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Research Article

Antimicrobial, antioxidant and essential oil studies on *Veratrum album* L. (Melanthiaceae)

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Abstract: In this study, essential oil components of the *Veratrum album* L. and the antimicrobial and antioxidant properties of these components were determined. The chemical composition of the essential oils of dried aerial parts of *V. album* was analyzed using GC and GC-MS. Antimicrobial activity was determined with the disk diffusion method. Total antioxidant status (TAS), total oxidant status (TOS) and 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity were detected for the antioxidant activity of the plant. According to the analysis results, the major essential oil components of the *V. album* were determined as hexacosane (39.5%), myristic (tetradecanoic) acid (22.8%), heptane (6.5%), anethole (4.9%) and 1,8- cineole (4.8%). The findings showed that the methanol extracts of the stem and leaf parts of the plant inhibited the growth of pathogenic microorganisms at different rates (14±0.1 - 34±0.3 mm). The TAS values of methanol extracts of stem and leaf parts of *V. album* were calculated as 3.75±0.07 and 3.91±0.01 mmol, while TOS values were calculated as 6.14±0.13 and 6.54±0.05 µmol. The scavenging activity of the DPPH radical increased depending on increasing concentrations of the plant extract.

1. INTRODUCTION

Plants have continued to play a dominant role in the protection of human health and to be an important alternative treatment method to alleviate the symptoms of diseases since ancient times due to various active substances they contain. Today, with the renewed interest in traditional medicine and the need and demand for more herbal medicines, the importance of studies with medicinal plants has increased even more. This revival of interest in plant-derived medicines stems from the current widespread belief that "green medicine" is safe and more reliable than expensive synthetic drugs, many of which have negative side effects (Nair & Chanda 2007). This has accelerated the search for new antimicrobial agents from various sources, such as medicinal plants (Cordell, 2000). Synthetic drugs are not only expensive and inadequate for the treatment of diseases, but also often have adulteration and side effects.

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Therefore, there is a need to investigate new infection control strategies (Sieradzki *et al.*, 1999; Dabur *et al.*, 2007).

Although the genus *Veratrum* is included in the Liliaceae family in the Flora of Türkiye (Edmondson, 1984), it has been included in the Melanthiaceae family in recent years with systematic and molecular studies (Reveal & Chase 2011; Seberg *et al.*, 2012). The genus *Veratrum*, the largest genus of the Melanthieae tribe is represented by 45 species globally and only one species in Türkiye. Zomlefer *et al.*, (2001; 2003) divided the genus *Veratrum* into two sections; *Veratrum* and *Fuscoveratrum; Fuscoveratrum* has two subsections: *Pseudoanticlea* and *Asiaveratrum*. The phylogenetic study of flower colors by Liao *et al.*, (2007) also supports this grouping. Zomlefer *et al.*, (2001; 2003) also examined the tepal shapes of the species, the nectariums in the tepals and the characteristics of the seeds in detail and evaluated them with the results of the molecular study. The most common *Veratrum* species in the world; *V. album* and *V. nigrum* are distributed in Central, Southern and Southeastern Europe. Species of the genus have a wide distribution range, mostly from temperate North America to Arctic Eurasia. The origin and largest center of diversity of the genus *Veratrum* is East Asia. About 20 of the *Veratrum* species grow naturally in China. Thus, it is very well known and widely used in traditional medicine in China. Medicines prepared from the dry stems and rhizomes of *Veratrum* species are known as "Li-lu" in Chinese sources (Atalay, 2013).

In ancient Chinese medical sources, Li-lu is mentioned as an herb that treats ailments, such as high blood pressure, inflammation, coagulation and spasm (Hollman, 2003; Wen *et al.*, 2005; Tang *et al.*, 2010). Among Indians and pre-industrial Europeans, *Veratrum* species have been used in the treatment of various diseases, including cough, sore throat, tonsillitis, mental illnesses, epilepsy, jaundice, scabies, bacterial infections, snake bites, venereal diseases and injuries (Li *et al.*, 2006; Li *et al.*, 2007; Tanaka *et al.*, 2011). *Veratrum* species are known by local names, such as “hellebore”, “American litter”, “false litter” and “white litter” (Li *et al.*, 2007). The pharmacological activities of *Veratrum* species have attracted attention for over 300 years. Extracts obtained from *Veratrum* species have been used as an insecticide against some harmful insect species until the 1950s (Jacobson, 1958). Thus, phytochemical studies have been conducted on *Veratrum* species since the 1930s, and over 100 alkaloid-type metabolites with different pharmacological properties have been identified (Rahman *et al.*, 1992). In addition to alkaloids, *Veratrum* species have also been reported to contain flavonoids and stilbenoids (Dai *et al.*, 2009; Hanawa *et al.*, 1992; Zhou *et al.*, 1999; 2001; Huang *et al.*, 2008). Among these, the metabolites with the highest biological activity were steroidal alkaloids (Ivanova *et al.*, 2011; Rahman *et al.*, 1992; Sener *et al.*, 1996; Zhou *et al.*, 1999; Zhu *et al.*, 2011). Exposure to alkaloids of the plant *Veratrum* causes similar toxic effects in animals and humans. The symptoms observed with exposure to *Veratrum* alkaloids include hypotension, vomiting (for species with this reflex), salivation, weakness, irregular pulse and slow breathing (Krayer & Acheson, 1946; Mulligan & Munro, 1987; Swiss & Bauer, 1951).

In line with these scientific studies in the literature, the present study aimed to investigate the antimicrobial and antioxidant activities and the mechanism of action of the extracts obtained from the stem and leaves of *Veratrum album*, about which we have little information on its use in folk medicine, despite being widely distributed in Türkiye. In addition, essential oil characterization of the extracts was performed.

2. MATERIAL and METHODS

2.1. Collection of the plant material

The plant material used in the present research was collected from the Kabaca plateau, Okuzyatagi locality in Artvin province (Lat: 41°7’58.019” N, Lon: 41°31’31.899” E, Alt: 2313 m). In addition, the general view of the plant is shown in Figure 1. It was collected from its

natural habitat during its vegetative period in June 2021, and dried in the shade. For this purpose, the plant specimens collected from nature were brought to the laboratory environment in cloth bags with frequent ventilation. Then, they were laid on blotting paper in a sun-free environment and allowed to dry in the shade. Most of the dried samples were used for essential oil studies, while a small amount of samples were used for antimicrobial and antioxidant studies. A group of plant specimens, which were turned into herbarium material, are kept in the Herbarium (FUH) of the Faculty of Science, Fırat University.

Figure 1. General appearance of *Veratrum album*.

2.2. Isolation of the Essential Oils

Air-dried aerial parts (stem and leaves) of the plant materials (250 g) were subjected to hydrodistillation using a Clevenger-type apparatus for five hours to yield essential oils. The obtained essential oil was analyzed using GC and GC-MS. A Shimadzu GC-MS (QP2020 model) with an FID detector was used. The system was equipped with a RXI-5MS (30m x 0.25 mm x 0.25 µm) capillary column through which helium was flowing as carrier gas. The column oven temperature program was as follows, the temperature was set to 40ºC and held for 2 min then heated to the final temperature of 240ºC at a ramp rate of 3ºC/min. The injection volume was selected as 1 µL in the split mode (This process was repeated two times). The column and analysis conditions were the same as in GC-MS, expressed above. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors. The MS results were compared with the Wiley-Nist W9N11 libraries in the device memory (Table 1).

2.3. Determination of Antimicrobial Effect

2.3.1. Test microorganisms

In this study, *Escherichia coli* ATCC25322, *Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae* ATCC 700603, *Bacillus megaterium* DSM32, *Candida albicans* FMC17, *Candida glabrata* ATCC 66032, *Trichophyton* sp. and *Epidermophyton* sp. microorganisms were used as test microorganisms. All microorganism cultures were obtained from Firat University, Faculty of Science, Department of Biology, Microbiology Laboratory culture collection.

2.3.2. Preparation of microorganism cultures and testing for antimicrobial effect

The antimicrobial activity of the plant extracts in methanol was determined according to the disk diffusion method (Collins & Lyne 2004). Bacteria strains (*E. coli*, *S aureus*, *K. pneumoniae*, *B. megaterium*) were incubated in Nutrient Broth (Difco) for 24 hours at 35 ± 1°C, yeast strains (*C. albicans* and *C. glabrata*) were incubated in Malt Extract Broth (Difco) for 48 hours at 25 ± 1°C and dermatophyte fungi (*Trichophyton* sp. and *Epidermophyton* sp.) were inoculated in Glucose Sabouroud Buyyon (Difco) and incubated at 25 ± 1°C for 48 hours. The bacteria, yeast and fungi cultures prepared in broth were inoculated into Mueller Hinton Agar, Sabouraud Dextrose Agar and Potato Dextrose Agar, respectively, at a rate of 1% (10^6 bacteria ml, 10^4 yeast ml, 10^4 fungi ml). After shaking well, 25 ml of the cultures were placed in sterile petri dishes of 9 cm diameter. Homogeneous distribution of the medium was achieved. Antimicrobial discs (Oxoid) of 6 mm diameter, each impregnated with 100 µL (1000 µg) extracts, were lightly placed on the solidified agar medium. After keeping the petri dishes prepared in this way at 4°C for 1.5-2 hours, the plates inoculated with bacteria were incubated at 37 ± 0.1°C for 24 hours, and the plates inoculated with yeast at 25 ± 0.1°C for 72 hours. As controls, different standard discs were used for bacteria (Streptomycin sulfate 10 µg/disc) and yeasts (Nystatin 30 µg/disc). Dimethyl sulfoxide (DMSO) was used for negative control. The zones of inhibition were measured in mm.

2.4. Determination of Antioxidant Activity

2.4.1. Total antioxidant assay (TAS) and total oxidant assay (TOS)

Total antioxidant (TAS) and total oxidant status (TOS) of the plant extracts were determined with Rel Assay kits (Rel Assay Kit Diagnostics, Türkiye). The TAS value was expressed as mmol Trolox equiv./L and Trolox was used as the calibrator (Erel, 2004). The TOS value was expressed as µmol H_2O_2 equiv./L and hydrogen peroxide was used as the calibrator (Erel, 2005).

2.4.2. 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity

The antioxidant activity of the methanol extract of the plant extracts was determined according to the 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity method (Cuendet *et al.*, 1997). The solution was prepared in methanol at a concentration of 25 mg/ml of the extract obtained. The prepared solution was diluted four times and the calibration curve of DPPH was obtained. For this purpose, 40 µL of the prepared solution was taken and added with 160 µL of DPPH solution. After thorough mixing, the vial was closed and kept in the dark for 30 minutes. The same procedures were repeated for all concentrations, and butylated hydroxyanisole (BHA) and methanol was used as a control. Afterwards, the absorbance of each mixture was read at 570 nm in the spectrophotometer. The percentage inhibition values were calculated according to the following equation:

$$I\ (\%) = (A_{control} - A_{sample}/A_{control}) \times 100$$

2.5. Statistical Analyses

Data were presented as mean ± standard deviation (SD) based on three replicates, and the significant difference ($p<0.05$) was determined by independent t-test, one-way and two-way analysis of variance with Duncan's test using SPSS v25.0.

3. RESULTS

The composition of the aerial parts essential oils of *V. album* was analyzed and a total of 21 compounds were identified. According to the results of the analysis, hexacosane (39.5%), myristic (tetradecanoic) acid (22.8%), heptane (6.5%), anethole (4.9%) and 1,8- cineole (4.8%) were the major components. The hydrodistillation of the aerial parts of *V. album* yielded 0.12 % of light yellowish oil. Table 1 lists the components identified from *V. album* corresponding to 87.5 % of the total essential oil and their retention index and percentage composition.

Table 1. Constituents of the essential oils from *V. album*.

No	RT	RI	Area	Name	Aerial parts %
1	5.1	930	48718	α-thujene	1.2
2	5.6	935	14757	α-pinene	0.1
3	5.7	960	58489	β-pinene	1.7
4	5.8	1017	52298	p-cymene	0.9
5	**5.9**	**1033**	**65509**	**1,8-cineole**	**4.8**
6	**6.0**	**1208**	**534856**	**Anethole**	**4.9**
7	62.9	1228	175344	Furan, 2-pentyl-	2.7
8	9.4	1280	55310	2-Heptanone	1.2
9	**9.9**	**1286**	**73828**	**Heptanal**	**6.5**
10	5.9	1404	25972	α-Ionone	2.3
11	8.6	1480	112848	1-Hexanol	0.6
12	12.2	1565	45719	Hydroperoxide, 1-ethylbutyl	0.4
13	12.4	1620	96863	Undecanone	1.0
14	61.9	1680	30506	2,3-Octanedione	0.6
15	10.3	2000	68987	Linoleic acid ethyl ester	0.4
16	12.6	2020	29708	Benzaldehyde	0.2
17	**55.5**	**2476**	**2281061**	**Hexacosane**	**39.5**
18	13.6	2508	43030	Dodecanoic acid	0.9
19	**58.6**	**2680**	**698636**	**Myristic acid**	**22.8**
20	63.4	2740	827808	Pentacosane	1.2
21	64.0	2798	182118	Heptacosane	1.7
				Total	87.5

** RI: retention indices; RT: retention time

The antimicrobial effects of methanol extracts of stem and leaf parts of *V. album* are seen in Table 2. In the results obtained, the antimicrobial effects of the stem and leaf parts of *V. album* against *E. coli*, *S. aureus*, *K. pneumoniae*, *B. megaterium*, *C. albicans*, *C. glabrata*, *Trichophyton* sp., *Epidermophyton* sp. were determined as 29 ±0.5 mm, 20±0.1 mm, 25±0.1 mm, 23±0.2 mm, 25±0.3 mm, 27±0.5 mm, 22±0.1 mm and 15±0.5 mm, respectively. Inhibition zones of the leaf part against *E. coli*, *S. aureus*, *K. pneumoniae*, *B. megaterium*, *C. albicans*, *C. glabrata*, *Trichophyton* sp., *Epidermophyton* sp. were detected as 34±0.3 mm, 25±0.5 mm, 24±0.6 mm, 23±0.0 mm, 19.7±0.6 mm, 25±0.2 mm, 21±0.5 mm and 14±0.1 mm, respectively (Table 2).

Antimicrobial effects of Streptomycin sulfate used as a control against *E. coli*, *S. aureus*, *K. pneumoniae* and *B. megaterium* ranged from 19±0.1 to 30±0.4 mm. The antimicrobial effects of Nystatin against *C. albicans*, *C. glabrata*, *Trichophyton* sp., *Epidermophyton* sp. were determined in the range of 20±0.2 - 25±0.7 mm (Table 2). The effects of extracts prepared from stem and leaf parts on some bacteria, yeast and dermatophyte species were found to be statistically significant ($p<0.05$). Extracts prepared from leaf parts showed a higher effect on *E. coli* and *S. aureus* when compared to extracts prepared from stem parts, while antimicrobial effects on other pathogenic microorganisms were found to be low. In addition, it was observed that the extracts obtained from both stem and leaf parts were more effective on *K. pneumoniae* and *C. glabrata* when compared to the control group (Table 2).

Table 2. Antimicrobial effect of *V. album.*

Microorganism	Stem	Leaf	Control	*F*	*p*
Escherichia coli	29.0±0.5 ^{fx}	34.0±0.3 ^{gy}	30.0±0.4 ^{ez}	*126.0*	*0.0*
Staphylococcus aureus	20.0±1.0 ^{bx}	25.0±0.5 ^{fy}	20.0±0.3 ^{bx}	*55.9*	*0.0*
Klebsiella pneumoniae	25.0±1.0 ^{dx}	24.0±0.6 ^{ex}	19.0±0.1 ^{ay}	*67.8*	*0.0*
Bacillus megaterium	23.0±0.2 ^{cx}	23.0±0.0 ^{dx}	25.0±0.0 ^{dy}	*300.0*	*0.0*
Candida albicans	25.0±0.3 ^{dx}	19.7±0.6 ^{by}	25.0±0.4 ^{dx}	*150.3*	*0.0*
Candida glabrata	27.0±0.5 ^{ex}	25.0±0.2 ^{fy}	20.0±0.2 ^{bz}	*354.5*	*0.0*
Trichophyton sp.	22.0±1.0 ^{cx}	21.0±0.5 ^{cx}	22.0±0.1 ^{cx}	*2.3*	*0.1*
Epidermophyton sp.	15.0±0.5 ^{ax}	14.0±1.0 ^{ax}	25.0±0.7 ^{dy}	*191.3*	*0.0*
F-value	117.9	336.8	341.0		
p-value	0.00	0.00	0.00		

Streptomycin sulfate (10 mg/disc) and Nystatin *(30 mg/disc) were used as standard antibiotic discs. The diameter of the paper discs was 6 mm.

The letters $^{a-g}$ indicate the comparisons in each column, and $^{x-z}$ the comparisons between the rows. Values with the same letters are not different from each other. Each value is expressed as the mean ± SD of three replicates (n=3, $p<0.05$)

TAS and TOS values of leaf parts were found to be higher than stem parts. In the results obtained, the TAS value of the stem and leaf parts of *V. album* was very high, and the TOS value was between normal values (Table 3).

Table 3. TAS and TOS values of parts of *V. album.*

	TAS (mmol Trolox equiv./L)	TOS (μmol H_2O_2 equiv./L)
Stem	3.8±0.7	6.2±0.1
Leaf	3.9±0.1	6.5±0.5
p-value	0.023	0.008

The inhibition percentages of DPPH radical scavenging activity of different concentrations of methanol extracts of stem and leaf parts of *V. album* were determined (Table 4). It was observed that the DPPH radical scavenging activity of stem parts of *V. album* was above 50% at 1000 µg/mL concentration (58.7±0.2) but below 50% at other concentrations. While the DPPH radical scavenging effect of BHA used as positive control was determined as 83.7±0.3%, the DPPH radical scavenging effect of methanol used as negative control was determined as 1.7±0.7%. The findings showed that the percentage of inhibition of the scavenging activity of DPPH radical in all concentrations of the leaf part of the same species was over 50%.

When the obtained results were compared with the controls, it was determined that the closest antioxidant effect to the control was in the methanol extract of the leaf part of *V. album* at 1000 µg concentration (75.5±0.4) (Table 4).

Table 4. Percent inhibition of the DPPH radical of stem and leaf parts of *V. album.*

Concentration	Stem	Leaf
1000 µg/mL	58.7±0.2^{a}	75.5±0.4^{x}
500 µg/mL	38.0±0.8^{b}	64.5±0.4^{y}
250 µg/mL	19.2±0.3^{c}	53.1±0.3^{z}
125 µg/mL	9.6±0.0^{d}	47.3±0.4^{t}
BHA (control I)	83.7±0.3^{e}	
MetOH (control II)	1.7±0.2^{f}	
F-value	20624.6	22928.8
p-value	0.00	0.00

Each value is expressed as the mean ± *SD* of three replicates (n=3, $p<0.05$)

The letters $^{a-f}$ indicate the comparisons in each column, and $^{x-t}$ the comparisons between the rows.

DPPH effects of different concentrations (125-1000 µg/mL) prepared from stem and leaf parts were found to be statistically significant (p<0.05). In addition, it was observed that the mean DPPH values of stem and leaf were statistically different for each concentration value (p<0.05). Depend on increasing concentrations, DPPH values of stem and leaf parts were found to be low when compared to control I (BHA). In terms of DPPH values, it was observed that the values obtained from the leaf were higher than the stem parts (Table 4).

4. DISCUSSION and CONCLUSION

The essential oil components of *V. album* are shown in Table 1. To our best knowledge, the essential oil components of this species were determined for the first time in this study. The essential oils isolated were a complex mixture of monoterpenes, sesquiterpenes and hydrocarbon. On the other hand, it was determined that hydrocarbons made up the higher contribution in *V. album* essential oil. Tabanca *et al.*, (2018) reported that when the active fractions of *V. lobelianum extracts* were further analyzed by GC-MS, ethyl palmitate and ethyl linoleate were identified. The mass spectra and linear retention indices (LRI) values that they determined were comparable with purchased authentic compounds of ethyl palmitate and ethyl linoleate (Tabanca *et al.*, 2018). In our study, the ethyl linoleate (Linoleic acid ethyl ester) content was 0.4%.

The reason for this variability in the composition of essential oils can be attributed to the differences in the geographical regions from which the species are collected and methodological conditions used. The extract of this plant is used in the treatment of various diseases in different countries (URL-1; URL-2). However, the essential oil content of the sample grown in the environment and conditions of our country was revealed for the first time by us in this study. Our aim is to determine the essential oil content of the sample grown in Türkiye in the first place. The environment and seasonal conditions in which the plant grows will of course affect the essential oil content. In addition, the essential oil contained in the plant can differ qualitatively and quantitatively in different phenological periods (vegetative, flowering and fruiting) of the plant. However, in order to standardize this content, it is necessary to determine the essential oil composition of the plant and to determine whether it contains valuable components. Based on the results obtained from this study, we can say that the *V. album* plant has very valuable components and the plant in question can be grown and produced consistently in a greenhouse environment created by imitating its natural habitat. Therefore, this study constitutes a basis for more comprehensive studies.

In another study conducted by Lin *et al.*, (2003) volatile oil of *Hemerocallis flava*, which for close *V. album* species, was obtained by simultaneous distillation–solvent extraction. Afterwards, the essential oil was analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) and 51 components were identified, constituting approximately 92% of the oil. The main components of the essential oil were 3-furanmethanol (47.9%), 2-furancarboxaldehyde (10.4%) and Furan, 2-pentyl (2.7%). When comparing the essential oil composition of the genus *Veratrum* with Hemerocallis genus studies, some similarity was found to be evident (Lin *et al.*, 2003).

It has been determined that the rhizome parts of *V. album* show antiviral effect against HSV at a concentration of 250 µg/mL, and against SINV virus at a concentration of 500 µg/mL (Hudson *et al.*, 2000). It has been reported that the ethanol extract of the same species inhibited the growth of *Mycobacterium tuberculosis* at concentrations lower than 100 µg/mL (Tosun *et al.*, 2005). The antimicrobial effects of *Veratrum* alkaloids against *P. ovale*, *T. mentagrophytes* and *S. cerevisiae* have been tested and found that only jerveratrum alkaloids showed antimicrobial activity (Wolters, 1970) Alkaloids obtained from the *V. album* have antioxidant effects (Atalay *et al.*, 2019).

In this study, hexacosane found at a high rate is a substance with antimicrobial properties. According to a study in which the inhibition zones of isolated terpenoids have also been recorded, the results indicated that hexacosane was more effective against *E. coli* and hexacosanoic acid had a greater activity against *A. flavus* (Singh & Singh 2003). Another study investigating the antimicrobial effect of hexacosane has reported that it was a highly effective compound with inhibition zone of 29, 27, 26 and 25 cm against *Klebsiella pneumoniae, Salmonella typhi, Mithecithinne staphaureus* and *Proteus vulgaris*, respectively (Rukaiyat *et al.*, 2015). These results are consistent with the findings obtained in the present study.

Veratrum is one of the most critical genera that are rich in a pharmaceutical alkaloids worldwide. This study showed that the essential oil obtained from *V. album* might have a potential to be used in subsequent pharmacological and biological screening tests. In addition, it is thought that such studies will be useful in comparing essential oil compositions and providing basic data for taxonomic and essential oil evaluation studies of the genus, and contribute scientific agriculture and product diversity as well as various industries including medicine, cosmetics, landscaping, flavor and food.

In the present study, the leaf parts of the *V. album* showed a better antimicrobial effect against bacteria, while the stem parts showed a better antimicrobial effect against yeast and dermatophyte fungi. The antioxidant activity of the leaf parts of the plant was better. Especially the plant's leaf parts extract showed a better effect at increased concentrations. We think that the antimicrobial and antioxidant effects of the plant are due to the alkaloids in its structure. In particular the antimicrobial and antioxidant effects of alkaloids called jervine have been reported in previous studies (Wolters, 1970; Atalay *et al.*, 2019). However, biological studies on the species are limited in the literature, and the results of this study provide valuable insights into the literature.

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.

Authorship Contribution Statement

Pelin Yılmaz Sancar: Investigation, Resources, Visualization, Formal Analysis and Writing-original draft. **Şule İnci:** Antimicrobial and Antioxidant studies, Writing-original draft. **Azize Demirpolat:** Essential oil studies, Writing-original draft. **Sevda Kırbağ:** Antimicrobial-Antioxidant studies, Statistical analysis, Supervision, Validation and Writing-original draft. **Şemsettin Civelek:** Supervision, Validation and Writing-original draft.

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