compound detected at the highest rate in the sample was anethole with 49,68%. This compound is followed by 9,12-octadecadienoic acid, methyl ester (E,E) with 17,13% and 9-octadecenoic acid (Z)-, methyl ester with 9,27%, respectively. Hexadecanoic acid, methyl ester with 6.19% and n-hexadecanoic acid with 4,21% represent the fatty acid group. Other compounds found at moderate levels include butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester with 3,85% and methyl stearate with 3,65%. The remaining compounds were detected at lower rates (less than 1%). These results reveal that the chemical profile of the sample includes particularly phenylpropene compounds such as anethole and methyl ester derivatives at significant levels.

4.7 Phenolic Contents

Phenolic compounds in the plant samples used in the study were determined using LC-MS/MS. The obtained data are shown in Table 4.8.

Table 4.8 Phenolic compounds of plants (µg/kg)

Compound	1	2	3	4	5	6	7
Alizarin	3326,70	-----	-----	-----	-----	-----	-----
Thymoquinone	-----	-----	-----	-----	-----	-----	-----
Vanilic Acid	1970,28	-----	-----	-----	-----	-----	-----
Acetohydroxamic Acid	775,31	-----	6199,97	1517,19	13406,36	5434,05	10083,04
Catechinhyrate	-----	-----	-----	-----	-----	-----	8994,91
Syringic Acid	-----	6149,75	2177,47	4743,43	-----	1510,78	-----
Resveratrol	2880,89	13477,99	4789,9	18681,78	2949,64	2407,71	929,81
Kaempferol	528,78	52196,05	79366,01	4861,61	22163,63	84241,77	6668,31
Ellagic acid	-----	-----	-----	-----	-----	-----	431477,01

Silymarin	-----	-----	-----	724,07	-----	-----	-----
Chlorogenic Acid	-----	217522,42	898,99	3154,75	459,84	306779,29	1130,48
Fumaric Acid	29231,31	74058,94	18246,41	20142,55	34850,11	21918,92	10693,58
Gallic Acid	-----	-----	-----	-----	-----	-----	65235.21
Protocatechuik Acid	3732,50	6947,11	11752,04	22426,86	3781,55	16065,54	-----
Caffeic Acid	1818,66	124257,24	4668,84	6811,59	-----	9956,21	-----
Oleuropein	-----	-----	-----	-----	-----	-----	-----
Phloridzindyhrate	-----	-----	2802,94	462,39	2473,34	862,24	2581.08
Myricetin	-----	-----	6260,99	-----	11260.69	-----	1318.36
Hydoxycinamic Acid	7910,29	11461,24	53909,11	11429,71	33258,60	19927,30	10583,41
Naringenin	-----	281,68	277,37	1256,10	-----	414,02	415,30
Quercetin	10650,14	1252,85	11798,70	1857,53	15321,20	3379,99	960,97
hyroxy1,4 naphthaquinone	-----	-----	-----	-----	-----	-----	-----
Luteolin	111,13	13304,76	26598,50	5798,97	-----	86469,19	1143,73
Salisilik Acid	546,36	10104,16	5722,87	20364,10	25962,96	16697,90	-----
4-Hydroxybenzoic Acid	16841,78	5480,43	11196,42	3031,67	2889,22	36290,65	5684,25
Curmin	-----	-----	-----	-----	-----	-----	-----

1: *Anthemis cotula*, 2: *Achillea falcata*, 3: *Scorzonera kotschyi*, 4: *Artemisia absinthium*, 5: *Tanacetum parthenium*, 6: *Senecio othonnae*, 7: *Tussilago farfara*

When the phenolic compound analysis results are evaluated, it is clearly evident that each of the studied plant species has unique chemical profiles. Despite its limited phenolic compound content, *A. cotula* stands out with its high levels of fumaric acid (29231,31 µg/kg), hydroxycinnamic acid (7910,29 µg/kg), and quercetin (10650,14 µg/kg). Additionally, compounds with high antioxidant potential such as Vanillic acid, alizarin, and resveratrol are also present. However, the failure to detect many compounds in this species suggests a simpler phenolic structure profile.

A. falcata is particularly rich in phenolic compounds, including chlorogenic acid (217522,42 µg/kg), caffeic acid (124257,24 µg/kg), fumaric acid (74058,94 µg/kg), and kaempferol (52196,05 µg/kg). Furthermore, the presence of numerous biologically active compounds, such as luteolin, salicylic acid, hydroxycinnamic acid, syringic acid, and protocatechuic acid, indicates that this species has a strong profile in terms of phenolic diversity and density.

S. kotschyi stands out with its particularly high kaempferol (79366,01 µg/kg), hydroxycinnamic acid (53909,11 µg/kg), fumaric acid, resveratrol, and salicylic acid contents. Furthermore, the detection of antioxidant compounds in the polyphenol

structure, such as myricetin and catechinhydrate, supports the biopharmacological potential of this species. The presence of compounds such as quercetin, 4-hydroxybenzoic acid, and protocatechuic acid at certain levels also reveals that this plant possesses a rich phenolic structure, both in terms of quantity and composition.

A. absinthium contains very high levels of resveratrol (18681,78 µg/kg) and salicylic acid (20364,10 µg/kg). The presence of compounds such as acetohydroxamic acid, hydroxycinnamic acid, 4-hydroxybenzoic acid, syringic acid, and quercetin is also noteworthy. Moderate levels of caffeic acid, phloridzin dihydrate, and kaempferol were also detected in this species.

T. parthenium is particularly notable for its high levels of acetohydroxamic acid (13406,36 µg/kg), quercetin (15321,20 µg/kg), salicylic acid (25962,96 µg/kg), and hydroxycinnamic acid (33258,60 µg/kg). It also contains various phenolic ester and acid compounds, albeit in lower amounts. The presence of components such as phloridzin dihydrate, myricetin, and 4-hydroxybenzoic acid further enhances the chemical diversity of this species.

S. othonnae is particularly notable for its kaempferol (84241,77 µg/kg) and chlorogenic acid (306779,29 µg/kg) contents. Additionally, the high levels of fumaric acid, protocatechuic acid, hydroxycinnamic acid, and salicylic acid indicate that this plant has a very rich phenolic composition.

T. farfara stands out from other species, particularly with its exceptional ellagic acid content (431477,01 µg/kg). High amounts of acetohydroxamic acid, catechinhydrate, gallic acid, hydroxycinnamic acid, quercetin, 4-hydroxybenzoic acid, phloridzin dihydrate, and myricetin were also detected in this species.

CHAPTER 5
DISCUSSION AND CONCLUSION

This study determined the antioxidant, antimicrobial, antiproliferative, anticholineterase activities, phenolic contents, and chemical compositions of ethanol and methanol extracts of *A. cotula, A. falcata, S. kotschyi, A. absinthium, T. parthenium, S. othonnae*, and *T. farfara* collected from Osmaniye province.

5.1 DPPH and FRAP Activities

The variations in antioxidant capabilities of the plants analyzed in this study can be attributed to the diversity of phenolic compounds, flavonoids, and other bioactive constituents present. *A. absinthium* demonstrated the highest antioxidant activity, signifying its abundance of phenolic compounds and its robust defense against free radicals. The methanol extract exhibited the greatest DPPH and FRAP values, indicating that methanol may serve as a more efficacious solvent for the extraction of phenolic chemicals. The literature indicates that the methanol extract of *A. absinthium* possesses the ability to efficiently scavenge superoxide anions, hydrogen peroxide, hydroxyl radicals, and nitric oxide radicals (Bora and Sharma, 2011). Moreover, its ethyl acetate and aqueous extracts have been documented to demonstrate significant antioxidant potential when evaluated using DPPH and FRAP methodologies (Hbika et al., 2022). These results align with the superior antioxidant activity of *A. absinthium* seen in our investigation.

The elevated antioxidant activity of *T. parthenium* and *A. cotula* corroborates their richness in bioactive chemicals. Research indicates that *T. parthenium* extracts demonstrate significant antioxidant properties in multiple in vitro assays, including DPPH free radical scavenging activity and Fe^{2+} chelation capability (Yada et al., 2020). A study on *A. cotula* indicated that essential oils extracted from the leaves and flowers of the plant cultivated in Jordan demonstrated antioxidant activity in DPPH, ABTS, and iron ion chelation (FIC) assays (Al-Momani et al., 2022). Our investigation

confirms that the elevated antioxidant potential of *A. cotula*, particularly its ethanol extract, aligns with existing literature.

The moderate antioxidant activity of *A. falcata* and *T. farfara* indicates that their phenolic content may be comparatively lower or that their phenolic compounds may exhibit reduced efficacy in antioxidant activity. Research indicates that the essential oil of *A. falcata* demonstrates minimal free radical scavenging activity as assessed by the DPPH technique (Younos et al., 2020). This aligns with our study's findings and may explain why *A. falcata* demonstrates less antioxidant capability relative to other plants. The ethyl acetate and n-butanol fractions of *T. farfara* have demonstrated the ability to scavenge free radicals and prevent pyrogallol auto-oxidation (Chang-Tian et al., 2012; Wei et al., 2020). Our investigation revealed that the ethanol extract of *T. farfara* demonstrated superior antioxidant activity, suggesting that various solvents may substantially influence the extraction of bioactive components.

Conversely, the observation that *S. kotschyi* and *S. othonnae* demonstrate the lowest antioxidant activity indicates that these plants possess a restricted potential for free radical scavenging. The literature indicates that methanol extracts from the aerial and root components of *S. kotschyi* exhibit antioxidant activity using various methodologies (Sezer Senol et al., 2014). Notwithstanding previous investigations, our research established that *S. kotschyi* possesses a comparatively diminished antioxidant capacity. This may result from the natural conditions in which the plant develops or the various extraction techniques employed. *S. othonnae* had the most diminished antioxidant properties, evidenced by the lowest DPPH and FRAP values. The presence of poisonous alkaloids in the *Senecio* genus necessitates a more comprehensive evaluation of the phytochemical profile of *S. othonnae*.

The impact of solvent choice on antioxidant activity is significant. The study demonstrated that methanol extracts of *A. absinthium* and *T. parthenium* had superior antioxidant activity relative to ethanol extracts. This research indicates that methanol extracts phenolic chemicals and flavonoids with greater efficacy. Nonetheless, certain investigations have observed that the influence of ethanol and methanol solvents on the kind and quantity of extracted phenolic chemicals differs. For instance, although polyphenols exhibit superior solubility in methanol, it has been documented that

certain flavonoids and tannins may be more abundant in ethanol (Ammar et al., 2018; Sim et al., 2019).

The elevated antioxidant capacity of the ethanol extracts from *A. falcata*, *T. farfara*, and *S. kotschyi* indicates that certain phenolic chemicals are more effectively solubilized or stabilized in ethanol. Ethanol is recognized for its efficacy in removing molecules of medium polarity (Hamburger et al., 2004). Consequently, selecting the suitable solvent based on the plant type is essential for maximizing antioxidant activity. In this regard, fractionation experiments utilizing solvents of varying polarity may enhance the comprehension of the antioxidant capacity of herbal extracts.

In conclusion, of the plants analyzed in this study, *A. absinthium*, *T. parthenium*, and *A. cotula* demonstrated the most potent antioxidant activity, whilst *S. kotschyi* and *S. othonnae* revealed the least antioxidant capacity. The choice of solvent is a crucial element influencing the antioxidant activity of plant extracts. Literature studies predominantly corroborate our findings, indicating that various extraction procedures and solvents significantly influence antioxidant capacity. These findings significantly contribute to the identification of natural antioxidant sources and the expansion of their application areas through the utilization of the antioxidant capabilities of herbal extracts. Moreover, additional research on the impact of various solvent extractions on the phytochemical profile would enhance the evaluation of the pharmacological potential of herbal products. Consequently, the prospective application of natural antioxidant sources in the dietary supplement, cosmetics, and pharmaceutical sectors can be comprehensively comprehended and enhanced.

5.2 TAS, TOS, and OSI Values

Our research established the TAS, TOS, and OSI values of ethanol and methanol extracts from *A. cotula*, *A. falcata*, *S. kotschyi*, *A. absinthium*, *T. parthenium*, *S. othonnae*, and *T. farfara* for the inaugural time.

The TAS, TOS, and OSI values of the plants analyzed in our study exhibit notable parallels and discrepancies when juxtaposed with findings from other plant species in the literature. *A. absinthium*, notably, exhibited the highest TAS values. The elevated TAS values observed in both ethanol and methanol solvents suggest that this plant possesses powerful antioxidant chemicals. Literature indicates that plants like

Origanum laevigatum (8,609 mmol/L) and *Hypericum spectabile* (9,306 mmol/L) exhibit significant antioxidant capacity (Gürgen et al., 2024; Sevindik et al., 2024). The elevated TAS value of *A. absinthium* corresponds with these observations. The methanol extract of *A. absinthium* (9,220 mmol/L) had the highest TAS value, indicating that the plant is abundant in antioxidant chemicals and possesses significant protective capabilities against oxidative stress. In this context, the elevated TAS value of *A. absinthium* parallels that of *O. laevigatum*, which was determined to be approximately 8,609 mmol/L (Korkmaz et al., 2024). This indicates that both plants possess potent antioxidant capabilities. Conversely, species like *T. parthenium* and *A. cotula* demonstrated moderate TAS levels. The TAS value of 6,840 mmol/L in the methanol extract of *A. cotula* and the TAS value of 5,732 mmol/L in the ethanol extract of *T. parthenium* suggest that these plants possess moderate antioxidant ability. The TAS value of *Tagetes patula* is documented as 5,386 mmol/L in the literature (Gürgen et al., 2024), suggesting that *T. parthenium* possesses a comparable antioxidant ability. Despite *T. parthenium*'s moderate antioxidant capacity being inferior to that of *O. laevigatum* and *H. spectabile*, it nonetheless exhibits considerable antioxidant potential relative to existing literature. In terms of TOS values, *A. falcata* and *S. othonnae* are distinguished as the plants exhibiting the greatest TOS values. The methanol extract of *A. falcata* (17,173 μmol/L) demonstrates that this plant is significantly abundant in oxidant chemicals. This indicates that *A. falcata* have a significant capacity to produce oxidative stress. The literature indicates that the TOS value of *H. spectabile* is 13,065 μmol/L (Sevindik et al., 2024), implying that *A. falcata* possesses a comparable capacity to generate oxidative stress due to its elevated TOS value. Likewise, the methanol extract of *S. othonnae* (15,354 μmol/L) is notable for its elevated TOS values. These plants possess elevated concentrations of oxidant chemicals, signifying the presence of powerful substances capable of inducing oxidative stress. *S. kotschyi*, a plant exhibiting elevated TOS values, is distinguished as a pro-oxidant species with minimal antioxidant compounds (TAS: 3,474 mmol/L) and significantly strong oxidant compounds (TOS: 14,413 μmol/L). This indicates that the plant has a pronounced oxidative stress profile. Moreover, the TOS value of *T. patula* is documented in the literature as 8,287 μmol/L (Korkmaz et al., 2024), while *S. kotschyi* exhibits a significantly elevated TOS value compared to *T. kotschyi*, suggesting a higher buildup of pro-oxidant chemicals. Upon examining the equilibrium between TOS and TAS, distinct oxidative stress profiles can be identified

across various plants. Certain plants exhibit elevated TAS values, whilst others demonstrate markedly higher TOS levels. This indicates that the equilibrium of antioxidant and oxidant chemicals in plants differs, thereby affecting their propensity to induce oxidative stress.

In comparison to the OSI values documented in the literature, our work reveals that plants like *A. absinthium* and *T. parthenium* exhibit a more equilibrated antioxidant-oxidant profile, characterized by lower OSI values. The OSI value of *A. absinthium* (0,102-0,117) is comparable to the 0,124 value of *O. laevigatum* documented in the literature, suggesting that both plants exhibit significant low levels of oxidative stress (Seğmenoğlu et al., 2024). The OSI values of *T. parthenium* were established at 0,144 for the ethanol extract and 0,101 for the methanol extract, signifying the plant's little capacity to provoke oxidative stress. The OSI value of *Mentha longifolia* was documented as 0,231 (Gürgen et al., 2024), signifying a greater oxidative stress potential relative to *T. parthenium* and *A. absinthium*. Conversely, *S. othonnae* (0,520) and *S. kotschyi* (0,415-0,446) are distinguished as plants with elevated OSI values. These plants are abundant in pro-oxidant chemicals and demonstrate significant ability to generate oxidative stress. The literature indicates that the OSI value of *Equisetum ramosissimum* is 0,217 (Uysal et al., 2024), and the elevated OSI levels of *S. kotschyi* imply that this plant possesses a heightened capacity to generate oxidative stress. In conclusion, our study's findings reveal that substantial antioxidant-oxidant imbalances occur in plants, and this equilibrium directly influences their capacity to provoke oxidative stress. Species including *A. absinthium*, *T. parthenium*, and *A. cotula* exhibit significant antioxidant capabilities, whereas *A. falcata*, *S. othonnae*, and *S. kotschyi* are notable for their elevated oxidative stress levels. Literature comparisons suggest that the antioxidant and oxidant profiles of these plants may differ according on the solvent utilized, and that extracts derived from these solvents may affect biological activity. Nonetheless, these results necessitate in vivo validation and a deeper comprehension of their mechanisms.

5.3 Antimicrobial Activity

This study evaluated the antibacterial properties of ethanol and methanol extracts from *A. cotula*, *A. falcata*, *S. kotschyi*, *A. absinthium*, *T. parthenium*, *S. othonnae*, and *T. farfara*.

The plants had diverse effects against the evaluated bacterial and fungal strains. The efficacy of plant extracts made with various solvents against microorganisms significantly varies according on the plant content and the solvents employed (Tiwana et al., 2024). *A. cotula* demonstrated significant antibacterial efficacy against the examined pathogens using ethanol and methanol extracts. The literature indicates that *A. cotula* is efficient against *E. faecium* at various concentrations (Şener et al., 2017). Our results corroborate this effect. Both ethanol and methanol extracts demonstrated efficacy against Gram-positive bacteria, specifically *S. aureus* and *E. faecalis*. The results indicate that *A. cotula* displays considerable biological activity against Gram-positive bacteria. Ethanol and methanol extracts of *A. absinthium* exhibit a wide range of antibacterial activity, consistent with existing literature. Monika (2016) conducted a study indicating that water, methanol, ethanol, and acetone extracts of *A. absinthium* exhibited efficacy against bacteria including *S. aureus*, *E. coli*, *S. typhii*, and *S. pneumoniae.* Our results indicate that ethanol and methanol extracts demonstrate comparable efficacy against both Gram-positive and Gram-negative bacteria. Significant results were achieved against bacteria such as *S. aureus* and *P. aeruginosa*, suggesting that this plant may serve as a potential antibacterial agent. Research on *S. othonnae* corroborates its antibacterial properties. Research conducted by Üçüncü et al. (2010) indicated that essential oils from *S. othonnae* shown antibacterial efficacy against bacteria including *B. cereus*, *S. aureus*, and *E. faecalis*. This investigation found that S. othonnae was efficient against comparable bacteria using its ethanol and methanol extracts. Moreover, *S. othonnae* demonstrated notable efficacy against fungi, indicating its potential as a therapeutic option for fungal infections. Research on *T. parthenium* also emphasizes the antibacterial properties of its essential oils. Polatoglu et al. (2010) and further research (Mohsenzadeh et al., 2011; Shafaghat et al., 2017) highlighted that this plant exhibits significant activity against Gram-positive bacteria, including *S. aureus* and *B. subtilis*. In our investigation, ethanol and methanol extracts of *T. parthenium* demonstrated efficacy against Gram-positive bacteria, including *S. aureus*. The plant's efficacy against Gram-negative bacteria suggests it possesses a potentially extensive range of antibacterial activities. A literature review of *A. falcata* indicated that hydroalcoholic extracts demonstrate bactericidal properties against *S. pneumoniae*, *B. cereus*, and *K. pneumoniae* (Hammad et al., 2014). Nonetheless, it has been documented that this plant demonstrates minimal efficacy against Gram-negative bacteria and fungus, including *C. albicans*. Our research demonstrates that *A. falcata*

is especially potent against Gram-positive bacteria, including *S. aureus*, but ineffective against fungi. This indicates that the plant possesses significant potential for biological action against Gram-positive bacteria, although its efficacy against fungal illnesses is constrained. Research on *T. farfara* indicates that ethanolic extracts demonstrate significant antibacterial efficacy, especially against Gram-positive bacteria like *L. rhamnosus* (Petrov et al., 2013). Additionally, an independent investigation indicated its efficacy against germs like *E. coli* and *S. aureus* (Boucher et al., 2020). Our research indicates that *T. farfara* demonstrates modest yet significant antibacterial efficacy, particularly against bacteria such as *E. faecalis* and *S. aureus*. The data suggest that *T. farfara* is an efficacious antibacterial agent, especially against Gram-positive bacteria. No literature contains information regarding the antibacterial properties of *S. kotschyi*. A study on the species *S. papposa* indicated its efficacy against pathogens including *S. aureus*, MRSA *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *A. baumannii*, *C. albicans*, *C. krusei*, and *C. glabrata* (Mohammed et al., 2020). Our research, however, demonstrates that *S. kotschyi* is especially efficacious against fungi. This indicates that *Scorzonera* species may exhibit varying activity profiles, with *S. kotschyi* potentially serving as a therapeutic agent, especially for fungal infections. The results indicate that the antibacterial efficacy of plants is contingent upon the species, solvents employed, and microorganisms evaluated. In comparison to other research in the literature, the antibacterial efficacy of these plants is notably elevated, exhibiting significant action against Gram-positive bacteria and some fungi. These findings illustrate the potential of these plants as natural antibacterial agents. Nonetheless, due to the distinct spectrum of activity exhibited by each plant, and their varying efficacy against specific illnesses, it is crucial to orient future research appropriately.

5.4 Total Phenolic and Total Flavonoid Contents

Our study quantified the total phenolic and total flavonoid concentrations in the ethanol and methanol extracts of *A. cotula, A. falcata, S. kotschyi, A. absinthium, T. parthenium, S. othonnae, and T. farfara*.

Marked disparities were noted in the total phenolic levels (TPS) and total flavonoid levels (TFS) concentrations of the plants analyzed in the study. *A. absinthium* was found to possess the greatest levels of phenolic and flavonoid compounds. This outcome corroborates the literature's acknowledgment of *Artemisia* species for their

significant antioxidant activities. Prior research has indicated that *Artemisia* species are abundant in phenolic chemicals. Hbika et al. (2022), documented the total phenolic content of the ethyl acetate extract of *A. absinthium* as 60,34 mg/g and the total flavonoid content as 25,84 mg/g. The values derived from our investigation exceed those documented in the literature, indicating that variations in extraction methods and solvents employed may influence these outcomes. *A. cotula* has been recognized as a plant with elevated levels of phenolic and flavonoid compounds. While direct information regarding the TPS and TFS contents of *A. cotula* is absent in the literature, methanol extracts of *A. tinctoria* var. *tinctoria* from the *Anthemis* genus have been documented to possess elevated total phenolic and flavonoid concentrations. According to Yıldırım et al. (2024), the ATiEA-II subfraction exhibited a superior total flavonoid content of 743,86 µg/g dry weight and a total phenolic content of 4659,36 µg/g dry weight in comparison to the other subfractions. The elevated TPS and TFS values of *A. cotula* in our study corroborate that *Anthemis* species often possess a substantial phenolic compound content. *A. falcata* and *T. farfara* were assessed to possess modest levels of phenolic and flavonoid compounds. The research indicates that the total phenolic content of water and aqueous alcohol extracts of *A. falcata* ranges from 25,64 to 28,13 mg/g, while the total flavonoid content ranges from 0.93 to 1,32 mg/g (Hammad et al., 2014). The values derived from our investigation are predominantly aligned with the findings in the literature. In the literature, the total phenolic content of *T. farfara* is documented as 278,2 mg/g, while the total flavonoid content is noted as 47,0 mg/g (Norani et al., 2019). The results indicate that variations in solvent and extraction procedure may influence the quantities of plant chemicals. The plants exhibiting the lowest total phenolic and flavonoid concentrations in the study were identified as *S. kotschyi* and *S. othonnae*. While direct information regarding the total phenolic and flavonoid contents of *S. kotschyi* is absent in the literature, a study by Sarıaltın and Acıkara (2022), reported that the total phenolic contents of *S. sandrasica*, *S. ahmet-duranii*, and *S. coriacea* ranged from 16,7452 to 50,2917 mg/g, and the total flavonoid contents ranged from 24,4208 to 99,2750 mg/g. The phenolic and flavonoid values of *S. kotschyi* observed in our study were at the lower thresholds of this range, suggesting that this plant had a low concentration of these compounds. Likewise, while there is no explicit data regarding the total phenolic and flavonoid concentrations of *S. othonnae*, research conducted by Alruwad et al. (2023), indicated that the total phenolic and flavonoid levels in *S. leucanthemifolius*

were 91,82 mg/g and 14,90 mg/g, respectively. Our analysis revealed that the phenolic and flavonoid concentrations in *S. othonnae* were very low, suggesting that the chemical profiles among various *Senecio* species may differ. *T. parthenium* was assessed to possess a moderate phenolic content. Shahhoseini et al. (2019), indicated that the total phenolic content of *T. parthenium* was 21,42 mg/g, whereas the total flavonoid content was 1,9 mg/g. The values acquired in this investigation surpass those documented in the literature, and it is posited that the discrepancies in the extraction process and solvent employed affected these outcomes. The research established that the choice of solvent markedly influenced the extraction levels of phenolic and flavonoid chemicals. Methanol was identified as a more efficacious solvent for *A. absinthium* and *T. parthenium*, but ethanol yielded superior extraction results for *A. cotula*, *A. falcata*, and *T. farfara*. Literature indicates that the extraction efficiency of phenolic and flavonoid chemicals correlates with solvent polarity. Our research corroborates this knowledge, indicating that solvent selection is a crucial element in enhancing the extraction efficiency of target chemicals. The plants analyzed in the study shown notable variations in their phenolic and flavonoid concentrations. The acquired data demonstrate that certain plants possess significant antioxidant capability, whilst others have diminished levels of phenolic and flavonoid compounds. This indicates that evaluating the biological activity of herbal extracts necessitates evaluation of both overall phenolic and flavonoid concentration as well as particular constituent profiles.

5.5 Anticholinesterase Activities

In this investigation, the activities of acetylcholinesterase and butyrylcholinesterase were assessed in ethanol and methanol extracts of *A. cotula*, *A. falcata*, *S. kotschyi*, *A. absinthium*, *T. parthenium*, *S. othonnae*, and *T. farfara.* This study investigated the inhibitory effects of various plant extracts on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes, identifying the most effective species.

The results indicated that *A. absinthium* possessed the lowest IC50 values and demonstrated the most significant enzyme inhibition relative to the other plants examined in our study. In comparison to galantamine, utilized as the standard in the study, the IC50 values of *A. absinthium* were markedly elevated. This indicates that while herbal extracts may block enzymes, they are inferior to synthetic inhibitors like

galantamine in terms of efficacy. The ethanol extract of *A. absinthium* is distinguished as a highly effective inhibitor, with IC50 values of 51,88 µg/mL for acetylcholinesterase (AChE) and 78,76 µg/mL for butyrylcholinesterase (BChE). The methanol extract demonstrated considerable inhibitory potency, with IC50 values of 95,40 µg/mL for AChE and 71,96 µg/mL for BChE. The results demonstrate that the ethanol extract is notably efficacious. The differing solubility of phenolic and flavonoid chemicals in various solvents, attributable to polarity variations, may directly influence the efficacy of the extracts. In comparison to other plants, species such as *A. cotula*, *T. parthenium*, and *A. falcata* exhibited modest inhibitory action. The ethanol extract of *A. cotula* exhibited an AChE activity of 80,23 µg/mL and a BChE activity of 119,73 µg/mL, but the ethanol extract of *T. parthenium* had marginally reduced activity with 101,71 µg/mL for AChE and 158.65 µg/mL for BChE. These data suggest that these species possess inhibitory potential, albeit with reduced activity relative to *A. absinthium*. Species such as *S. kotschyi* and *S. othonnae* had the most feeble inhibitory characteristics. The IC50 values for *S. othonnae* were determined to be 202,10 µg/mL for AChE and 287,24 µg/mL for BChE, which are significantly elevated in comparison to the other plants examined in the study. This indicates that *S. othonnae* exerts a diminished influence on cholinesterase enzymes. The results underscore the substantial impact of solvent type on inhibitory action. Typically, portions of plant extracts derived using ethanol solvents had lower IC50 values compared to those obtained with methanol extracts. This suggests that ethanol is a better appropriate solvent for extracting certain active chemicals. Nevertheless, the more pronounced suppression of methanol extracts in certain plants indicates that the active chemicals may vary according to the solvent used.

A. cotula has been documented to inhibit the enzymes acetylcholinesterase and butyrylcholinesterase (Sut et al., 2019). This investigation corroborates our findings, indicating that the plant had a modest inhibitory capacity. The impact of *A. falcata* on cholinesterase activity was examined via the chemical constituents of its essential oils, with some components identified as contributing to enzyme inhibition (Radulović et al., 2015). The IC50 values of this plant in our investigation exhibit a profile analogous to the literature findings. While there is no specific literature about the cholinesterase activity of *S. kotschyi*, *S. ketzkhowelii*, a member of the same genus, has been documented as a potent AChE inhibitor (Göç et al., 2025). Consequently, the

diminished inhibitory impact of *S. kotschyi* indicates phytochemical disparities between these species. There is no direct study on *S. othonnae*; however, *S. hoggariensis*, a related species, is shown to block both enzymes (Arab et al., 2022). The minimal inhibition of *S. othonnae* may suggest compositional disparities. The capacity of *A. absinthium* to inhibit AChE and BChE enzymes has been highlighted in the literature, with one study indicating IC50 values of 32 µg/mL for AChE and 36 µg/mL for BChE (Khan et al., 2022). The IC50 values derived from our investigation were marginally elevated in comparison to the literature. This discrepancy may result from variations in the extraction process employed, the geographical region of cultivation, or the chemical composition of the plants. *T. parthenium* has been documented in the literature to exert an inhibitory impact on both enzymes (Orhan et al., 2015), and our results indicate that the plant serves as a moderate inhibitor. The ethyl acetate extract of *T. farfara* has been identified as a strong inhibitor (Uysal et al., 2019). Our analysis revealed that methanol and ethanol extracts exhibited elevated IC50 values. This reiterates the substantial impact of solvent type on inhibitory action. This study indicates that herbal extracts may possess inhibitory capability against AChE and BChE. The potent inhibitory efficacy of *A. absinthium* is especially significant. In comparison to the IC50 values of galantamine, these herbal extracts have significantly reduced enzyme inhibitory activity. Consequently, the isolation and purification of more effective inhibitors is a crucial step in enhancing the future pharmacological accessibility of these plants. A comprehensive examination of the synergistic or antagonistic effects of multicomponent herbal inhibitors may enhance the understanding of cholinesterase inhibition processes. Ultimately, identifying the biologically active constituents of the plants exhibiting significant inhibition, as established in this study by advanced chemical analysis, would substantially enhance drug development procedures.

5.6 Antiproliferative Activities

Our research assessed the antiproliferative effects of ethanol and methanol extracts from *A. cotula*, *A. falcata*, *S. kotschyi*, *A. absinthium*, *T. parthenium*, *S. othonnae*, and *T. farfara* on the A549 lung cancer cell line.

Our investigation demonstrates that, overall, ethanol extracts display more significant antiproliferative effects than methanol extracts. Nevertheless, methanol extracts

demonstrated a more pronounced effect in certain species. The chemical structures of the plant components, which may differ based on the extraction solvent, help elucidate this phenomenon. The bioavailability of extracts is contingent upon the efficacy of the solvent in extracting the phytochemical constituents (Hikmawanti et al., 2021). Ethanol extracts of *A. cotula*, *A. falcata*, *S. kotschyi*, and *T. farfara* shown a marked reduction in cell viability at elevated concentrations (200 µg/mL). The results substantiate the idea that ethanol may more efficiently destroy phenolic molecules and flavonoids. Previous investigations have suggested that phenolic compounds exhibit cytotoxic and antiproliferative effects on cancer cells. Phenolic chemicals and flavonoids are recognized for their antiproliferative effects on cancer cells, either through the enhancement of oxidative stress or the initiation of apoptotic processes (Kuete et al., 2016). The literature lacks direct information concerning the efficacy of *A. cotula* against the A549 cancer cell line. *A. mirheydari* has demonstrated significant cytotoxic effects against LS180, MCF-7, and MOLT-4 human cancer cell lines (Jassbi et al., 2016). Likewise, although there is no explicit data about the impact of *S. kotschyi* on the A549 cell line, *S. calyculata* has been documented to demonstrate efficacy against the A549 lung cancer cell line (Ayromlou et al., 2019). *A. falcata* has demonstrated efficacy against HeLa and Caco-2 cell lines (Younos et al., 2020). The data indicate that varying biological activities can be found among species within the same genus. Nonetheless, methanol extracts shown superior antiproliferative action relative to ethanol extracts in *A. absinthium* and *T. parthenium*. The literature indicates that the methanol extract of *A. absinthium* shown negligible efficacy against the A549 cell line (Erel et al., 2011). Parthenolide derived from *T. parthenium* has been identified as a potential anticancer drug against the A549 lung cancer cell line (Parada-Turska et al., 2007). This mismatch may arise from variations in extraction methods, bioavailability, or cell lines. A dose-dependent antiproliferative effect was consistently observed across all species. This indicates that the capacity of phytochemical substances to suppress cell development escalates with concentration. Certain chemicals are recognized to induce lethal effects, especially at elevated doses, by modifying cell membrane permeability or disrupting cell signaling pathways (Cook et al., 2019). Nonetheless, both extracts of the *S. othonnae* species exhibited predominantly low antiproliferative properties. The literature lacks direct information on the efficacy of *S. othonnae* against the A549 cancer cell line. *S. scandens* has been documented to demonstrate efficacy against the A549 lung cancer cell line (Dou et al.,

2017). Likewise, several sesquiterpenoids derived from the flower buds of *T. farfara* have been documented to provide antiproliferative activities in the A549 cell line (Song et al., 2021). The findings of our investigation suggest that herbal extracts may serve as viable biological agents in cancer therapy. Nonetheless, they require validation through in vitro research, in vivo models, and mechanistic investigations. Distinguishing between ethanol and methanol extracts is crucial for isolating certain chemicals and doing further research on their biological activity. Future research should encompass more thorough analysis to elucidate the mechanisms of action of these extracts. Moreover, evaluating the impacts of the extracts on various cancer cell lines would be crucial for the generalizability of the findings. The impact of these extracts on healthy cells must be assessed to evaluate potential toxicity risks and establish their safety profile.

5.7 Chemical Compositions

The chemical contents of the plant samples included in the study were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analysis identified 23 chemicals in the *A. cotula* sample, categorized into diverse phytochemical categories, such as fatty acid derivatives, terpenoids, and phenylpropanoids. The predominant chemical was linoleic acid, methyl esterat 30,36%, succeeded by palmitic acid, methyl ester at 17.78% and oleic acid, methyl ester at 12,35%. The three primary chemicals collectively account for almost 60% of the total composition, defining the sample's unique component profile. These fatty acid methyl esters are recognized for their anti-inflammatory, antioxidant, and antibacterial properties (Krishnaveni et al., 2014; Odchimar et al., 2016; Umoh et al., 2024). Compounds belonging to the sesquiterpene class, including caryophyllene (3,65%), α-terpinenyl acetate (2,66%), and anethole (2,27%), are present in substantial quantities, and the literature frequently highlights their anti-inflammatory, analgesic, and antimicrobial properties (Peana et al., 1999; Marinov and Valcheva-Kuzmanova, 2015; Gyrdymova and Rubtsova, 2022). Monoterpenes, including α-pinene, β-myrcene, D-limonene, and o-cymene, are found in minimal quantities yet may contribute to potential therapeutic benefits (Koziol et al., 2014; Zielińska-Błajet and Feder-Kubis, 2020). Fatty acid esters are the predominant constituents of the essential oil of *A. cotula*, succeeded by terpenoid chemicals, which possess significant biological action. This content profile suggests

that the plant possesses antibacterial and antioxidant properties. GC-MS analysis identified 37 chemicals in the *A. falcata* sample. The predominant component was 13-epimanool at 11,67%, succeeded by α-pinene at 8,29% and α-terpinenyl acetate at 7,33%. Furthermore, 6,85% caryophyllene and 6,44% anethole were identified. The studies reveal that the essential oil profile of the plant predominantly comprises monoterpene and sesquiterpene derivatives. Monoterpenes, specifically α-pinene, D-limonene, β-myrcene, o-cymene, and eucalyptol, are compounds recognized for their aromatic characteristics as well as their antimicrobial, bronchodilatory, and anti-inflammatory effects (Saddiq and Khayyat, 2010; de Cássia da Silveira e Sá et al., 2013; Alamoudi et al., 2025). Sesquiterpenes, including caryophyllene, germacrene-D, and Δ-cadinene, have been linked to anti-inflammatory, analgesic, and antiproliferative properties (Bakır et al., 2008; Li and Wang, 2016; Casiglia et al., 2017). The presence of phenylpropanoid chemicals, including anethole, indicates that the plant may possess antioxidant and anticancer characteristics (Freire et al., 2005; Raposo et al., 2024). Fatty acid derivatives are a minor component of the essential oil makeup. These comprise compounds including hexadecanoic acid, methyl ester (1,42%), methyl stearate (0.83%), 9-octadecenoic acid, methyl ester (4,23%), and 9,12-Octadecadienoic acid, methyl ester (0,82%), which are typically utilized in antibacterial, neuroprotective, and dermatological applications (Chandrasekaran et al., 2008; Sanguanphun et al., 2022; Salih and Faeq, 2023). Furthermore, specifically structured aromatic derivatives like 3-Methyl-5-(2,6,6-Trimethyl-1-Cyclohexen-1-yl)-1-Pentyn-3-O at 4,30% enhance the chemical variety of the essential oil, suggesting that its potential biological effects may encompass a wider range. The essential oil of *A. falcata* possesses a significant concentration of biologically active terpenoid chemicals, substantiating the plant's traditional applications. This chemical profile indicates that the plant possesses considerable medicinal potential, especially for its antibacterial, antioxidant, and anti-inflammatory properties.

GC-MS study of the essential oil derived from the *S. kotschyi* plant discovered a total of 16 components. The chemical with the largest proportion among these is n-hexadecanoic acid (palmitic acid) with 30,43%, representing a substantial component of the plant's essential oil makeup. Palmitic acid is a prevalent saturated fatty acid documented in the literature for its diverse antibacterial, anti-inflammatory, and neuroprotective properties (Bentrad et al., 2017; de Souza et al., 2018; Lee et al.,

2019). The second most prevalent molecule is α-terpinenyl acetate at 16,57%, recognized for its aromatic characteristics, as well as its antioxidant and antibacterial effects (Peana et al., 1999; Chowdhury and Kumar, 2020). Caryophyllene, comprising 11,92%, is a prominent sesquiterpene in the essential oil; this chemical is particularly significant pharmacologically due to its analgesic and anti-inflammatory properties (Dahham et al., 205; Fidyt et al., 2016). Moderate amounts of compounds include anethole (6,56%), caryophyllene oxide (5,99%), germacrene-D (5,46%), and bicyclogermacrene (4,32%), all classified as bioactive terpenoids. Caryophyllene oxide is recognized for its antifungal properties, whereas anethole is distinguished by its anticancer, antispasmodic, and estrogenic effects (Albert-Puleo, 1980; Yang et al., 2000; Shahbazian et al., 2015; Lima et al., 2020). This profile also includes copaene, β-elemene, aromadendrene, Δ-cadinene, and several cyclic aromatic derivatives, which, although present in minor quantities, enhance the chemical diversity of the essential oil. *S. kotschyi* essential oil presents a diverse composition of fatty acids and terpenoid constituents. This chemical composition establishes a scientific foundation for the plant's traditional application and suggests considerable pharmacological potential, especially for anti-inflammatory, antibacterial, and antioxidant properties.

GC-MS research revealed 16 components in *A. absinthium* essential oil, predominantly including terpenoids and aromatic chemicals. Caryophyllene oxide, an oxygenated sesquiterpene recognized for its antibacterial and antifungal properties, constituted the largest percentage at 20,82% (Jassal et al., 2021; Gyrdymova and Rubtsova, 2022). Subsequently, D-limonene (16,05%), L-(+)-ascorbic acid 2,6-dihexadecanoate (12,60%), and 1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]- (10,79%) are included. These molecules are primary determinants of the biological activity potential of the essential oil. D-limonene is notable for its anti-inflammatory, anticancer, and respiratory system benefits, whereas the ascorbic acid ester derivative is distinguished by its structural attributes that enhance antioxidant capacity (Yu et al., 2017; Santana et al., 2020; Mandal and Parija, 2022). The essential oil comprised 7,18% caryophyllene, 6,55% benzene-derived aromatic chemicals, 5,22% 3-Methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-pent-1-yn-3-ol, and 5,02% 1-p-Menthen-8-yl acetate. Each of these compounds is esteemed for its aromatic characteristics, antibacterial qualities, and pharmacological efficacy (Loizzo et al., 2007; Nogueira et al., 2020). Despite their

lower concentrations, chemicals like as α-pinene, germacrene-D, geranyl acetate, and α-humulene contribute to the essential oil's total pharmacological effectiveness through synergistic effects. The essential oil of *A. absinthium* comprises a significant concentration of oxygenated sesquiterpenes, monoterpene derivatives, and antioxidant ester chemicals. This content profile may possess antibacterial, antioxidant, anti-inflammatory, and possibly anticancer activities. These findings endorse the plant's employment in traditional medicine and indicate its significance as a natural resource for pharmacological and aromatherapeutic purposes.

The GC-MS investigation of *T. parthenium* essential oil detected 15 chemicals in total. The principal component of the essential oil is n-hexadecanoic acid (palmitic acid) at 29,75%, recognized for its anti-inflammatory, antibacterial, and cell membrane-stabilizing properties (Zavodnik et al., 1996; Bravo-Santano et al., 2019; Korbecki and Bajdak-Rusinek, 2019). This outcome signifies that palmitic acid is a key component of the plant's oil composition. Subsequently, eicosane constitutes 10,04%, caryophyllene 7,60%, tetracosane 6,97%, and 9-tricosene (Z) 6,65%. These hydrocarbons and sesquiterpenes are typically linked to antibacterial and insect repellent properties (Suzuki et al., 1975; Sabulal et al., 2006; Nerio et al., 2010). Caryophyllene (7,60%) and its oxidized derivative, caryophyllene oxide (5,72%), are prominent terpenoids in the oil, recognized for their anti-inflammatory and antineurosedative properties (Rigano et al., 2020; Moghrovyan et al., 2022). Furthermore, 5,61% of 3-Cyclohexene-1-methanol, α, α, 4-trimethyl-, acetate, and 4,98% of aromatic benzene derivative compounds contribute to the plant's distinctive aroma while also exhibiting antioxidant and antimicrobial properties (Althagafi and Gaffer, 2019; Sosa et al., 2019; Ferdosi et al., 2022). Compounds such 2-Isopropyl-5-methyl-1-heptanol, α-copaene, α-muurolene, and benzene, 1-methoxy-4-(1-propenyl)-, present in lesser quantities, augment the chemical diversity and enhance the potential pharmacological effects of the plant. *T. parthenium* essential oil possesses a diverse composition of fatty acids, terpenoids, and saturated hydrocarbons, signifying that the plant serves as a substantial natural source of anti-inflammatory, antibacterial, antioxidant, and repelling attributes. These chemicals enhance the plant's utility in traditional medicine and are also significant for their future pharmacological and cosmetic applications.

GC-MS investigation of *S. othonnae* essential oil revealed nine components, predominantly consisting of one main constituent. Esdragole, exhibited a notably high concentration of 78,67%, signifying a predominant single constituent in the essential oil's chemical composition. This molecule is classified as an aromatic phenylpropanoid and has been linked to antibacterial, antifungal, and antioxidant properties in the literature (Park et al., 2003; Salih et al., 2017). The substantial occurrence of this chemical in the essential oil of *S. othonnae* species correlates with its biological effects. Additionally, butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester, comprising 8,36% of the composition, is likely an ester of anethole-like structures and may be significant for lipophilicity and pharmacological effects. n-hexadecanoic acid (palmitic acid), comprising 4,51%, is a saturated fatty acid that contributes to structural stability and exhibits antibacterial and anti-inflammatory properties (Huang et al., 2011; Charlet et al., 2022). Furthermore, molecules like estragole (2,08%) and D-limonene (1,42%), identified in lesser percentages, enhance the aromatic and medicinal properties of the essential oil. Phenolic derivatives, including phenol, 2-methoxy-3-(2-propenyl)-, and benzaldehyde, despite their minimal concentrations, may induce synergistic effects. *S. othonnae* essential oil comprises a significant concentration of phenylpropanoid aromatic chemicals, suggesting that the plant is notably recognized for its antibacterial, antioxidant, and phytoestrogenic attributes. The plant's high concentration of chemicals renders it a compelling subject for pharmacological investigation and the creation of natural medicinal products.

The GC-MS investigation of *T. farfara*'s essential oil discovered 16 chemicals, primarily comprising phenylpropanoid derivatives and fatty acid methyl esters. Anethole is the predominant constituent, comprising 49,68%, significantly influencing the chemical profile of the essential oil. Anethole is an aromatic molecule recognized in the literature for its significant antioxidant, antibacterial, anticancer, and estrogenic properties (Esfandyari-Manesh et al., 2013; Senatore et al., 2013; Contant et al., 2021). Moreover, unsaturated fatty acid esters, including 9,12-octadecadienoic acid methyl ester (linoleic acid methyl ester) at 17,13% and 9-octadecenoic acid (oleic acid methyl ester) at 9,27%, are significant constituents regarding the lipophilic properties and biopermeability of the essential oil. Saturated fatty acids, including hexadecanoic acid, methyl ester (palmitic acid methyl ester) at 6,19% and free n-hexadecanoic acid at 4,21%, are typically linked to antibacterial and anti-inflammatory properties (Ibrahim

et al., 1991; Laurenza et al., 2008; Saeed et al., 2012; Davoodbasha et al., 2018). Nonetheless, butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester at 3,85% and methyl stearate at 3,65% enhance both the pharmacological and aromatic characteristics of the essential oil. Aromatic and monoterpenic chemicals, including estragole, eugenol, and D-limonene, albeit in minimal concentrations, have synergistic actions that may specifically augment the antibacterial efficacy of the essential oil (Donati et al., 2015; Zahi et al., 2015; Marchese et al., 2017). These components augment the functionality of the essential oil in medicinal and cosmetic applications. In summary, *T. farfara* essential oil presents a pharmacologically significant profile, especially regarding its antioxidant, antibacterial, and cytoprotective properties, attributed to its elevated levels of aromatic phenylpropanoids and physiologically active fatty acids. This chemical composition underpins the plant's application in traditional medicine and illustrates its potential for incorporation into contemporary phytotherapeutic products.

5.8 Phenolic Contents

Analysis of phenolic compounds revealed that each plant species studied displayed a distinct phenolic profile. This serves as a measure of plants' ability for secondary metabolite production, which varies based on their taxonomic distinctions, metabolic pathway variety, and environmental variables. Although possesses a restricted quantity of phenolic chemicals, *A. cotula*, notably, is distinguished by its elevated concentrations of several essential chemicals. The peak concentration was recorded for fumaric acid (29231,31 µg/kg), suggesting the plant's potential antioxidant and anti-inflammatory properties (Linker et al., 2011). Moreover, the substantial concentrations of prevalent phenolic chemicals, including hydroxycinnamic acid (7910,29 µg/kg) and quercetin (10650,14 µg/kg), indicate that this species may exhibit notable pharmacological effects. Furthermore, the existence of chemicals recognized for their antioxidant capabilities, such vanillic acid, alizarin, and resveratrol, albeit in diminished concentrations (Gülçat, 2010; Zengin et al., 2016; Sharma et al., 2020), may enhance the functional potential of *A. cotula*. The inability to identify several phenolic chemicals in this species indicates a more simplistic and restricted phenolic structural profile overall. This may indicate either a fundamental constraint due to the plant's genetic composition or the influence of sampling time, cultivation

circumstances, and environmental stressors on the production of phenolic compounds. Consequently, reassessing the phenolic content of *A. cotula* investigating under varying conditions, while considering intricate environmental and genetic factors, could yield a more thorough comprehension.

The phenolic analysis results from the study indicated that *A. falcata* possesses substantial concentrations of phenolic chemicals. The elevated amounts of substances including chlorogenic acid (217522,42 µg/kg), caffeic acid (124257,24 µg/kg), fumaric acid (74058,94 µg/kg), and kaempferol (52196,05 µg/kg) signify that this species possesses a notably rich phenolic makeup. These compounds are recognized for their potent antioxidant, anti-inflammatory, and oxidative stress-reducing capabilities, demonstrating efficacy in numerous pharmaceutical trials. Chlorogenic and caffeic acid are significant chemicals in plant defense and therapeutic uses for human health because of their free radical scavenging properties (Gülçin, 2006; Linker et al., 2011; Moschona et al., 2017; Olszowy-Tomczyk and Wianowska, 2023). Moreover, the existence of substantial concentrations of various physiologically active chemicals, including luteolin, salicylic acid, hydroxycinnamic acid, syringic acid, and protocatechuic acid, suggests that *A. falcata* is a plant abundant in both chemical variety and phenolic diversity. This variety may improve biological activity via synergistic actions. Luteolin and kaempferol, both belonging to the flavonoid class, are often assessed in anticancer research for their antioxidant and cytotoxic properties (Dimas et al., 2000; Yazar et al., 2024). Likewise, salicylic acid and protocatechuic acid are significant in modulating inflammatory responses and alleviating cellular stress (Masella et al., 2012; Rosheen et al., 2023). Taking all these facts into account, *A. falcata* is believed to have a diverse phenolic profile, rendering it a viable candidate for pharmacological purposes. This phenolic abundance may be linked to the species' chemical defense mechanisms against environmental stress.

Based on the examination of phenolic compounds, *S. kotschyi* is distinguished by its abundant phenolic profile. The identification of elevated concentrations of substances like kaempferol (79366,01 µg/kg) and hydroxycinnamic acid (53909,11 µg/kg) indicates that this species may possess significant antioxidant potential. Kaempferol, a member of the flavonoid class, is a significant chemical known for its anticancer and cardioprotective properties, operating through mechanisms including cytoprotection,

oxidative stress reduction, and apoptosis induction (Imran et al., 2019). Hydroxycinnamic acid and fumaric acid are secondary metabolites that effectively resist free radicals and possess anti-inflammatory activities (Nagasaka et al., 2007; Kaur et al., 2020). Substantial concentrations of resveratrol and salicylic acid were also identified in this species. Resveratrol is a polyphenolic stilbene commonly linked in the literature to anti-aging, anti-inflammatory, and anticancer properties (Bejenaru et al., 2024). Salicylic acid is involved in plant defense mechanisms and the regulation of inflammatory processes in human pharmacology (Rosheen et al., 2023). The study notably includes substances with established antioxidant properties, such as myricetin and catechinhydrate (Yao et al., 2014; Kaur et al., 2017). These two chemicals are notable for their capacity to inhibit oxidative damage and their potential to facilitate cellular regeneration. Quercetin, 4-hydroxybenzoic acid, and protocatechuic acid, integral constituents of the phenolic profile, exhibit a wide array of biological effects due to their free radical scavenging and metal chelating capabilities (Li et al., 2011; Muangsri et al., 2022; Mohammed et al., 2023). The existence of these chemicals, in both diversity and abundance, indicates the polyphenolic composition of *S. kotschyi* is rich both qualitatively and numerically. This structural complexity offers a significant foundation for the further exploration of the pharmacological and therapeutic possibilities of this species. In the contemporary landscape, where natural antioxidant resources are more significant, phenolic compounds derived from indigenous plants such as this species may prove advantageous in the formulation of functional meals and phytotherapeutic goods.

Analyses of phenolic compounds have established that *A. absinthium* is a plant characterized by a notable phenolic density. The elevated concentrations of two pharmacologically significant chemicals, resveratrol (18681,78 µg/kg) and salicylic acid (20364,10 µg/kg), suggest that this species possesses considerable potential for biological action. Resveratrol is a significant chemical in numerous scientific investigations owing to its antioxidant, cardioprotective, anti-inflammatory, and anticancer attributes (Bejenaru et al., 2024). This stilbene derivative, recognized for its capacity to mitigate oxidative stress and delay cellular aging, holds considerable medicinal potential, especially in polyphenol-abundant flora. Salicylic acid is a phenolic acid recognized for its ability to modulate inflammation in both plant defense mechanisms and human health (Arif, 2015). Furthermore, the identification of

significant phenolic chemicals, including acetohydroxamic acid, hydroxycinnamic acid, 4-hydroxybenzoic acid, syringic acid, and wuercetin in the *A. absinthium* species demonstrates that the phenolic structure of this plant is both flexible and useful. Quercetin is a notable flavonoid recognized for its antioxidant and anti-inflammatory actions, as well as its cell-protective characteristics (Mohammed et al., 2023). Compounds like hydroxycinnamic acid and syringic acid provide beneficial effects through their free radical scavenging and hepatoprotective activities (Srinivasulu et al., 2018). Moderate concentrations of caffeic acid, phloridzin dihydrate, and kaempferol were also identified in the plant. Caffeic acid is a prevalent phenolic acid known for its significant antioxidant properties and its ability to inhibit lipid peroxidation (Yazar et al., 2025). Phloridzin dihydrate is a flavonoid glycoside typically present in fruits like apples, recognized for its antidiabetic properties; Kaempferol is a flavonoid often investigated in cancer research for its apoptosis-inducing capabilities that facilitate cell regeneration (Londzin et al., 2018; Imran et al., 2019). The simultaneous presence of these chemicals indicates that *A. absinthium* exhibits a robust composition of antioxidant and anti-inflammatory activities, which may empirically validate the species' traditional applications. Consequently, the phenolic composition of this plant presents a pathway for additional investigation regarding its biological efficacy and prospective phytotherapeutic uses.

The study of phenolic compounds indicated that *T. parthenium* exhibits a notable phenolic profile characterized by both high content density and structural variety. The elevated concentrations of pharmacologically significant compounds, including acetohydroxamic acid (13406,36 µg/kg), quercetin (15321,20 µg/kg), salicylic acid (25962,96 µg/kg), and Hydroxycinnamic acid (33258,60 µg/kg), serve as crucial indicators substantiating the antioxidant and anti-inflammatory potential of this plant. Hydroxycinnamic acid is a phenolic acid recognized for its free radical scavenging properties and its involvement in preventing oxidative damage (Razzaghi-Asl et al., 2013). Salicylic acid serves as a precursor molecule in both traditional medicine and contemporary pharmaceuticals, owing to its analgesic and antipyretic properties (Singh and Vijayan, 1974). Quercetin, a potent flavonol antioxidant, is linked to various biological activities, including the stabilization of cell membranes, the suppression of inflammation, and the inhibition of cancer cell proliferation (Mohammed et al., 2023). Flavonoid-rich plants are often assessed in pharmacological

research because of their abundant polyphenolic composition. Acetohydroxamic acid, a compound that inhibits specific enzymes, is recognized for its antibacterial and antiurolytic properties (Musher et al., 1974; Griffith et al., 1978; Raikwar and Patel, 2022). Additional phenolic substances, including phloridzin dihydrate, myricetin, and 4-hydroxybenzoic acid, were identified in *T. parthenium*, although present in minimal quantities, enhances the chemical variety of this species. Phloridzin dihydrate is recognized for its support of the antioxidant defense system and modulation of glucose absorption due to its flavonoid glycoside structure, whereas myricetin is a flavonoid acknowledged for its DNA damage prevention and neuroprotective attributes (Song et al., 2021; Tian et al., 2021). 4-hydroxybenzoic acid possesses antibacterial properties and is regarded as a natural preservative (Kosová et al., 2015). The amalgamation of these chemicals indicates the phenolic content of *T. parthenium* exhibits equilibrium in both quantity and diversity, which may augment biological activity via potential synergistic effects. Consequently, this species may be regarded as a potential candidate for additional pharmacological investigations concerning therapeutic applications of phenolic chemicals.

Analyses of phenolic content indicate that *S. othonnae* possesses a highly intricate and abundant phenolic profile. The exceptionally elevated concentrations of chlorogenic acid (306779,29 µg/kg) and kaempferol (84241,77 µg/kg) serve as significant markers for assessing the biological activity potential of this plant. Chlorogenic acid is a prominent and powerful phenolic acid recognized for its significant antioxidant activities, with its anti-inflammatory, hepatoprotective, and antibacterial effects. This chemical may demonstrate regulatory effects on lipid metabolism and has been linked to antidiabetic action in certain studies (Naveed et al., 2018). Kaempferol is a flavonoid molecule that participates in various biological processes, including cellular regeneration, mitigation of oxidative stress, and modulation of apoptotic mechanisms. Numerous in vitro and in vivo investigations have corroborated the anticancer, anti-inflammatory, neuroprotective, and cardiovascular protective properties of kaempferol (Chen and Chen, 2013). The significant cohabitation of these two chemicals indicates that *S. othonnae* is a plant that warrants meticulous evaluation from a therapeutic standpoint. Moreover, substances including fumaric acid, protocatechuic acid, hydroxycinnamic acid, and salicylic acid, identified in elevated concentrations in this species, underscore the plant's phenolic abundance. Fumaric acid is an intermediate

molecule implicated in mitochondrial energy metabolism and has been noted for its potential in immune system modulation and the therapy of neurological disorders (Lee et al., 2012). Protocatechuic acid is a phenolic acid recognized for its antioxidant, antibacterial, and cytoprotective characteristics. This chemical may also provide protective actions at the cellular level through its free radical scavenging properties (Kakkar and Bais, 2014). Compounds like hydroxycinnamic acid and salicylic acid establish a foundation for mitigating inflammatory reactions and diminishing oxidative damage (Sova and Saso, 2020; Rosheen et al., 2023). This multifaceted and compact phenolic framework suggests that *S. othonnae* is a species deserving of pharmacological scrutiny. The elevated concentrations of these chemicals, both in quality and quantity, indicate that this species may possess significant synergistic biological effects and could be a vital candidate among natural antioxidant sources. Future investigations using biological activity assessments and cellular-level mechanistic analyses of this species may elucidate the therapeutic potential of phenolic compounds more distinctly.

Based on examinations of phenolic compounds, *T. farfara* is distinguished among all examined species for its elevated ellagic acid concentration (431477,01 µg/kg). This remarkable concentration indicates that the species may have substantial antioxidant and biopharmacological potential. Ellagic acid is a polyphenolic molecule that has been extensively studied in recent years for its antioxidant, anticancer, anti-inflammatory, and antiproliferative activities. It is recognized for its capacity to prevent DNA damage, regulate the cell cycle, and induce apoptosis (Vattem and Shetty, 2005). The substantial presence of this chemical markedly enhances the biological activity potential of *T. farfara*. Other notable substances found in this species encompass phenolic structures with potent antioxidant effects, including acetohydroxamic acid, catechin hydrate, gallic acid, hydroxycinnamic acid, quercetin, 4-hydroxybenzoic acid, phloridzin dihydrate, and myricetin. Catechinhydrate is a powerful flavonoid that actively mitigates oxidative stress via its free radical scavenging ability (Kaur et al., 2017). Gallic acid is a phenolic compound that has become significant in cancer research, especially for its antibacterial, antioxidant, and antiproliferative properties (Şabik et al., 2024). Quercetin, a flavonoid that mitigates cellular stress, is recognized for its ability to stabilize cell membranes, modulate inflammation, and exhibit specific toxicity towards tumor cells (Mohammed et al.,

2023).Moreover, numerous studies have demonstrated the regulation of glucose absorption and anti-aging potential of phloridzin dihydrate, the neuroprotective and anticancer properties of myricetin, as well as the hepatoprotective and antimicrobial activities of hydroxycinnamic acid and 4-hydroxybenzoic acid (Manuja et al., 2013; Sova and Saso, 2020; Song et al., 2021; Tian et al., 2021). The simultaneous presence of several chemicals in the same plant suggests that *T. farfara* exhibits a substantial content of phenolic chemicals, both in abundance and variety. The data indicate that *T. farfara* is an exceptional natural resource due to its phenolic content and possesses the potential to enhance traditional applications while being assessed in contemporary phytotherapeutic methods. The complex chemical composition of this species, particularly ellagic acid, warrants additional biological and clinical research to explore its synergistic effects.

CHAPTER 6
CONCLUSION AND RECOMMENDATIONS

In this study, ethanol and methanol extracts of seven different medicinal plant species (*A. cotula, A. falcata, S. kotschyi, A. absinthium, T. parthenium, S. othonnae,* and *T. farfara*) collected from Osmaniye province were evaluated. Their antioxidant, antimicrobial, antiproliferative, anticholinesterase activities, total phenolic and flavonoid contents, and essential oil compositions were comprehensively determined. The findings demonstrated that *A. absinthium* is a versatile medicinal plant, particularly notable for its high antioxidant capacity, anticholinesterase activity, and phenolic richness. Species such as *T. parthenium* and *A. cotula* stand out for both their antioxidant activity and antimicrobial potential, while *T. farfara*, with its particularly high ellagic acid content, exhibited a phenolic compound-rich profile, supporting its antiproliferative activity. On the other hand, although *S. kotschyi* and *S. othonnae* were rich in some phenolic compounds, their overall biological activity was found to be relatively weaker. However, their antifungal potential, essential oil components, and phenylpropanoid concentrations are particularly noteworthy for pharmacological research.

The solvent types used in the study were found to cause significant differences in the biological activities of the herbal extracts. In general, extracts prepared with ethanol solvents were found to have higher antiproliferative and anticholinesterase activities, while methanol extracts exhibited stronger antioxidant activity in some species. This demonstrates that solvent polarity is a determining factor in the variety and quantity of phenolic compounds extracted, and that selecting appropriate extraction strategies based on the targeted biological effect is crucial. For example, while the methanol extract of *A. absinthium* was notable for its high TAS, DPPH, and FRAP values, the ethanol extract of the same plant was found to be more effective in terms of anticholinesterase and antiproliferative effects. These data indicate that optimizing the solvent selection for each plant species is critical for increasing biological activity.

The essential oil components obtained through GC-MS analyses provided important clues about the chemical basis of the pharmacological effects of the plants studied. Various fatty acid methyl esters, sesquiterpenes, phenylpropanoids, and monoterpene derivatives were identified as key compounds in many species, and consistent relationships were established between the known antioxidant, antimicrobial, and anti-inflammatory effects of these compounds and the biological activities observed in the study. Species with higher concentrations of compounds such as caryophyllene, α-terpinenyl acetate, anethole, d-limonene, and quercetin were found to have higher biological activity. However, variations in chemical composition were evident among species. For example, the predominance of anethole in the essential oil of *S. othonnae* suggests that this plant may exhibit antimicrobial and phytoestrogenic effects due to the effects of its phenylpropanoid building blocks.

Phenolic compound analyses provided the opportunity to more thoroughly assess the antioxidant and pharmacological potential of plant species. Species *S. kotschyi, T. farfara, A. absinthium*, and *A. falcata* in particular, have been found to possess a compound profile rich in various phenolic acids (chlorogenic acid, fumaric acid, hydroxycinnamic acid), flavonoids (kaempferol, quercetin, myricetin), and stilbenes (resveratrol). Each of these compounds exerts antioxidant, anti-inflammatory, antiproliferative, and neuroprotective effects through different biological mechanisms. The high ellagic acid content of *T. farfara*, in particular, suggests that this species could be a valuable candidate for anticancer research. However, further fractionation and isolation studies are required to better understand the bioavailability and synergistic effects of these compounds.

Antimicrobial activity tests have shown that some plant extracts, in particular, are effective against Gram-positive bacteria and some fungal species. The significant antimicrobial activity of ethanol and methanol extracts of *A. cotula, A. absinthium*, and *T. parthenium* against *S. aureus, E. faecalis* and some *Candida* species suggests that these plants can be considered natural antibacterial and antifungal agents. However, some plants exhibited limited activity against only certain microorganisms, suggesting that each species may have unique activity profiles against different infectious agents.

Anticolinesterase activity findings revealed the inhibitory capacity of the plant extracts on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), with *A. absinthium*

in particular, demonstrating strong inhibitory properties against these enzymes. However, the IC_{50} values obtained were quite high compared to the standard drug galantamine, suggesting that these plants should be considered as a source of inhibitory compounds rather than as direct therapeutic agents. In this context, it is recommended to isolate more effective compounds by fractionating plant extracts with high inhibitory activity and to elaborate on their inhibitory mechanisms.

Antiproliferative activity analyses were determined through in vitro tests on the A549 lung cancer cell line, and ethanol extracts, in particular, were found to exhibit high cytotoxic effects in some species. *A. cotula, A. falcata, S. kotschyi*, and *T. farfara* species significantly reduced cell viability at high concentrations, and this efficacy is thought to be related to the phenolic and flavonoid compounds present in the extracts. However, the concentration-dependent nature of the antiproliferative effects and the low efficacy profile of some species suggest that the therapeutic potential of these extracts must be confirmed by further in vivo and mechanistic studies.

Overall, this study has detailed the biological potential of plant species from the Osmaniye flora, demonstrating that these plants possess scientifically significant biological effects beyond their commonly known uses. However, since the findings are only valid in vitro, these effects need to be confirmed in vivo, including bioavailability determinations, toxicological safety tests, and trials on target disease models. Furthermore, based on the data obtained, it is anticipated that some plants could potentially be considered dietary supplements, natural preservatives, cosmetic raw materials, or pharmaceutical precursors, and that formulations with enhanced bioavailability could be developed within this scope.

In conclusion, this study has scientifically demonstrated the versatile pharmacological potential of seven different plants from the Osmaniye flora, providing a strong basis for evaluating certain species as natural antioxidants, antimicrobials, and anticholinesterase agents. In future studies, purifying the active compounds of these species, evaluating them in new formulations, and supporting their production with biotechnological approaches will enable these natural resources to be made available to modern medicine in a sustainable manner.

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