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Nobel's harvest in Türkiye: Delving into *Artemisia*'s spirit - essential oil content and antimicrobial potential of seven species

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ABSTRACT: The 2015 Nobel Prize in Physiology or Medicine was awarded due to the isolation of the active ingredient of artemisinin, a sesquiterpene lactone, from the plant *Artemisia annua* L. and proving its effectiveness in the treatment of malaria, and the chemical contents and biological activities of other *Artemisia* L. species aroused great interest. In this study, it was aimed to investigate the chemical content of essential oils obtained by hydrodistillation from the aerial parts of seven *Artemisia* species (*A. abrotanum* L., *A. absinthium* L., *A. annua* L., *A. austriaca* Jacq., *A. chamaemelifolia* Vill., *A. incana* (L.) Druce, *A. tournefortiana* Rchb.) growing in different regions of Turkiye and to evaluate their antimicrobial activities. The essential oils were analyzed by GC and GC-MS. The main components are chrysanthenone (55.9%) for *A. abrotanum*, sabinyl acetate (23.0%) for *A. absinthium*, artemisia ketone (53.7%) for *A. annua*, camphor (34.2%) for *A. austriaca*, selin-11-en-4-α-ol (29.1%) for *A. chamaemelifolia*, camphor (29.7%) for *A. incana*, and (*Z*)-*β*-farnesene (71.5%) for *A. tournefortiana*. *In vitro* antimicrobial activity of essential oils against five microorganisms (*Bacillus subtilis, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa*) was investigated using the microdilution method. The highest activity against all species was observed in *A. incana* essential oil. *Staphylococcus aureus* was found to be the most sensitive bacteria to all essential oils.

KEYWORDS: Antimicrobial; Artemisia; essential oil; GC; GC-MS.

1. INTRODUCTION

Artemisia L., belonging to the Asteraceae family and Anthemideae tribe, comprises over 500 species, predominantly distributed in Asia, Europe, North America and North Africa such as South Algeria [1,2] *Artemisia* genus is divided into four subgenera: *Artemisia* Less., *Dracunculus* (Bess.) Rydb., *Seriphidium* (Bess.) Rouy, and *Tridentatae* (Rydberg) McArthur. While taxa of the first three subgenera are found in Türkiye, no taxon belonging to the Tridentatae subgenus is present in our country. In Türkiye, *Artemisia* genus is identified as 26 taxa, including 21 species, 3 subspecies, and 2 varieties, belonging to three subgenera [3].

Artemisia annua L., is notably one of the popular plants in traditional Chinese medicine, frequently employed in the treatment of diseases such as malaria, hepatitis, cancer, inflammation, as well as fungal, bacterial, and viral infections [4]. The isolation of artemisinin, the principal sesquiterpene produced by the plant, from this species has been recommended by the World Health Organization for the treatment of quinine-resistant malaria [5]. The recognition of Tu You You with the 2015 Nobel Prize in Physiology or Medicine for the isolation of the active compound in artemisinin and the validation of its efficacy in malaria treatment have stimulated significant interest in the chemical compositions and biological activities of other species within the *Artemisia* genus [6].

Ethnobotanical studies conducted in Turkiye have revealed that *Artemisia* species are utilized for various medicinal purposes, including anthelmintic, antipyretic, hypotensive, appetite stimulant, wound healing, gastric, diuretic, tonic, and sedative effects. These species have been traditionally employed in the management of diseases such as malaria, diabetes, asthma, ulcers, and the common cold [7]. Numerous

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pharmacological activity studies based on ethnobotanical principles have been conducted using *Artemisia* species.

When examining the chemical analyses of species belonging to the *Artemisia* genus, it is observed that they generally contain a variety of secondary metabolites, including terpenoids, predominantly monoterpenes in volatile oils [1], sesquiterpene lactones [8], steroids [9], phenolic compounds such as flavonoids [10,11], phenolic acids [12], lignans [8,13], coumarins [9,14,15], and alkaloids [16]. All of these compounds precede various biological activities [17].

In this study, the aim is to investigate the chemical composition of the essential oils obtained through hydrodistillation from the aerial parts of seven *Artemisia* species (*A. abrotanum* L., *A. absinthium* L., *A. annua* L., *A. austriaca* Jacq., *A. chamaemelifolia* Vill., *A. incana* (L.) Druce, *A. tournefortiana* Rchb.) grown in different regions of Türkiye. Considering the essential oil content and ethnobotanical uses of *Artemisia* species, their *in vitro* antimicrobial properties against five different species (*Bacillus subtilis, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa*) were investigated In addition to species that have been previously studied and whose activity has been proven, this study examined for the first time the antimicrobial activity in essential oils of *A. abrotanum, A. chamamelifolia* and *A. tournefortiana* species growing in Türkiye.

2. RESULTS

The essential oil yields were determined as follows: *A. abrotanum* (0.58%), *A. absinthium* (1%), *A. annua* (2.33%), *A. austriaca* (0.74%), *A. chamaemelifolia* (0.65%), *A. incana* (0.35%), and *A. tourfortiana* (0.5%). The essential oils underwent simultaneous analysis using GC and GC-MS to determine their chemical characterization. Components were given in Table 2. As a result of the subsequent antimicrobial analysis, the MIC values of the essential oils were given in Table 3.

3. DISCUSSION

Essential oils can be found in various parts of plants, including flowers and inflorescences (e.g., chamomile, lavender, rose, ylang-ylang), leaves (e.g., basil, laurel, lemongrass, mint, rosemary), fruits (black pepper, coconut), fruit peels (orange, bergamot, lemon, mandarin), seeds (anise, cumin, cardamom, fennel), roots and rhizomes (ginger, turmeric), and resins (myrrh, frankincense) [18]. The composition of essential oils can vary due to factors such as plant species or cultivar, geographical origin, environmental conditions, agricultural practices, and extraction method [19].

In the scope of this study, the compositions of the essential oils of seven *Artemisia* species were determined using GC and GC-MS methods. The obtained results revealed significant variations in the major components among the studied species. For *A. abrotanum*, chrysanthenone (%55.9), 1,8-cineole (%5.9), filifolone (%3.2), and *β*-bourbonene (%3.0) were identified as the predominant components. *A. absinthium* exhibited notable components such as sabinyl acetate (%23), artemisia ketone (%22.1), myrcene (%12.8), camphor (%5.5), and additionally chamazulene (%0.5). *A. annua* demonstrated significant levels of artemisia ketone (%53.7), camphor (%12.3), 1,8-cineole (%4.2), and artemisia alcohol (%4.1). In the case of *A. austriaca*, camphor (%34.2), trans-sabinol (%16.8), 1,8-cineole (%10.9), and terpinen-4-ol (%3.5) were the major constituents. *A. chamamolliaefolia* exhibited prominent compounds such as selin-11-en-4-*α*-ol (%29.1), 1,8-cineole (%16.3), presilphiperpholone-9-*α*-ol (%8.6), and artemisia ketone (%5.4). For *A. incana*, camphor (%29.7), borneol (%12.1), piperitone (%10.1), and 1,8-cineole (%8.7) were identified as significant components. Lastly, *A. tournefortiana* displayed high levels of (*Z*)-*β*-farnesene (%71.5), intermedol (%8.4), α-thujene (%1.9), and chrysanthenone (%1.5).

In a 2020 study conducted by Lithuanian researchers, the composition of essential oil extracted from *A. abrotanum*, grown in Lithuania, was analyzed at various developmental stages. The primary compound identified in the highest concentration was (+)-piperitone (20.38–38.48%). Additionally, significant quantities of the following compounds were isolated: 1,4-cineole, lavandulyl butanoate, aromadendrene, and isogermacrene D. The essential oil content was found to be highest during the end of the flowering vegetation stage, measuring 38.48%, whereas it was lowest during the growth and leaf production, at 20.38%[20]. In a study conducted in Türkiye in 2011, the mosquito-repellent activity of the essential oil obtained from the *A. abrotanum* was investigated. The main components of the essential oil were found to be: 1.8-cineole (32.6%), borneol (13.5%), presylphiperfolan-9α-ol (10.2%), and p-cymene (8.0%) [21]. In our study, the highest content of the *A. abrotanum* essential oil was chrysanthenone (55.9%). The differences between the studies may be due to the different places and times of collection of the plants.

In a study conducted in Türkiye in 2014, the essential oils of *A. absinthium* and *A. austriaca* species were analyzed. The major components were identified as β-myrcene (44.32 %) in *A. absinthium* and camphor in *A. austriaca* (43.27 %), respectively [22]. In our study, the major component for *A. austriaca* was camphor (34.2%), but at a lower level than in this study. For *A. absinthium*, major components were sabinyl acetate (23%), artemisia ketone (22.1%), and the myrcene ratio was found to be lower (12.8%). In a review conducted on *A. absinthium* in 2020, it was shown by many studies that the essential oil content of the plant varies in quality and quantity depending on the geographical region and environmental conditions [23].

Upon examining research on the volatile oils of *Artemisia* species cultivated in Türkiye, a particular study focused on the chemical compositions of the volatile oils obtained from different vegetative stages of *A. annua* (pre-flowering (PF), 50% flowering (50 F), full flowering (FF), and post-flowering (PF)). The contents of volatile oils during PF, 50 F, FF, and PF stages were found to be 0.8%, 0.96%, 1.22%, and 1.38%, respectively. A total of 20 components were identified, with artemisia ketone (28.30% to 37.15%), camphor (18.00% to 23.30%), and 1,8-cineole (9.00% to 10.39%) determined as the major constituents [24]. These data were consistent with the major components found in *A. annua* essential oil in our study. However, if we compare the FF stage of *A.annua* of the previous study with our study, the amount of artemisia ketone was higher (53.7% vs 37.15%) and the amount of camphor was lower (12.3% vs 23.8%).

It was observed that previous studies on *A. chamamolliaefolia* essential oil were generally conducted in Iran. In a study of *A. chamaemelifolia* collected at two phenological stages from five distinct natural habitats in northern Iran, the highest essential oil yield was observed in the Shahkoh population, with 1.10 ml/100g of dry matter at the 50% flowering stage. The Pelor population yielded the highest concentration of 1,8-cineole, reaching 31.82%, also at the 50% flowering stage. Notably, the Kandovan population exhibited the highest percentages of artemisia ketone (12.27%), camphor (17.21%), and borneol (13.50%) when harvested before flowering. Additionally, the Gadok population, when harvested before flowering, produced the highest content of chrysanthenone, which was 18.14%. For the Kandovan population at the 50% flowering stage, the essential oil contained the highest levels of Davanone D (28.44%) and Davanone (28.88%). These findings suggest that *A. chamaemelifolia* contains three distinct chemotypes: 1,8-cineole, Davanone and/or Davanone D, and chrysanthenone [25]. In another study conducted in Iran, the composition of the essential oil obtained from the dried aerial parts of *A. chamaemelifolia* was analyzed by GC and GC/MS. 49 components were identified, the main components were vulgarone B (38.8%), santolinyl acetate (10.5%) and 14-hydroxy-9-epi-β-caryophyllene (8.4%) [26]. In another study examining the essential oils of the aerial parts, stem, leaf and flower parts of *A. chamaemelifolia*, thirty-one compounds were identified, representing 96.6%, 94.6%, 93.2% and 91.0%, respectively. Menthyl acetate (26.5%, 22.0%, 20.5% and 20.5%) and (Z)-nerolidol (20.8%, 26.3%, 14.7% and18.1%) were the main constituents in the aerial parts, stem, leaf and flower oils, respectively [27]. Our study was the first to investigate *A. chamaemelifolia* essential oil in Turkey. Our findings differ from previous studies and the major components found were as follows: selin-11-en-4-α-ol (%29.1), 1,8-cineole (%16.3), presilphiperpholone-9-α-ol (%8.6), and artemisia ketone (%5.4). There are few studies on this subject and updated studies are needed.

In a study analyzing the volatile oil of *A. incana*, the yield of volatile oil was reported as 0.36%, and major components were identified as camphor (19.0%), borneol (18.9%), 1,8-cineole (14.5%), bornyl acetate (7.8%), camphene (4.9%), and *a*-thujone (4.8%) [28]. In our study, for *A. incana,* major components were found as camphor (29.7%), borneol (12.1%), piperitone (10.1%), and 1,8-cineole (8.7%), which was parallel to previous study.

In a study conducted in Iran in 2010, the volatile oil obtained from the aerial parts of *A. tournefortiana* was meticulously analyzed, leading to the identification of twenty-nine compounds, collectively representing 97.3% of the total components. The major components reported in this investigation were *trans*-thujone (47.0%), sabinene (16.5%), and *β*-pinene (8.3%) [29]. Another study conducted in India provided further insights into the essential oil derived from *A. tournefortiana*. Nineteen components, constituting 93.47% of the total oil composition, were identified. Notably, oxygenated monoterpenes (54.46%) were found to dominate over other compound classes. The primary components reported included *cis*-spiroether (47.66%), (*Z*)-*β*-farnesene (22.83%), *trans*-nerolidol (3.89%), and camphor (3.80%). It is noteworthy that *cis*-spiroether was identified for the first time in this region through this study [30]. *A.tournefortia* essential oils were analyzed for the first time in Türkiye with this study. The highest compound found in the essential oil was (Z)-β-farnesene (71.5%), which is different from previous studies conducted in other countries.

The literature data generally align well with our findings. However, a notable discrepancy arises concerning the amount of chamazulene detected in studies analyzing the volatile oil of *A. absinthium* in Türkiye, where the observed quantities were higher than our results [31-33]. This variance could potentially be attributed to differences in the collection region (Erzurum, Malatya, and Antalya) or the time of

collection. Upon evaluating existing literature studies, our research stands out as the first GC and GC-MS analysis conducted on the essential oils of *A. abrotanum, A. chamamelifolia* and *A. tournefortiana* plants growing in Türkiye.

The antimicrobial activities of essential oils have been studied against numerous microorganisms over the years, but their mechanisms of action are not yet fully understood. Considering the diverse chemical compound groups they contain, attributing their antibacterial activities to a specific mechanism is challenging, as they likely have multiple targets within the cell [34]. Essential oils disrupt the membrane integrity of both Gram-negative and Gram-positive bacteria, demonstrating their effectiveness. Due to their lipophilic nature, they can easily pass through the cell membrane and cell wall. The interaction of essential oils and their components with polysaccharides, fatty acids, and phospholipids makes the bacterial membrane more permeable, leading to the loss of cellular content and ions, eventually resulting in cell death [19]. Additionally, the phenolic structure of essential oils induces an antimicrobial response against bacteria. Phenolic compounds disrupt the cell membrane, effectively inhibit the functional properties of the cell, and ultimately cause leakage of the cell's internal materials [35]. It has also been reported that essential oils affect proteins embedded in the cytoplasmic membrane of the cell and enzymes involved in energy regulation or the synthesis of structural components [34].

In this study, essential oils obtained from seven different *Artemisia* species were evaluated for their antimicrobial activities against *Bacillus subtilis* NRRL B-4378, *Escherichia coli* NRRL B-3008, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, and *Staphylococcus aureus* ATCC 6538 strains using the microdilution method. When assessing the Minimum Inhibitory Concentrations, it was found that the plant exhibiting the highest antimicrobial activity against all strains was *A. incana*, while the lowest activity was observed for *A. annua*. Among the tested strains, *Escherichia coli* NRRL B-3008 and *Salmonellatyphimurium* ATCC 13311 were identified as the most resistant, while *Staphylococcusaureus* ATCC 6538 was the most susceptible. Recent studies have proven the antimicrobial activities of various *Artemisia* species similar to our study [36-40].

The antimicrobial analyses revealed that all seven species studied exhibited antibacterial effects against the examined strains. This finding holds significance for future comprehensive studies that aim to elucidate the antibacterial and antifungal effects of all *Artemisia* species growing in our country on different strains.

4. CONCLUSION

In this study, the essential oil contents of seven *Artemisia* species grown in Turkiye and their antimicrobial activity of essential oils against five microorganisms (*Bacillus subtilis, Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa*) was investigated *in vitro* using the microdilution method. The total number of compounds detectable in the essential oil for each species and its ratio to all compounds: *A. abrotanum* (28, 82.2%), *A. absinthium* (26, 94.1%), *A. annua* (42, 92.2%), *A. austriaca* (27, 93.8%), *A. chamaemelifolia* (31, 77.8%), *A. incana* (52, 93.9%), and *A. tourfortiana* (22, 86.8%). The main components are chrysanthemum (55.9%) for *A. abrotanum*, sabinyl acetate (23.0%) for *A. absinthium*, artemisia ketone (53.7%) for *A. annua*, camphor (34.2%) for *A. austriaca*, selin- 11-en-4-*α*-ol (29.1%) for *A. chamaemelifolia*, camphor (29.7%) for *A. incana*, and (*Z*)-*β*-farnesene (71.5%) for *A. tournefortiana*. *In vitro* antimicrobial activity showed that the highest activity against all species was observed in *A. incana* essential oil. *S. aureus* was found to be the most sensitive bacteria to all essential oils. These findings contribute valuable insights into the chemical diversity and potential biological activities of *Artemisia* species. Further investigations on the antimicrobial activities and other pharmacological properties of these species may enhance our understanding of their medical and industrial applications.

5. MATERIALS AND METHODS

5.1. Obtaining plant materials and essential oils

The localities where the studied species were collected, collection dates, voucher numbers, and the plant identifier are given in Table 1. Dried aerial parts of the collected plants were powdered, approximately 100 g each. Subsequently, essential oils were obtained through steam distillation using the Clevenger apparatus for a duration of three hours. The analysis of the essential oils were conducted simultaneously using Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) systems.

Table 1. Localities where *Artemisia* L. species were collected, collection dates and herbarium numbers

Species	Locality and Date of Collection	Voucher number*	Identified by
ABR	C5, Bitlis: Güroymak, between Muş- Güroymak, 500 m before Güroymak district, roadside, streamside, 1290 m, 05.09.2019	AEF28823	Murat Kursat
ABST	B5, Kars: 1 km before the city center, Tuzluca-Kars road, among roadside piles of rubble, 1765 m, 10.08.2019	AEF28821	Murat Kursat
ACHA	B5, Kars: Susuz, Kısır Dağı, the slopes north of Kiziroğlu Village, 2450 m, 10.08.2019	AEF28826	Murat Kursat
ANN	B1, Sakarya, Akyazı, Kuzuluk road, roadside. 13. 10.2019	AEF28820	Murat Kursat
AINC	C5, Muş: Malazgirt, Between Aktuzla village and Karıcalı village, roadside, slopes, 1560 m, 09.08.2019	AEF28822	Murat Kursat
ATOR	B5, Kars: 1 km before the city center, Tuzluca-Kars road, among the piles of rubble on the roadside, 1765 m, 10.08.2019	AEF28824	Murat Kursat
AUS	C5, Van: Between Çaldıran and Doğubeyazıt, 4 km after Çaldıran, the slopes, 08.08.2019	AEF28825	Murat Kursat

ABST: *A. absinthium* L., ABR: *A. abrotanum* L., ACHA: *A. chamaemelifolia* Vill., ANN: *A. annua* L., AINC: *A. incana* (L.) Druce, ATOR: *A. tournefortiana* Rchb., AUS: *A. austriaca* Jacq. *AEF: Ankara University Faculty of Pharmacy Herbarium.

5.2. Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS)

The GC method was employed to determine the relative percentages of the essential oils. The GC system used was the Agilent 6890N, with helium as the carrier gas (at a flow rate of 0.8 mL/min), and a polar column, HP-Innowax (60 m × 0.25 mm; 0.25 µm film thickness). The injection port temperature was set at 250°C, and a FID-type detector was utilized at a temperature of 300°C. The temperature program was applied as same as GC-MS conditions.

The mass spectra of the volatile compounds were analyzed using the GC-MS system. The GC-MS system employed was the Agilent 5975, with helium as the carrier gas (at a flow rate of 0.8 mL/min) and a polar column, HP-Innowax (60 m × 0.25 mm Ø; 0.25 µm film thickness). The injection port temperature was set at 250°C. The analysis of substances with a mass-to-charge ratio (m/z) ranging from 35 to 450 was carried out with 70 eV electron energy. A temperature program was applied, starting at 60°C for 10 minutes, then increasing to 220°C at a rate of 4°C/min, holding at 220°C for 10 minutes, and finally increasing to 240°C at a rate of 1°C/min, with a total duration of 80 minutes. During data evaluation, the "Başer Volatile Oil Compound Library," Wiley GC/MS, and MassFinder 4.0 Library Search Software were utilized (Table 2).

5.3. Microorganisms

The strains utilized for the assessment of antimicrobial activity included lyophilized cultures of *Bacillus subtilis* NRRL B-4378, *Escherichia coli* NRRL B-3008, *Pseudomonas aeruginosa* ATCC 27853 from NRRL (ARS Culture Collection), *Salmonella typhimurium* ATCC 13311, and *Staphylococcus aureus* ATCC 6538, all sourced from the American Type Culture Collection (ATCC).

5.4. Culture

Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) culture were obtained ready-made and were appropriately diluted with distilled water.

Table 2. Volatile components (%) of the studied *Artemisia* L. species by GC and GC-MS

[a]RRI	Component	ABR [b]%	ABST %	ACHA %	AINC %	ANN %	AUS %	ATOR %
1014	Tricyclene	0.2	-	-	0.2	0.1	-	-
1018	Ethyl-2-methyl butyrate	-	-	-	-	0.4	-	-
1032	*a*-Pinene	0.7	2.0	0.1	2.8	3.1	1.3	0.8
1035	*a*-Thujene	0.2	0.4	-	0.2	0.2	-	1.9
1076	Camphene	0.8	-	0.1	3.7	1.7	3.3	0.1

1093	Hexanal	-	-	-	[c]tr	-	-	-
1118	*β*-Pinene	0.1	1.9	-	0.7	0.5	0.5	0.1
1132	Sabinene	0.4	1.3	0.6	-	0.3	2.3	-
1151	Propyl-2-methyl butyrate	-	-	-	-	tr	-	-
1174	Myrcene	-	12.8	-	-	1.0	2.6	-
1188	α-Terpinene	-	-	0.3	0.1	0.1	0.5	-
1203	Limonene	0.1	-	-	-	-	-	-
1213	1,8-Cineole	5.9	2.0	16.3	8.7	4.2	10.9	0.7
1234	Isochrysanthenone	0.3	-	-	-	-	-	-
1255	*γ*-Terpinene	0.2	0.3	0.8	0.2	tr	0.9	tr
1280	*p*-Cymene	0.7	0.5	1.0	1.0	tr	0.8	tr
1290	Terpinolene	-	-	-	-	tr	-	-
1358	Artemisia ketone	-	22.1	5.4	-	53.7	-	-
1403	Yomogi alcohol	-	-	0.2	-	1.6	-	-
1405	Santolina alcohol	-	-	1.9	-	0.2		
1429	Artemisyl acetate	-	-	-	-	0.1	-	-
1445	Filifolone	3.2	-	-	-	-	-	-
1450	*trans*-linalool oxide (*Furanoid*)	-	-	-	tr	-	-	-
1452	1-Octen-3-ol	0.7	-	-	tr	-	-	-
1465	Eucarvone	0.5	-	-	-	-	-	-
1466	α-Cubebene	-	-	-	-	-	-	0.6
1474	*trans*-Sabinene hydrate	0.2	-	0.2	0.1	0.1	0.6	-
1479	Longipinene	-	-	0.1	-	-	-	-
1479	Linalool-7-oxide-3-one	-	-	-	1.9	-	-	-
1482	(Z)-3-Hexenyl-2-methyl butyrate	-	-	-	-	tr	-	
1497	*α*-Copaene	-	-	0.1	0.1	0.3	-	-
1510	Artemisia alcohol	-	2.1	0.3	-	4.1	-	-
1522	Chrysanthenone	55.9	-	-	-	-	-	1.5
1532	Camphor	-	5.5	2.5	29.7	12.3	34.2	
1535	β-Bourbonene	3.0	-	-	-	-	-	-
1547	Dihydroachillene	-	-	-	0.1	-	-	-
1553	Linalool	-	5.7	0.1	0.2	-	-	-
1556	*cis*-Sabinene hydrate	0.2	-	0.3	0.3	tr	0.7	-
1571	*trans*-*p*-Ment-2-en-1-ol	0.2	-	0.2	0.6	-	0.3	-
1582	*cis*-Chrysanthenyl acetate	0.9	5.3	-	-	-	-	-
1586	Pinocarvone	0.4	-	-	1.2	0.7	0.2	-
1590	Bornyl acetate	-	-	-	1.1	-	0.3	-
1600	*β*-Elemene	-	1.6	-	-	tr	-	-
1611	Terpinen-4-ol	0.8	-	2.1	1.7	0.6	3.5	-
1612	*β*-Caryophyllene		0.4	tr	tr	1.3	-	0.4
1617	Lavandulyl acetate	-	0.7	-	-	-	-	-
1628	Aromadendrene	-	-	-	-	-	-	0.2
1638	*cis*-p-Ment-2-en-1-ol	-	-	-	0.5	-	-	-
1648	Myrtenal	-	0.9	-	0.2	-	-	-
1658	Sabinyl acetate	-	23.0	-	-	-	10.4	-
1661	Alloaromadendrene	-	-	-	0.2	-	-	-
1668	(Z)-*β*-Farnesene	-	-	-	-	0.2	0.3	71.5
1670	*trans*-Pinocarveol	0.9	-	-	0.8	1.0	-	-
1682	*δ*-Terpineol	-	-	-	tr	-	tr	-
1683	*trans*-Verbenol	1.2	-	-	0.2	-	-	-
1704	Myrtenyl acetate	-	-	-	0.2	-	-	-
1706	*α*-Terpineol	-	-	0.2	-	0.3	0.2	-
[a]RRI	**Component**	**ABR [b]%**	**ABST %**	**ACHA %**	**AINC %**	**ANN %**	**AUS %**	**ATOR %**
1719	Borneol	-	-	-	12.1	0.1	-	-
1720	*trans*-Sabinol	-	0.8	-	-	-	16.8	-
1726	Germacrene D	2.3	0.9	0.9	-	0.7	1.1	0.3
1742	*β*-Selinene	-	0.6	-	-	-	-	-
1743	Chrysanthenyl isovalerate I	0.5	-	-	-	-	-	-
1748	Piperitone	-	-	-	10.1	-	-	-
1760	Chrysanthenyl isovalerate II	0.8	-	-	-	-	-	-
1764	*cis*-Chrysanthenol	-	0.6	-	-	-	-	-

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1770	trans-Linalool oxide *(Pyranoid)*	-	-	-	0.3	-	-	-
1773	*δ*-Cadinene	-	-	-	-	tr	-	0.4
1793	Campholene alcohol	-	-	-	0.7	-	-	-
1802	Cumin aldehyde	-	-	0.3	-	-	-	-
1804	Myrtenol	-	-	-	1.3	0.2	0.1	-
1845	*trans*-Carveol	-	-	-	0.3	0.1	-	-
1864	*p*-Cymen-8-ol	-	-	-	0.1	-	-	-
1878	2,5-Dimethoxy-*p*-cymene	-	-	-	-	0.1	-	0.3
1880	Benzyl 2-methylbutyrate	-	-	-	-	0.1	-	-
1941	*a*-Calacorene	-	0.1	-	-	-	-	-
1945	1,5-Epoxy-salvial(4)14-ene	-	-	0.2	-	-	-	-
1950	Palustrol	-	-	-	0.1	-	-	-
1958	(*E*)-*β*-Ionone	-	-	-	-	-	-	0.3
1969	*cis*-Jasmone	-	-	-	0.1	-	-	-
2001	Isocaryophyllene oxide	-	-	-	-	0.1	-	-
2008	Caryophyllene oxide	-	1.2	-	0.9	1.1	-	1.1
2030	Presilphiperfolan-9-ol	-	-	8.6	-	-	-	-
2030	Methyl eugenol	-	-	-	0.4	-	-	-
2037	Salvial-4(14)-en-1-one	-	-	-	-	0.1	-	-
2050	(*E*)-Nerolidol	-	-	1.2	-	-	-	0.2
2104	Viridifluorol	-	-	-	4.8	-	-	-
2144	Spathulenol	0.6	-	-	2.5	0.1	0.1	-
2186	Eugenol	-	-	-	-	0.1	0.1	-
2187	T-Cadinol	-	-	-	-	-	-	0.1
2200	*a*-Bisabolol oxide A	0.3	-	-	-	-	-	-
2210	Copaborneol	-	-	-	0.1	-	-	-
2232	*a*-Bisabolol	-	-	1.3	-	-	-	0.3
2247	*trans*-*a*-Bergamotol	-	-	-	0.1	-	-	-
2257	*β*-Eudesmol	-	-	-	0.4	-	1.8	-
2264	Intermedeol	-	-	-	0.6	-	-	6.0
2265	Longiverbenone	-	-	2.1	-	-	-	-
2369	Eudesma-4(15)7-dien-1*β*-ol	-		0.5	-	-	-	-
2273	Selin-11-en-4-α-ol	-	-	29.1	-	-	-	-
2312	9-Geranyl-*p*-cymene	-	0.9	-	-	-	-	-
2316	Caryophylladienol-I	-	-	-	0.2	tr	0.1	-
2324	Caryophylladienol-II	-	-	-	0.6	-	-	-
2392	Caryophyllenol-II	-	-	-	0.2	0.1	-	
2430	Chamazulene	-	0.5	-	-	-	-	-
2931	Hexadecanoic acid	-	-	0.8	tr	-	-	-
	Total	**82.2**	**94.1**	**77.8**	**92.6**	**91.0**	**93.9**	**86.8**

[a] RRI: Relative retention indices calculated against *n*-alkanes.
[b] %: calculated from the FID chromatograms.
[c] tr.: trace amount (< 0.1%).

ABST: *A. absinthium* L., ABR: *A. abrotanum* L., ACHA: *A. chamaemelifolia* Vill., ANN: *A. annua* L., AINC: *A. incana* (L.) Druce, ATOR: *A. tournefortiana* Rchb., AUS: *A. austriaca* Jacq.

5.5. Sterilization

The culture media, laboratory equipment, and contaminated materials used in antimicrobial activity experiments were sterilized in an autoclave at 121°C and 1.5 atmospheres for 20 minutes.

5.6. Incubation of microorganisms

The culture media (MHA) prepared for strain development were stored in a refrigerator at +4°C for a maximum of two weeks. After verifying their purity, they were preserved in a 15% glycerol solution at -85°C. Subsequently, microorganisms were inoculated into the culture media, and their proliferation was ensured by incubating them in a bacteriological incubator at 37°C for 24 hours. The developed cultures were adjusted for turbidity using a turbidimeter based on McFarland No: 0.5 tube (approximately 10^8 CFU/mL for bacteria).

5.7. Microdilution Method

In the analyses, 96-well "U"-shaped microplates were utilized. For standard substances and essential oils, the first well of the eight wells used had an initial concentration of 20 mg/mL, and 100 µL was transferred from the tested stock solution. Double-fold serial dilution was performed by taking 100 µL. Subsequently, the density-adjusted microorganisms were diluted with MHB (1:100) and applied to the wells in 100 µL aliquots. The prepared microplates were incubated at 37°C. After 24 hours of incubation, 20 µL of Resazurin solution was added to the wells, and they were incubated again at 37°C for 3 hours. At the end of these procedures, wells without a pink color indicated no growth. The results were expressed as minimum inhibitory concentration (mg/mL). Wells with only culture media were used for sterility control, wells with culture media and microorganisms were used for growth control, and wells with chloramphenicol antibiotic served as a positive control. The experiments were conducted in triplicate, and the results were presented as mean values (Table 3).

Table 3. Minimum Inhibition Concentration (MIC) (mg/mL)

	E. coli	***B. subtilis***	***P. aeruginosa***	***S. typhimurium***	***S. aureus***
ABR	19.5	9.6	9.6	19.5	2.4
ABST	>12.5	>12.5	>12.5	>12.5	>12.5
ACHA	14	14	14	14	14
ANN	50	50	50	50	25
AINC	3.5	3.5	3.5	3.5	1.7
ATOR	>7.5	>7.5	>7.5	>7.5	3.7
AUS	>13.7	13.7	13.7	>13.7	>13.7
Chloramphenicol	0.0002	0.0008	0.0004	0.0002	0.0008

ABST: *A. absinthium* L., ABR: *A. abrotanum* L., ACHA: *A. chamaemelifolia* Vill., ANN: *A. annua* L., AINC: *A. incana* (L.) Druce, ATOR: *A. tournefortiana* Rchb., AUS: *A. austriaca* Jacq.

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