International Journal of Secondary Metabolite
2025, Vol. 12, No. 4, 886–897
https://doi.org/10.21448/ijsm.1629765
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

Investigation of antimicrobial effects of essential oil and secondary metabolite extraction from *Ruscus aculeatus* L. (Asparagaceae)

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ARTICLE HISTORY
Received: Jan. 30, 2025
Accepted: June 21, 2025

KEYWORDS
Antimicrobial,
Disc diffusion method,
Ruscus aculeatus,
Secondary metabolite,
Oil extraction.

Abstract: *Ruscus aculeatus* L. (Asparagaceae) is an important species with medicinal, industrial, and ornamental uses. In this study, the oil extract and secondary metabolites obtained from the fruit of *R. aculeatus* were tested against some bacterial and fungal pathogens to investigate their antimicrobial properties. *R. aculeatus* plant was collected from Yalıncak/Trabzon in May 2024. To get essential oil, the ripe fruits of the *R. aculeatus* plant dry weight were weighed, crushed with a mortar and transferred to a Soxhlet cartridge. And for getting secondary metabolites, 30 grams of ground leaves were placed in two different jars containing 100 mL of ethanol and methanol. The crude extracts and the solvents were evaporated in a rotary evaporator to obtain crude extracts. The extract obtained was used for antimicrobial tests by the disc diffusion method. Firstly, each pathogen was inoculated into Mueller-Hinton Agar (MHA) medium by smear inoculation. Then, antibiotic discs were placed in the medium. Each disc was impregnated with 30 µl of extract. The same volume of kanamycin was used as a control. After 24 and 48 hours, the diameters of the zones formed around the discs were measured and recorded. In the antimicrobial experiment, the analysis of the ES column revealed that the compound demonstrated no efficacy against *E. cloaceae, S. epidermidis, K pneumoniae, and C. albicans* ($p>0.01$). However, it was observed to form a significant inhibition zone in other bacterial groups. At the end of the study, it was determined that the extraction was effective on *Bacillus subtilis* ATCC 6633.

1. INTRODUCTION

Ruscus aculeatus, commonly known as butcher's broom, is a perennial shrub from the Asparagaceae family, native to Europe and parts of Asia. *R. aculeatus* is a plant recognized for its diverse array of secondary metabolites, particularly steroidal saponins, which have attracted considerable attention in pharmacological research. These compounds, primarily found in the rhizomes and roots of the plant, exhibit various biological activities, including anti-inflammatory, vasoprotective, and cytotoxic effects (Masullo *et al*., 2016; Sobolewska *et al*., 2020; Walasek-Janusz *et al*., 2022). Steroidal saponins such as ruscogenin and neoruscogenin

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e-ISSN: 2148-6905

are found especially in the roots and rhizomes; these saponins have been associated with anti-inflammatory, venotonic, and diuretic effects (Petrova *et al*., 2021). In addition, other secondary metabolites such as flavonoids, sterols, and triterpenoids are also initially located in different parts, especially in the leaves and fruits (Schulz *et al*., 2004). Its significance in traditional and modern medicine is well-documented, primarily due to its rich composition of bioactive compounds, particularly steroidal saponins and flavonoids, which confer various therapeutic properties. Recent investigations have utilized advanced analytical techniques, such as high-performance liquid chromatography (HPLC), to extract and quantify these bioactive saponins from *R. aculeatus* (Walasek-Janusz *et al*., 2022). The variability in the concentration of these metabolites is influenced by several factors, including the plant's age, geographical origin, and extraction methods (D'Antuono & Lovato, 2003; Walasek-Janusz *et al*., 2022). The concentration and composition of bioactive metabolites in *Ruscus aculeatus* are influenced by several factors, including plant age, geographical origin, and extraction methods. For instance, older plants often accumulate higher levels of steroidal saponins such as ruscogenin and neoruscogenin, which are key pharmacologically active compounds (Sticher, 2008). Additionally, geographical variation plays a significant role, as environmental conditions such as soil type, altitude, and climate can modulate secondary metabolite biosynthesis (Pavlović *et al*., 2013). Extraction techniques also critically affect metabolite yield and profile; methods such as ultrasound-assisted or supercritical fluid extraction have been shown to enhance the recovery of saponins compared to traditional maceration or Soxhlet extraction (Wang *et al*., 2018). Such variability underscores the importance of standardizing extraction protocols to ensure consistent quality and efficacy in therapeutic applications. Additionally, the structural diversity of saponins, including both spirostanol and furostanol types, contributes to the pharmacological versatility of *R. aculeatus*, allowing for a range of potential health benefits (Masullo *et al*., 2016; Sobolewska *et al*., 2020). Historically, *R. aculeatus* has been utilized in folk medicine for its purported benefits in treating conditions such as chronic venous insufficiency, hemorrhoids, and edema, making it a valuable resource in herbal pharmacotherapy (Bihari *et al*., 2022; Nath & Kültür, 2022; Urbanek, 2017; Vanscheidt *et al*., 2011). *R. aculeatus* has various ethnobotanical uses in traditional medicine. It is widely used in the treatment of skin diseases, rheumatic disorders, and digestive system decline due to its anti-inflammatory, analgesic, and antiseptic properties (Kaya & Demirci, 2018; Öztürk *et al*., 2015). In addition, the wound healing effects of extracts obtained from its initial leaves and roots also have an important place in folk medicine (Yılmaz *et al*., 2020). The ethnobotanical applications of *R. aculeatus* are diverse, with documented uses ranging from culinary to medicinal. In regions like Türkiye, it has been traditionally employed for ailments such as prostate issues and kidney stones (Nath & Kültür, 2022). In Southern Italy, the young shoots of *R. aculeatus* have been harvested for food, reflecting its dual role as both a medicinal and edible plant (Dolina & Łuczaj, 2014; Sanzo *et al*., 2013). This versatility underscores the importance of *R. aculeatus* in local diets and traditional healing practices, highlighting its cultural significance alongside its pharmacological potential. Recent studies have elucidated the pharmacological mechanisms underlying the therapeutic effects of *R. aculeatus*. For instance, its extracts have been shown to exert venotonic effects, which help alleviate symptoms associated with chronic venous disorders (Bihari *et al*., 2022; Urbanek, 2017). It has been reported that extracts with high saponin content obtained from the roots of *R. aculeatus* (butcher's broom) increase venous tone, disperse capillary permeability, and improve lymphatic drainage, especially thanks to the components ruscogenin and neoruscogenin (Belcaro *et al*., 2011; Pittler & Ernst, 2004). Thanks to these effects, *R. aculeatus* extracts are used to alleviate chronic venous insufficiency but also to alleviate the feeling of heaviness, fatigue, and pain (Schaefer *et al*., 2003). This herbal-based treatment offers an attractive option, especially for those seeking alternatives to traditional pharmacotherapies. Clinical trials have demonstrated that formulations containing *R. aculeatus* can significantly improve patients' quality of life for those suffering from chronic venous insufficiency by reducing leg heaviness and discomfort (Jj *et al*., 2009; Vanscheidt *et al*., 2011).

Furthermore, the presence of anthocyanins in its berries suggests potential applications in the food industry as natural colorants, expanding its utility beyond medicinal uses (Longo & Vasapollo, 2004). In addition to its medicinal properties, *R. aculeatus* faces ecological challenges due to overharvesting and habitat loss, which threaten its populations in the wild (Buse–Dragomir, 2023; Khiem *et al*., 2018). *R. aculeatus* is an important species that is distributed in the Mediterranean climate forest understory and is also evaluated as a therapeutic and ornamental plant. However, this species is facing decreasing expansion pressure due to habitat destruction, deforestation, excessive rainfall, and climate change-related stress factors (Ionkova, 2009; Petrova *et al*., 2015). Especially excessive use of roots and rhizomes with pharmaceutical products threatens the natural distribution of the species. Therefore, in recent years, various conservation biology proposals have been put forward for the sustainable use of *R. aculeatus*, preservation of its genetic diversity, and habitat restoration (Demirtaş *et al*., 2020; Petrova *et al*., 2015). *R. aculeatus* has garnered significant attention in recent years for its bioactive compounds. These compounds are primarily extracted from the leaves and stems of the plant and are known for their diverse chemical composition, including various terpenes and phenolic compounds. The pharmacological potential of these extracts has been explored in numerous studies, highlighting their antimicrobial, antifungal, and antioxidant properties (Rodrigues *et al*., 2021; Vanscheidt *et al*., 2011). Research has demonstrated that the extracts of *R. aculeatus* exhibit notable antimicrobial activity against a range of pathogens, including bacteria and fungi. For instance, the extracts have been shown to possess significant antifungal properties, which may be attributed to their high content of volatile compounds (Rodrigues *et al*., 2021; Vanscheidt *et al*., 2011). The efficacy of these extracts in combating microbial infections positions *R. aculeatus* as a promising candidate for natural antimicrobial agents, particularly in the context of increasing resistance to conventional antibiotics (Rodrigues *et al*., 2021; Vanscheidt *et al*., 2011). Furthermore, the antioxidant activity of *R. aculeatus* extracts has been a focal point of research, with studies indicating that these extracts can effectively scavenge free radicals and reduce oxidative stress (Rodrigues *et al*., 2021). This property is particularly relevant in the context of chronic diseases, where oxidative damage plays a critical role in disease progression. The phenolic compounds identified in the extracts are believed to contribute significantly to this antioxidant capacity, thereby enhancing the therapeutic potential of *R. aculeatus* in managing oxidative stress-related conditions (Rodrigues *et al*., 2021). In addition to their antimicrobial and antioxidant properties, the extracts of *R. aculeatus* have been investigated for their potential applications in food preservation and cosmetic formulations. The natural antimicrobial properties of these extracts can be harnessed to extend the shelf life of food products and to enhance the safety and efficacy of cosmetic products (Rodrigues *et al*., 2021; Vanscheidt *et al*., 2011). As consumer demand for natural and plant-based products continues to rise, the exploration of *R. aculeatus* extracts presents a valuable opportunity for innovation in these industries. Conservation efforts, including micropropagation techniques, are being explored to ensure the sustainability of this valuable species while meeting the growing demand for its medicinal applications (Khiem *et al*., 2018; Moyano *et al*., 2006). Thus, *R. aculeatus* represents a critical intersection of traditional knowledge, modern pharmacology, and conservation biology, warranting further research and sustainable practices to preserve its benefits for future generations. The extracts of *R. aculeatus* represent a rich source of bioactive compounds with significant antimicrobial, antioxidant, and potential preservative properties. Ongoing research into their chemical composition and biological activities will further elucidate their therapeutic potential and pave the way for their application in various fields, including medicine, food science, and cosmetics. In this context, this study aimed to test the antimicrobial effects of secondary metabolites and essential oils extracted from locally collected *R. aculeatus* on some pathogens.

2. MATERIAL and METHOD

2.1. Collecting and Drying the Plant

Ruscus aculeatus specimens were collected in May 2024 with stems, leaves, and fruits from a deeded garden in the Yalıncak neighbourhood of Ortahisar district of Trabzon province. The coordinates of the location are 400 57 44.77' N; 390 48' 41.21' E, and its altitude is 209 m. The species identification of the plant was carried out by Dr Hanife Erdoğan Genç, a staff member of the Eastern Black Sea Forestry Research Institute. *R.aculeatus* is selected for structural adaptations that are modified, spine-like, and replace the central photoneuronal function. After the leaves and fruits were removed from the branches, all of these organs of the plant (branch, leaf, fruit) were prepared for secondary metabolite and essential oil extraction.

2.2. Secondary Metabolite Extraction

Secondary metabolite extraction was performed according to the adaptation of the methods described in Jones and Kinghorn (2012). For this, a total of 60 grams of *Ruscus* leaves were ground with a food processor. Then, 30 grams of ground leaves were placed in two different jars containing 100 mL of ethanol and 100 mL of methanol. The branch of the plant was finely cut with garden shears, and 30 grams each was placed in two different jars containing 100 mL each of ethanol and methanol. The samples were kept in glass jars with lids for seven days in the dark and at room temperature. After incubation, the filtrate was separated from mycelia by filtration through 11 mm filter paper (grade 1 filter paper, Whatman Inc., Picataway, NJ) and then with sterile 8-mm and 2-mm filters (Millipore, Billerica, MA) to obtain a cell-free filtrate. The crude extracts were then filtered through filter paper, and the solvents were evaporated in a rotary evaporator (Model Heidolph, Germany) at 40°C and 80 rpm to obtain crude extracts.

2.3. Essential Oil Extraction

The extracted fruits of *Ruscus aculeatus* were first dried in an oven at 40°C for 1 day. Then the weight of the dried fruits was weighed, and the fruits were thoroughly crushed in a mortar and pestle. The crushed dried fruit samples were transferred to the Soxhlet cartridge, and 250 mL n-hexane was added to the 500 mL flask of the device, and the Soxhlet device (Model Eflab, Turkey) was operated at temperature setting number 1. The flask containing the mixture of n-Hexane and oil was placed in a rotary evaporator, and the n-Hexane was evaporated at 40°C and 80 rpm, and the volume of the remaining oil was calculated (Adıgüzel *et al*., 2009; Awang *et al*., 2016). For the extraction of essential oil from the leaves and stems of the plant, the materials were weighed and finely ground. Then, they were transferred to a Soxhlet cartridge as in the fruit, and extraction was performed using n-Hexane at a temperature setting of 1. After the solvent was evaporated in a rotary evaporator at 40°C and 80 rpm, the volume of the remaining oil was calculated (Adıgüzel *et al*., 2009; Awang *et al*., 2016).

2.4. Antimicrobial Effects of Extracts

Crude secondary metabolites obtained from the branches and leaves of the plant and crude essential oil extracts obtained from the branches, leaves, and fruits of the plant were tested on nine pathogenic microorganisms (*Staphylococcus aureus* ATCC25923, *Enterococcus faecalis* ATCC51299, *Serratia marcescens* ATCC13880, *Salmonella typhimurium* ATCC13311, *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC2471, *Staphylococcus epidermidis* ATCC14990, *Klebsiella pneumoniae* ATCC700603, and *Candida albicans* ATCC10351) by the disc diffusion method. Firstly, each pathogen was inoculated into Mueller-Hinton Agar (MHA) medium by smear inoculation. Then, antibiotic discs were placed in the medium. Each disc was impregnated with 30 µl of extract. Kanamycin (for bacteria, 1 mg mL^{-1}) antibiotic, and fluconazole (10 µg/disc, for fungi) were used as positive controls, and the pure solvent DMSO was used as a negative control. After 24 and 48 hours, the diameters of the zones formed around the discs were measured and recorded.

2.4. Statistical Analysis

One-way analysis of variance (ANOVA) was run to determine any significant differences in the study groups. A Duncan multiple range test was performed using SPSS (Statistical Package for Social Sciences, version 28, Chicago, IL, USA), and the significance level was set at $p < 0.05$.

3. RESULTS

After the experiment, the secondary metabolites obtained from ethanol were derived from 1.46 g/mL of leaves and 1.07 g/mL of stems, while the secondary metabolites obtained from methanol were derived from 2.2 g/mL of leaves and 0.57 g/mL of stems. At the end of the process, 0.4 g (from leaf), 0.83 g (from branch), and 1.1 g (from fruit) oil were obtained from 40 g of leaf, 40 g branch/stem and 30 g fruit, respectively. In the antimicrobial experiment, the analysis of the ES column revealed that the compound demonstrated no efficacy against *E. cloaceae*, *S. epidermidis*, *K pneumoniae* and C. *albicans* (p>0.01). However, it was observed to form a significant inhibition zone in other bacterial groups (Table 1).

Table 1. Antimicrobial activity of secondary metabolites against human pathogens bacteria and fungi.

Bacteria/Fungi	EtOH: stem (ES)	EtOH: leaf (EL)	MeOH: stem (MS)	MeOH: leaf (ML)	DMSO	Control*
Escherichia coli ATCC 2471	11.28±0.95^{g}	6.57±1.22^{j}	6.85±1.01^{j}	NZ	NZ	17.60±0.89^{e}
Enterobacter cloaceae ATCC2468	NZ	6.74±0.49^{j}	NZ	10.01±0.72^{gh}	NZ	25.94±0.20^{a}
Bacillus subtilis ATCC 6633	8.40±0.78^{i}	11.72±1.12^{g}	16.45±0.90^{ef}	9.42±0.70^{h}	NZ	23.33±0.77^{b}
Serretia marcescens ATCC13880	10.23±0.94^{gh}	8.69±1.12^{i}	11.35±0.50^{g}	NZ	NZ	18.54±0.30^{de}
Staphylococcus aureus ATCC25923	11.93±1.00^{g}	8.34±0.90^{i}	NZ	NZ	NZ	19.82±1.15^{d}
Salmonella typhimurium ATCC13311	11.91±0.47^{g}	NZ	6.93±0.22^{j}	NZ	NZ	19.13±0.09^{d}
Enterococcus feacalis ATCC 51299	8.18±0.89^{i}	7.86±0.91^{i}	9.38±0.75^{h}	8.47±0.82^{i}	NZ	15.38±0.97^{f}
Staphylococcus epidermidis ATCC14990	NZ	NZ	NZ	NZ	NZ	23.32±0.75^{b}
Klebsiella pneumoniae ATCC700603	NZ	NZ	NZ	NZ	NZ	21.33±0.51^{c}
*Candida albicans*ATCC10351	NZ	NZ	5.86±1.00^{h}	NZ	NZ	10.79±1.04^{gh}

* Kanamycin for bacteria/ fluconazole for fungi.

There is no statistically significant difference ($p > 0.05$) between values bearing the same letter in the same row or column. Means represented by different letters are statistically significant at $p < 0.05$. Letters are assigned based on post-hoc test results for multiple comparisons. NZ: No effect observed (no zone of inhibition). Inhibition zones are shown in mm.

The examination of the EL column revealed that it was ineffective against *S. typhimurium, S. epidermidis, K. pneumoniae,* and *C. albicans* (p>0.01). However, it demonstrated greater efficacy against *B. subtilis* compared to the other strains tested (F (4,10) = 149.036, p<0.01) (see Table 1). The results demonstrated that the secondary metabolites derived from the stem exhibited greater efficacy than the leaf extract, with an inhibition zone of 11 mm against *E. coli, S. aureus* and *S. typhimurium* (see Table 1) The MS column was found to be ineffective against, E. *cloaceae*, *S. aureus*, *S. epidermidis* and *K. pneumonia* (p>0.01). However, it demonstrated a notable effect against *B. subtilis*, with a recorded zone diameter of 16.45 mm (p<0.01). An examination of the ML column reveals that it has been demonstrated to be effective against *E.*

cloaceae (F (4,10) = 2102.81, p<0.01), *B. subtilis* (F (4,10) = 149.036, p<0.01) and *E. feacalis* (F (4,10) = 38.63, p<0.01).

A comprehensive examination of the data revealed that all metabolites demonstrated efficacy against both *B. subtilis* and *E. feacalis*, while only MS exhibited activity against *C. albicans*. It was determined that EtOH: stem (ES) extraction was effective against *Salmonella typhimurium* ATCC13311. In particular, the high zone created by MS on *B. subtilis* in comparison to other strains indicates that secondary metabolites derived from this plant may have potential applications, particularly in the treatment of infections caused by *B. subtilis* (see Table 1). In the application of essential oil, only the oil obtained from the fruit was effective on *B. subtilis* and formed a 10.37 mm inhibition zone (F (4.10) = 21383.68, p<0.01) (see Table 2).

Table 2. Antimicrobial activity of essential oil against human pathogens, bacteria, and fungi.

Bacteria/Fungi	Stem (ES)	Leaf (EL)	Fruit	n-Hexane	Control*
Escherichia coli ATCC 2471	NZ	NZ	NZ	NZ	17.60±0.89^{e}
Enterobacter cloaceae ATCC2468	NZ	NZ	NZ	NZ	17.62±0.20^{a}
Bacillus subtilis ATCC 6633	8.40±0.78^{i}	11.72±1.12^{g}	16.45±0.90^{ef}	NZ	23.33±0.77^{b}
Serretia marcescens ATCC13880	NZ	NZ	NZ	NZ	18.54±0.30^{de}
Staphylococcus aureus ATCC25923	NZ	NZ	NZ	NZ	19.82±1.15^{d}
Salmonella typhimurium ATCC13311	NZ	NZ	NZ	NZ	19.13±0.09^{d}
Enterococcus feacalis ATCC 51299	NZ	NZ	NZ	NZ	15.38±0.97^{f}
Staphylococcus epidermidis ATCC14990	NZ	NZ	NZ	NZ	23.32±0.75^{b}
Klebsiella pneumoniae ATCC700603	NZ	NZ	NZ	NZ	21.33±0.51^{c}
*Candida albicans*ATCC10351	NZ	NZ	NZ	NZ	10.79±1.04^{gh}

*Kanamycin for bacteria/ fluconazole for fungi.

There is no statistically significant difference (p > 0.05) between values bearing the same letter in the same row or column. Means represented by different letters are statistically significant at p < 0.05. Letters are assigned based on post-hoc test results for multiple comparisons. NZ: No effect observed (no zone of inhibition). Inhibition zones are shown in mm.

4. DISCUSSION and CONCLUSION

Total secondary metabolites obtained from *Ruscus aculeatus* using two different solvents and two different parts of the plant were tested on different human pathogenic bacteria and fungi, and it was determined that the most effective group was *Bacillus subtilis*. In previous studies, few publications on the effect of the *Ruscus* species on this pathogen were found. Research indicates that secondary metabolites from various plant species, including *Ruscus*, exhibit broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. For instance, studies have shown that extracts containing these metabolites can inhibit the growth of pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli* (Allemailem, 2021; Bahadori *et al*., 2019). The mechanisms of action are believed to involve disruption of bacterial cell membranes and interference with metabolic processes, which are common pathways through which plant-derived compounds exert their antimicrobial effects (Allemailem, 2021). *Ruscus*, particularly *Ruscus aculeatus*, is known for its diverse phytochemical profile, which includes flavonoids, saponins, and terpenoids, all of which have been implicated in antimicrobial activity (Bahadori *et al*., 2019). Studies have indicated that extracts from *Ruscus aculeatus* exhibit significant antimicrobial activity against a range of pathogens. For instance, the antimicrobial properties of *Ruscus* extracts have been linked to their ability to disrupt bacterial cell membranes and inhibit bacterial growth (Thambiraj, 2017). This is consistent with findings from other studies that demonstrate the effectiveness of various

plant extracts against *Bacillus subtilis*, a Gram-positive bacterium known for its resilience and ability to form spores (Thambiraj, 2017). The mechanism of action often involves the interaction of these secondary metabolites with bacterial cell walls or membranes, leading to increased permeability and eventual cell lysis (Vanscheidt *et al*., 2011).

Moreover, the specific compounds within *Ruscus aculeatus* extracts, such as flavonoids, have been shown to possess strong antibacterial properties. Flavonoids can interfere with bacterial metabolism and inhibit the growth of bacteria by affecting their cellular functions (Ambarwati *et al*., 2017). The minimum inhibitory concentration (MIC) values for various plant extracts against *Bacillus subtilis* have been documented, providing a quantitative measure of their antimicrobial potency (Jemil *et al*., 2018). For example, extracts with MIC values below 100 µg/mL are considered to have significant antibacterial activity, which aligns with the observed effects of *Ruscus*-derived compounds (Ambarwati *et al*., 2017).

Ethanol extraction is a widely utilized method for isolating these secondary metabolites, as it effectively dissolves a broad range of polar and non-polar compounds. Studies have shown that ethanol extracts of *Ruscus* leaves exhibit significant antimicrobial activity against a variety of pathogenic bacteria. For instance, the presence of steroidal glycosides, particularly furostanol glycosides, has been identified in these extracts, which may play a crucial role in their antimicrobial efficacy (Napolitano *et al*., 2011). The antimicrobial activity of these compounds is often attributed to their ability to disrupt bacterial cell membranes, leading to cell lysis and death (Anand *et al*., 2019). Ethanol extracts from *Ruscus* leaves have demonstrated notable inhibitory effects against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, while showing varying degrees of activity against Gram-negative bacteria (Anand *et al*., 2019). This selective antimicrobial activity is significant, as Gram-positive bacteria are generally more susceptible to plant-derived antimicrobials due to their simpler cell wall structure. The effectiveness of these extracts can also be influenced by the concentration of active compounds, which varies depending on the extraction method and the specific plant part used (Kaya *et al*., 2020). These secondary metabolites play a crucial role in the plant's defense mechanisms against pathogens, suggesting that they may possess significant antimicrobial properties.

The antimicrobial effects of essential oils derived from various plant sources have garnered significant attention in recent years, particularly regarding their efficacy against Gram-positive bacteria such as *Bacillus subtilis*. Essential oils are complex mixtures of volatile compounds that can exert antimicrobial properties through various mechanisms, including disruption of cell membranes, inhibition of enzymatic activity, and interference with cellular respiration. Research has demonstrated that essential oils possess notable antibacterial activity against *Bacillus subtilis*. For instance, a study indicated that certain essential oils exhibited strong to moderate antimicrobial activity against this bacterium, with inhibition zone diameters ranging from 12 ± 0.00 mm to 22 ± 1.41 mm, and minimum inhibitory concentrations (MICs) between 0.5% and 8% (v/v) (Semde *et al*., 2018). This suggests that essential oils can effectively inhibit the growth of *Bacillus subtilis*, which is crucial for food safety and preservation. The antimicrobial action of essential oils can be attributed to their chemical composition, which often includes monoterpenes and phenolic compounds. These constituents can disrupt the lipid bilayer of bacterial cell membranes, leading to increased permeability and eventual cell lysis (Rapper *et al*., 2016). Specifically, the essential oils from *Psidium guajava* have shown significant antibacterial activity against *Bacillus subtilis*, with inhibition ranges of 6-25 mm, indicating a strong potential for these oils in antimicrobial applications (Hussin *et al*., 2021). Similarly, essential oils from *Juniperus excelsa* have also demonstrated a robust antimicrobial effect against *Bacillus subtilis*, further supporting the notion that plant-derived essential oils can serve as effective natural antimicrobials (Saruhan & Öz, 2023). Moreover, the effectiveness of essential oils can vary based on their chemical profiles. For example, the presence of specific compounds such as thymol and eugenol has been linked to enhanced antimicrobial activity

against various pathogens, including *Bacillus subtilis* (Dadalioglu & Evrendilek, 2004). The synergistic effects of blended essential oils have also been noted, where combinations of different oils can lead to increased antimicrobial efficacy compared to individual oils (Tadtong *et al*., 2016).

The antimicrobial effects of *Ruscus* fruit extracts, particularly when using n-hexane as a solvent, have garnered attention due to their potential applications in treating bacterial infections. *Ruscus aculeatus* contains various bioactive compounds, including ruscogenin, which has been shown to exhibit anti-inflammatory properties and may contribute to its antimicrobial activity (Vieira, 2010; Bahadori *et al*., 2019). The extraction process using n-hexane is significant because it selectively isolates non-polar compounds that may possess enhanced antimicrobial properties compared to other solvents (Edziri *et al*., 2020). Research indicates that n-hexane extracts from various plant sources, including *Ruscus* species, demonstrate broad-spectrum antibacterial activity. For instance, studies have shown that extracts from *Ruscus hypophyllum* exhibit antimicrobial effects against both sensitive and multi-resistant bacterial strains (Edziri *et al*., 2020). This is particularly relevant in the context of rising antibiotic resistance, where natural extracts could serve as alternative therapeutic agents. The efficacy of n-hexane extracts is often attributed to their rich phytochemical profile, which includes saponins and flavonoids that are known for their antimicrobial properties (Bahadori *et al*., 2019). Moreover, the antimicrobial activity of n-hexane extracts is not limited to *Ruscus* alone; other studies have highlighted similar effects in extracts from different plant species. For example, n-hexane extracts of *Annona squamosa* have shown significant antibacterial activity against various bacterial strains (Adesanwo *et al*., 2020). These findings suggest that the antimicrobial effects of n-hexane extracts could be a common characteristic among many plant species, including *Ruscus*, reinforcing the potential of these extracts in pharmacological applications. This highlights the importance of understanding the chemical composition of essential oils to optimize their use in combating bacterial pathogens.

There are many species of medicinal and aromatic plants in the world. Since ancient times, people have used plants both as a nutrient and for therapeutic purposes to relieve health problems. Today, plants have started to be used for medicinal purposes all over the world and in Türkiye. According to World Health Organization (WHO) research, the number of medicinal plants used for therapeutic purposes is around 20,000 (Faydaoğlu & Pürücüoğlu, 2011). Generally, herbal medicines are prepared by obtaining active ingredients from plants included in the group of medicinal plants. Herbal products are often used in the treatment of diseases with chronic medical conditions, including breast cancer (2%), liver diseases (21%), HIV (22%), asthma (24%), and rheumatologic disorders (26%) (Şarışen & Çalışkan, 2005).

The *Ruscus* plant used in this study is of medicinal and commercial importance. This herb has been used in many health studies before. *Ruscus aculeatus* is notable for its rich content of bioactive compounds, particularly steroidal saponins, which contribute to its anti-inflammatory, antihypertensive, and anti-venous properties (Naido *et al*., 2024). Recent studies have isolated new biphenyl derivatives from their rhizomes, demonstrating significant anti-inflammatory and antibacterial activities (Niu *et al*., 2023). Additionally, extracts of *Ruscus aculeatus* have shown protective effects against ischemia-reperfusion injury, enhancing microcirculation and reducing leukocyte adhesion (Souza *et al*., 2024). Its applications extend to traditional medicine, landscape architecture, and cosmetic products (Kebeli & Çelikel, 2024) (CABI "*Ruscus aculeatus* (Butcher s-broom)", 2022). The main phenolics of *Ruscus aculeatus* leaves and stems were identified as quercetin and p-coumaric acid (Luis *et al*., 2011).

In conclusion, the study underscores the therapeutic potential of secondary metabolites derived from the plant, particularly MS and ES extracts, as promising candidates for further research and development in antimicrobial therapies. Essential oils derived from the fruit also warrant attention for their selective efficacy. Further studies are recommended to isolate and characterize the bioactive compounds responsible for the observed activities.

Acknowledgments

We would like to thank to Trabzon Regional Directorate of Forestry and Biological Control Laboratory against Forest Pests for making use of the infrastructure facilities.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Availability of data and material: All the data/information can be obtained with the corresponding author by email, if necessary.

Authorship Contribution Statement

A.S.: Methodology, Writing-original draft and editing Funding acquisition; **R.A.:** Methodology, Writing-original draft and editing Funding acquisition Conceptualization; **S.B.:** Conceptualization, Data curation, Formal analysis, Methodology, Validation, Resources, Writing-review and editing; **M.U.:** Conceptualization, Data curation, Methodology, Validation, Resources, Writing-review and editing, Supervising.

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