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Chemical Composition and Antimicrobial Effect of Essential Oil of *Anthemis pauciloba* Boiss. var. *pauciloba* from Türkiye

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ABSTRACT
Anthemis pauciloba Boiss. var. *pauciloba* is one of four recognized varieties in Türkiye, locally known as "bol papatya." Its flowers are traditionally used as a cold infusion to treat asthma. The aim of the research was to determine the chemical composition of essential oil (EO) of *A. pauciloba* var. *pauciloba* aerial parts obtained by hydrodistillation using a Clevenger-type apparatus, examined by GC-FID, and GC-MS, simultaneously. The EO was evaluated for antibacterial and antifungal activities against microbial strains utilizing the broth-microdilution technique. *α*-Thujone (28.7%), *α*-pinene (26.7%), and *β*-thujone (9.0%) were found as the main constituents of EO. The antimicrobial activity (Minimum Inhibitory Concentration) against gram-negative, gram-positive, and yeast was observed by the essential oil. The essential oil demonstrated the highest antimicrobial activity against *Candida krusei* (MIC: 1.25 mg/mL). The antimicrobial activity of the essential oil from the aerial parts of *A. pauciloba* var. *pauciloba* was evaluated for the first time in this study.

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Türkiye'den *Anthemis pauciloba* Boiss. var. *pauciloba*'nın Uçucu Yağının Kimyasal Bileşimi ve Antimikrobiyal Etkisi

ÖZET
Anthemis pauciloba Boiss. var. *pauciloba*, Türkiye'de tanımlanan dört varyeteden biridir ve halk arasında "bol papatya" olarak bilinmektedir. Bitkinin çiçekleri, astım tedavisinde soğuk çay şeklinde kullanılmaktadır. Araştırmanın amacı, *A. pauciloba* var. *pauciloba*'nın toprak üstü kısımlarından Clevenger tipi aparat kullanılarak hidrodistilasyon yöntemiyle elde edilen uçucu yağın (EO) kimyasal bileşimini belirlemektir. Uçucu yağın bileşenleri, GC-FID ve GC-MS teknikleriyle eşzamanlı olarak analiz edilmiştir. Uçucu yağının mikrobiyal suşlara karşı antibakteriyel ve antifungal aktivitesini mikrodilüsyon tekniği kullanarak değerlendirmiştir. Uçucu yağın ana bileşikleri olarak *α*-tuyon (%28.7), *α*-pinen (%26.7) ve *β*-tuyon (%9.0) bulunmuştur. Gram-negatif, gram-pozitif ve mayaya karşı antimikrobiyal aktivite (Minimum İnhibitör Konsantrasyon) değerlendirilmiştir. Uçucu yağ, *Candida krusei*'ye karşı en yüksek antimikrobiyal aktiviteyi göstermiştir (MİK: 1.25 mg/mL). Bu çalışmada, *A. pauciloba* var. *pauciloba*'nın toprak üstü kısımlarından elde edilen uçucu yağın antimikrobiyal aktivitesi ilk kez değerlendirilmiştir.

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INTRODUCTION

Anthemis L. belongs to the Asteraceae family, tribe Anthemideae, and is the second largest genus in the family, with more than 210 species. It is distributed widely across Europe, Southwest Asia, and North and East Africa (Hamzaoğulu et al., 2011; Özbek et al., 2021).

Anthemis includes 51 species and 81 taxa in Türkiye (Davis, 1975; Güner et al., 2000). *Anthemis* species are known to have various biological activities, and they are commonly used in folk medicine. Essential oil from *Anthemis nobilis* flowers is commonly used for pharmaceuticals, and it is also an important source of oil in food additives, cosmetics, and aromatics. Some *Anthemis* species essential oils possess anti-ageing activity and antioxidants (Sarogluo et al., 2006). Species belonging to *Anthemis* genus are commonly referred to as "Papatya" in Türkiye. Papatya is a popular name given to plants whose flowers resemble those of German and Roman chamomile (Orlando et al., 2019).

A. pauciloba Boiss. is represented by four varieties in Türkiye, and var. *pauciloba* known local name "bol papatya", and the flowers of the plant are used as cold tea in the treatment of asthma (Melikoğlu et al., 2015; Bizim Bitkiler, 2024). *A. pauciloba* is an erect or rarely decumbent herb. Stems are simple or commonly branched near the base, (15-)30-45 cm. Leaves are variable in dissection; basal leaves are petiolate, and ± ovate in outline, and upper leaves are cuneate-spathulate. Capitula is radiate or discoid. Ligules, when present yellow (Davis, 1975).

Anthemis pauciloba var. *pauciloba* is characterized by simple stems or stems branched near the base, with basal leaves ranging from linear-oblanceolate to linear-obovate, typically bearing 3 or 7 pairs of lateral lobes. This variety has been recorded in various regions of Türkiye, particularly in mountainous and steppe ecosystems. Notable collection sites include Manisa, Isparta, Antalya, Gaziantep, Şanlıurfa, and Mardin, indicating a broad ecological distribution. The presence of this taxon across diverse habitats, including limestone rocky valleys, macchie clearings, and steppe environments, underscores its adaptability to varying climatic and edaphic conditions (Davis, 1975).

Phytochemical studies on various Anthemis species have revealed significant variations in essential oil (EO) composition due to factors such as geographic location, genetic differences, and extraction methods. For instance, the major components of *A. pauciloba var. microstephana* were identified as α-pinene (62.0%), 1,8-cineole (11.6%), and α-caryophyllene alcohol (8.0%), while *A. pauciloba var. sieheana* contained 1,8-cineole (8.27%) and β-pinene (4.97%) (Kürkçüoğlu et al., 2009; Keskin et al., 2017). Studies on *A. pauciloba var. pauciloba* have shown variations in major constituents, with camphor (36.7%), camphene (13.9%), and α-pinene (13.6%) in one report, while another study found *α*-thujone (28.7%), *α*-pinene (26.7%), and *β*-thujone (9.0%) as dominant compounds (Kürkçüoğlu et al., 2009). Such differences highlight the influence of environmental and ecological factors on EO composition.

Among these constituents, thujone -a monoterpene ketone- is particularly notable due to its neurotoxic and bioactive properties, including potential anticancer effects (Pelkonen et al., 2013; Radulović et al., 2017). *α*-Thujone has also been reported as a primary compound in other *Anthemis* species, such as *A. carpatica* (40.2%), *A. montana*, and *A. cretica* ssp. *carpatica* (Bulatovic et al., 1997; Pavlovic et al., 2010), as well as in plants from related genera like *Artemisia* and *Salvia* (Pelkonen et al., 2013). In addition to their diverse chemical compositions, various *Anthemis* species have been reported to exhibit significant antimicrobial and anti-inflammatory properties (Radulović et al., 2017; Zámboriné et al., 2020), which further underscores their pharmacological potential. These findings emphasize the phytochemical diversity within the *Anthemis* genus and the need for further investigation into the chemical composition and biological effects of their essential oils.

The aim of this study was to determine the chemical composition of the essential oil obtained from the aerial parts of *Anthemis pauciloba* var. *pauciloba*, and to evaluate its antibacterial and antifungal activities against selected microorganisms. To the best of knowledge, this is the first study to investigate both the antimicrobial potential of this species.

MATERIALS and METHODS

Plant Material

The aerial parts of *Anthemis pauciloba* var. *pauciloba* were collected on 26 June 2014 during the flowering stage, with all specimens bearing fully developed flowers. The plant material was gathered from a stony area near Kılan village, located in Ulukışla district, Niğde province, Türkiye, at an altitude of 1390 meters. The collection was carried out by Süleyman Doğu, and a voucher specimen was deposited in the Herbarium of the Department of Biology, Necmettin Erbakan University (Herbarium number: S.D. 3560).

After collecting, the aerial parts were transported to the laboratory in paper bags, dried in a dry, shaded, well-ventilated room at ambient temperature, and subsequently ground into powder. The powdered samples were then placed in airtight zip-lock bags, the air was removed, and the bags were stored at +4 °C until further analysis.

Extraction

The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus for 3h. EO of *Anthemis pauciloba* var. *pauciloba* and examined by GC-FID and GC-MS, simultaneously.

Gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS)

Anthemis pauciloba var. *pauciloba* essential oil was analysed by GC using a Hewlett-Packard 6890 (Sem Ltd., Istanbul, Turkey) system, and an HP Innowax FSC column (60 m × 0.25 mm ∅, with 0.25 µm film thickness) was used with nitrogen at 1 ml/min. The initial oven temperature was 60 °C for 10 min, and increased at 4 °C/min to 220 °C, then remained constant at 220 °C for 10 min and increased at 1 °C/min to 240 °C. Injector temperature was set at 250 °C. Percentage composition of the individual components was obtained from electronic integration using flame ionization detection (FID) at 250 °C. *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI).

GC/MS analysis was performed with a Hewlett-Packard GCD (Sem Ltd., Istanbul, Turkey), system, and Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness) was used with helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 ml/min, and the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 425 (Demirci et al., 2008).

Components of Essential Oil Identification

The volatile components were identified by comparing their relative retention times (RRI) to those of authentic samples or by comparing their relative retention index to a series of *n*-alkanes. For identification, an in-house (Library's Başer) and computer matching against commercial databases (Library's MassFinder software 4.0 and Wiley GC/MS Library (Wiley, NY, USA) were built up, from actual components of known essential oil was employed (Demirci et al., 2022).

Microbial Cultures

The test organisms used in the study were as follows: *Staphylococcus aureus* American Type Culture Collection (ATCC) 6538, *Salmonella Tymphirium* ATCC 14028, *Staphylococcus aureus* ATCC 700699, *Escherichia coli* Northern Regional Research Laboratory (NRRL) B-3008, *Candida albicans* ATCC 90028, and *Candida krusei* ATCC 6258.

Antimicrobial Activity

The microdilution broth susceptibility assay was tested for the antibacterial and antifungal evaluation of the EO of *A. pauciloba* var. *pauciloba* aerial parts. Stock solutions of the EO were prepared in dimethylsulfoxide (DMSO) and sterile distilled water. Overnight-grown microorganism suspensions in MHA (for bacteria) and *Candida albicans* yeast suspension in yeast medium (for fungus) were standardized to 108 CFU/mL. The wells were then filled with 100 µL of each culture suspension. The final row, which was devoid of microbes, served as a sterility control. In another row, the microbe and MHA medium were used as a growth control. The minimum inhibitory concentration (MIC, mg/mL) was obtained after a 24-hour incubation at 37°C. 20 µL of resazurin (Sigma) reagent was put on plates for visualization and incubated at 37°C for 3 hours. Ketoconazole (Fluka), itraconazole (FAGEM), Fluconazole (FAGEM), and ciprofloxacin (Merck), ampicillin (Sigma) were used as standard components (CLSI, 2006; Saltan et al., 2018). All experiments were repeated three times, and average MICs are presented in Table 2.

Statistical analysis

GraphPad Prism Software Version 9.0 was used for data analysis to evaluate differences in results between the experimental and standard groups. The findings are displayed as the average ± standard deviation (S.D.).

RESULTS and DISCUSSION

Essential Oil Yield and Composition

The present research aimed the identifying the volatile components of *A. pauciloba* var. *pauciloba* aerial parts. The essential oil was subjected to hydrodistillation to obtain it, and it was analyzed by both GC-FID and GC-MS simultaneously. The volatile components of the essential oil were listed in Table 1. The essential oil's yield was determined to be 0.15%.

A total of 85 volatile components were determined in the EO's composition of *A. pauciloba* var. *pauciloba* aerial parts, representing 94.7% of the total EO. The components of EO were grouped into six main chemical classes: oxygenated monoterpenes, monoterpene hydrocarbons, oxygenated sesquiterpenes, sesquiterpene hydrocarbons, fatty acids, and others. The essential oil of *A. pauciloba* var. *pauciloba* was defined by a high concentration of oxygenated monoterpenes (45.4%) and monoterpene hydrocarbons (30%). The essential oil was identified major components as *α*-thujone (28.7%), *α*-pinene (26.7%) and *β*-thujone (9.0%), respectively.

Table 1. The chemical composition of the essential oil of *Anthemis pauciloba* var. *pauciloba*
Çizelge 1. Anthemis pauciloba var. pauciloba uçucu yağının kimyasal kompozisyonu

RRI[a]	KI[b]	Compound	%	Identification method
1032	1008-1039[c]	*α*-Pinene	**26.7**	t_R, MS
1035	1012-1039[c]	*α*-Thujene	0.3	t_R, MS
1076	1043-1086[c]	Camphene	0.1	t_R, MS
1118	1085-1130[c]	*β*-Pinene	1.1	t_R, MS
1132	1098-1140[c]	Sabinene	tr	t_R, MS
1135	1109-1137[c]	Thuja-2,4(10)-diene	0.4	MS
1151	1122-1169[c]	*δ*-3-Carene	0.1	MS
1188	1154-1195[c]	*α*-Terpinene	0.1	t_R, MS
1213	1186-1231[c]	1,8-Cineole	0.2	t_R, MS
1224	1224[d]	*o*-Mentha-1(7)5,8-triene	0.1	MS
1255	1222-1266[c]	*γ*-Terpinene	0.2	t_R, MS
1278	1244-1279[c]	*m*-Cymene	0.1	MS
1280	1246-1291[c]	*p*-Cymene	0.8	t_R, MS
1285	1277-1317[d]	Isoamyl isovalerate	0.1	MS
1400	1370-1414[c]	Nonanal	tr	MS
1430	1385-1441[c]	*α*-Thujone	**28.7**	MS
1451	1400-1452[c]	*β*-Thujone	**9.0**	MS
1466	1438-1480[c]	*α*-Cubebene	tr	MS
1497	1462-1522[c]	*α*-Copaene	1.7	MS
1499	1486-1500[e]	α-Campholene aldehyde	1.0	MS
1535	1496-1546[c]	*β*-Bourbonene	0.4	MS
1536	1504-1548[c]	Pinocamphone	0.2	t_R, MS
1586	1545-1590[c]	Pinocarvone	0.4	t_R, MS
1611	1564-1630[c]	Terpinen-4-ol	0.5	t_R, MS
1612	1570-1685[c]	*β*-Caryophyllene	0.4	t_R, MS
1628	1583-1668[c]	Aromadendrene	0.1	MS
1648	1597-1648[c]	Myrtenal	0.8	MS
1663	1647-1668[c]	*cis*-Verbenol	0.2	MS
1670	1643-1671[c]	*trans*-Pinocarveol	0.6	t_R, MS
1683	1665-1691[c]	*trans*-Verbenol	1.1	t_R, MS
1687	1637-1689[c]	*α*-Humulene	0.1	t_R, MS
1704	1655-1714[c]	*γ*-Muurolene	0.5	MS
1725	1696-1735[c]	Verbenone	0.3	t_R, MS
1773	1722-1774[c]	*δ*-Cadinene	0.5	MS
1776	1735-1782[c]	*γ*- Cadinene	0.2	MS
1804	1743-1808[c]	Myrtenol	0.2	MS
1830	1782-1833[d]	Tridecanal	tr	MS
1838	1789-1842[c]	*(E)-β*-Damascenone	tr	MS
1845	1805-1850[c]	*trans*-Carveol	0.4	t_R, MS
1849	1836-1837[f]	Calamenene	0.1	MS
1864	1813-1865[c]	*p*-Cymen-8-ol	0.1	MS
1929	1929[d]	2-Methyl butyl benzoate	0.1	MS
1941	1893-1941[c]	*α*-Calacorene	0.1	MS
1981	1916-1993c	Heptanoic acid	0.1	t_R, MS
2008	1936-2023[c]	Caryophyllene oxide	1.3	t_R, MS
2037	2016-2043[c]	Salvial-4(14)-en-1-one	0.3	MS
2041	1980-2060[c]	Pentadecanal	0.2	MS
2057	2014-2062[c]	Ledol	0.2	MS
2071	2003-2071[c]	Humulene epoxide II	0.2	MS
2080	2052[e]	Junenol	0.1	MS
2084	2011-2089[c]	Octanoic acid	0.1	t_R, MS
2098	2049-2104[c]	Globulol	0.2	MS
2100	2100[f]	Heneicosane	0.1	t_R, MS

2130	2130[d]	Salviadienol	0.1	MS
2131	2089-2131[d]	Hexahydrofarnesyl acetone	1.4	MS
2144	2074-2150[c]	Spathulanol	2.4	MS
2178	2134-2191[d]	*T*-Cadinol	0.2	MS
2179		*nor*-Copaonone	0.2	MS
2198	2100-2205[c]	Thymol	0.4	t_R, MS
2209	2143-2230[d]	*T*-Muurolol	0.1	MS
2210		Copaborneol	0.2	MS
2219	2142-2219[d]	Torreyol	0.1	MS
2239	2140-2246[c]	Carvacrol	0.2	t_R, MS
2247	2241-2247[d]	*trans*-*α*-Bergamotol	0.2	MS
2250	2186-2250[c]	*α*-Eudesmol	0.9	MS
2255	2180-2255[c]	*α*-Cadinol	0.3	MS
2278	2231-2278[d]	Torilenol	0.3	MS
2289		4-*oxo*-*α*-Ylangene	0.2	MS
2298	2227-2301[c]	Decanoic acid	0.1	t_R, MS
2300	2300[f]	Tricosane	0.7	t_R, MS
2312		9-Geranyl-*p*-cymene	0.9	MS
2316	2316-2320[d]	Caryophylladienol I	0.1	MS
2329		14-Acetoxy-*α*-humulene	0.1	MS
2369	2351-2402[c]	Eudesma-4(15)7-dien-1-*β*-ol	0.3	MS
2389		Caryophyllenol I	0.2	MS
2392		Caryophyllenol II	0.3	MS
2400	2339-2421[c]	Undecanoic acid	0.3	t_R, MS
2430	2334-2452[c]	Chamazulene	0.1	t_R, MS
2500	2500[f]	Pentacosane	0.1	t_R, MS
2503	2442-2524[c]	Dodecanoic acid	0.3	t_R, MS
2617	2573-2678[c]	Tridecanoic acid	0.2	t_R, MS
2670	2634-2719[c]	Tetradecanoic acid	0.5	t_R, MS
2700	2700[f]	Heptacosane	0.2	t_R, MS
2900	2900[f]	Nonacosane	tr	t_R, MS
2931	2862-2945[c]	Hexadecanoic acid	2.2	t_R, MS
		Monoterpene hydrocarbons	30	
		Oxygenated monoterpenes	45.4	
		Sesquiterpene hydrocarbons	4.1	
		Oxygenated sesquiterpenes	10	
		Fatty acid	3.8	
		Others	1.3	
		Yield (%)	**0.15**	
		Total	**94.6**	

[a]RRI: Relative retention indices calculated against *n*-alkanes; [b]KI from literature ([c, d, e, f]), [c]Babushok et al., 2011; [d]Pubchem, 2024; [e]NIST Chemistry WebBook, 2024; [f]The Pherobase, 2024; t_R: Identification based on the retention times of genuine compounds on the HP Innowax FSC column; MS: Tentative identification on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data. tr: Trace (<0.1 %);,%: calculated from FID data.

The oil of *A. pauciloba* var. *pauciloba* was characterized by a high number of oxygenated monoterpenes and monoterpene hydrocarbons. The major components of EO were determined as *α*-thujone, *α*-pinene, and *β*-thujone.

In an earlier study, Kürkçüoğlu et al. (2009) reported that essential oils of *A. pauciloba* var. *microstephana* and *A. pauciloba* var. *pauciloba* were obtained from the aerial parts by two techniques, hydrodistillation in a Clevenger-type apparatus and microdistillation using an Eppendorf MicroDistiller®. Major components of the oil of *A. pauciloba* var. *microstephana* were found as *α*-pinene (20.1%), *α*-caryophyllene alcohol (8.0%) and *α*-pinene (62.0%), 1,8-cineole (11.6%), respectively. Major components of the volatiles of *A. pauciloba* var. *pauciloba* were found as camphor (36.7%), camphene (13.9%), α-pinene (13.6%), guaiol (16.8%), β-bisabolenal (8.6%), and spathulenol (7.5%), respectively (Kürkçüoğlu et al., 2009).

In another study, Keskin et al. (2017) reported that the main components of *Anthemis pauciloba* var. *sieheana* EO were 1,8-cineol (8.27 %), and *β*-pinene (4.97 %). The main constituents of *A. pauciloba* var. *sieheana*'s fatty acids were 9,12-octadecadienoic acid methyl ester (48.46%), 9-octadecanoic acid methyl ester (16.17%), and hexadecenoic acid methyl ester (13.3%) (Keskin et al., 2017).

In the present study, the essential oil (EO) of *A. pauciloba var. pauciloba* was characterized by a high concentration of *α*-thujone (28.7%), *α*-pinene (26.7%), and *β*-thujone (9.0%) as the major components. In contrast, Kürkçüoğlu et al. (2009) reported that the predominant constituents of *A. pauciloba var. pauciloba* EO were camphor (36.7%), camphene (13.9%), and *α*-pinene (13.6%). These differences suggest that environmental factors, ecological conditions, and extraction methods may significantly influence the chemical composition of the essential oil.

Furthermore, Keskin et al. (2017) identified 1,8-cineole (8.27%) and *β*-pinene (4.97%) as the major constituents of *A. pauciloba var. sieheana* EO. The variations observed in the chemical profiles of different *A. pauciloba* varieties highlight the phytochemical diversity within the species and emphasize the potential impact of genetic and environmental factors on essential oil composition.

According to the literature, thujone is a type of monoterpene ketone that occurs naturally in different amounts within various plant species (Plkonen et al., 2013). According to the search results, *α*-thujone was determined as the main component in the EO *of A. carpatica, A. montana*, and *A. cretica* ssp. *carpatica* (Bulatovic et al., 1997; Bulatovic et al., 1998; Pavlovic et al., 2010). The essential oil of *A. carpatica* was found to contain 40.2% *α*-thujone.

Thujone is a volatile compound widely debated due to its behaviour-modulating and toxic properties (Bulatovic et al., 1997). However, a study in 2016 found that *α*-thujone stimulates an anticancer immune response. Chemotypes of *Anthemis* were identified with thujone and *cis*-epoxycimene, as the main components (Radulović et al., 2017; Zámboriné et al., 2020). Also, Thujone is a major component of EOs derived from plants like *Salvia officinalis, Salvia sclarea, Tanacetum vulgare, Artemisia absinthium,* and *Thuja occidentalis* (Pelkonen et al., 2013).

Antimicrobial Effects

The antimicrobial effects of the EO of *A. pauciloba* var. *pauciloba* aerial parts were tested against reference *S. aureus* (gram-positive bacteria), *E. coli* (gram-negative bacteria), *S. tymphirium* (gram-negative bacteria), *C. albicans* (yeast), and *C. krusei* (yeast) strains. The results of the antimicrobial effects of the EO are listed in Table 2. The EO demonstrated the highest antimicrobial activity against *C. krusei* (1.25 mg/mL). Among the tested microorganisms *C. krusei* was observed to be more sensitive to the EO. In this study, the antimicrobial activity of the essential oil of *A. pauciloba* var. *pauciloba* aerial parts was used for the first time.

Table 2. MIC values (mg/mL) of the essential oil of *Anthemis pauciloba* var. *pauciloba*
Çizelge 2. Anthemis pauciloba var. pauciloba uçucu yağının MİK değerleri (mg/mL)

	E. coli NRRL B-3008	*S. aureus* ATCC 6538	*S. Tymphirium* ATCC 14028	*S. aureus* ATCC 700699	*C. albicans* ATCC 90028	*C. krusei* ATCC 6258
EO	>10±0.00	>10±0.00	>10±0.00	>10±0.00	10±2.89	1.25±0.72
Ampicilin	0.01±0.01	0.63±0.36*	1.3±0.75*	0.02±0.01	-	-
Clarithromycin	0.02 ±0.01	0.63±0.36*	0.04±0.2	0.16±0.09	-	-
Ketoconazole	-	-	-	-	0.01±0.01	-
Itraconozole	-	-	-	-	0.04±0.02	0.01±0.01
Fluconazole	-	-	-	-	-	0.04±0.02

EO: Essential oil; *: µg/mL; -: not dedected

Although the essential oil of *A. pauciloba* var. *pauciloba* demonstrated some antifungal activity against *C. krusei* (MIC: 1.25 mg/mL), its overall antimicrobial potential against the tested microorganisms was relatively low compared to standard antifungal agents. This limited activity may be attributed to the complex chemical composition of the oil, where the presence of both active and inactive constituents may influence its bioactivity.

While it is not appropriate to directly compare the antimicrobial effects of different plant species, previous studies on *Artemisia herba-alba* have shown that essential oils rich in oxygenated monoterpenes, particularly α-thujone and β-thujone, exhibit varying antimicrobial activities depending on their relative proportions (Mighri et al., 2010). In the present study, the antifungal effect observed against *C. krusei* suggests that *α*-thujone and *α*-pinene, as key components, may play a role in the bioactivity of *A. pauciloba* var. *pauciloba* oil. However, the significantly lower activity compared to standard agents indicates that these compounds alone may not be sufficient to achieve potent antimicrobial effects.

Further studies focusing on the isolation and testing of individual constituents and their synergistic interactions are required to clarify the specific compounds responsible for the observed antifungal activity. Additionally, investigating the effects of geographic variation and environmental factors on the chemical composition could provide deeper insights into the bioactive potential of this species.

The oils from roots and aerial parts of *Anthemis mixta* and *A. tomentosa* were evaluated for their antibacterial effect against ten bacterial species. Notably, the essential oils obtained from the aerial parts of both species were particularly effective against Gram-positive bacteria (Formisano et al., 2012). This aligns with previous findings suggesting that the lipophilic nature of essential oil components allows them to interact with the lipid bilayer of Gram-positive bacteria, increasing membrane permeability and causing cellular disruption (Burt, 2004; Bassolé & Juliani, 2012).

In comparison, the essential oil of A. pauciloba var. pauciloba in the present study exhibited limited activity against Gram-positive bacteria, except for C. krusei. This discrepancy may be attributed to differences in chemical composition between species, particularly the relative abundance of oxygenated monoterpenes such as α-thujone and α-pinene, which are known to contribute to antimicrobial activity. Furthermore, variations in extraction methods and geographic origin could also explain the observed differences in antibacterial efficacy. In another study, the essential oils of three *Anthemis* species from Türkiye were analyzed for their chemical composition and antimicrobial activity. Although the antibacterial effects reported by Kurtulmuş et al. (2009) were relatively stronger than those observed in the present study, the variability may be attributed to differences in chemical composition, likely influenced by environmental conditions.

These findings indicate that Anthemis species may possess some antimicrobial potential, though further studies are needed to clarify their efficacy. Investigations into their mode of action, synergistic effects with other antimicrobial agents, and clinical applicability could provide valuable insights for developing targeted antibacterial therapies.

CONCLUSIONS

The essential oil of *A. pauciloba* var. *pauciloba* aerial parts was identified. Also, the essential oil was found to have high antimicrobial activity against *C. krusei.* However, this is the first time that the antimicrobial activity of this essential oil has been reported.

Conflict of Interest

The authors declare that they do not have any competition and any conflicts of interest.

Author Contributions

Execution research project, Experimental design, Data analysis, Manuscript preparation- DK, AK, SD, BD; Experimental design, Data analysis, Manuscript preparation- DK, BD; Materials, Supervision, Writing - review & editing. DK, AK, SD, BD; Experimental design, Data analysis, Manuscript preparation. DK, BD; Materials AK, SD.

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