Kastamonu Uni., Orman Fakültesi Dergisi, 2023, 23(1): 31-46 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.1269485

Chemical Composition of Essential Oils Obtained from Abies taxa in Türkiye and Investigation of Antimicrobial Activities

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Received Date: 20.09.2022 Accepted Date: 29.11.2022

Abstract

Aim of study: The aim of this study is to reveal the biochemical component and antimicrobial effects of essential oils obtained from different parts of Abies taxa in Türkiye.

Material and methods: Essential oils were analyzed for their antibacterial and antifungal activities by using Steam Distillation Method and MIC test against nineteen microorganisms, In the wells where the effect was observed according to the MIC test, the MBC test was performed to determine that the effect was bactericidal or inhibitory (bacteriostatic). For determination of chemical composition; samples of essential oils obtained from plants with Hydrodistillation were analyzed with GC MS QP 2010 Ultra (Shimadzu).

Main results: Beta-pinene, cis-Ocimene and Beta-Phellandrene were found to be the main components in all parts of the taxon when looking at the chemical compounds of A. cilicica subsp. isaurica taxon. There are differences in essential oil components in the branches and leaves of A. nordmanniana subsp. nordmanniana and A. cilicica subsp. cilicica taxa in the main components.

Highlights: The results of the study reveal that the oils obtained from the leaves, branches and cones of *Abies* taxa can be used as a supportive health product and for medical purposes with additional studies.

Keywords: *Abies*, Antimicrobial Activity, Biochemical Content, Essential Oils, Türkiye

Türkiye'de Abies Taksonlarından Elde Edilen Uçucu Yağların Kimyasal Bileşimi ve Antimikrobiyal Aktivitelerinin Araştırılması

Öz

Çalışmanın amacı: Bu çalışmanın amacı, Türkiye'deki *Abies* taksonlarının yaprak dal ve kozalaklarından elde edilen uçucu yağların biyokimyasal bileşeni ve antimikrobiyal etkilerini ortaya koymaktır.

Materyal ve yöntem: Uçucu yağlar, 19 mikroorganizmaya karşı su buharı distilasyonu metodu ve MİK testi kullanılarak, antimikrobiyal ve antifungal aktiviteleri açısından araştırılmıştır. MİK testine göre etki gözlenen kuyucuklarda, saptanan etkinin bakteri öldürücü (Bakterisidal) ya da gelişmesini durdurucu (Bakteriostatik) olduğunu saptamak için MBK testi yapılmıştır. Kimyasal kompozisyonun tespiti için; clevenger cihazı ile bitkilerden elde edilen uçucu yağlara ait numunelerin GC-MS QP 2010 Ultra (Shimadzu) ile analizleri yapılmıştır.

Temel sonuçlar: A.cilicica subsp. *isaurica* 'nin kimyasal bileşiklerine bakıldığında taksonun tüm kısımlarında beta-pinene, cis-Ocimene ve Beta-Phellandrene'nin ana bileşenler olduğu bulunmuştur. *A. nordmanniana* subsp. *nordmanniana* ve *A.cilicica* subsp.*cilicica* taksonlarının dallarında ve yapraklarında uçucu yağ ana bileşenlerinde farklılıklar vardır.

Önemli vurgular: Çalışmanın sonuçları, *Abies* taksonlarının yaprak dal ve kozalaklarından elde edilen yağların destekleyici sağlık ürünü olarak ve ilave çalışmalarla medikal amaçlı olarak da kullanılabileceğini ortaya koymaktadır.

Anahtar Kelimeler: *Abies*, Antimikrobiyal Aktivite, Biyokimyasal İçerik, Uçucu Yağlar, Türkiye

Citation (Atıf): Ozturk Pulatoglu, A., Guney, K., Ceter, T., & Yilmaz, E. S. (2023). Chemical Composition of Essential Oils Obtained from Abies taxa in Türkiye and Investigation of Antimicrobial Activities. *Kastamonu University Journal of Forestry Faculty*, 23 (1), 31-46.

Introduction

The number of microorganisms that are resistant to synthetic drugs is increasing every day (Dulger et al., 1999; Rawat & Uniyal, 2003). Interest in alternative and complementary medicine is growing due to the fact that natural products are more harmless and more reliable than synthetic products (Parvathi & Brindha, 2003). Between 50000 and 70000 plant species are used in traditional and modern medical methods (Schippmann et al., 2006). Around 80% of the world's population is estimated to use traditional medicine and to date, 170 of the 194 WHO Member States have reported the use of traditional medicine (WHO, 2022).

Essential oils derived from aromatic and medicinal plants are known to have many biological activities, especially antibacterial, antifungal and antioxidant properties (Tumen et al., 2010).

Due to the continued development of microbial resistance to antibiotics, potential new effective compounds against pathogenic microorganisms are being investigated. Plants whose chemical composition or pharmacological activities are not fully known but used in folk medicine are primarily noted in this sense (Kizil et al., 2002a). For this purpose, researchers in different parts of the world have focused on determining the biochemical contents of plants and examining the biological activities of the determined substances (Butnariu et al., 2012; Barbat et al., 2013; Butu et al., 2014a, 2014b; Samfira et al., 2015;)

Conifers are more vulnerable to biological and abiotic factors due to the secretion of oleoresin. The resinous substance contains basic phytochemicals such as turpentine (volatile fraction) and rosin (non-volatile fraction) (Ulukanlı et al., 2014). It has been demonstrated by many studies that plant extracts have the ability to prevent the growth of many pathogenic organisms (Mitrokosta et al., 1993; Ilçim et al., 1998; Yeşilada et al., 1999; Kelmanson et al., 2000; Çelik & Çelik, 2007; WHO, 2012; Padiyara et al., 2018).

The antimicrobial activity of plant oils and extracts has been known for many years (Hammer et al., 1999). For thousands of years, plant oils and extracts, antimicrobial activity, protection of raw and processed food, pharmacological products, has been used for a variety of purposes such as alternative medicine and natural therapies (Mishra & Dubey, 1994; Lawless, 1995; Jones, 1996; Reynolds, 1996; Lis-Balchin & Deans, 1997). The antimicrobial activities of the plants were evaluated by many studies on the various vegetative and generative organs of many plants with antimicrobial properties (Leven et al. 1979; Erturk & Demirbag, 2003; Sudharameshwari & Radhika, 2007; Benli et al., 2008; Altuner et al., 2010; Altuner et al., 2018; Bader et al., 2018; Çiçek & Çeter, 2019; Souliman et al., 2019).

Due to its climate and geographical location, Türkiye is rich in coniferous trees, which occupy about half of the country's total forest area (Muthoo ,1997; Hafizoglu & Usta, 2005). In this study, taxa belonging to the *Abies* Mill. which is naturally common in Türkiye was studied. *Abies*, which is the second-largest genus in the family Pinaceae, is represented by 48 species, 78 taxa in the world, and 3 species and 5 taxa in Türkiye (URL-1, 2018); Güner et al., 2012; Xiang et al., 2015). Among these, 2 taxa are endemic and according to the Red Book of Turkey Plants, they are in the *Abies cilicica* subsp. *isaurica* Coode & Cullen lr (lc), *Abies nordmanniana* subsp. *equi-trojani* (ASC. & Sint. ex Boiss.) Coode & Cullen are in the LR (nt) category (Ekim et al., 2000).

Abies taxa, which grow naturally in Türkiye, are spread in high mountainous areas in the south of the country, at lower elevations and even at sea level in the north of the country in the form of pure or mixed forests in total 670389.6 ha (Kayacık, 1967; Yaltırık & Efe, 2000; OGM, 2007).

About essential oil constituents of the family Pinaceae, there have been some studies on the antioxidant activity, terpenoids, steroids, anti-HIV activity, procyanidins, etc. of all the Pinaceae (Sakagami et al., 1991; Sakar et al. 1991; Bağcı et al. 1999; Dığrak et al., 1999; Tanaka et al., 1999; Kizil et al., 2002a, 2002b; Dayisoylu et al., 2009; Tumen et al., 2010; Ulukanlı et al., 2014). Studies were carried out on the essential oils from rosin of *A. cilicica* subsp. *cilicica* cones that the studies on essential oils of the root, stems (Kizil et al., 2002a) and leaves (Bagci & Digrak, 1994; 1996) indicated the

antibacterial and antifungal activities of nine *Abies* species. It is also known that conifers of *Abies* species are used to treat bronchitis when used through an infusion. The leaves have been used as an expectorant and antidiarrheal. Oils, available in the leaves of fir species, contain compounds such as sesquiterpenes, diterpenes, triterpenes, flavonoids, condensed tannins, lignans, organic acids and waxes (Sakar et al., 1996). The rosin of *Abies cilicica* has traditionally been used as antiseptic, anti-inflammatory, antipyretic, antibacterial and antiviral medicines and as chewing gum against some stomach diseases (e.g., ulcer), lip-dryness and asthma and curing the wound in the form of ointment and plaster (Baytop, 1999).

This study aims to determine the biochemical content and antimicrobial activities of essential oils obtained from different parts of taxa belonging to the *Abies* genus collected from different regions of Türkiye.

Material and Methods

Plant Material

In the study, the locality information, collection dates and the parts used for the plant taxa studied in essential oil are shown in Table 1. We carried out the diagnosis of used plant samples at Kastamonu University B. Bilgili Herbarium.

Table 1. Information on plant samples used.

Plant	Province	Used parts	Collection date
Abies nordmanniana subsp. *equi-trojani*	Kastamonu	leaf, branch and cone	01.08.2017
Abies nordmanniana subsp. *nordmanniana*	Ordu	leaf and branch	22.09.2016
Abies cilicica subsp. *isaurica*	Antalya	leaf, branch and cone	03.04.2018
Abies cilicica subsp. *cilicica*	Adana	leaf and brunch	01.04.2018

Microbial Material (fungi and bacteria)

The strains of fungi and bacteria used in this study were obtained from the Research Laboratory of Kastamonu University, Faculty of Science and Letters, Department of Biology. Gram-positive strains of bacteria used in the study: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Bacillus subtilis* DSMZ 1971 *Enterococcus faecium*, *Enterococcus faecalis* ATCC 29212, *Enterococcus durans*, *Listeria innocua*, *Listeria monocytogenes*. Gram-negative bacterial strains are: *Serratia marcescens, Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 13048, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella typhimurium* SL 1344, *Salmonella kentucky*, *Salmonella infantis*, *Salmonella enteritidis* ATCC 13075, *Klebsiella pneumoniae* ATCC 7544 and fungi: *Candida albicans* DSMZ 1386.

Method

Obtaining Essential Oil

The plants were cut into small pieces of appropriate size and their oils were extracted. The essential oils of the leaves, branches and cones of the plant parts were then obtained by using Clevenger Apparatus using hydrodistillation method. Essential oils samples are preserved in free amber sample containers and store in refrigerator at +4^{o}C untill further required.

GC-MS Analysis

For determination of chemical composition; samples of essential oils obtained from plants with Clevenger apparatus were analyzed separately with GCMS QP 2010 Ultra (Shimadzu) equipped with Rtx-5ms capillary column (30m·0.25 mm; coating thickness 0.25 μm). Analytical conditions: injector temperature 250°C, 1 mL/min Helium as carrier gas injection mode: split ratio 1:10; injected volume: 1μ L of oil dissolved in the hexane; and oven temperature set 4°C/min 40°C. from 240°C. pressure: 100 kPa, the evacuation flow: 3 mL/min. The MS scan conditions are: transfer line temperature 250°C, interface temperature 250°C, ion source temperature 200°C. The determination of the components is based on the comparison of the duration of detention and the Wiley database mapping. As far as possible, the reference compounds were chromatographed

to confirm GC retention times (Canlı et al., 2017).

Preparation of Microorganisms

During the preparation stages of inoculum from the strains of bacteria to be used in the study, the same-looking pure colonies from bacteria and fungi cultures developed for 24 hours in Nutrient Agar media were taken with sterile loop and transferred into the sterile saline solution in the sterile sample tube. The turbidity of prepared bacterial and fungal samples has been adjusted to 0.5 McFarland standards. Thus, approximately $1x10^7$ kob/ml fungal suspensions and approximately $1.0x10^8$ kob/ml. bacterial suspensions were mixed to find microorganism and their standards were set. Subsequently, the names of fungi and bacteria were written on the tubes and the prepared concentrations were mixed with the vortex prior to use.

Minimum Inhibition Concentration (MIC)

Essential oils obtained primarily by distillation method have been sterilized with a filter of 0.45 µm. In MIC test, effective concentration was tried to be found using 96 sterile well plates with microdilution tests. 100 µl Mueller Hinton Broth (MHB) pipetting for each well and 100 µl oil obtained from plants were transferred into the first well, after mixing the content of the well thoroughly 100 µl were transferred into the second well. This serial dilution process continued to 10th well, after the contents of the 10th well were thoroughly mixed and then removed by taking 100 µl. Thus, wells numbered 1-10 were prepared as serial dilution wells in which plant essential oils would be tested. 10 µL standardized microorganism stock solution, has been added to all wells except the 12th well. The activity of plant extract has been tested in 1-10 Wells. While 11th well (MHB broth and microorganisms) was positive control, the 12th well was prepared as negative control containing only culture medium (MHB broth only). Each sample was studied in the same way in three parallel. Bacteria samples in the plaques studied were incubated at 37ºC for 24 hours and fungal samples (*Candida albicans*) at 27ºC for 48 hours after incubating at incubator for the lowest concentration MIC value where reproduction is not visible when viewed with the eye (Balouiri et al. 2016).

Minimum Bactericidal / Fungicidal Concentration (MBC, MFC)

Fungal samples and bacteria samples taken with sterile loop from wells where reproduction was not observed in MIC test were transferred to Nutrient agar media by line cultivation method. Bacterial samples incubated for 24 hours at 37ºC, fungal samples incubated for 48 hours at 27ºC, the lowest concentration where reproduction was not observed was determined as MFC value for fungi and MBC value for bacteria.

Results

GC-MS Findings

Table 2 shows the chemical components and percentages of essential oils belonging to different parts of *Abies* taxa grown naturally in Türkiye according to GS MS analysis results.

Table 2. The chemical compositions (%) of the essential oils belonging to the plant taxa

Name*	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	4-1	4-2
(-)-beta-selinene					0.44					
(-)-ISOLEDENE	0.17	0.15	-		0.1					
(-)-Isolongifolol, acetate		0.11	-							
(-)-Myrtenol					0.41				0.17	
(-)-Neoclovene-(I), dihydro-										0.47
(-)-trans-Pinocarveol	0.24	0.38	2.78	8.75	0.29	2.93	1.55		0.22	
(-)-Verbenol			0.23	0.28						
(+)-alpha-thujone				0.34						
(+)-Pinanediol				0.64						
(12Z)-ABIENOL									0.87	
(1R,2R,3S,5R)-(-)-2,3-Pinanediol						0.12				
(1R,6S)-gamma-himachalene		0.19	-							
(1S,2S,3R,5S)-(+)-2,3-PINANEDIOL										0.42

Table 2. *(Continued)*

Name*	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	4-1	4-2
(1S,2S,3R,5S)-(+)-Pinanediol									0.49	
(1S,5R)-Myrtenal						1.07				
(1S,5S)-2(10)-Pinene								14.66		
(S)-cis-Verbenol						0.46	0.27	0.12		0.44
.alfa.-Copaene	0.77	-	-							
.alpha.-Amorphene						0.2			0.68	
.alpha.-Chamigrene						0.39				
.alpha.-Humulene (CAS)		0.67	-		0.63			1.88		1.99
.alpha.-Longipinene (CAS)		0.74	-					1.18		
.ALPHA.-PINENE, (-)-									0.62	12.83
.ALPHA.-TERPINOLENE	0.24	0.13	-		0.13			0.92		0.81
.beta.-bisabolol		0.16	-							0.64
.beta.-Gurjunene				1.15						
.beta.-HIMACHALENOXIDE										0.33
.beta.-Phellandrene	6.4	9.98	1.75		6.06			5.28		1.58
.DELTA.3-Carene							0.21		2.72	
.delta.-Cadinene (CAS)									0.6	
.gamma.-Muurolene	0.46	-	-							
.gamma.-Terpinene										36.62
1,3,6-Octatriene, 3,7-dimethyl-, (E)- (CAS)								41.11		
1,3-Dioxolane, 2,2-dimethyl-4,5-bis(1-methylethenyl)-							0.96		1.18	
1,4-dihydroxy-p-menth-2-ene			0.18							
1,5-epoxysalvial-4(14)-ene									0.93	
1,6-Dimethylhepta-1,3,5-triene				0.41						
10s,11s-Himachala-3(12),4-diene								0.56		
11-Hexadecyn-1-ol							0.22			
15-Isobutyl-(13.alpha.H)-isocopalane									0.53	
1A,2,4,5,6,7,7A,7B-OCTAHYDRO-1,1,7,7A-TETRAMETHYL-, [1AS-(1A.ALPHA						0.42				
1-Acetyl-1-cyclohexene			0.17			0.19				
1-Aromadendrene					0.14					
1-Dodecanol										0.36
1-Epi-alpha-gurjunene										1.27
1H-Benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-, (R)-										0.87
1-TERPINEOL		0.15	-	0.43						
2,2,7-Trimethylbicyclo[5.4.0]undec-11-en-10-one									0.27	
2,3-Pinanediol							0.31			
2,5-Furandione, 3-(dodecenyl)dihydro-				0.32						
2,6-Dimethyl-1,6-heptadien-4-ol acetate		0.18	-							
2-.BETA.-PINENE					27.84					
2-Acetoxy-1,8-cineole				0.3					0.3	0.22
2-Carene					0.1					0.44
2-Heptyl acetate		0.14	-							
2-Hexenoic acid, 3,4,4-trimethyl-5-oxo-, (Z)-									0.35	
2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene					0.68					
2-METHYLISOBORNEOL									2.27	
2-Octanol, acetate								0.13		
2-Pinen-10-yl acetate						1.46				
3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone							0.18	0.11		
3-Isopropenyl-2-methylcyclohexyl acetate							0.21			
3-Pinanone		0.24	0.65			0.59				
3-Tetradecyn-1-ol			0.11							
4-Acetyl-1-methylcyclohexene					0.09					
4-isopropylbenzyl alcohol			1.05			0.81		0.14	30.11	2.49
4-TERPINENYL ACETATE	0.12	0.18	1.13		0.17			0.32	3.81	1.65
5,5,8-Trimethyl-nona-3,6,7-trien-2-one								0.39		
5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol								0.12		
6,6-Dimethylhepta-2,4-diene				0.52						
67860-11-1				0.35						

Table 2. *(Continued)*

Name*	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	4-1	4-2
6-epi-shyobunol							1.21			
8,9-DEHYDRO-NEOISOLONGIFOLENE								0.16		0.23
9-Decen-2-one, 5-methylene-							0.27			
9-Undecenal, 2,10-dimethyl-				0.85						
AC1LBWRG				0.43						
AC1LBX6Q			0.21	0.25						
AC1LCRRH	0.16	-	-							
AC1NSI42									0.27	
Acetic acid, 5-methylhex-2-yl ester					0.19					
Alloaromadendrene		0.11	-							
ALPHA.-CAMPHOLENE ALDEHYDE						0.91				
ALPHA.-PINENE, (-)-							0.31			
ALPHA.-TERPINENYL ACETATE					0.89					
alpha-Bisabolol	0.34	0.32	-		0.08			0.61		1.59
alpha-Cadinol	0.28	-	-			0.19			0.24	
alpha-Himachalene	1.69	0.22	-					0.3		0.29
alpha-Longipinene	0.94	0.13	-					0.2		0.96
ALPHA-PINENE				0.92						
alpha-Pinene-oxide			1.41		0.1	1.75				
ALPHA-TERPINENE		0.13	-							
alpha-TERPINEOL	0.96	1.34	0.77	14.65	0.94	0.88	7.79	0.9	1.12	1.29
Androst-4-ene-3,17-dione (CAS)							0.66			
anoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1S-(1.alpha.,3a.beta.,4.alpha.,8a.								0.43		
ARHRNUWNRNFXBZ-UHFFFAOYSA-N								0.22		
Benzene, methyl(1-methylethyl)- (CAS)										0.65
Benzenemethanol, .alpha.,.alpha.,4-trimethyl				9.26						
Benzenemethanol, 4-(1-methylethyl)- (CAS)							36.75			
BETA. BOURBONENE						0.12				
beta-Cedrene			0.1							
beta-Himachalene		0.45	-		0.09			0.67		
Beta-Pinene	30.05	49.11	15.03	0.53		11.89				3.78
Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-					0.11					
Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-				6.54						
Bicyclo[3.2.0]heptan-3-ol, 2-methylene-6,6-dimethyl-						0.23				
Bornyl acetate	0.13	2.25	0.15	2.07	0.59	1.15	0.84	3.01		0.28
BORONAL									0.28	
Cadin-4-en-10-ol	0.59	0.18	-							
Cadinene <delta->	2.23	0.18	0.17			0.17				
Cadinene <gamma->	2.82	-	0.25			0.19			0.39	
Camphene	1.54	2.96	0.9		8.12	1.12		9.18		3.46
Campholenic aldehyde			0.76							
Carotol								0.77		
Carvenone							0.42		0.58	
Caryophyllene	1.75	1.44	-		1.4			3.58	0.18	3.49
Caryophyllene oxide	0.2	0.43	0.11	1.51	0.59	0.17	3.87	1.28	0.26	3.39
Cedranoxide, 8,14-									0.44	
Cembrane						0.24				
Cembrene	0.57	-	-							
Chrysanthenone							12.26	0.24	8.76	
Chrysanthenyl acetate			0.1							
CIS PIPERITONE OXIDE				0.51						
CIS-.ALPHA.-BISABOLENE			0.11							
CIS-LIMONENE OXIDE									0.21	
CIS-PINONSAEURE							0.35			
cis-6-Nonenyl Acetate		0.43	-							
cis-Abienol	1.61	-	-							
cis-Carvyl acetate			0.41	1.36		0.38	0.46			
cis-Ocimene	23.56	16.2	57.79							
cis-p-menth-2-en-1-ol							0.17			
Clovanediol							1.65			

Table 2. *(Continued)*

Name*	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	4-1	4-2
Cryptone (CAS)	0.16	0.47	-		0.42					
Cubebene <alpha->			0.14			0.23				0.22
Cubedol										1.52
Cubenol					0.13					
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-(.+/-.)-							0.98			
Cymen-8-ol <para->				0.22						
Cymene <para->					0.39			0.69		
DEHYDROABIETINE			0.16			0.14				
Dehydroabietan	0.96	-	0.16						0.19	
d-Ledol				9.92			0.2			
D-Limonene			0.78			0.76				
Driman-8,11-diol							0.25			
Elemene <delta->						0.12				
endo-Borneol	0.16	-	-							
EPOXY (1,11)HUMULENE							0.58			
Espatulenol			0.12							
EUCALYPTOL (1,8-CINEOLE)			0.18	0.23	0.37					
Eucarvone							0.71		2.06	0.24
exo-methyl-camphenilol				0.56						
Farnesene <(E,E)-, alpha->								0.18		
Fenchol <alpha->		0.13	-	0.82			0.3			
fencholenic aldehyde			0.15			0.17				
gamma-Pyronene							0.88			
GERMACRENE-D	5.1	-	-							
Globulol					0.1					
Grandiflorenic acid	0.16	-	-							
Himachala-2,4-diene	0.24	0.17	-		0.16			1.59		0.9
hthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1.alpha.,4a.beta.,8a						0.17				
Humulane-1,6-dien-3-ol							0.52			
Humulen-(v1)		1.31	-					0.54		0.36
Humulene	0.73	-	-							
Humulene epoxide II									0.72	
HUMULENE OXIDE		0.15	-		0.18			0.41		1.14
hyl-3-[3,7,12-trimethyl-14-(1,4,4-trimethylcyclohex-2-enyl)tetradeca-3,7,11-trienyl]ox					0.08					
Isoaromadendrene epoxide									0.8	
Isomenthol	0.16	-	-	1.8						
IUIFSUPCQCUASG-UHFFFAOYSA-N							0.26			
Juniper camphor	0.36	2.04	-	4.68	10.24					
KDKRHPHEWUSWKM-UHFFFAOYSA-N									1.7	
L-Bornyl acetate			0.46		0.11					
Ledol							0.25			
LGIKFBYSSJHCQR-ALLKAYEBSA-N									0.2	
Limonene oxide <cis->			0.24			0.26				
Linalool		0.33	-		0.21					1.49
Linalyl acetate		0.28	-		0.21					
Longiborneol	0.47	0.92	-		0.15			0.97		1.93
Longifolene	1.2	0.45	-		0.08			0.53		0.47
M-CYMENE	0.58	-	-							
Menth-2-en-1-ol <trans-, para->		0.15	-	0.34						
Menthol					0.34					
Methyl dehydroabietate				0.39						
MGUPDCNFBFALJD-UHFFFAOYSA-N								0.19		
Muurolene <alpha->	1.87	-	-						0.21	
MYRCENE	7.45	1.61	0.17		1.07	0.16		1.5		2.38
Myrtenal		0.46	-							
Myrtenol	0.23	-	1.41				1.57			
Neryl acetate										0.49
NFSVGWISRPLYOZ-UHFFFAOYSA-N							8.12			

Table 2. *(Continued)*

Name*	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	4-1	4-2
Nona-3,5-dien-2-ol				0.26						
Nopinone			0.17	1.02						
Nopol			0.1							
Norinone							0.32			
NSC156908							0.55			
o-Cymene	0.17	-	0.57			0.19				
P-CYMENE		0.21	-							
Pentacosane										0.42
Pentadecane, 2-methyl-2-phenyl- (CAS)								0.26		
Phellandrene <alpha->								0.48		
Phoracanthal				0.24						
PINANEDIOL			0.13	1.99						
Pinocarvone		0.25	0.31	2.5	0.2	0.25	0.32			
Platambin				1.3					14.1	
p-Menth-8-ene-1,2-diol				0.25						
p-Mentha-1,5-dien-8-ol (CAS)				0.78			0.63	0.32	2.67	1.23
renecarboxylic acid, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-6-hydroxy-1,4a,7-									0.74	
salvial-4(14)-en-1-one									0.73	
Santene	0.71	1.36	-		1.94			0.55		
Santolina triene					31.43	60.86				
SBRYOYBBQHELIA-CMDGGOBGSA-N				0.23						
SCLARAL				0.4			0.25			
Sclaral (sclareolide lactol)									0.48	
SCLAREOLIDE				0.31			0.29			
Selina-6-en-4-ol					0.57	0.3				
Selinene <beta->					0.13					
sesquisabinene hydrate		0.15	-					0.17		0.3
Seychellene									8.16	
Spathulenol						0.43			0.57	
Terpinen-4-ol				0.95		0.27	5.29			
Tetracosane			0.14		0.09	0.15		0.12		0.63
tetramethyl-, cis-								0.86		
Thujene <alpha->			0.13					0.18		
Thujopsene-(I2)						0.16				
Thymoquinone							1.21		0.42	
T-Muurolol	0.28	-	-							
Torreyol									0.19	
TRANS(.BETA.)-CARYOPHYLLENE								0.18		
Trans-Sobrerol			0.13	0.7						
Tricyclene	0.17	0.26	-		0.91	0.18		1.09		0.41
Trivertal							0.48			
UNII-7N44SFN4SS			1.19							
UPQOJPOSKCDZFM-STQMWFEESA-N	0.19	-	-							0.46
Verbenene			0.39			0.51				
Verbenol			2.57	2.82	0.24	3.57				
VERBENONE			3.72	14.92	0.09	2.96	5.16		1.36	0.76
Viridiflorol		0.17	-					0.54	4.71	1.78
WGTRJVCFDUCKCM-SCUASFONSA-N			0.14							
XVLIQMJSPZLBQW-UHFFFAOYSA-N									1.13	
Ylangene	0.24	-	-					0.19		
yran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3R-(3.alpha.,4a.beta.,6a.alpha.,10a						0.13				
YUBSLXLMZVQYAG-UHFFFAOYSA-N	0.61	-	-							

*: 1-1: *Abies nordmanniana* subsp. *equi-trojani* leaf,1-2: *Abies nordmanniana* subsp. *equi-trojani branch*, 1-3: *Abies nordmanniana* subsp. *equi-trojani* cone, 2-1: *Abies nordmanniana* subsp. *nordmanniana* leaf, 2-2: *Abies nordmanniana* subsp. *nordmanniana* branch, 2-3: *Abies nordmanniana* subsp. *nordmanniana* cone, 3-1: *Abies cilicica* subsp. *isaurica* leaf, 3-2: *Abies cilicica* subsp. *isaurica* branch, 4-1: *Abies cilicica* subsp. *cilicica* leaf, 4-2: *Abies cilicica* subsp. *cilicica* branch.

Beta-pinene, cis-Ocimene and Beta-Phellandrene were found to be the main components in all parts of the taxon when looking at the chemical compounds in essential oils derived from branches, leaves and conifers of *A. cilicica* subsp. *isaurica*

taxon. (-)- trans-Pinocarveol, beta - Phellandrene, 4-Terpinenyl Acetate, alpha-terpineol, Beta-Pinene, Bornyl acetate, Cadinene <delta->, Camphene, Caryophyllene oxide, cis-Ocimene, Myrcene are common components for all parts of the taxon.

When looking at the chemical compounds in essential oils derived from branches, leaves and conifers of *A. nordmanniana* subsp. *equi-trojani* taxon; it is observed that (-)-trans-Pinocarveol, alpha-Terpineol, Bornyl acetate, Caryophyllene oxide, Pinocarvone, Verbenol and Verbenone all have different percentages, although they are common components. Essential oils belonging to different parts of taxa have differences in the main components. D-Ledol, Caryophyllene oxide, Verbenone, Terpineol <alpha ->, 38102-55-5, Pinocarveol <trans-> and Bicyclo[3.1.1]hept-2-ene-2-methanol, 6.6-dimethyl - are seen as the main components in the essential oil components belonging to the branch of the taxon; and the essential oils obtained from its leaves; Santolina triene, 2- .Beta.- Pinene, Juniper camphor, Camphene (CAS) and .beta.-Phellandrene are the main ingredients. In essential oil components obtained from conifers, Santolina triene and Pinene <beta -> are the main components.

There are differences in essential oil components in the branches and leaves of *A. nordmanniana* subsp. *nordmanniana* taxon in the main components. In the branches, NFSVGWISRPLYOZ-if uhfffao-N, Benzenemethanol, 4-(1-Benzen)- (CAS), the caryophylle oxide, Driman-for 8.11-diol, Chrysanthemen, Terpineol <alpha> and terpinen-4-ol are the main components; in the leaves 1.3,6-octatrie to 3.7-dimethyl-, (E)-(CAS), (1S,5S)-2(10)-Pinene, Camphene (CAS) and beta. -Phellandrene are seen as the main component. (S)-cis-Verbenol, 3-hydroxy-2-Methyl-5-(prop-1-en-2-yl) cyclohexanone, alpha-terpineol, Bornyl acetate, oxide caryophylle, Chrysanthemen and P-Mentha-1.5-Dien-8-ol (CAS) components have the low percentage and they are common taxa in both branches and in leaves.

When looking at the essential oil components of the branches and leaves of the *A. cilicica* subsp. *cilicica* taxon, it is seen that there are differences in its main components. In its branches, 4-Isopropylbenzyl alcohol, p-Mentha-1.5-dien-8-ol (CAS), Eucarvone, Platambine, Chrysanthenone and Seychellene have the most percentage components; in its leaves, gamma.Terpinene and .Alpha.-Pinene, (-) - are the main components. Compounds that are common in branches and leaves but have a low percentage are; Alpha-Pinene is (-) -, 2-Acetoxy-1.8-cineole, 4-Isopropylbenzyl alcohol, 4-Terpinenyl Acetate, alpha-terpineol, Caryophyllene, Caryophyllene oxide, Eucarvone, p-Mentha-1.5-dien-8-ol (CAS), Verbenone, and Viridifluorol.

MIC Values

MIC test was performed to determine the lowest concentration of essential oils belonging to different parts of *Abies* taxa. The results of the MIC test are given in Table 3.

Table 3. MIC values of plant taxa µg/µl.

Microorganisms	Plant extracts									
	Abies nordmanniana subsp. *equi-trojani*			*Abies nordmanniana* subsp. *nordmanniana*		*Abies cilicica* subsp. *isaurica*			*Abies cilicica* subsp. *cilicica*	
	leaf	branch	cone	leaf	branch	leaf	Branch	cone	leaf	branch
Enterobacter aerogenes (ATCC 13048)	25	100	25	50	12.5	-	100	12.5	12.5	50
Salmonella infantis (9)	0.39	25	3.125	12.5	1.562	100	25	1.562	1.562	50
Listeria monocytogenes	1.562	25	3.125	12.5	1.562	100	50	3.125	1.562	25
Klebsiella pneumoniae (13)	12.5	100	12.5	50	12.5	100	100	1.562	50	50
Pseudomonas aeruginosa (DSMZ 50071)	6.25	0.195	6.25	0.195	100	1.562	0.195	0.195	0.195	0.195
Pseudomonas fluorescens	100	0.781	3.125	25	6.25	-	25	1.562	25	12.5
Salmonella kentucky (10)	12.5	0.39	0.781	12.5	0.781	-	100	0.781	12.5	6.25
Enterococcus faecalis (ATCC 29212)	0.195	0.195	0.195	6.25	1.562	100	6.25	0.195	0.781	-

Table 3. *(Continued)*

Microorganisms	Plant extracts									
	Abies nordmanniana subsp. *equi-trojani*			*Abies nordmanniana* subsp. *nordmanniana*		*Abies cilicica* subsp. *isaurica*			*Abies cilicica* subsp. *cilicica*	
	leaf	branch	cone	leaf	branch	leaf	Branch	cone	leaf	branch
Listeria innocua	0.195	0.195	0.781	1.562	0.781	3.125	100	0.195	0.195	-
Salmonella enteritidis (ATCC 13075)	12.5	0.195	1.562	12.5	0.39	100	100	0.781	12.5	3.125
Enterococcus durans	0.781	0.195	1.562	12.5	6.25	6.25	100	6.25	12.5	3.125
Salmonella typhimurium	25	0.781	6.25	50	6.25	100	100	3.125	50	100
Candida ablicans (DSMZ 1386)	0.195	100	3.125	0.195	0.195	0.195	100	3.125	50	100
Enterococcus faecium (4)	25	3.125	12.5	50	6.25	-	100	6.25	100	100
Staphylococcus aureus (ATCC 25923)	12.5	0.39	0.781	0.195	0.195	25	25	0.195	0.39	6.25
Staphylococcus epidermidis(DSMZ 20044)	100	100	25	25	12.5	-	100	12.5	50	100
Bacillus subtilis (DSMZ 1971)	3.125	0.39	0.195	50	25	3.125	12.5	0.195	50	25
Escherichia coli (ATCC 25922)	100	-	3.125	12.5	3.125	100	100	0.781	25	50
Saratia marcescens (ATCC 13048)	-	-	12.5	100	-	100	-	12.5	50	100

When the results of the MIC test were examined; *A. nordmanniana* subsp. *equi-trojani* taxon leaf and branch extracts were found to show MIC value against microorganisms strains between 0,195-100 mg/100 µL concentrations. In the extracts of leaves, the lowest inhibitory concentration (MIC) value against *P. fluorescens, P. epidermidis* and *E. coli* is 100 µg/ µl, while the strongest antimicrobial effect has been detected against the *E. faecalis, L. innocua* and *C. ablican* strain. The lowest antimicrobial effect in branch extracts of the same species was detected against *E. aerogenes, K. pneumoniae, C. ablicans* and *S. epidermidis* strains, the strongest antimicrobial effect was detected against *P. aeruginosa, E. faecalis, L. innocua, P. enteritidis* and *E. durans* strains. The lowest inhibitory concentration (MIC) value of cone extract against *E. aerogenes* and *S. epidermidis* was 25 µg / µl, and the highest inhibitory concentration (MIC) value against *E. faecalis* and *B. subtilis* was 0.195 µg/µl. There were no antimicrobial effects of leaf extract of the species against the *S. marcescens*, and there were no antimicrobial effects of branch extract against the *E. coli* and *S. marcescens* in the studied concentration.

The leaf and branch extracts of *A. nordmanniana* subsp. *nordmanniana* taxon were found to show MIC value against microorganisms strains between 0.195-100 mg/100 µL concentrations. The lowest antimicrobial effect in extracts of the species' leaves was against the *S. marcescens* strain, the strongest antimicrobial effect was against P. *aeruginosa, C. ablicans* and *S. aureus* strains. The lowest antimicrobial effect in extracts from the branches of the species was against *P. aeruginosa* strain, the strongest antimicrobial effect was against the *C. ablicans* and *S. aureus* strains. No antimicrobial effects were detected in the branch extract concentration applied against *S. marcescens*.

When the results of the MIC test were examined; the leaf and branch extracts of *A. cilicica* subsp. *isaurica* taxon showed MIC value against microorganisms strains between 0.195-100 mg/100 µL and cone extract between 0.195-12.5 mg/100 µL concentrations. It has been detected that the lowest antimicrobial effect in extracts of the species ' leaves was against the *S. infantis, L. monocytogenes, K. pneumoniae, E. faecalis, P. enteritidis, P. typhimurium, E. coli* and *S. marcescens* strains, and the strongest antimicrobial effect was against *C. ablicans* strain. It has been detected that the lowest antimicrobial effect in extracts of the species' branches was against *E. aerogenes, K. pneumoniae, P. kentucky, L. innocua, P.*

enteritidis, E. durans, P. typhimurium, C. albicans, E. faecium S. epidermidis and *E. coli* strains; the strongest antimicrobial effect was against *P. aeruginosa* strain. It has been detected that the lowest antimicrobial effect on the extract of the species' conifer was against the *E. aerogenes, S. epidermidis* and *S. marcescens* strains; the strongest antimicrobial effect was against *P. aeruginosa, E. faecalis, L. innocua, S. aureus* and *B. subtilis* strains. No antimicrobial effects were detected in the concentration of the leaf extract of the species against *E. aerogenes, P. fluorescens, S. kentucky, E. faecium* and *S. epidermidis*; No antimicrobial effects were detected in the concentration of the branch extract against *S. marcescens*.

In extracts of *A. cilicica* subsp. *cilicica* leaves, the lowest inhibitory concentration (MIC) value against *E. faecium* was 100 µg/µl, while the strongest antimicrobial effect was 0.195 µg / µl against *P. aeruginosa* and *L. innocua* strains. In branch extracts, the lowest inhibitory concentration (MIC) value against *S. typhimurium, C. ablicans, E. faecium, S. epidermidis* and *S.* marrescens was 100 µg/µl, while the strongest antimicrobial effect was 0.195 µg/µl against *P. aeruginosa* strain. No antimicrobial effects were detected in the branch extract concentration against *E. faecalis* and *L. innocua*. In the previous study on cones of the same taxa, *S. cerevisiae, K. pneumoniae* and *M. smegmatis* were the most sensitive microorganisms to the essential oil due to their low MIC values of 0.5 µg/µl that the results revealed that limonene was the most effective constituent on the microbial activities, followed by β-pinene, myrcene and bpinene. The most effective antifungal activities were also determined for myrcene (Dayisoylu et al., 2009).

MBC, MFC values

In the wells where the effect was observed according to the MIC test, the MBC test was performed to determine that the effect was bactericidal or inhibitory (bacteriostatic). The MBC, MFC values are given in detail in Table 4.

Table 4. MBK, MFK values of plant taxa µg/µl.

Microorganisms	Plant extracts									
	Abies nordmanniana subsp. *equi-trojani*			*Abies nordmanniana* subsp. *nordmanniana*		*Abies cilicica* subsp. *isaurica*			*Abies cilicica* subsp. *cilicica*	
	leaf	branch	cone	leaf	branch	leaf	branch	cone	leaf	branch
Enterobacter aerogenes (ATCC 13048)	50	100	25	100	12.5	-	100	12.5	50	50
Salmonella infantis (9)	25	100	50	50	1.562	100	50	12.5	25	50
Listeria monocytogenes	25	100	25	25	25	-	100	6.25	25	50
Klebsiella pneumoniae (13)	25	100	25	100	50	-	100	1.562	50	50
Pseudomonas aeruginosa (DSMZ 50071)	6.25	6.25	12.5	6.25	100	12.5	6.25	1.562	12.5	3.125
Pseudomonas fluorescens	100	6.25	3.125	25	6.25	-	100	3.125	25	25
Salmonella kentucky (10)	50	6.25	25	25	25	-	100	0.781	25	100
Enterococcus faecalis (ATCC 29212)	6.25	3.125	12.5	25	6.25	100	100	12.5	25	-
Listeria innocua	6.25	3.125	6.25	12.5	12.5	100	100	1.562	12.5	-
Salmonella enteritidis (ATCC 13075)	25	3.125	6.25	50	6.25	100	100	0.781	25	6.25
Enterococcus durans	1.562	0.781	12.5	12.5	12.5	-	100	6.25	12.5	12.5
Salmonella typhimurium	50	6.25	25	50	25	100	100	12.5	50	100
Candida ablicans (DSMZ 1386)	0.781	100	25	12.5	6.25	100	100	12.5	100	100
Enterococcus faecium (4)	25	6.25	12.5	50	25	-	-	6.25	-	100
Staphylococcus aureus (ATCC 25923)	25	6.25	6.25	1.562	0.781	50	25	1.562	1.562	50
Staphylococcus epidermidis(DSMZ 20044)	-	-	25	50	50	-	-	12.5	100	100
Bacillus subtilis (DSMZ 1971)	3.125	0.39	0.195	50	25	3.125	12.5	0.195	100	25
Escherichia coli (ATCC 25922)	100	-	6.25	50	25	100	-	3.125	100	100
Saratia marrescens (ATCC 13048)	-	-	50	100	-	-	-	12.5	50	100

When the effect of *A. nordmanniana* subsp. *equi-trojani* extracts on *E. aerogenes* was examined, it was determined that MIC value was bactericidal for branches and conifers, MIC value for Leaf was bacteriostatic for 25 µg/µl, concentrations of

50 µg/µl and above showed bactericidal effect. When the effect on *K. pneumoniae* was examined, it was observed that the MIC value for the branch was bactericidal and the MIC value for the other parts was bacteriostatic. Bactericidal action is observed on *P. aeruginosa* only for leaves. When the effects of the extracts from the same species on *P. fluorescens* were examined, bactericidal effect of the MIC value was observed for leaves and cones. It has been found that, MIC value is bactericidal for the branch on *C. ablicans*, for leaf and cones on *E. faecium*, for cones on *S. epidermidis*, for all parts of the plant on *B. subtilis* and for leaves on *E. coli*. The effects of *A. nordmanniana* subsp. *equi-trojani* extracts on other used microorganisms for all parts of the plant the MIC values were determined to be bacteriostatic.

When we examined at the effect of *A. nordmanniana* subsp. *nordmanniana* extracts on *E. aerogenes*, MIC value for branch was bactericidal and MIC value for leaf was 50 µg/µL bacteriostatic. Bactericidal effect of extracts' MIC value was observed on branch for *S. infantis, P. aeruginosa,* on leaves for *E. durans, S. typhimurium, E. faecium* and *S. marrescens,* on both branch and leaves for *P. fluorescens* and *B. subtilis*.The effect of *A. nordmanniana* subsp. *nordmanniana* extracts on other used microorganisms, the MIC values were determined to be bacteriostatic for all parts of the plant.

When the effect of *A. cilicica* subsp. *isaurica* extracts on the microorganisms used was examined; the MIC value showed bactericidal effect on branches and cones for *E. aerogenes, K. pneumoniae, S. kentucky* and *E. durans;* on all parts of the plant for *S. enteritidis* and *B. subtilis.* The MIC value was determined to be bactericidal on cones for *E. faecium, S. epidermidis* and *S. marrescens;* on leaves for *S. infantis, E. faecalis, S. typhimurium* and *E. coli*, on the branches *for L. innocua, C. albicans, S. typhimurium* and *S. aureus.*

When the effect of *A. cilicica* subsp. *cilicica* extracts on microorganisms used was examined; it was found that the MIC value had bactericidal effect on leaves *for K. pneumoniae, P. fluorescens, E. durans, S. typhimurium* and *S. marcescens*. In the branches of the same species, it was observed that the MIC value had bactericidal effect on *E. aerogenes, P. infantis, K. pneumoniae, S. typhimurium, C. albicans, E. faecium, S. epidermidis, B. subtilis* and *S. marcescens*. It appears that this species MIC values are bacteriostatic on, *L. monocytogenes, P. aeruginosa, S. kentucky, E. faecalis, L. innocua, S. enteritidis, S. aureus* and *E. coli.*

Discussion

Studies have shown that the antimicrobial activity of essential oils is largely dependent on oil concentration and bioactive compounds (Dayisoylu et al., 2009). Extracts belonging to different parts of *Abies* taxa, which were used in this work, were found to be effective against the microorganisms used in the study. The study showed that taxa belonging to the genus *Abies* and different parts of these taxa exhibited different antimicrobial activity. Looking at previous studies, it was found that *A. balsamea*, had no effect on *E. coli* and it has been found to be effective against *S. aureus* with 56 µg/µl MIC (Pichette et al., 2006).

In another study on cones of fir species, α-Pinene was found to be the main component of more than 50%, followed by β-pinene. In this study, looking at the chemical compounds in essential oils obtained from cones of *A. cilicica* subsp. *isaurica taxon*; cis-Ocimene with a ratio of 57.79%, in *A. equi-trojani* cones, Santolina triene was the most identified, compared to 60.86%, and in cones of both species, beta-pinene was the second largest (Tumen et al., 2010).

According to previous studies, the highest essential oil in the Pinaceae family has been observed in the *A. equi trojani* taxon. Essential oils of *Abies* species have been found to have less content against *Pinus* species in terms of other compounds except α-pinene and β-pinene. In the study on cones of taxa belonging to the family Pinaceae, the highest level of monoterpene hydrocarbon levels has been identified in *A. cilicica* (93.14%) species, the highest monoterpene alcohol has been identified in *A. equi-trojani* (10.70%) (Tumen et al., 2010).

In another study on the seeds of *A. nordmanniana*, it was also indicated to be effective against microorganisms (Digrak et al., 2002). The methanol extract of leaves

belonging to the species *A. webbiana* shows a broad spectrum of antimicrobial activity (Vishnoi et al., 2007), the methanol extract of *A. cilicia* leaves has been found to be effective against *B. subtilis* and *S. aureus* in earlier studies. (Dığrak et al., 1999). In this study, when mic test results for *A. cilicica subsp. isaurica* taxon were examined, it was found that leaf and branch extracts showed MIC value against microorganisms strains between 0.195-100 mg/100 µL and cone extract between 0.195-12.5 mg/100 µL concentrations. When the effect of extracts of the same species on the microorganisms were examined; it was found that MIC value had bactericidal effect on all parts of the plant for *S. enteritidis and B. subtilis*. When the effect of *A. cilicica* subsp. *cilicica* extracts on the microorganisms were examined; it appeared that MIC values were bacteriostatic on *L. monocytogenes, P. aeruginosa, S. kentucky, E. faecalis, L. innocua, S. enteritidis, P. aureus and E. coli*.

In a study involving *Abies* species that do not grow naturally in Türkiye, *A. Pinsapo* and *A. alba* species have been identified as having the least impact against bacteria among *Abies* species. Oils belonging to the *A. koreana, A. nordmanniana* subsp. *nordmanniana, A. nordmanniana* subsp. *bornmiielleriana, A. cilicica* subsp. *isaurica* and *A. cilicica* subsp. *cilicica* species are more effective against bacteria than other *Abies* species, *A. concolor* and A. *pinsapo* species are not active against most bacteria. (Bagci & Digrak, 1994). It has been noted in previous studies that essential oil components can vary depending on plant species, hemotypes and climatic conditions, and therefore antimicrobial effects can cause differences (Lawrence, 1985). As a matter of fact, collected from different environments in our country this is supported by the fact that *A. nordmanniana* species have different effects on bacteria (Bagci & Digrak, 1994).

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: A.O.P., K.G.; Investigation: A.O.P., K.G.; Material and Methodology: A.O.P., K.G., T.Ç.; Supervision: K.G., T.Ç.; Visualization: A.O.P.; Writing-Original Draft: A.O.P.; Writing-review & Editing: A.O.P., K.G., T.Ç.; Laboratory Work: E.S.Y.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

The authors declared that this study has received no financial support.

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