CHEMICAL COMPOSITIONS AND ANTIBACTERIAL ACTIVITIES OF ESSENTIAL OILS FROM *AQUILARIA AGALLOCHA, ARTEMISIA ABSINTHIUM, HYSSOPUS OFFICINALIS,* AND *MENTHA PULEGIUM*

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Abstract. The study evaluated the chemical composition and antibacterial activity of essential oils from *Aquilaria agallocha, Artemisia absinthium, Hyssopus officinalis,* and *Mentha pulegium.* Gas chromatography/gas chromatography mass spectrometry (GC/GC-MS) was used to analyze the essential oils' volatile components; their antibacterial activity against multidrug-resistant clinical bacterial isolates was assessed using a disk diffusion susceptibility assay together with the determination of minimum inhibitory concentration (MIC). The major volatile chemical compound of *Aq. agallocha, Ar. absinthium, H. officinalis,* and *M. pulegium* oils were dimethyl ester dodecanedioic acid (19.65%), β-caryophyllene (18.51%), camphor (14.2%), and pulegone (58.5%), respectively. All essential oils had varying bactericidal activity against the test isolates of *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Streptococcus pyogenes*, but not *Pseudomonas aeruginosa.* The essential oil with the overall highest antibacterial activity was that of *M. pulegium,* followed by *H. officinalis, Aq. agallocha* and *Ar. absinthium.* The findings confirm that medicinal plant essential oils can play a role as an alternative treatment option against current clinical multidrug-resistant microorganisms.

Keywords: antibacterial activity, essential oil, gas chromatography/mass spectrometry, human pathogen, medicinal plant, minimum inhibitory concentration

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INTRODUCTION

Plants have not only played an important role in human food supply for thousands of years, but also have great economic value due to their therapeutic effects, such as antibacterial, antifungal, anticancer, anti-inflammatory, antimutagenic, and antioxidant (Sitarek *et al*, 2017). For millions, traditional herbal treatments are the main and sometimes the only source of health care.

In recent years, the rapid increase in health care costs and the availability of traditional medicines to individuals at the lower economic level have made these traditional treatments more attractive (WHO, 2013). In addition, the side effects of synthetic drugs have led to a resurgence in the popularity of herbal medicine, which is considered safe based on a long history of use (Sitarek *et al*, 2017). As of 2022, 170/194 of the World Health Organization (WHO) members, representing approximately 80% of the world's population, reported that they continue to use traditional medicine (WHO, 2022).

Currently, bacterial resistance to antibiotics has reached an alarming level worldwide, particularly the rapid spread of multi- and pan-antibiotic-resistant bacteria (superbugs) (WHO, n.d.). According to O'Neill (2014), antibiotic-resistant infections are estimated to cause approximately 700,000 deaths each year, and if current trends continue, this number could rise to 10 million annually by 2050. In this context, the WHO reported an urgent need for new antibiotics to combat pathogens resistant to current antibacterials (WHO, n.d.).

This increasing threat of antibacterial resistance has raised the scientific community's interest in herbal medicines with antimicrobial properties. Medicinal plants have a significant potential due to their low toxicity, pharmacological activity and low cost of production. Essential oils isolated from such plants are of particular interest as they show antibacterial, antiviral, antifungal, antioxidant, antiparasitic, and insecticidal properties (Chouhan *et al*, 2017).

Genus *Aquilaria*, belonging to the Thymelaeaceae Family, is a raw material in traditional and modern medicine (Hashim *et al*, 2016). Agarwood oil is an extremely rare and a valuable oil found in Bhutan, northeastern India and some parts of Southeast Asia. The different extracts of this plant are reported to have an antinociceptive, antimicrobial, antioxidant, and sedative effects as well as antihyperglycemic activity (Rahman *et al*, 2016).

Genus *Artemisia* (wormwood), Family Asteraceae, is typically used as antianorexia, anticancer (breast, leukemia and liver), antidepressant, antihelminthic, anti-inflammatory, antioxidant, antiparasitic, antiseptic, antispasmodic, balsamic, and insecticidal agent, and for treatment of prolonged fever and sclerosis (Ali *et al*, 2021).

Genus *Hyssopus*, Family Lamiaceae, is widespread from the eastern Mediterranean to central Asia and Mongolia. Hyssopus spp are known for their antiseptic, antitussive and expectorant properties; antiasthmatic, antidiabetic, anti-inflammatory, antioxidant, and antiviral effects have also been identified (Sharifi-Rad *et al*, 2022).

Genus *Mentha* (pennyroyal), Family Lamiaceae, a native plant of Europe, North Africa, Asia Minor, and the Near East, has been used in medical practice for its antiseptic, antispasmodic, carminative, and sudorific properties. Pennyroyal essential oil has been shown to have strong antibacterial properties against Gram-positive and -negative bacteria (Boukhebti *et al*, 2011).

Thus, we investigated the chemical compositions and antibacterial activities of the essential oils of *Aq. agallocha*, *Ar. absinthium*, *H. officinalis*, and *M. pulegium* against Gram-negative and -positive clinical bacteria isolates from a hospital in Türkiye.

The significance of this study lies in its contribution to the development of alternative antimicrobial agents from natural sources. By demonstrating the antibacterial potential of essential oils, particularly *M. pulegium*, against multidrug-resistant clinical isolates, this research provided valuable insights into their possible roles as complementary or substitute therapies in the global fight against antibiotic resistance.

MATERIALS AND METHODS

Essential oils source

Aq. agallocha, *Ar. absinthium*, *H. officinalis*, and *M. pulegium* essential oils purified by the steam distillation method were obtained from Katyani Exports (Delhi, India) and stored at 4 °C in the dark until use. Each essential oil containing 2.5% Tween 20 was sterilized via passage through a 0.2 µm syringe filter.

Gas chromatography-mass spectrometry (GC-MS) method

The chemical compositions of essential oils were analyzed using gas chromatography/ gas chromatography-mass spectrometry (GC/GC-MS) (GC-MS Clarus 500; Perkin Elmer Inc, Waltham, MA) equipped with a capillary column (60.0 m × 0.25 mm × 0.25 µm) (HP Innowax Capillary; Agilent Technologies, Santa Clara, CA). Helium at a flow rate of 0.8 ml/min was used as the carrier gas, and 1-µl aliquots of the samples were injected using a 40:1 split ratio. The temperature of the injector system was kept constant at 250 °C, with the column temperature programmed at 60°C for 10 minutes, then from 60 °C to 220 °C at 4°C/minute and maintained at 220°C for 10 minutes; the analysis was completed in 60 minutes. For the detection of mass,

a scanning range (m/z) of 35-450 atomic mass units was used with an electron bombardment ionization at 70 eV. Essential oil components were identified employing Wiley and Oil Adams libraries (Adams, 2007).

Bacterial samples and cultivation

Bacteria from respiratory tract samples were obtained from Kahramanmaraş Sütçü Imam University Hospital Medical Microbiology Laboratory. Samples were inoculated onto blood Eosin Methylene-blue (EMB) agar (Becton, Dickinson and Company, Sparks, MD) and incubated at 37 °C for 24-48 hours. The isolated strains were identified using conventional methods (Cheesbrough, 2006) and the BD Phoenix 100 automated identification system (BD Phoenix System; Beckton Dickinson, Franklin Lakes, NJ). *In vitro* antibiotic susceptibilities of the strains were determined using the Phoenix TM 100 automated identification system based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (EUCAST, 2024).

Disk diffusion sensitivity assay

Dehydrated Mueller Hinton agar (MHA) (Becton, Dickinson and Company, Sparks, MD) (34.0 g/l) was melted by heating, sterilized at 121 °C for 15 minutes, cooled to 50 °C, and then poured into sterile petri dishes (9 cm diameter). A bacterial suspension (containing 10^8 CFU/ml of bacteria) was spread on the MHA, then a sterile blank disc (6 mm diameter) was placed on the medium and a 20-µl aliquot of essential oil preparation was added to the disc. An antibiotic-containing disc served as a positive control and an untreated disc as a negative control. The petri dishes were incubated at 37 °C for 24 hours, and inhibition zones were measured using a caliper.

Minimum inhibitory concentration (MIC) assay

A 100-µl aliquot of Mueller Hinton Broth (Becton, Dickinson and Company, Sparks, MD) and a 100-µl aliquot of essential oil

preparation, serially diluted from 200 to 0.39 mg/ml (2.5% Tween 20), were added to each well of a sterile 96-well microplate. Finally, a 10-µl aliquot of each test microorganism (1.5×10^4 cells) was added to each well. Positive control well contained only MHB and negative control well contained MHB and the bacterial inoculum. Plates were incubated at 37 °C for 24 hours, and $A_{600\ nm}$ of each well was measured using a microplate reader (SpectraMax Plus 384, Molecular Devices, San Jose, CA). A minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism after overnight incubation (EUCAST and ESCMID, 2000)

Statistical analysis

Each experiment was carried out in triplicate and the result was reported as mean ± standard deviation (SD).

Ethical approval

Approval was received for this study from the Medical Research Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine (Session no: 2021/21, Decision no: 01).

RESULTS

Chemical composition of the essential oils

From the GC/GC-MS analyses, the essential oil of *Ar. agallocha* contained 23 volatile chemical compounds, with the major constituents identified as dodecanedioic acid, dimethyl ester (19.65%), 1-(4-isopropylphenyl)-2-methylpropyl acetate (16.61%) and Patchouli alcohol (8.46%) (Table 1); that of *Ar. absinthium* 13 volatile chemical components, with the major compounds identified as caryophyllene (18.51%), 1.8-cineole (13.14%), β-pinene (12.61%), and (+/-)trans-nerolidol (12.35%) (Table 2); that of *H. officinalis* 37 chemical compounds, with the major constituents identified as 2-nornanone (14.2%), trans-pinocamphone (10.2%) and 1.8-cineole (8.77%) (Table 3); and

Table 1

Chemical composition of *Aquilaria agallocha* essential oil

Peak No.	Name	Retention time (minutes)	Area (AU)	Height (AU)	A/H ratio	Concen-tration (%)	Area (%)	Height (%)	Simi-larity (%)	CAS no.
1	α-Guaiene	20.568	8366160	1635739	5.11	2.66	2.66	3.05	97	01.12.3691
2	α-Himachalene	22.775	2492398	496475	5.02	0.79	0.79	0.93	94	3853-83-6
3	α-Patchoulene	23.058	5929876	937956	6.32	1.89	1.89	1.75	91	560-32-7
4	Seychellene	23.699	4013769	718785	5.58	1.28	1.28	1.34	96	20085-93-2
5	Aciphyllene	24.145	2379575	471577	5.05	0.76	0.76	0.88	88	87745-31-1
6	δ-Guaiene	24.287	13652663	2559254	5.33	4.34	4.34	4.77	96	3691-11-0
7	β-Himachalene	24.627	8223682	1609943	5,11	2.61	2,61	3.00	95	1461-3-6
8	Cyclohexanol <4-tertbutyl-> acetate	26.243	5832325	1237523	4.71	1.85	1.85	2.31	96	32210-23-4
9	Cyclohexanol <4-tertbutyl-> acetate	27.723	1512382	368822	4.10	0.48	0.48	0.69	97	32210-23-4
10	Benzoic acid, methyl ester	28.137	6418300	1390684	4.62	2.04	2.04	2.59	98	93-58-3
11	(E)-4-tert-Butylcyclohexanol	30.568	10144541	2014072	5.04	3.22	3.22	3.75	98	21862-63-5
12	2,6,6-Trimethyl-1-crotonoyl-1-cyclohexene	34.747	1793209	405693	4.42	0.57	0.57	0.76	94	35044-68-9

Table 1 (cont)

Peak No.	Name	Retention time (minutes)	Area (AU)	Height (AU)	A/H ratio	Concentration (%)	Area (%)	Height (%)	Similarity (%)	CAS no.
13	Benzyl alcohol	37.767	2647729	551923	4.8	0.84	0.84	1.03	97	100-51-6
14	Guaiol	39.649	13552077	2415550	5.61	4.31	4.31	4.50	91	489-86-1
15	1-(4-Isopropylphenyl)-2-methylpropyl acetate	41.076	5026222	1042363	4.82	1.60	1.60	1.94	85	0-0-0
16	1-(4-Isopropylphenyl)-2-methylpropyl acetate	41.710	52254991	7124711	7.33	16.61	16.61	13.28	79	0-0-0
17	δ-Iraldeine	42.893	14947868	2878927	5.19	4.75	4.75	5.37	81	79-89-0
18	Bulnesol	43.523	12750564	2590232	4.92	4.05	4.05	4.83	94	22451-73-6
19	δ-Iraldeine	44.569	16261254	2599749	6.25	5.17	5.17	4.85	80	79-89-0
20	Patchouli alcohol	45.014	26606114	4453640	5.97	8.46	8.46	8.3	92	5986-55-0
21	Acetylcedrene	48.151	16186254	3024846	5.35	5.15	5.15	5.64	93	0-0-0
22	Dodecanedioic acid, dimethyl ester	52.201	61822289	9567468	6.46	19.65	19.65	17.83	92	1731-79-9
23	Methyl-8 -(14)-pimaren-18-oate	57.880	21765245	3559514	6.11	6.92	6.92	6.63	78	3582-27-2

A/H ratio: area to height ratio; AU: arbritary unit; CAS no: Chemical Abstracts service number

Table 2

Chemical composition of *Artemisia absinthium* L. essential oil

Peak No.	Name	Retention time (minutes)	Area (AU)	Height (AU)	A/H ratio	Concentration (%)	Area (%)	Height (%)	Similarity (%)	CAS no.
1	β-Pinene	12.221	52422996	10348739	5.07	12.61	12.61	13.96	96	127-91-3
2	Myrcene	12.438	2969914	700703	4.24	0.71	0.71	0.95	93	123-35-3
3	D-Limonene	13.015	40098698	8805050	4.55	9.64	9.64	11.88	95	5989-27-5
4	Eucalyptol	14.505	54648248	9554620	5.72	13.14	13.14	12.89	96	470-82-6
5	*p*-Cymene	15.348	27577784	5841755	4.72	6.63	6.63	7.88	97	99-87-6
6	β-Linalool	22.151	2780251	613949	4.53	0.67	0.67	0.83	97	78-70-6
7	β-Caryophyllene	23.141	76942235	10200126	7.54	18.51	18.51	13.76	97	87-44-5
8	L-α-Bornyl acetate	25.682	29820261	4918698	6.06	7.17	7.17	6.63	97	5655-61-8
9	Camphor	27.416	5260263	898367	5.86	1.27	1.27	1.21	97	464-49-3
10	Isoborneol	27.726	1618107	292918	5.52	0.39	0.39	0.40	96	124-76-5
11	Nerolidol	35.437	41576688	7885788	5.27	10.00	10.00	10.64	95	7212-44-4
12	(+/-)Trans-Nerolidol	36.515	51333723	9153084	5.61	12.35	12.35	12.35	95	40716-66-3
13	Caryophyllene oxide	41.569	28709157	4930030	5.82	6.91	6.91	6.65	92	1139-30-6

A/H ratio: area to height ratio; AU: arbritary unit; CAS no: Chemical Abstracts service number

Table 3

Chemical composition of *Hyssopus officinalis* L. essential oil

Peak No.	Name	Retention time (minutes)	Area (AU)	Height (AU)	A/H ratio	Concentration (%)	Area (%)	Height (%)	Similarity (%)	CAS no.
1	Camphene	11.824	23123377	5115719	4.52	5.16	5.16	6.4	95	79-92-5
2	β-Pinene	12.225	32422682	6016893	5.39	7.24	7.24	7.5	95	127-91-3
3	L-β-Pinene	12.431	12233211	2425616	5.04	2.73	2.73	3.0	95	18172-67-3
4	D-Limonene	13.012	10351849	2465974	4.20	2.31	2.31	3.1	95	5989-27-5
5	*cis-Ocimene*	13.368	411796	110738	3.72	0.09	0.09	0.1	95	08.10.6874
6	β-Phellandrene	13.696	16848505	3586608	4.70	3.76	3.76	4.5	95	555-10-2
7	α-terpinolene	14.227	1736917	442082	3.93	0.39	0.39	0.6	96	586-62-9
8	Eucalyptol	14.504	39277397	7140725	5.50	8.77	8.77	8.9	94	470-82-6
9	o-Cymene	15.347	3289099	789513	4.17	0.73	0.73	1.0	96	99-87-6
10	2,2'-Biazulene, 1,1',4,4'-tetramethyl-7,7'-bis (1-methylethyl)-	17.440	8053576	1594319	5.05	1.80	1.80	2.0	80	17509-80-7
11	α-Gurjunene	18.548	1260229	222044	5.68	0.28	0.28	0.3	94	489-40-7
12	(-)-β-Bourbonene	19.078	2434579	384544	6.33	0.54	0.54	0.5	96	5208-59-3
13	trans Sabinene hydrate	21.131	886570	186639	4.75	0.20	0.2	0.2	97	17699-16-0

Table 3 (cont)

Peak No.	Name	Retention time (minutes)	Area (AU)	Height (AU)	A/H ratio	Concentration (%)	Area (%)	Height (%)	Similarity (%)	CAS no.
14	Cadinene <gamma->	21.540	718958	122141	5.89	0.16	0.16	0.2	91	39029-41-9
15	1,3,3-Trimethyl-norcamphor	22.055	1442278	324934	4.44	0.32	0.32	0.4	94	1195-79-5
16	β-Linalool	22.153	4495598	918465	4.89	1.00	1.00	1.2	94	78-70-6
17	Linalyl acetate	22.255	1406331	339120	4.15	0.31	0.31	0.4	90	115-95-7
18	β-Thujone	22.922	24841901	4283233	5.80	5.54	5.54	5.4	97	471-15-8
19	β-Caryophyllene	23.094	20009677	2869772	6.97	4.47	4.47	3.6	94	87-44-5
20	Thujone	23.403	21272993	3805208	5.59	4.75	4.75	4.8	97	546-80-5
21	Bornyl acetate	25.115	16789427	3009561	5.58	3.75	3.75	3.8	96	76-49-3
22	α-Humulene	25.743	14030771	2523969	5.56	3.13	3.13	3.2	93	6753-98-6
23	trans-Pinocamphone	26.429	45730433	7131124	6.41	10.2	10.2	8.9	96	547-60-4
24	Germacrene-D	26.627	7490090	1481404	5.06	1.67	1.67	1.9	95	23986-74-5
25	Camphor	27.445	63483530	9088574	6.98	14.2	14.2	11	97	76-22-2
26	Isocamphopinone	27.700	33870018	5576218	6.07	7.56	7.56	7.0	95	15358-88-0
27	bicyclogermacrene	27.864	7253075	1372173	5.29	1.62	1.62	1.7	92	100762-46-7

Table 3 (cont)

Peak No.	Name	Retention time (minutes)	Area (AU)	Height (AU)	A/H ratio	Concen-tration (%)	Area (%)	Height (%)	Similarity (%)	CAS no.
28	α-Terpineol	28.692	2519628	548595	4.59	0.56	0.56	0.7	97	98-55-5
29	L-Borneol	29.519	15872325	2858219	5.55	3.54	3.54	3.6	96	464-45-9
30	Myrtenol	31.722	968899	197362	4.91	0.22	0.22	0.3	96	515-0-4
31	Palmitic acid methyl ester	39.647	1482534	393969	3.76	0.33	0.33	0.5	94	112-39-0
32	Elemol <α->	40.460	3257433	689216	4.73	0.73	0.73	0.9	94	639-99-6
33	Caryophyllene oxide	41.544	859569	178242	4.82	0.19	0.19	0.2	92	1139-30-6
34	Stearic acid, methyl ester	44.242	1944832	471467	4.13	0.43	0.43	0.6	95	112-61-8
35	Oleic acid methyl ester	45.700	2392657	586390	4.08	0.53	0.53	0.7	94	112-62-9
36	Oleic acid, methyl ester	45.894	546184	95573	5.71	0.12	0.12	0.1	93	112-62-9
37	Linoleic acid, methyl ester	47.813	3046430	762287	4.00	0.68	0.68	1.0	95	112-63-0

A/H ratio: area to height ratio; AU: arbritary unit; CAS no: Chemical Abstracts service number

that of *M. pulegium* 11 volatile chemical components, with the major compounds identified as pulegone (58.5%), 3-*p*-menthanol (13.3%) and isomenthone (9.91%) (Table 4).

Antibiograms of the clinical bacterial isolates

The test microorganisms isolated from the human respiratory tract were the Gram-positive bacteria *Staphylococcus aureus (S. aureus)* and *Streptococcus pyogenes (S. pyogenes)*, and the Gram-negative bacteria *Acinetobacter baumannii (A. baumannii), Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae),* and *Pseudomonas aeruginosa (P. aeruginosa). Staphylococcus aureus* was resistant to ampicillin, cefoxitin, clindamycin, erythromycin, oxacillin, penicillin G, and tetracycline; intermediate susceptible to ciprofloxacin and levofloxacin; and susceptible to daptomycin, fusidic acid, gentamicin, linezolid, teicoplanin, trimethoprim/sulfamethoxazole, and vancomycin (Table 5); whereas *S. pyogenes* was resistant to clindamycin, erythromycin, gentamicin, and tetracycline; intermediate susceptible to levofloxacin; and susceptible to cefepim, cefuroxime, chloramphenicol, daptomycin, linezolid, penicillin G, teicoplanin, and vancomycin.

Of the Gram-negative bacteria, *K. pneumoniae* was resistant to nearly all the antibiotics tested (16/17) (amikacin, amoxicillin/clavunate, ampicillin, cefazolin, cefepime, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, levofloxacin, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole), with the exception for the intermediate susceptibility to meropenem; *A. baumannii* was similarly resistant to nearly all antibiotics tested (7/8) (amikacin, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, trimethoprim/sulfamethoxazole) but susceptible to colistin; *P. aeruginosa* was resistant to a lower proportion of the antibiotics tested (11/15) (amikacin, amoxicillin/clavunate, ampicillin,

Table 4

Chemical composition of *Mentha pulegium* L. essential oil

Peak No.	Name	Retention time (minutes)	Area (AU)	Height (AU)	A/H ratio	Concen-tration (%)	Area (%)	Height (%)	Similarity (%)	CAS no.
1	Menthyl acetate	23.002	6942127	1195896	5.8	2.69	2.69	3.34	97	16409-45-3
2	Isomenthone	24.239	25562307	4509988	5.67	9.91	9.91	12.61	92	491-7-6
3	Isopulegol 2	25.162	9816958	1996354	4.92	3.81	3.81	5.58	97	0-0-0
4	3-*p*-Menthanol	25.491	1099087	248968	4.41	0.43	0.43	0.70	96	1490-4-6
5	*p-Menthone*	25.726	7593741	1538599	4.94	2.94	2.94	4.30	97	89-80-5
6	3-*p*-Menthanol	26.079	34309892	6420779	5.34	13.3	13.3	17.96	98	1490-4-6
7	Limona ketone	28.607	4315887	908164	4.75	1.67	1.67	2.54	92	01.09.6090
8	trans-Isopulegone	28.994	6943732	1372292	5.06	2.69	2.69	3.84	92	29606-79-9
9	Pulegone	30.180	1,51E+08	15446361	9.77	58.5	58.5	43.2	97	89-82-7
10	Methoxycitronellal	31.687	8386047	1695059	4.95	3.25	3.25	4.74	91	3613-30-7
11	Methoxycitronellal	32.474	2066453	425960	4.85	0.80	0.80	1.19	92	3613-30-7

A/H ratio: area to height ratio; AU: arbritary unit; CAS no: Chemical Abstracts service number

cefazolin, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, ertapenem, levofloxacin, and tigecycline), intermediate susceptible to cefepim, imipenem, and piperacillin/tazobactam, but susceptible to meropenem; while *E. coli* was the least resistant to the antibiotics tested (10/17) (ampicillin, cefazolin, cefepim, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, levofloxacin, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole) and susceptible to the remaining (amikacin, amoxicillin/clavunate, ertapenem, gentamicin, imipenem, meropenem, and tigecycline) (Table 5). Thus, all the test clinical bacteria isolates were multidrug-resistant, but, as expected, to different sets of antibiotics.

Antibacterial activities of the essential oils

The disk diffusion assay (Table 6) demonstrated that *Aq. agallocha* essential oil was most effective against *S. aureus*, followed by against *E. coli*, then equally effective against *A. baumannii, K. pneumoniae* and *S. pyogenes* but showed no activity against *P. aeruginosa*. *Ar. absinthium* essential oil was effective only against *S. aureus*. *H. officinalis* essential oil was most effective against *A. baumannii* and *S. aureus*, followed by *K. pneumoniae* and *S. pyogenes* and then *E. coli*, but ineffective against *P. aeruginosa*. *M. pulegium* essential oil was most effective against *A. baumannii*, followed by against *S. aureus* and *S. pyogenes*, then against *E. coli* and *K. pneumoniae*, but was inactive against *P. aeruginosa*. The positive antibiotic control against each bacterial isolate was based on the respective antibiogram (Table 5).

MIC values (Table 7) of *Aq. agallocha* and *Ar. absinthium* essential oils were the same (25 mg/ml) for *A. baumannii, E. coli, K. pneumoniae, S. aureus,* and *S. pyogenes*, but were two times higher for *P. aeruginosa*. MIC values of *H. officinalis* and *M. pulegium* essential oils for the five bacteria isolates were lower: that of *H. officinalis* being 0.8, 1.6, 1.6, 1.6, and 3.1 mg/ml for *A. baumannii,*

Table 5

Antibiograms of Gram-negative and Gram-positive clinical pathogenic bacteria used in the study

Test antibiotic	Gram-negative bacteria				Gram-positive bacteria	
	Acinetobacter baumannii	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus pyogenes*
Amikacin	R	S	R	R	S	(-)
Amoxicillin/clavunate	(-)	S	R	R	(-)	(-)
Ampicillin	(-)	R	R	R	R	(-)
Cefazolin	(-)	R	R	R	(-)	(-)
Cefepim	(-)	R	R	I	(-)	S
Cefoxitin	(-)	(-)	(-)	(-)	R	(-)
Ceftazidime	(-)	R	R	R	(-)	(-)
Ceftriaxone	(-)	R	R	R	(-)	(-)
Cefuroxime	(-)	R	R	R	(-)	S
Chloramphenicol	(-)	(-)	(-)	(-)	(-)	S
Ciprofloxacin	R	R	R	R	(-)	(-)
Clindamycin	(-)	(-)	(-)	(-)	R	R
Colistin	S	(-)	R	(-)	(-)	(-)
Daptomycin	(-)	(-)	(-)	(-)	S	S
Ertapenem	(-)	S	R	R	(-)	(-)
Erythromycin	(-)	(-)	(-)	(-)	R	R

Table 5 (cont)

Test antibiotic	Gram-negative bacteria				Gram-positive bacteria	
	Acinetobacter baumannii	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus pyogenes*
Fusidic Acid (Stafine)	(-)	(-)	(-)	(-)	S	(-)
Gentamicin	R	S	R	(-)	S	R
Imipenem	R	S	R	I	(-)	(-)
Levofloxacin	R	R	R	R	I	I
Linezolid	(-)	(-)	(-)	(-)	S	S
Meropenem	R	S	I	S	(-)	(-)
Oxacillin	(-)	(-)	(-)	(-)	R	(-)
Penicillin G	(-)	(-)	(-)	(-)	R	S
Piperacillin/ Tazobactam	(-)	R	R	I	(-)	(-)
Teicoplanin	(-)	(-)	(-)	(-)	S	S
Tetracycline	(-)	(-)	(-)	(-)	R	R
Tigecycline	(-)	S	(-)	R	(-)	(-)
Trimethoprim/ Sulfamethoxazole	R	R	R	(-)	S	(-)
Vancomycin	(-)	(-)	(-)	(-)	S	S

In vitro antibiotic susceptibilities were determined using the Phoenix TM 100 automated identification system (Beckton Dickinson, Franklin Lakes, NJ) based on EUCAST (2024) guideline.

I: intermediately susceptible; R: resistant; S: susceptible; (-): not tested

Table 6

Disk diffusion zone diameters of essential oils from *Aquilaria agallocha, Artemisia absinthium, Hyssopus officinalis,* and *Mentha pulegium* against a set of Gram-negative and Gram-positive clinical pathogenic bacteria

Clinical pathogenic bacteria	Inhibition zone diameter of essenmtil oil, (mean ± SD mm)				Positive control antibiotic (10 mg/ml)	Zone diameter (mm)
	Aquilaria agallocha	*Artemisia absinthium*	*Hyssopus officinalis*	*Mentha pulegium*		
Staphylococcus aureus	13.3 ± 2.9	23.0 ± 1.7	18.7 ± 1.1	21.7 ± 2.1	Linezolid	30.0
Streptococcus pyogenes	8.0 ± 0	(-)	12.3 ± 2.5	22.3 ± 2.5	Bacitracin	10.0
Acinetobacter baumannii	8.0 ± 1.73	(-)	17.0 ± 3.0	27.3 ± 2.3	Colistin	20.0
Escherichia coli	9.3 ± 2.0	(-)	9.0 ± 1.9	15.9 ± 0	Meropenem	30.0
Klebsiella pneumoniae	8.0 ± 0	(-)	13.3 ± 1.1	17.7 ± 2.5	Meropenem	14.0
Pseudomonas aeruginosa	(-)	(-)	(-)	(-)	Meropenem	30.0

mm: millimeters; SD: standard deviation; (-): no inhibition zone

Table 7

Minimum inhibitory concentrations of essential oil from *Aquilaria agallocha*, *Artemisia absinthium*, *Hyssopus officinalis*, and *Mentha pulegium* against a set of Gram-negative and Gram-positive clinical pathogenic bacteria

Clinical pathogenic bacteria	Minimum inhibitory concentration of various essential oils,			
	Aquilaria agallocha	*Artemisia absinthium*	*Hyssopus officinalis*	*Mentha pulegium*
Gram-positive bacteria				
Staphylococcus aureus	25.0	(-)	3.1	3.1
Streptecoccus pyogenes	25.0	(-)	1.6	0.8
Gram-negative bacteria				
Acinetobacter baumannii	25.0	(-)	0.8	0.1
Escherichia coli	25.0	(-)	1.6	0.2
Klebsiella pneumoniae	25.0	(-)	1.6	0.1
Pseudomonas aeruginosa	50.0	(-)	(-)	(-)

mg/ml: milligrams per milliliter; (-): not determined

E. coli, *K. pneumoniae*, *S. pyogenes*, and *S. aureus*, respectively; and that of *M. pulegium* being 0.1, 0.1, 0.2, 0.8, and 3.1 mg/ml for *A. baumannii*, *K. pneumoniae*, *E. coli*, *S. pyogenes*, and *S. aureus*, respectively.

DISCUSSION

We analyzed the chemical components of essential oils of *Aq. agallocha*, *Ar. absinthium*, *H. officinalis*, and *M. pulegium* and found them to be effective with varying in vitro efficacy to some, but not all, of a reprentative group of multidrug-resistant Gram-negative and -positive bacteria isolated from patients at a hospital in Türkiye.

We found *A. agallocha* essential oil contained 23 volatile chemical compounds, the majority being dodecanedioic acid dimethyl

ester, 1-(4-isopropylphenyl)-2-methylpropyl acetate and Patchouli alcohol. Bhuiyan *et al* (2009), in Bangladesh, reported 29 components with the highest proportions being octacosane (19.8%), 5-isobutyramido-2-methyl pyrimidine (13.5%) and Naphthalene, 1,2,3,5,6,7,8,8a-octahydro1,8a-dimethyl-7-(1-methylethenyl)-, [1R- (1.alpha.,7.beta.,8a.alpha.)]- (12.7%). In India, Talukdar (2014) identified 17 of the 35 chemical compounds, with the highest proportion belonging to three furanoids, namely 2-isobutyl-3-methylfuran, 3-methyl-2-(2-methyl-2-butenyl)-furan, and 3-methyl-2-(2-oxopropyl)-furan. Alam *et al* (2020), in Bangladesh, identified 38 chemical compounds with the major components being n-hexadecanoic acid (17.34%), 1(11)-spirovetiven-11-ol (10.42%) and guai-1(5)-en-11-ol (8.98%).

We noted that *Ar. absinthium* essential oil consisted of 13 chemical components, with the major chemical compounds being β-caryophyllene, eucalyptol (1,8-cineole) and β-pinene (12.61%). Basta *et al* (2007) in Greece identified 68 compounds, with the main components being caryophyllene oxide (25.3%), *p*-cymene (16.8%) and eucalyptol (1,8-cineole) (8.9%). Kordali *et al* (2005), in Türkiye, found 111 compounds with the main components being chamazulene (17.8%), nuciferol butanoate (8.2%), nuciferol propionate (5.1%), and caryophyllene oxide (4.3%).

Our study of *H. officinalis* essential oil revealed 37 volatile compounds, with the major components being camphor (2-bornanone), 1,8-cineole (eucalyptol), β-pinene, and β-trans-pinocamphone. Glamoclija *et al* (2005), in Serbia and Montenegro, reported 24 volatile compounds, the most abundant being isopinocamphone (43.29%), pinocamphone (16.79%) and β-pinene (16.31%). Wesołowska *et al* (2010), in Poland, obtained essential oil from *H. officinalis* using three methods: steam distillation (31 compounds), simple hydrodistillation (36 compounds),

and Dean-Stark hydrodistillation (27 compounds). GC-MS analysis showed that isopinocamphone was the major component in all samples (40.07-45.45%). Said-Al Ahl *et al* (2015), in Egypt, identified 33 volatile compounds, with the main components being cis-pinocamphone (26.85%), β-pinene (20.43%), and trans-pinocamphone (15.97%). Stappen *et al* (2015), in India, identified 44 volatile components, with the major compounds being pinocarvone (23.4%), cis-pinocamphone (20.3%) and β-pinene (17.8%). Zawislak (2016), in Poland, reported 52 volatile components, including 1 unidentified, with the major compounds being isopinocamphone (22.53-28.74%), pinocamphone (11.41-17.99%), β-pinene (6.69-12.01%).

We identified 11 volatile compounds in *M. pulegium* essential oil, with the main components being 3-*p*-menthanol, isomenthone and pulegone. Lorenzo *et al* (2002), in Uruguay, reported 22 volatile compounds, with pulegone (73.4%), isomenthone (12.9%) and menthone (3.6%) as the main components. Agnihotri *et al* (2005), in India, reported 24 volatile compounds, with the main components being pulegone (65.9-83.1%), menthone (8.3-8.7%) and piperitone (1.3-3.2%). Beghidja *et al* (2007) identified 41 volatile compounds in *M. pulegium* oils grown in different regions of eastern Algeria, with the major proportions being pulegone (43.5-87.3%), piperitenone (14.4-26.7%), isomenthone (10.3-22.6%), and menthone (3.96-6.78%). Mahboubi and Haghi (2008), in Iran, identified 16 volatile compounds, with piperitone (38.0%), piperitenone (33.0%), α-terpineol (4.7%), and pulegone (2.3%), comprising the major components. More recently, Oualdi *et al* (2023), in Morocco, identified 10 volatile components, with the major compounds being pulegone (74.88%), d-limonene (8.69%) and 2-(2,2,4-trimethyl-3-cyclopenten-1-yl) ethanol (5.43%).

These findings highlight the differences in the chemical composition of essential oils, which

are determined by geographic origin, climate, harvest time, plant parts used, isolation process, and detection method (Sharifi-Rad *et al*, 2022).

The main antibacterial mechanism of plant essential oils is due to the ability of their chemical constituents to interact with the bacterial cell membrane, affecting membrane properties, or to enter the cell cytoplasm disrupting metabolic processes, and ultimately leading to Gram-negative and -positive bacteria cell death (Moo *et al*, 2020; Balakrishnan *et al*, 2021; Farhanghi *et al*, 2022; Liu *et al*, 2025). Other studies indicate inhibition of biofilm formation and reduction in bacterial virulence factors are other bactericidal properties of medicinal plant essential oils (Alcici *et al*, 2025).

Respiratory tract infection is one of the most important infectious diseases worldwide, and is the leading cause of morbidity and mortality in critically ill patients in developing countries (Kousalya *et al*, 2010). Hence, we chose representative multidrug-resistant Gram-negative (*A. baumannii, E. coli*, *K. pneumoniae*, and *P. aeruginosa*) and Gram-positive (*S. aureus* and *S. pyogenes*) bacteria isolated from patients' respiratory tract to evaluate the in vitro bactericidal efficacy of the four plant essential oils. Based on the disc diffusion assay, all four essential oils were effective against the five clinical multidrug-resistant bacteria isolates, but, as expected, their efficacies varied. Our sample of *Ar. Absinthium* essential oil was only effective against *S. aureus*, and all four essential oils were ineffective against *P. aeruginosa*. The latter bacterium contains a lipopolysaccharide outer coat, which restricts the penetration of lipophilic compounds, and the presence of multiple efflux pumps (*eg,* MexAB-OprM) efficiently expels drugs that penetrate the cell, while detoxification enzymes protect against the damage of drugs retained within the cell's cytosol (Elfadadny *et al*, 2024; Langendonk *et al*, 2021; Verdial *et al*, 2023).

In addition, the bacterium's strong biofilm-forming ability reduces the diffusion of antibacterial agents.

As experimental conditions, such as the assay methods and the concentrations tested, play decisive roles in determining antibacterial outcomes (Kiray, 2023), it is difficult to compare our results with those reported in the literature. In general, the bactericidal properties of similar essential oils are consistent with our findings (Lopes-Lutz *et al*, 2008; Mahboubi and Haghi, 2008; Hajlaoui *et al*, 2009; Kizil *et al*, 2010; Baj *et al*, 2011; Boukhebti *et al*, 2011; Ghosh *et al*, 2013; Bekka-Hadji *et al*, 2022; Ez-Zriouli *et al*, 2022). Of note, low levels of phenolic compounds in essential oils have been attributed to their low antibacterial effectiveness (Bayaz, 2014).

One limitation of our study was that only a single isolate of each bacterial species was tested. Future studies should include multiple clinical isolates obtained from different patients and from other hospitals to help better assess the reproducibility and generalizability of the observed antibacterial activity.

In conclusion, our studies showed that essential oils from the medicinal plants *Aquilaria agallocha, Artemisia absinthium, Hyssopus officinalis,* and *Mentha pulegium* had bactericidal properties against multidrug-resistant clinical isolates of Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative (*Acinetobacter baumannii, Escherichia coli* and *Klebsiella pneumoniae*) bacteria, but not against *Pseudomonas aeruginosa*. These findings suggest that medicinal plant essential oils may serve as potential alternatives or additions to current synthetic antimicrobials, especially in combating multidrug-resistant bacteria in Türkiye and elsewhere.

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CONFLICTS OF INTEREST DISCLOSURE

The authors declare no conflict of interest

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