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Research Article

Comparison of volatile profiles of *Achillea sintenisii*, an endemic species from Türkiye, and the evaluation of its essential oil's antibacterial and antibiofilm activities

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Abstract: *Achillea sintenisii* Hub-Mor is an endemic species in Türkiye, characterized by 1–10 capitula and ray flowers, and it thrives on calcareous rocks or gypsum-rich soils. This study aimed to determine the chemical composition and assess the antibacterial and antibiofilm activity of the essential oil of *A. sintenisii* (AS-EO). The essential oil compounds of the flowering aerial part of *A. sintenisii* were extracted using both hydrodistillation and solid-phase microextraction (SPME) techniques and identified by gas chromatography-mass spectrometry (GC-MS). Two different adsorbents were employed in the SPME approach. The yield of the essential oil of flowering aerial parts of *A. sintenisii* was 0.4% h/h. A total of 74, 112, and 78 compounds were identified from the samples obtained by hydrodistillation (S1) and SPME extraction using the CAR-PDMS (S2) and PDMS-DVB (S3) adsorbents, respectively. The AS-EO exhibited the highest antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *S. aureus* ATCC 43300 (MRSA), with a minimum inhibitory concentration (MIC) of <1/512 for each bacterium. This strong antibacterial effect may be attributed to the high content of 1,8-cineole and l-borneol. However, AS-EO did not exhibit any antibiofilm activity. These findings suggest that the essential oil of *A. sintenisii* has potential as a natural antibacterial agent, warranting further research to uncover its full potential.

1. INTRODUCTION

Belonging to the *Asteraceae* family, the genus *Achillea* comprises 50 species and 15 subspecies in Türkiye, widely recognized for their significant health benefits in traditional medicine, particularly for the respiratory and digestive systems (Güner *et al.*, 2012; Tuzlacı, 2016). The primary habitats of this genus span various regions including Iran, Türkiye, Serbia, and Eastern Europe (Başer, 2016; Mohammadhosseini *et al.*, 2017). *Achillea* species have traditionally been

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used to treat various diseases related to the digestive, genital, and urinary systems. Additionally, they have been reported to be effective in alleviating headaches, migraines, colds, bronchitis, and wounds. The herba of *A. millefolium*, the most well-known species of the *Achillea* genus, is documented in EMA monographs as a remedy for loss of appetite, skin disorders, and minor injuries, minor spasms associated with menstrual periods, and gastrointestinal issues (EMA, 2020). The most commonly identified secondary metabolites in *Achillea* species include flavonoids, phenolic acids, terpenes, phytosterols, organic acids, fatty acids, and alcohols (Bader *et al*., 2022; Becker *et al*., 2016; Conforti *et al*., 2005; Maggi *et al*., 2009; Strzępek-Gomółka *et al.*, 2021; Tuzlacı, 2016; Yeşilada, 2023).

The chemical diversity of the essential oils from the genus *Achillea* is remarkable. The plant part used and the extraction technique significantly influence the chemical composition of the essential oil, along with other factors like geographic location and seasonal variations (Bader *et al.*, 2022; Mazandarani *et al.*, 2013; Mohammadhosseini *et al.*, 2017; Raut & Karuppayil, 2014). *Achillea sintenisii* Hub-Mor is an endemic species within this genus characterized by 1-10 capitula and ray flowers, and it thrives on calcareous rocks or gypsum-rich soils (Aytaç *et al.*, 2016). As previously mentioned, many *Achillea* species have a long history of use in traditional medicine; however, no records exist regarding the medicinal use of *A. sintenisii* in Anatolian medicine.

Antibiotic resistance poses a significant threat to public health, creating substantial challenges in the prevention and treatment of infectious diseases (WHO, 2015). Various studies have been conducted to identify novel therapeutic agents and approaches to address this issue. The difficulty of discovering new antibiotics and the increasing resistance to existing ones have prompted researchers to explore alternative therapeutic strategies. Biofilm formation is one of the key mechanisms contributing to antibiotic resistance, as bacteria within biofilms exhibit high resistance to antibiotics and disinfectants, making them particularly difficult to eradicate. Biofilm formation plays a crucial role for pathogenicity, protecting antimicrobial agents, the immune system, and environmental variations, such as humidity, temperature, and exposure to harmful substances like antibiotics and disinfectants (Asfour, 2018; Karaca *et al*., 2020; Tunca-Pınarlı *et al*., 2023). Since biofilm production results from interactions between bacterial and material surfaces, influenced by the surrounding environmental conditions, appropriate strategies must be developed to effectively control biofilm formation. Specifically, the impact of various conditions on biofilm formation should be examined for each microorganism to determine targeted and efficient control measures.

The antibacterial properties of essential oils have been widely recognized and studied for a considerable time; however, their efficacy against biofilms remains incompletely understood. Nevertheless, there has been a growing interest in exploring the effectiveness and mechanisms of action of essential oils, particularly against antibiotic-resistant pathogens. While headspace solid-phase microextraction (HS-SPME) is a rapid and efficient method for extracting volatile components, the Clevenger apparatus, a well-established technique for obtaining essential oils, is time-consuming. Therefore, this study aims to analyze, for the first time, the essential oil of *A. sintenisii* for its potential antibacterial and antibiofilm activities. Additionally, it seeks to compare the chemical composition of the essential oil extracted from the flowering aerial parts of *A. sintenisii* using solid-phase microextraction and hydrodistillation.

2. METHOD

2.1. Plant Material

The flowering aerial parts of *A. sintenisii* were collected from Sivas in June 2021. The voucher specimen was registered at the Herbarium of Ankara University Faculty of Pharmacy under the accession number AEF30920.

2.2. Extraction of Essential Oil

2.2.1. Hydrodistillation

The air-dried plant material was comminuted immediately before hydrodistillation using a Clevenger apparatus. The essential oil (**S1**) was obtained with a 0.4% (v/v) yield, dried using anhydrous sodium sulphate, and stored in air-tight, amber-colored glass vials at 4 °C until further analysis.

2.2.2. *Headspace-solid phase microextraction*

A total of 205.6 mg and 211.5 mg of samples were used for volatile extraction using two different phases: **(i) S2** was extracted with a StableFlexTM SPME fiber coated with 75 µm carboxen/polydimethylsiloxane (CAR/PDMS) and **(ii) S3** was extracted using SPME fibers of StableFlexTM coated with 65 µm polydimethylsiloxane/divinylbenzene (PDMS-DVB), respectively. All samples were placed in a glass-sealed 20 mL vial and secured with a crimper. Each sample was subjected to the headspace extraction for 40 minutes at a constant temperature of 80°C. Subsequently, the SPME fiber was directly introduced into the injection port of GC-MS where it was held for three minutes to facilitate the release of the analytes.

Finally, the fiber was baked out for 10 minutes in a GC-MS injector after each extraction and desorption cycle to minimize contamination.

2.3. GC/MS Analysis

The analysis of volatile compounds was performed using the Shimadzu GC/MS Ultra QP2010 system, was equipped with a mass selective detector (MSD) and a gas chromatography-flame ionization detector (GC-FID). A Teknokroma TRB-5MS capillary column (30m× 0.25 mm i.d., 0.25 film thickness) was used, with helium as the gas carrier at a flow rate of 1.72 mL/min. The oven temperature program began with an initial hold at 40°C for two-minutes, followed by a gradual increase of 3°C/minute until reaching 200°C, where it was held for 10 minutes. Subsequently, the temperature was raised at a 5°C/min ramp to 250°C and maintained for 5 minutes, resulting in a total analysis time of 70 minutes. The injector temperature was maintained at 250°C, and a split injection mode with a 1:100 ratio was employed.

GC-MS analyses were performed using electron impact ionization (EI) and a quadrupole mass selective detector. The temperature program was identical to that of GC-FID, except for the detector settings. The MS conditions were as follows: EI ion source temperature of 200°C with an ionization energy of 70 eV, quadrupole detector temperature of 150°C, scan rate of 3.2 scans per second at *m/z* scan range (50–650), and MS transfer line temperature of 240°C. Volatile compounds were identified by comparing their spectra and retention times with reference standards. Component detection was based on a comparison of relative retention times to a C8–C26 *n*-alkanes mixture, as well as mass spectra from NIST27, NIST147, WILEY7, WILEY W9N11 library data of the GC-MS system, literature data, and standards of the main components.

2.4. Antibacterial Activity Test

The minimum inhibitory concentration (MIC) values of AS-EO were determined using the broth microdilution method (CLSI, 2009; Akkaoui *et al.*, 2020). The following bacterial strains were used as test organisms: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Staphylococcus aureus* ATCC 25923, methicillin-resistant *S. aureus* ATCC 43300 (MRSA), and *Enterococcus faecalis* ATCC 29212. Serial two-fold dilutions of the AS-EO were prepared in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA) supplemented with Tween 80 (Merck, Germany) (final concentration of 0.5%, v/v). The inoculum concentration was adjusted to 5×10^5 CFU/mL, prepared from a 24-hour bacterial culture. Microplates were then incubated at 35 °C for 18-24 hours. The MIC value (v/v) was determined as the lowest concentration that completely

inhibited visible microbial growth. A set of wells containing only inoculated broth supplemented with Tween 80 served as the negative control.

2.5. Antibiofilm Activity Test

The antibiofilm activity was assessed using an *in-vitro* microplate-based biofilm model against *Pseudomonas aeruginosa* PAO1 employing the crystal violet assay. Prior to conducting the antibiofilm activity test, the MIC value of the AS-EO against *P. aeruginosa* PAO1 was determined (Eryılmaz *et al.*, 2019; Bali *et al.*, 2019; Jardak *et al.*, 2021).

2.5.1. Biofilm formation

P. aeruginosa PAO1 was incubated for 24 hours at 37 ºC in Brain Heart Infusion (BHI) Broth. Following incubation, final inoculum suspensions containing ~10^6 CFU/mL of *P. aeruginosa* were prepared in BHI enriched with 2% sucrose. For each test condition, 100 µL of the inoculum suspension were added to the wells of 96-well microtiter plates. The plates were then incubated at 37°C for 72 hours to allow the formation of mature biofilms.

2.5.2. Treating of the biofilm cells with the AS-EO

After biofilm formation, the medium was aspirated, and non-adhered cells were removed by washing the wells with sterile phosphate-buffered saline (PBS, pH 7.2). The essential oil was then added to the wells containing mature *P. aeruginosa* biofilms. The plates were incubated at 37°C for 24 hours. Following incubation, the well content was aspirated, and the wells were washed with PBS. The plates were then dried at room temperature for one hour. To stain biofilm cells, 100 µL of 0.5% crystal violet solution was added to each well. After 30 min, the wells were washed three times with PBS. Subsequently, an acetone-alcohol (30:70 v/v) solution was added to dissolve the bound dye within the biofilm matrix. BHI Broth enriched with 2% sucrose was used as a control. The optical density of the dissolved crystal violet dye was measured by a microplate reader (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Vantaa, Finland) at 620 nm (OD620nm). The percentage of biofilm inhibition values was calculated using the following formula:

Biofilm inhibition% = [(OD (growth control) – OD (sample))/OD (growth control)] x 100

3. RESULTS

3.1. Chemical Profile of Essential Oil

The essential oil compounds from the flowering aerial part of *A. sintenisii* were extracted using both distillation and SPME techniques and subsequently identified by GC/MS. The yield of the essential oil extracted from the flowering aerial parts of *A. sintenisii* was 0.4% h/h. A total of 74, 112, and 78 compounds were identified from the samples obtained by hydrodistillation (S1), SPME extraction using CAR-PDMS absorbent (S2) and SPME extraction using PDMS-DVB adsorbent (S3), respectively. The chemical structures of these compounds were determined based on MS and retention index data. Table 1 presents the volatile compounds detected in *A. sintenisii*.

We observed that all three samples contained significant amount of monoterpenes. Furthermore, it was noted that the samples (S2 and S3) obtained using SPME extraction had a higher monoterpene content than S1 which was obtained through hydrodistillation. The main monoterpenes and major components of the essential oil were identified as 1,8-cineole (11.05–15.11%), and camphane (10.43-11.94%). A notably high concentration of piperitone (24.29%) was detected in S2, whereas lower concentrations were observed in S1 and S3. Conversely, borneol was found at 2.65% in S2, while it was present in significantly higher amounts in S1 (8.28%) and S3 (11.57%). Caryophyllene oxide, a sesquiterpene, was found to at notably high concentrations in all three samples in amounts of 4.60% in S1, 4.44% in S2, and 4.50% in S3. Additionally, a 4.12% quantity of sesquisabinane hydrate was detected only in the essential oil obtained by hydrodistillation. *β*-eudosmol was identified in S1 and S3 at 4.12% and 3.86%, but

was not detected in S2. Meanwhile, artemisia ketone was found in all samples, though it was present in particularly high concentrations in S3 (9.04%).

Table 1. Volatile compounds of *A. sintenisii*.

Volatile compounds of *A. sintenisii*				S1	S2	S3	IM
No	LRI (cal)	LRI (lit)	Compound	%	%	%	
1	386	385	Acetaldehyde	-	0.02	-	a
2	477	475	2-propanone	-	0.35	-	a
3	533	532	Isobutanal	-	0.02	-	a
4	554	552	2-propenal, 2-methyl-	-	0.32	-	a
5	583	582	2-butanone	-	0.03	-	a
6	620	619	Crotonaldehyde	-	0.04	-	a
7	635	633	Acetic acid	-	0.24	-	a
8	645	643	Butanal, 2-methyl-	-	0.08	-	a
9	653	652	1-butanol	-	0.03	-	a,b
10	676	675	*n*-pentanal	-	0.03	-	a,b
11	723	722	1-butanol, 2-methyl-	-	0.07	-	a
12	732	730	Methyl isobutyl ketone	-	0.05	-	a
13	733	731	Pyrrole		0.09	-	a
14	750	748	2-butenal, 3-methyl-	-	0.02	-	a
15	752	754	1-Pentanol	-	0.02	-	a,b
16	774	775	Ethyl pyruvate	-	0.01	-	a
17	776	777	Hexanal	0.07	0.25	-	a,b
18	797	798	4-Pentenal, 2-methyl-	-	0.01	-	a
19	825	827	2-Hexenal	-	0.02	-	a
20	836	837	3-Hexen-1-ol, (*E*)-	-	0.01	0.01	a
21	855	855	1-Hexanol	0.04	0.01	-	a,b
22	857	856	3-Methyl-3-butenyl acetate	-	0.01	-	a
23	867	868	2-Methyl butyl acetate	-	0.11	-	a
24	876	878	2,5-Octadiene, 3,4,5,6-tetramethyl-	-	-	0.04	a
25	880	881	Heptanal	-	0.03	-	a,b
26	885	886	Cyclofenchene	-	0.03		a
27	928	926	*α*-thujene	0.07	0.18	0.16	a
28	936	935	*α*- pinene	1,81	2.29	3.41	a,b
29	937	937	Benzaldehyde	0.02	0.8	0.01	a,b
30	940	938	Valeric acid	-	0.03	-	a
31	945	947	Camphene	1.5	1.3	1.95	a
32	956	956	1-heptanol	0.01	-	0.01	a,b
33	965	966	3-octanone	0.01	-	-	a
34	966	968	Sabinene	0.68	-	0.02	a
35	971	973	***β*-pinene**	**6.4**	**4.74**	**8.27**	a
36	982	982	Octanal	0.01	0.01	-	a,b
37	984	983	*α*-Myrcene	-	-	0.02	a
38	987	986	3-Hexenyl acetate, (*Z*)-	-	0.01	-	**a**
39	993	995	Hexanal, 2,2-dimethyl-	-	0.05	-	**a**
40	998	999	*α*-Phellandrene	0.11	0.55	-	a
41	1000	1001	Yomogi alcohol	-	-	0.15	a
42	1005	1007	(+)-4-carene	0.13	-	-	a
43	1010	1011	*α*-terpinene	-	0.1	0.07	a
44	1021	1022	**1,8-cineole**	**13.49**	**11.05**	**15.11**	**a**
45	1023	1024	l-limonene	0.28	0.5	-	a
46	1024	1025	*p*-Cymol	-	1.19	0.25	a
47	1042	1043	Cyclohexanol, 3,3,5-trimethyl-	-	-	0.12	a
48	1045	1047	*γ*-Terpinene	0.33	0.24	0.19	a
49	1046	1048	**Artemisia ketone**	1.69	0.09	**9.04**	a
50	1065	1066	*cis*-linaloloxide	0.25	-	-	a
51	1073	1072	Artemisia alcohol	1.27	0.03	0.63	a
52	1076	1074	3,5-Heptadien-2-one, 6-methyl-(*E*)-	-	1.29	-	a
53	1081	1079	*α*-terpinolene	0.17	0.08	0.05	a,b

54	1083	1081	Ethyl heptanoate	0.33	-		a
55	1085	1086	Linalool	-	0.72	0.58	a,b
56	1087	1088	Phenethyl alcohol	-	0.06	-	a
57	1089	1090	Pentanoic acid, 3-methylbutyl ester	0.02	-	-	a
58	1092	1090	isopentyl pentanoate	-	-	0.03	a
59	1094	1093	*trans*-sabinene hydrate	0.51	0.53	1.48	a
60	1095	1093	Pentyl 3-methylbutanoate	-	0.13	-	a
61	1104	1103	*cis*-pinene hydrate	-	0.05	-	a
62	1106	1108	Bicyclo[3,1,1]heptan-2-one, 6,6-dimethyl-, (1R)-	-	0.07	-	a
63	1108	1109	Nopinone	-	-	0.07	a
64	1112	1110	*cis*- verbenol	-	-	0.47	a
65	1119	1117	2,3,3-trimethyl-3-cyclopentene acetaldehyde	-	0.11	0.15	a
66	1124	1125	*p*-menth-2-en-1-ol	0.49	-	-	a
67	1126	1127	*β*-terpineol	0.16	-	-	a,b
68	1132	1131	**Camphane (Bornane)**	**10.43**	**11.94**	**11.49**	a
69	1135	1134	*cis*-limonene oxide	0.44	0.04		a
70	1137	1138	4,5-Heptadien-2-one, 3,3,6-trimethyl-	0.23	-	-	a
71	1141	1140	Pinocarvone	0.49	-	-	a
72	1150	1152	Pinocarveol	0.37	1.37	0.53	a
73	1152	1153	**l-borneol**	**8.28**	2.65	**11.57**	a,b
74	1160	1162	*p*-methyl acetophenone	-	0.08	0.01	a
75	1162	1165	Butanoic acid, 3-hexenyl ester, (*Z*)-	0.07	-	-	a
76	1164	1165	Terpinen-4-ol	0.86	0.22	0.27	a
77	1166	1165	*cis*-*p*-mentha-1(7),8-dien-2-ol	-	0.02	-	a
78	1167	1165	*Z*-3-hexenyl butanoate	-	-	0.08	a
79	1168	1165	Thujol	3.54	-		a
80	1171	1170	Myrtenal	-	0.79	0.70	a
81	1174	1175	*n*-caprylic acid	-	0.02	-	a
82	1178	1177	Methyl chavicol	-	0.10		a
83	1179	1178	Anethofuran	-	0.24	0.37	a
84	1180	1179	*cis*-piperitol	-	0.28	0.24	a
85	1182	1189	2,6-dimethyl-3,7-octadiene-2-ol	0.17	-	-	a
86	1193	1192	*α*-terpineol	-	2.43	1.98	a
87	1195	1194	*trans*-3(10)-Caren-2-ol	0.16	0.18	0.14	a
88	1197	1198	2,7-Dimethyl-2,6-octadiene	-	-	0.09	a
89	1199	1200	n-dodecane	-	0.03	0.01	a,b
90	1205	1206	trans-carveol	-	0.26	0.16	a
91	1207	1206	*cis*-sabinene hydrate acetate	-	1.26	0.55	a
92	1210	1212	Myrtenol	-	0.97	0.86	a
93	1213	1214	Cuminic aldehyde	-	0.12		a
94	1214	1215	cis-geraniol	-	0.04	0.15	a
95	1226	1228	Bornyl formate	-	-	0.06	a
96	1230	1233	**Piperitone**	1.69	**24.29**	3.89	a
97	1232	1234	Cyclohexanone, 2,6-bis(2-methylpropylidene)-	0.24	-	-	a
98	1234	1235	Tetracyclo[6,3,2,0(2,5),0(1,8)] tridecan-9-ol, 4,4-dimethyl-	-	2.32	0.91	a
99	1235	1237	Isogeraniol	-	-	0,03	a
100	1236	1237	Chavicol	-	0.27	0.14	a
101	1245	1247	Myrcenyl acetate	-	0.05	-	a
102	1250	1251	3-cyclohexen-1-one, 2-isopropyl-5-methyl-	0.07	-	-	a
103	1251	1252	Perilla aldehyde	-	0.08	-	a
104	1255	1257	Linalyl acetate	-	0.02	0.01	a
105	1265	1267	Lavandulyl acetate	-	-	0.04	a
106	1267	1268	Isobornyl acetate	0.04	0.05	-	a
107	1270	1273	Ascaridole	-	0.21	-	a

108	1279	1281	Pinocarvyl acetate	-	0.01	0.01	a
109	1281	1282	Perilla alcohol	-	0.17	-	a
110	1284	1285	Isopulegol acetate	-	0.09	-	a
111	1320	1322	Myrtenyl acetate	-	0.04	-	a
112	1334	1336	Citronellyl acetate	-	-	0.01	a
113	1338	1340	Eugenol	-	-	0.75	a,b
114	1340	1342	*trans*-carvyl acetate	-	0.05	0.05	a
115	1342	1344	*n*-decanoic acid	-	0.07	-	a
116	1350	1352	*α*-cubebene	-	-	0.03	a
117	1352	1353	6-dodecanone	-	-	0.04	a
118	1357	1359	*cis*-jasmone	0.15	1.23	1.07	a
119	1360	1362	Phenol, 2-methoxy-3-(2-propenyl)-	-	1.00	-	a
120	1362	1364	Linalyl propionate	1.36	-	-	a
121	1374	1376	Methyleugenol	0.17	1.68	1.22	a,b
122	1375	1376	*α*-copaene	0.33	0.34	-	a
123	1387	1389	6,8-Nonadien-2-one, 6-methyl-5-(1-methyl ethylidene)	-	-	0.09	a
124	1400	1400	*n*-tetradecane	-	0.04	0.02	a,b
125	1404	1405	(-)-*α*-cedren	-	-	0.62	a
126	1410	1411	Iso-amyl benzoate	-	0.04	-	a
127	1411	1412	3-methyl-3-butenyl benzoate	-	0.06	-	a
128	1425	1424	*trans*-caryophyllene	0.39	0.54	1.09	a
129	1430	1433	*α*-bergamotene	0.06	-	-	a
130	1435	1436	Isoeugenol	-	0.09	-	a
131	1444	1446	*cis*-*β*-santalene	0.5	-	-	a
132	1447	1449	*α*-caryophyllene	-	-	0.04	a,b
133	1450	1449	*β*-farnesene	0.23	-	-	a
134	1452	1455	(+)-aromadendrene	0.25	-	0.52	a
135	1470	1472	*α*-curcumene	0.20	0.74	1.31	a
136	1474	1476	Germacrene D	0.23	-	-	a
137	1490	1493	Zingiberene	1.20	0.26	-	a
138	1492	1494	Elemene	0.34	1.17	0.15	a
139	1495	1497	*β*-bisabolene	-	0.08	0.06	a
140	1502	1504	6-methyl-3,5-heptadien-2-one	0.25	-	-	a
141	1515	1517	*β*-nerolidol	1.03	-	1.05	a
142	1525	1523	*α*-longipinene	-	0.40	-	a
143	1550	1551	*β*-sesquiphellandrene	-	0.29	0.36	a
144	1552	1554	Germacrene B	-	0.06	-	a
145	1573	1575	(+) spathulenol	2.52	0.42	0.11	a
146	1580	1581	(-)-**caryophyllene oxide**	**4.60**	**4.44**	**4.50**	a
147	1584	1586	***cis*-sesquisabinene hydrate**	**4.12**	-	-	a
148	1588	1590	Isoaromadendren-epoxid	0.54	-	0.25	a
149	1590	1592	Salvial-4(14)-en-1-one	-	0.15	-	a
150	1592	1593	Carotol	-	-	1.15	a
151	1595	1597	*α*-cedrol	-	0.41	-	a
152	1598	1600	Hexadecane	-	-	0.02	a,b
153	1616	1618	longipinocarveol, trans-	2.32	-	-	a
154	1618	1620	*δ*-cadinol	0.09	-	-	a
155	1647	1649	***β*-eudesmol**	**4.12**	-	**3.86**	a
156	1664	1665	*β*-neoclovene	0.41	-	-	a
157	1666	1668	*α*-bisabolol	0.43	0.02	0.39	a
158	1674	1675	Acetic acid, 4a-methyldecahydronaphthalen-1-yl ester	-	-	0.57	a
159	1698	1700	Bergamotol, *Z*-,alpha,-*trans*-	0.86	4.29	-	a
160	1704	1705	Farnesol	-	0.02	0.1	a,b
161	1764	1765	3(10)-Caren-4-ol, acetoacetic acid ester	0.53	-	-	a
162	1793	1795	Hexadecanal	0.23	-	-	a,b
163	1810	1812	2-decen-1-ol	-	0.04	-	a

164	1840	1842	Hexahydrofarnesyl acetone	0.17	-	-	a
165	1896	1897	Geranyl linalool	-	0.05	-	a
166	1940	1942	Palmitic acid	0.16	-	-	a
167	2000	2000	Eicosane	0.97	-	-	a
168	2036	2038	Stearaldehyde	-	0.02	0.08	a
169	2050	2052	9,12-octadecadien-1-ol, (*Z*,*Z*)-	0.23	-	-	a
170	2400	2400	Tetracosane	0.61	-	-	a
171	2452	2455	Fumaric acid, hexyl nonyl ester	0.41	-	-	a
172	-	-	Unknown	0.12	-		
173	-	-	Unknown	-	0.23	-	
			Monoterpenes	57.94	74.75	77.16	
			Sesquiterpenes	24.94	13.63	15.59	
			Others	4.18	8.59	3.34	
			TOTAL (%)	**87.06**	**96.97**	**96.09**	

a: Compounds are listed in order of elution from a DB-5 column. **b:** Identification of components based on standard compounds; All values are expressed as mean ± standard deviation of triplicate measurements; **LRI (cal):** Linear retention indices (DB-5 column) calculated against n-alkanes. % values are calculated from FID data using standard **LRI (lit):** https://pubchem.ncbi.nlm.nih.gov **S1:** sample 1 obtained by Clevenger; **S2:** sample 2 obtained by CAR-PDMS type of SPME fiber; **S3**: Sample 3 obtained by PDMS-DVB type of SPME

3.2. Antibacterial Activity Test

The MIC values (v/v) of the essential oil are presented in Table 2. The oil exhibited the highest antibacterial activity against *S. aureus* ATCC 25923 and *S. aureus* ATCC 43300 (MRSA), with MIC of <1/512 for each bacterium. However, no antibacterial activity was observed against *P. aeruginosa* ATCC 27853.

Table 2. MIC values of essential oil of *A. sintenisii* against tested bacteria.

	Test Bacteria					
	Escherichia coli ATCC 25922	*Klebsiella pneumoniae* ATCC 13883	*Staphylococcus aureus* ATCC 25923	*Staphylococcus aureus* ATCC 43300 (MRSA)	*Enterococcus faecalis* ATCC 29212	*Pseudomonas aeruginosa* ATCC 27853
EO of A. sintenisii	1/128	1/32	<1/512	<1/512	1/8	-

EO: Essential oil

3.3. Antibiofilm Activity Test

The essential oil of *A. sintenisii* did not exhibit antibiofilm activity against *P. aeruginosa* PAO1. To gain a more comprehensive understanding of its potential antibiofilm properties, further studies should be conducted using different test bacteria.

4. DISCUSSION and CONCLUSION

While distillation is a well-known and widely used classical method, the headspace solid-phase microextraction (HS-SPME) method is a simple, efficient and solvent-free technology that has gained increasing preference in analytical studies. SPME integrates sampling, extraction, concentration, and sample introduction into a single step without the need for solvents. The concentration of analytes in the sample is achieved through direct extraction onto fused silica fiber coated with an appropriate stationary phase. In this study, two adsorbents with different polarities, CAR-PDMS and PDMS-DVB, were employed in the SPME method to maximize the detection of chemical components present in essential oil. (Kataoka *et al*., 2000; Vas and Vekey, 2004).

Sökmen *et al*. (2003) investigated the essential oil extracted using the Clevenger apparatus from the herbal parts of *A. sintenisii* collected from Sivas in 2003. The essential oil was characterized by a high monoterpene content, with camphor, eucalyptol, β-pinene, borneol, and piperitone identified as the major components. The composition of the essential oil obtained using the

Clevenger apparatus, one of the extraction methods employed in this study, was compared to the findings of Sökmen *et al*. The results of our investigation align with those of Sökmen *et al*., confirming the consistency in major components. Previous studies have also indicated variations in the chemical composition of essential oils derived from the *Achillea* species. These reports highlight that the essential oils of *Achillea* species, which are primarily rich in oxygenated monoterpenes, contain various subgroups of terpenes, including artemisia ketone, linalool, camphor, 1,8-cineole, piperitone, α-terpineol, caryophyllene, caryophyllene oxide, aromadendrene, β-eudesmol, and spathulenol, as well as hydrocarbons and fatty acids. Besides, it is reviewed that the most common components of *Achillea* essential oils include camphor, 1,8-cineole (eucalyptol), cis- and trans-sabinene hydrate, borneol, α-thujone, β-thujone, linalool, and α-terpineol (Bader *et al*., 2022; Kordali *et al*., 2009; Maffei *et al*., 1993; Mohammadhosseini *et al*., 2017; Sökmen *et al*., 2003; Strzępek-Gomółka *et al*., 2021; Toplan *et al*., 2022; Turkmenoğlu *et al*., 2015). These findings partially align with our study. The variations in essential oil composition among *Achillea* species can be attributed to several factors, including geographical location, altitude, and climatic conditions, as well as differences in chemotypes and extraction methods used for obtaining essential oils.

The antibacterial activity exhibited by the essential oil can be attributed to its chemical composition, which includes 1,8-cineol, l-borneol, camphane, and β-pinene as the most abundant constituents. The main compound of essential oil obtained through distillation was 1,8-cineole, which is known for its pharmacological properties, particularly its anti-inflammatory and antioxidant effects. Furthermore, 1,8-cineole has been shown to possess antibacterial and anti-quorum sensing properties against a wide range of pathogenic bacteria (Cai *et al*., 2020; Wang *et al*., 2022). Similarly, l-borneol, another major compound of the essential oils, is a natural monoterpenoid with antimicrobial activity and has been reported to exhibit synergistic effects with ciprofloxacin (Dorman and Deans, 2000; Leite-Sampaio *et al*., 2022). The major sesquiterpenes identified in the essential oil were caryophyllene oxide, cis-sesquisabinene hydrate, and β-eudesmol. The presence of these bioactive compounds is believed to play a crucial role in the antibacterial activity of the essential oil.

The growing issue of antibiotic resistance necessitates the discovery of new antibacterial agents. In this study, the essential oil of *A. sintenisii* exhibited strong antibacterial activity against *S. aureus* ATCC 25923 and *S. aureus* ATCC 43300 (MRSA), yet it failed to inhibit the biofilm formed by *P. aeruginosa PAO1*. Methicillin-resistant *S. aureus* (MRSA) is widely recognized as a major cause of both hospital-acquired and community-acquired infections, largely due to its multidrug resistance. The significant activity of the essential oil against both *S. aureus* ATCC 25923 and *S. aureus* ATCC 43300 (MRSA) suggests its promising potential as an antimicrobial agent against serious, difficult-to-treat infections. Given these findings, further research is warranted to explore its full therapeutic potential. Conversely, the essential oil of *A. sintenisii*, demonstrated no efficacy against the preformed biofilm of *P. aeruginosa*. To further investigate its antibiofilm potential, additional studies should be conducted using other biofilm-forming microorganisms. It is also required to fully elucidate the medical potential of the essential oil and to provide a comprehensive profile of its bioactive properties.

Declaration of Conflicting Interests and Ethics

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

Authorship Contribution Statement

Aslı Can Ağca: Conception, Investigation, Methodology, Analysis and Interpretation, Literature review, Original draft, Critical Review**. Nilüfer Vural:** Investigation, Methodology, Analysis and Interpretation, Critical Review. **Suna Sibel Rızvanoğlu:** Investigation, Methodology, Analysis and Interpretation, Critical Review. **Müjde Eryılmaz:** Investigation,

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REFERENCES

Akkaoui, S., Johansson, A., Yagoubi, M., Haubek, D., El Hamidi, A., Rida, R., Claesson, R., & Ennibi, Q. (2020). Chemical composition, antimicrobial activity, *in vitro* cytotoxicity and leukotoxin neutralization of essential oil from *Origanum vulgare* against *Aggregatibacter actinomycetemcomitans*. *Pathogens, 9*(3), 192. https://doi.org/10.3390/pathogens9030192

Asfour, H.Z. (2018). Anti-quorum sensing natural compounds. *Journal of Microscopy and Ultrastructure, 6*, 1-10. https://doi.org/10.4103/JMAU.JMAU_10_18

Aytaç, Z., Duman, H., & Ekici, M. (2016). Two new *Achillea* L. (Asteraceae) species from Turkey. *Turkish Journal of Botany, 40*(4), 373-379. https://doi.org/10.3906/bot-1504-19

Bader, A., AlQathama, A., Cioni, P.L., Ceccarini, L., Abdelhady, M.I.S., Al-Shareef, W., Ascrizzi, R., & Flamini, G. (2022). Essential oil biodiversity of *Achillea ligustica* All. Obtained from mainland and island populations. *Plants, 11*, 1054. https://doi.org/10.3390/plants1108105

Bali, E.B., Türkmen, K.E., Erdönmez, D., & Sağlam, N. (2019). Comparative study of inhibitory potential of dietary phytochemicals against quorum sensing activity of and biofilm formation by *Chromobacterium violaceum* 12472, and swimming and swarming behaviour of *Pseudomonas aeruginosa* PAO1. *Food Technology and Biotechnology, 57*(2), 212-221. https://doi.org/10.17113/ftb.57.02.19.5823

Başer, K.H.C. (2016). Essential oils of *Achillea* species of Turkey. *Natural Volatiles & Essential Oils, 3*, 1-14.

Becker, L., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.G., Jr, Shank, R.C., Slaga, T.J., Snyder, P.W., & Andersen, F.A. (2016). Safety assessment of *Achillea millefolium* as used in cosmetics. *International Journal of Toxicology, 35* (Supplement 3), 5S-15S. https://doi.org/10.1177/1091581816677717

Cai, Z-M., Peng, J-Q., Chen, Y., Tao, L., Zhang, Y-Y., Fu, L-T., Long, Q-D., & Shen, X-C. (2020). 1,8-Cineole: a review of source, biological activities, and application. *Journal of Asian Natural Products Research, 23*(10), 938-954. https://doi.org/10.1080/10286020.2020.1839432

Clinical and Laboratory Standards Institute. (2009). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard (8th ed.)* (CLSI standard M07-A8). Clinical and Laboratory Standards Institute.

Conforti, F., Loizzo, M.R., Statti, G.A., & Menichini, F. (2005). Comparative radical scavenging and antidiabetic activities of methanolic extract and fractions from *Achillea ligustica* ALL. *Biological & Pharmaceutical Bulletin, 28*, 1791-1794. https://doi.org/10.1248/bpb.28.1791

Dorman, H.J.D., & Deans, S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*, 308-316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

European Medicine Agency-EMA. (2020, December 3). Millefolii herba. https://www.ema.europa.eu/en/medicines/herbal/millefolii-herba

Eryılmaz, M., Kart, D., & Gürpınar, S.S. (2019). Vajinal floradan izole edilen *Lactobacillus* sp. metabolitlerinin antibiyofilm aktivitelerinin araştırılması [Investigation of antibiofilm

activities of Lactobacillus sp. metabolites isolated from vaginal flora]. *Turk Mikrobiyoloji Cemiyeti Dergisi*, *49*(3), 169-174. https://doi.org/10.5222/TMCD.2019.169

Güner, A., Aslan, S., Ekim, T., Vural, M., & Babaç, M.T., (Eds.) (2012). *Türkiye Bitkileri Listesi (Damarlı Bitkiler)* [*List of Türkiye plants-vascular plants*]. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği.

Jardak, M., Mnif, S., Ayed, R.B., Rezgui, F., & Aifa, S. (2021). Chemical composition, antibiofilm activities of Tunisian spices essential oils and combinatorial effect against *Staphylococcus epidermidis* biofilm. *LWT - Food Science and Technology, 140*, 110691. https://doi.org/10.1016/j.lwt.2020.110691

Karaca, B., Cihan, A.Ç., Akata, I., & Altuner, E.M. (2020). Anti-biofilm and antimicrobial activities of five edible and medicinal macrofungi samples on some biofilm producing multi-drug resistant *Enterococcus* strains. *Turkish Journal of Agriculture - Food Science and Technology*, *8*, 69-80.

Kataoka, H., Lord, H.L., & Pawliszyn, J. (2000). Applications of solid-phase microextraction in food analysis. *Journal of Chromatography A, 880*(1-2), 35-62. https://doi.org/10.1016/s0021-9673(00)00309-5

Kordali, S., Cakir, A., Akcin, T.A., Mete, E., Akcin, A., Aydın, T., & Kiliç, H. (2009). Antifungal and herbicidal properties of essential oils and *n*-hexane extracts of *Achillea gypsicola* Hub-Mor. and *Achillea biebersteinii* Afan. (Asteraceae). *Industrial Crops and Products, 29*, 562-570. https://doi.org/10.1016/j.indcrop.2008.11.002

Leite-Sampaio, N.F., Gondim, C.N.F.L., Souza, C.E.S., & Coutinho, H.D.M. (2022). Antibiotic potentiating action of α-pinene and borneol against EPEC and ETEC serotypes. *Microbial Pathogenesis, 162*, 105371. https://doi.org/10.1016/j.micpath.2021.105371

Maffei, M., Germano, F., Doglia, G., & Chialva, F. (1993). Essential oils, chromosome numbers and karyotypes from Italian *Achillea* species. Part II. *Journal of Essential Oil Research, 5*, 61–70. https://doi.org/10.1080/10412905.1993.9698171

Maggi, F., Bramucci, M., Cecchini, C., Coman, M.M., Cresci, A., Cristalli, G., Lupidi, G., Papa, F., Quassinti, L., Sagratini, G., & Vittori, S. (2009). Composition and biological activity of essential oil of *Achillea ligustica* All. (Asteraceae) naturalized in central Italy: Ideal candidate for anti-cariogenic formulations. *Fitoterapia, 80*, 313-319. https://doi.org/10.1016/j.fitote.2009.04.004

Mazandarani, M., Mirdeilami, S.Z., & Pessarakli, M. (2013). Essential oil composition and antibacterial activity of *Achillea millefolium* L. from different regions in Northeast of Iran. *Journal of Medicinal Plants Research, 7*(16), 1063-1069. https://doi.org/10.5897/JMPR12.961

Mohammadhosseini, M., Sarker, S.D., & Akbarzadeh, A. (2017). Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. *Journal of Ethnopharmacology, 199*, 257-315. https://doi.org/10.1016/j.jep.2017.02.010

Raut, J.S., & Karuppayil, S.M. (2014). A status review on the medicinal properties of essential oils. *Industrial Crops and Products, 62*, 250-64. https://doi.org/10.1016/j.indcrop.2014.05.055

Sökmen, A., Vardar-Ünlü, G., Polissiou, M., Daferera, D., Sökmen, M., & Dönmez, E. (2003). Antimicrobial activity of essential oil and methanol extracts of *Achillea sintenisii* Hub. Mor. (Asteraceae). *Phytotherapy Research, 17*, 1005–1010. https://doi.org/10.1002/ptr.1274

Strzępek-Gomółka, M., Gaweł-Bęben, K., & Kukula-Koch, W. (2021). *Achillea* species as sources of active phytochemicals for dermatological and cosmetic applications. *Oxidative Medicine and Cellular Longevity, 2021*, ID 6643827. https://doi.org/10.1155/2021/6643827

Toplan, G.G., Taskın, T., İşcan, G., Göger, F., Kürkçüoğlu, M., Civaş, A., Ecevit-Genç, G., Mat, A., & Başer, K.H.C. (2022). Comparative studies on essential oil and phenolic content with in vitro antioxidant, anticholinesterase, antimicrobial activities of *Achillea biebersteinii* Afan. and *A. millefolium* subsp. *millefolium* Afan. L. growing in Eastern Turkey. *Molecules, 27*, 1956. https://doi.org/10.3390/molecules27061956

Tunca-Pınarlı, Y., Benek, A., Turu, D., Bozyel, M.E., Canli, K., & Altuner, E.M. (2023). Biological activities and biochemical composition of endemic *Achillea fraasii*. *Microorganisms, 11*, 978. https://doi.org/10.3390/microorganisms11040978

Turkmenoglu, F.P., Agar, O.T., Akaydin, G., Hayran, M., & Demirci, B. (2015). Characterization of volatile compounds of eleven *Achillea* species from Turkey and biological activities of essential oil and methanol extract of *A. hamzaoglui* Arabacı & Budak. *Molecules, 20*, 11432-11458. https://doi.org/10.3390/molecules200611432

Tuzlacı, E. (2016). *Türkiye bitkileri geleneksel ilaç rehberi* [*Traditional medicine guide to plants of Turkey*]. İstanbul Medikal Yayıncılık.

Vas, G., & Vekey, K. (2004). Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. *Journal of Mass Spectrometry, 39*, 233–254. https://doi.org/10.1002/jms.606

Wang, Y., Zhang, Y., Song, X., Fang, C., Xing, R., Liu, L., Zhao, X., Zou, Y., Li, L., Jia, R., Ye, G., Shi, F., Zhou, X., Zhang, Y., Wan, H., Wei, Q., & Yin, Z. (2022). 1,8-Cineole inhibits biofilm formation and bacterial pathogenicity by suppressing luxS gene expression in *Escherichia coli*. *Frontiers in Pharmacology, 13*, 988245. https://doi.org/10.3389/fphar.2022.988245

World Health Organization. (2015). *Antibiotic resistance: Multi-country public awareness survey*. https://iris.who.int/bitstream/handle/10665/194460/9789241509817_eng.pdf

Yeşilada, E. (2023). *Halk hekimlerinin hazinesi, bilimsel referanslar ışığında Anadolu halk ilaçları envanteri* [*Treasure of folk medicine, Anatolian folk medicines inventory in the light of scientific references*]. Hayygrup Yayıncılık.