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Chemical composition and antimicrobial activity of a newly identified chemotype of *Achillea wilhelmsii* K.Koch from Kashan, Iran

Mansureh Ghavam[1]✉, Ines Castangia[2], Maria Manconi[2], Gianluigi Bacchetta[2] & Maria Letizia Manca[2]

Achillea wilhelmsii **K.Koch (Asteraceae) is traditionally used in Kashan (Iran) to control diabetes, headaches, kidney stones and heartburn. Due to its beneficial properties, the aerial parts of the plant were collected from the area of Maragheh (Kashan, Isfahan, Iran), in June 2022, during its full flowering, and the essential oil was obtained by hydrodistillation (Clevenger). The yield, composition and antimicrobial activity of the extractive solution were measured. Qualitative evaluation was performed by means of gas chromatography–mass spectroscopy method, and antimicrobial activity was determined against 12 strains of microorganisms by measuring inhibition halo, minimum inhibitory concentration, and minimum bactericidal concentration (MBC). The yield of essential oil was ~ 0.1071% (w/w) and it mainly contained oxygenated monoterpenes (47.87%), being for the first time fragranol (33.22%), fragranyl acetate (16.18%) and oleic acid (6.33%) the most abundant. The highest inhibitory halo was found against** ***Candida albicans*** **and Gram-positive** ***Staphylococcus aureus*** **(~ 10 mm). The essential oil was also effective against gram-negative bacteria such as** ***Acinetobacter baumannii*** **and** ***Shigella dysenteriae*****, as the inhibition halo was ~ 9 mm and similar to that of rifampin, used as a reference. Therefore, it seems that this essential oil from and endemic species has a unique chemotype with potential antimicrobial activity, which may be a possible option for fragranol isolation and the production of natural antibiotics effective against various microorganisms.**

Keywords *Achillea*, Essential oil quantity, Fragranol, Antibacterial/yeast activity, Ethnobotany

Medicinal plants have long been used for various purposes such as manufacturing medicines, cosmetics, foods, and nutritional supplements. Moreover, indigenous populations often rely on herbal formulations for treating different disorders due to their effectiveness, affordability, accessibility, and limited side effects[1–3]. These products contain multiple valuable natural molecules with different biological and therapeutic activities, representing a significant and unexploited natural resources[4]. Research into the effects of medicinal plants and the discovery of new drugs holds great promise in countering the increasing resistance of pathogens to antibiotics, which complicates the treatment of microbial diseases[5].

Essential oils, often rich in natural molecules with antibacterial properties, have been used since ancient times to prevent bacterial growth and spoilage. Despite their potential, their effectiveness has not been fully exploited yet[6]. Previous studies have highlighted the antimicrobial potential of essential oils from aromatic plants against various pathogens, including foodborne pathogens, bacteria (both Gram-negative and Gram-positive), viruses, and yeasts responsible for human diseases[7,8]. The antibacterial properties of essential oils may vary depending on factors such as the plant family, climatic and soil conditions, harvesting time, and extraction method[9]. Plants of the Asteraceae family, known for their aromatic properties, often produce essential oils rich in antimicrobial molecules. These oils have been extensively used in both folk and modern medicine[10]. The genus *Achillea*, within the Asteraceae family, comprises numerous herbaceous species distributed across temperate regions of Asia and

[1]Department of Nature Engineering, Faculty of Natural Resources and Earth Sciences, University of Kashan, Kashan, Iran. [2]Department of Life and Environmental Sciences, University of Cagliari, University Campus, S.P. Monserrato-Sestu Km 0.700, 09042 Monserrato, CA, Italy. ✉email: mghavam@kashanu.ac.ir

Europe[11,12]. Several species of *Achillea*, including *Achillea wilhelmsii* K.Koch (*A. wilhelmsii*), are renowned for their medicinal properties and pleasant aroma[13,14]. The word "yarrow" is derived from the Greek word "Achill", signifying "hero", reflecting the historical use of the genus in treating diseases during wars[15]. In Iran, Achillea species, locally known as "Bomadaran", have a significant presence in traditional medicine, attributed to their various pharmacological effects, including reducing blood pressure and cholesterol, regulating menstrual cycles, and alleviating numerous ailments[16–19]. Additionally, different studies have confirmed the moderate to strong antibacterial properties of plants of this genus[20].

Among Achillea species, *Achillea wilhelmsii* K.Koch (*A. wilhelmsii*) is a chamaephyte native to the South and East Mediterranean, Central Asia, and Pakistan. It is characterized by stems with compact white felt hairs, lobed yellow flowers, and tubular flowers[18,21]. *Achillea wilhelmsii* is used for various medicinal purposes across different regions, including inducing abortion, alleviating stomach pain and fever, and treating jaundice in children[22]. Ethnobotanical studies reveal its various uses in different parts of Iran, ranging from treating heart aches and respiratory infections to regulating menstrual cycles and counteracting diarrhea[23–25]. Pharmacological investigations have reported its anti-ulcer, anti-proliferative, anti-anxiety, antioxidant, anti-inflammatory, and antimicrobial properties[26–31].

Previous studies have reported varying levels of inhibitory activity of *A. wilhelmsii* essential oil against bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and others[32–34]. The essential oil mainly contains camphor and 1,8-cineol[19,33,35,36].

To the best of our knowledge, the essential oil of *A. wilhelmsii* from the Kashan region has not been previously analyzed and tested. Considering the potential pharmacological effects of this species, the identification of its chemotype is crucial. Therefore, this study aimed at evaluating for the first time the qualitative and quantitative chemical composition and antimicrobial activity of the essential oil of *A. wilhelmsii* grown in the natural habitat of Maragheh region (Kashan, Isfahan province, Iran).

Materials and methods

Collection and preparation of plant materials

The Kashan region, located in Isfahan, Iran, particularly the Maragheh area (coordinates N 33° 41′ 42″ and E 51° 26′ 78″), situated at an altitude of 2000–2200 m above sea level, was chosen for sample collection. Permission to collect plant material was obtained from the Agricultural Jahad Office. In June 2022, during full flowering, three plots of plants were randomly selected in this area. Epigean parts were randomly collected from 150 individuals in each plot, then transferred to the laboratory and dried at room temperature (20 °C and 40% relative humidity). Additionally, an herbarium sample was collected and stored at the Faculty of Natural Resources and Earth Sciences, University of Kashan, Iran. The plant was identified by Gianluigi Bacchetta and recorded with code number 1413.

Extraction and separation of essential oil

All plant experiments were carried out in accordance with guidelines. Extraction was performed using hydrodistillation method, wherein 90 g of ground sample were placed in a 2000 mL flask with approximately 1400 mL of distilled water, and connected to the Clevenger apparatus (Merck, Germany). Essential oil extraction was carried out for 3 h. The collected essential oil underwent sodium sulfate dehydration, and its weight was accurately measured using a precision balance to calculate the extraction yield[37]. The essential oil was then stored in glass bottles at 4 °C in the dark until further use. This extraction process was repeated three times.

Qualitative and quantitative analysis of essential oil

The qualitative and quantitative analysis of the essential oil was performed using a GC–MS device (model 6890) with an ionization energy of 70 eV and coupled with a mass spectrometer (model 5973 N, Agilent, America). A HP-5MS capillary column with 5% methylphenylsiloxane stationary phase (Length 30 m, Internal Diameter 0.25 mm, Layer Static Thickness 0.25 µm) was used. A temperature gradient, starting from 60 °C and then increasing (at a rate of 3 °C/min) up to 246 °C, was chosen to ensure the detection and quantification of all molecules contained in the essential oil. The injector and the detector temperatures were set at 250 °C. The ionization energy was 70 eV. The sample injection volume was 1 µL with the split mode (1:50). The flow rate of the helium gas used as mobile phase was 1.5 mL/min. The injector and detector temperatures were set at 250 °C. The retention indices of the separated molecules was measures and compared with that of standards of *n*-alkane mixtures (C8–C20) and mass spectral data of each peak using a computer library (Wiley-14 and NIST-14 Mass Spectral Library). Obtained data have been compared with those already reported in the literature[38].

Antimicrobial activity measurement

Tested microorganisms

The antimicrobial activity of the essential oil was tested against 11 microorganisms provided by Iran Science and Technology Research Organization (IROST) and including seven Gram-negative bacteria, *Klebsiella pneumonia* (*K. pneumonia*, ATCC 10031), *Escherichia coli* (*E. coli*, ATCC 25922), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 27853), *Salmonella paratyphi-A serotype* (*S. paratyphi-A serotype*, ATCC 5702), *Shigella dysenteriae* (*S. dysenteriae*, PTCC 1188), *Acinetobacter baumannii* (*A. baumannii*, ATCC BAA-747), and *Proteus mirabilis* (*P. mirabilis*, ATCC 43071); three Gram-positive bacteria, *Staphylococcus epidermidis* (*S. epidermidis*, CIP 81.55), *Staphylococcus aureus* (*S. aureus*, ATCC 29737), and *Bacillus subtilis* (*B. subtilis*, ATCC 6633); and *Candida albicans* as yeast strain (*C. albicans*, ATCC 10231). Bacterial strains were cultured in Mueller-Hinton Agar growth medium and yeast in Sabouraud Dextrose Agar medium and incubated at 37 °C and 27 °C for 24 h, respectively.

Determination of inhibition halo by means of agar diffusion method
This method was performed based on CLSI standards[39]. Petri dishes of Mueller-Hinton Agar growth medium were used for the bacteria and Sabouraud Dextrose Agar medium for the yeast. Microbial suspensions were prepared for the different microbial variants and maintained for 24 h, with 0.5 McFarland turbidity. 100 µL of each was cultured in the same growth media conditions. The essential oil was dissolved in dimethyl sulfoxide (60 mg/mL) and 10 µL of obtained solution was poured into the petri dishes to reach a 600 µg/mL of oil. Petri dishes were incubated at 37 °C for 24 h with bacterial strains and at 27 °C for 48 h with the yeast strain. The test was repeated three times for each essential oil sample and for each strain. The diameter of the inhibition halo was measured. Antibiotics gentamicin (10 µg/disc) and rifampin (5 µg/disc) for bacteria and nystatin (100,000 unit/mL) for yeast were used as standard drugs for positive control under the same test conditions.

Determination of the minimum inhibitory concentration (MIC)
The minimum concentration capable of inhibiting the growth of bacteria was assessed using the microdilution method. Essential oil (8000 µg/mL) was dissolved in a mixture of tryptic soy broth and dimethyl sulfoxide and diluted to reach the following concentrations: 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25 and 15.63 µg/mL. Experiments were performed using 96-well microplates each filled with 95 µL of culture medium, 5 µL of bacterial suspension with 0.5 McFarland dilution and 100 µL of each diluted essential oil were. The plates were incubated at 37 °C for 24 h using bacterial strains and for 48 and 72 h at 27 °C using yeast. After leaving them, the first concentration that inhibited the growth of different strains was considered as the minimum inhibitory concentration. For each strain, the test was repeated 3 times and 3 different samples of essential oil were used.

Determination of minimum bactericidal concentration (MBC)
To determine the minimum concentration able to kill the bacteria, the same microdilution method described above was used. The various bacteria strains were incubated for 24 h with the essential oil at different concentrations (8000, 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25 and 15.63 µg/mL). After 24 h of incubation with both bacteria and oils at different concentrations, 5 µL of the content of each well was inoculated with nutrient agar medium and incubated at 37 °C for 24 h for bacterial strains. After incubation, the colony-forming units were enumerated. The MBC was the lowest concentration able to effectively reduce the growth of microorganisms (99.5%).

Statistical analysis
Analysis of variance (ANOVA) was performed to analyze the data. The difference between the mean values of the data was evaluated using Duncan's post hoc test at a significance level of 1%.

Results and discussion
Color and yield of essential oil
The essential oil extracted from *A. wilhelmsii* exhibited a distinct bluish-green color, unlike the predominantly yellow oils reported in previous studies[36,40,41]. This variance in color could be attributed to the specific harvesting time chosen for this study, as suggested by[35]. Their research indicated that the color of *A. wilhelmsii* essential oil varied with phenological stages, being yellow in May, colorless in June, and pale green in July. This highlights the influence of both phenological stage and habitat on the composition and color of the essential oil. The yield of essential oil obtained from *A. wilhelmsii* collected in the Maragheg area of the Kashan region, Iran, was approximately 0.1071% (w/w) based on the dry weight of plant material. This yield was notably lower than the highest reported yield of 2.47% obtained from plants cultivated in the Shahada Valley of West Azerbaijan province[35]. Such discrepancies in yield could be attributed to variations in habitat conditions across different Iranian regions. For instance, the yield of essential oil from plants collected in the natural habitat of Zanjan province was reported to be 0.89%[41], while it increased up to 1.48% when cultivated in Ghochi, West Azerbaijan province[42]. Additionally[11], observed decreasing yield values (from 5.6 to 1.1%) when *A. wilhelmsii* was cultivated in different regions of West Azerbaijan province. Similarly[19], reported varying yields of essential oil (ranging from 0.6 to 0.3%) when obtained from *A. wilhelmsii* cultivated in Hamedan province. These findings underscore the significant influence of ecological characteristics on the yield of essential oil extracted from *A. wilhelmsii* across different habitats[43,44].

Chemical compounds contained in the essential oil
The essential oil extracted from *A. wilhelmsii* revealed the presence of 55 different compounds, representing 100% of the oil composition (Table 1; Fig. 1). Comparatively[42], identified 56 compounds in the essential oil of this plant, while other studies reported varying numbers of molecules. For instance[36], identified 21 compounds[11], found 26 compounds[35], detected 33 compounds, and[45] identified 22 compounds. Studies conducted in Turkey reported the lowest number of molecules (16) when the plant was collected, while 46 compounds were isolated when collected from Anatolia[33,46]. This variability underscores the influence of growth habitat and environmental conditions on the composition of essential oil from *A. wilhelmsii*[8]. Among the compounds identified in the essential oil, oxygenated monoterpenes were the most abundant, comprising 47.87% of the oil. Fragranol emerged for the first time as the predominant terpene, constituting 33.22% of the oil. This contrasts with previous studies where fragranol content varied widely, ranging from 0.2 to 43.20%. Notably, fragranol is recognized for its unique cyclobutane ring structure and was firstly isolated from *Artemisia*[47,48]. Fragranyl acetate, detected at 16.18% in this study, was identified for the first time in *A. wilhelmsii* essential oil, along with oleic acid (6.33%), which was also newly detected. Oleic acid, an omega-9 unsaturated fatty acid, possesses various health-promoting properties including antioxidant, anti-inflammatory, and antimicrobial effects[49,50]. Additionally, α-pinene (3.61%), linalool (3.40%), and camphor (3.35%) were detected as less abundant compounds in the essential oil.

No	Compound	RI $_{Exp}$	RI $_{Lit}$	Concentration (%)	Molecular formula
1	1-Butanol, 3-methyl-, acetate	918.9	876	0.3	$C_7H_{14}O_2$
2	α-Pinene	983.7	934	3.6	$C_{10}H_{16}$
3	Camphene	1002.3	951	0.3	$C_{10}H_{16}$
4	Dehydrosabinene	1006.2	956	0.2	$C_{10}H_{14}$
5	Benzaldehyde	1015.8	975	0.2	C_7H_6O
6	α-Sabinene	1025.6	977	0.3	$C_{10}H_{16}$
7	β-Pinene	1031.6	979	0.2	$C_{10}H_{16}$
8	Yomogi alchool	1048.3	999	0.2	$C_{10}H_{18}O$
9	Butanoic acid	1058.2	1017	0.3	$C_9H_{18}O_2$
10	Pentyl isobutyrate	1064.0	1057	0.3	$C_9H_{18}O_2$
11	Isobutyric acid, isopentyl ester	1067.4	1018	0.4	$C_9H_{18}O_2$
12	α-Terpinene	1055.7	1017	0.3	$C_{10}H_{16}$
13	o-Cymene	1080.8	1015	1.3	$C_{10}H_{14}$
14	1,8-Cineole	1089.4	1031	2.1	$C_{10}H_{18}O$
15	γ-Terpinene	1116.0	1062	0.3	$C_{10}H_{16}$
16	Isopentyl 2-methylbutanoate	1156.8	1101	0.4	$C_{10}H_{20}O_2$
17	Linalool	1160.8	1100	3.4	$C_{10}H_{18}O$
18	Isovaleric acid	1163.7	1105	0.5	$C_{10}H_{20}O_2$
19	Amyl isovalerate	1165.9	1108	0.7	$C_{10}H_{20}O_2$
20	4-Isopropylbenzenethiol	1169.4	–	0.3	$C_9H_{12}S$
21	Crysanthenone	1184.1	1125	0.3	$C_{10}H_{14}O$
22	trans-2-Menthenol	1189.1	1142	0.7	$C_{10}H_{18}O$
23	cis-Verbenol	1207.8	1140	1.1	$C_{10}H_{16}O$
24	Camphor	1212.3	1145	3.3	$C_{10}H_{16}O$
25	Dill ether = Anethofuran	1216.8	1191.2	0.8	$C_{10}H_{16}O$
26	α-Pinocarvone	1228.4	1164.2	0.3	$C_{10}H_{14}O$
27	Cyclohexene,4-methylene	1239.5	–	1.2	C_7H_{10}
28	Terpinen-4-ol	1248.8	1178	0.8	$C_{10}H_{18}O$
29	(3E)-3,7-dimethylocta-3,6-dien-1-ol	1251.9	–	0.5	$C_{10}H_{18}O$
30	α-Terpineol	1264.4	1191	0.8	$C_{10}H_{18}O$
31	Fragranol	1288.6	1221.6	33.2	$C_{10}H_{18}O$
32	Bicyclo[10.1.0]tridec-1-ene	1297.2	–	0.3	$C_{13}H_{22}$
33	Fragranyl acetate	1327.4	1345	16.2	$C_{12}H_{20}O_2$
34	Thymol	1368.6	1294	0.3	$C_{10}H_{14}O$
35	Myrtenyl acetate	1393.7	1335	0.6	$C_{12}H_{18}O_2$
36	Geranyl acetate	1417.4	1383	0.3	$C_{12}H_{20}O_2$
37	Jasmone	1468.6	1406	0.3	$C_{11}H_{16}O$
38	Caryophyllene	1500.1	1423	0.2	$C_{15}H_{24}$
39	Fragranyl 2-methylbutyrate	1511.9	1574.9	3.2	$C_{15}H_{26}O_2$
40	Fragranyl isobutyrate	1557.4	1483.9	1.6	$C_{14}H_{26}O_2$
41	Germacrene D	1564.9	1485	1.6	$C_{15}H_{24}$
42	Sesquicineole	1594.3	1515	0.8	$C_{15}H_{26}O$
43	Nerolidol	1642.9	1565	1.8	$C_{15}H_{26}O$
44	(Z,E)-α-Farnesene	1662.6	1491	0.2	$C_{15}H_{24}$
45	Caryophyllene oxide	1673.5	1589	0.9	$C_{15}H_{24}O$
46	Succinic acid, di(1-(pentafluorophenyl)ethyl) ester	1699.4	2080	0.4	$C_{20}H_{12}F_{10}O_4$
47	τ-Cadinol	1699.4	1639	0.8	$C_{15}H_{26}O$
48	13-Tetradecanolide	1742.0	1643	0.4	$C_{14}H_{26}O_2$
49	Levomenol = Kamillosan	1777.0	2021	0.2	$C_{15}H_{26}O$
50	Palmitoleic acid	1801.2	1953	0.2	$C_{16}H_{30}O_2$
51	Diisobutyl phthalate = Phthalic acid, diisobutyl ester	1890.9	1868	0.2	$C_{16}H_{22}O_4$
52	α-Sinensal	2062.5	1752	0.7	$C_{15}H_{22}O$
53	Palmitic acid	2077.6	1964	2.3	$C_{16}H_{32}O_2$
54	Oleic acid	2638.9	2140	6.3	$C_{18}H_{34}O_2$
55	Stearic acid	2655.8	2188	1.1	$C_{18}H_{36}O_2$
	Total			99.5	

Continued

No	Compound	RI $_{Exp}$	RI $_{Lit}$	Concentration (%)	Molecular formula
	Monoterpenes hydrocarbons			6.5	
	Oxygenated monoterpenes			47.8	
	Sesquiterpenes hydrocarbons			2	
	Oxygenated sesquiterpenes			4.5	
	Others (Nonterpenoids)			38.7	

Table 1. Chemical name, retention time, concentration and structure formula of compounds found in the essential oil obtained from *A. wilhelmsii*. Compounds are listed in order of their retention time from an HP-5 column. RI $_{Exp.}$, linear retention indices on HP-5 column, experimentally determined using homolog series of n-alkanes (C8-C20). RI $_{Lit.}$, Linear retention index taken from Adams (2007), or NIST 14 (2014) and literature.

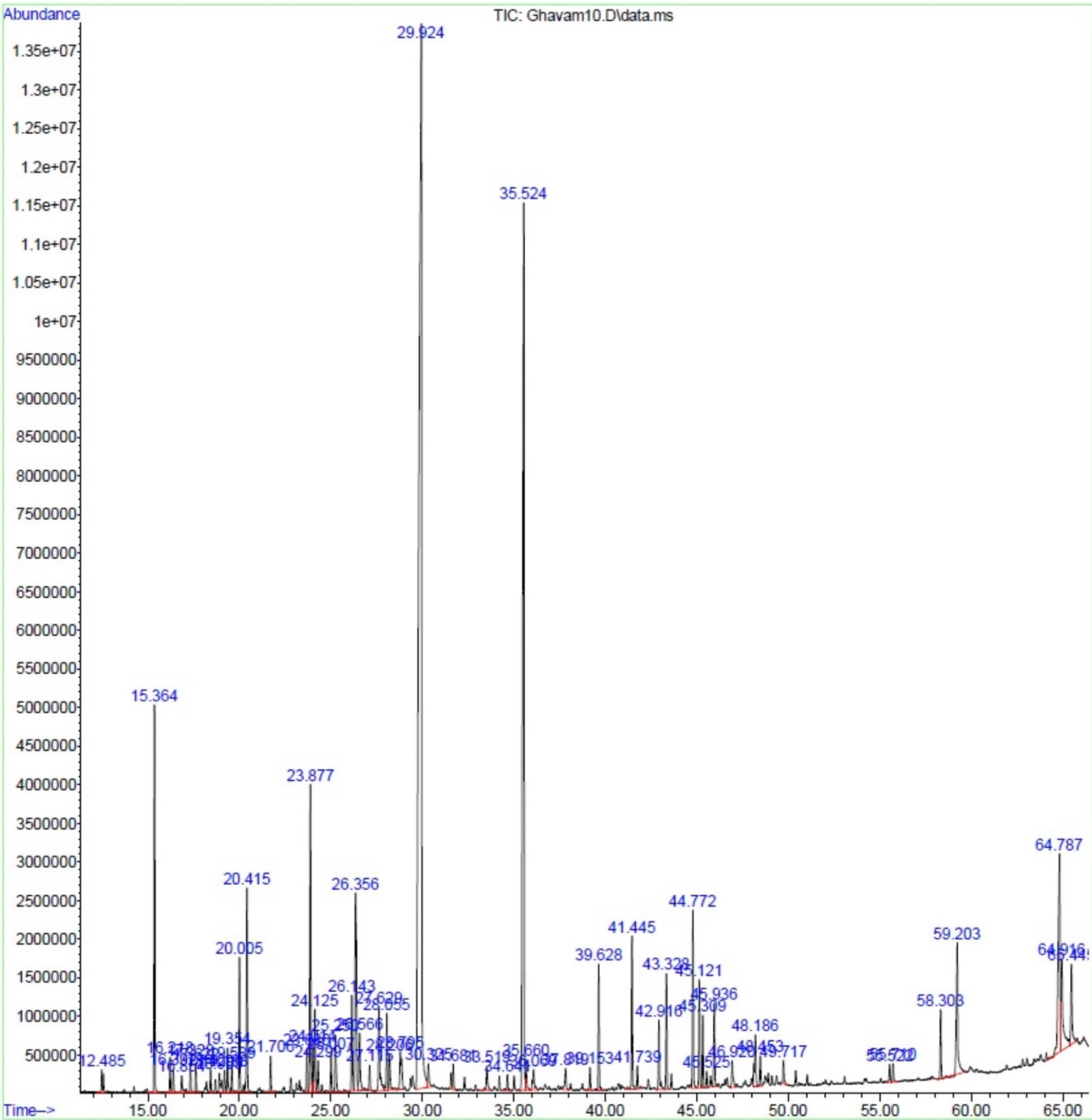

Fig. 1. Spectra of the essential oil obtained from *A. wilhelmsii* obtained with a gas-chromatograph coupled whit mass spectroscopy.

These findings are consistent with some studies but vary in concentration depending on the growth habitat. Notably, α-pinene is known for its antimicrobial properties[51,52]. Linalool, a monoterpene alcohol, has diverse pharmacological effects, including antioxidant, anti-inflammatory, and antimicrobial properties[53]. Camphor, widely used for its medicinal properties, exhibits antiseptic and antitussive effects and modulates various physiological functions[54]. Minor components detected in the essential oil included fragranyl 2-methylbutyrate (3.17%), palmitic acid (2.32%), and 1,8-Cineole (2.16%). Fragranyl 2-methylbutyrate was identified for the first time in *A. wilhelmsii* essential oil, while palmitic acid and 1,8-Cineole were detected in trace amounts in previous studies. The beneficial properties of 1,8-Cineole include anti-inflammatory and antioxidant activities[55]. Overall, these results highlight the significant impact of growth habitat on the composition and beneficial properties of *A. wilhelmsii* essential oil. The unique chemotype identified in the *A. wilhelmsii* cultivated in the Maragheg area of the Kashan region suggests its potential as a distinct and valuable resource in natural product research[56,57].

Antimicrobial activity of essential oil

The antimicrobial activity of the essential oil extracted from *A. wilhelmsii* against selected microorganisms was evaluated, resulting in varying inhibition halos (Table 2; Fig. 2). Notably, the largest inhibition halo (~ 10 mm) was observed against *Candida albicans*, though lower compared to nystatin (~ 33 mm) and consistent with previous findings[33]. *Candida albicans*, a common fungal pathogen, can lead to severe infections, particularly in immunocompromised individuals, highlighting the significance of effective antifungal agents[58,59]. The efficacy of the essential oil against *C. albicans* and other microorganisms can be attributed to its composition, which is influenced by the plant's growth habitat[60,61]. The inhibition halo against Gram-positive *Staphylococcus aureus* (~ 10 mm) was lower compared to rifampin (~ 21 mm) and gentamicin (~ 27 mm), indicating moderate antibacterial activity. Similarly, previous studies reported varying inhibition halos for *A. wilhelmsii* essential oil against *S. aureus*[31,33]. *Staphylococcus aureus* is a dangerous strain mainly responsible of hospital-acquired infections, necessitating effective antimicrobial agents[62]. Additionally, the essential oil exhibited inhibition against *Bacillus subtilis* (~ 9.5 mm), an opportunistic pathogen associated with eye infections and septicemia. This inhibitory effect, not previously reported by others, suggests the potential of *A. wilhelmsii* essential oil as an antibacterial agent[49,63]. Surprisingly, strong inhibitory activity was observed against Gram-negative *Acinetobacter baumannii* (~ 9 mm) and *Shigella dysenteriae* (~ 9 mm), outperforming rifampin. *A. baumannii* and *Sh. dysenteriae* are causative agents of various infections, highlighting the significance of effective treatments[64,65]. The novel inhibitory effect against these Gram-negative bacteria suggests the potential of *A. wilhelmsii* essential oil as a natural antibacterial agent. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil were measured, ranging from 1000 to 8000 µg/mL and consistent with previous studies[66]. While the MIC values were relatively high compared to control antibiotics, further investigation is warranted to elucidate the mechanism of action and optimize the antimicrobial efficacy of the essential oil[67]. Overall, the antimicrobial activity of *A. wilhelmsii* essential oil against a range of pathogenic microorganisms underscores its potential as a natural antimicrobial agent. Further research into its mode of action and formulation for clinical use is warranted to harness its therapeutic benefits effectively.

Conclusion

In this study, we successfully extracted a bluish-green essential oil from A. wilhelmsii cultivated in the natural habitat of Kashan, marking the first instance of such extraction. Our findings revealed a nearly unique qualitative composition of the essential oil, characterized by a predominance of oxygenated sesquiterpene

	A. wilhelmsii essential oil			Rifampin		Gentamicin		Nystatin	
Standard strains	**IH (mm)**	**MIC (µg/mL)**	**MBC (µg/mL)**	**IH (mm)**	**MIC (µg/mL)**	**IH (mm)**	**MIC (µg/mL)**	**IH (mm)**	**MIC (µg/mL)**
Gram-positive bacteria									
B. subtilis	9.50 ± 1.50[c]	1000	2000	19.00 ± 0.00[b]	31.25	30.00 ± 0.00[a]	3.90	NA	NA
S. aureus	10.00 ± 0.00[c]	2000	2000	21.00 ± 0.00[a]	31.25	27.00 ± 0.00[b]	1.95	NA	NA
S. epidermidis	ND	2000	2000	27.00 ± 0.00[b]	1.95	45.00 ± 0.00[a]	1.95	NA	NA
Gram-negative bacteria									
E. coli	ND	8000	8000	11.00 ± 0.00[b]	3.90	20.00 ± 0.00[a]	3.90	NA	NA
K. pneumoniae	ND	4000	8000	8.00 ± 0.00[b]	15.63	17.00 ± 0.00[a]	3.90	NA	NA
P. aeruginosa	ND	1000	2000	ND	31.25	20.00 ± 0.00[a]	7.81	NA	NA
S. paratyphi-A	ND	1000	2000	8.00 ± 0.00[b]	15.63	18.00 ± 0.00[a]	3.90	NA	NA
Sh. dysenteriae	9.00 ± 0.00[b]	2000	4000	9.00 ± 0.00[b]	15.63	17.00 ± 0.00[a]	3.90	NA	NA
A. baumannii	9.00 ± 0.00[b]	2000	2000	8.00 ± 0.00[c]	7.81	17.00 ± 0.00[a]	3.90	NA	NA
P. mirabilis	ND	2000	2000	9.00 ± 0.00[b]	15.63	20.00 ± 0.00[a]	31.25	NA	NA
Yeast strain									
C. albicans	10.00 ± 0.00[b]	4000	4000	NA	NA	NA	NA	33.00 ± 0.00[a]	125

Table 2. Inhibition halos (IH), minimum inhibitory concentration (MIC) and minimum bactericidal concentration of essential oil obtained from *A. wilhelmsii* or rifampicin or gentamicin or nystatin used as positive controls. Mean values ± standard deviations are reported. Values with different letters are statistically different (Duncan, $p \leq 0.01$).

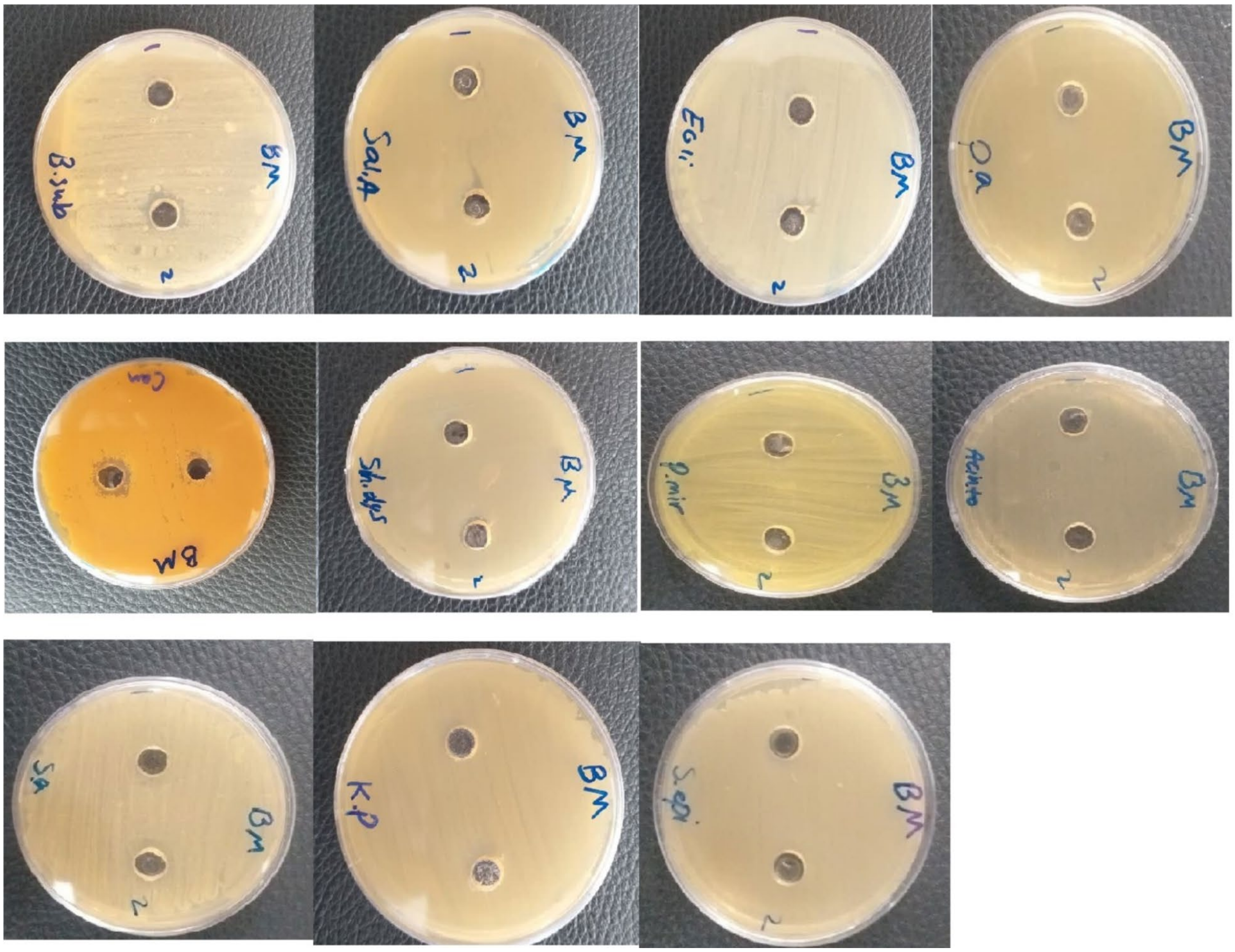

Fig. 2. Inhibition halos of essential oil obtained from *A. wilhelmsii* against selected microorganisms.

compounds, particularly fragranol, along with acidic compounds like fragranyl acetate, oleic acid, and fragranyl 2-methylbutyrate. This underscores the significant influence of the plant's growth habitat and environmental conditions on the synthesis of different chemical compounds. Moreover, the distinctive composition of the essential oil was associated with significant inhibition halos against some pathogenic microorganisms, notably the Gram-negative bacteria *A. baumannii* and *Sh. dysenteriae*. These findings highlight the potential of *A. wilhelmsii* essential oil as a natural antimicrobial agent, particularly against challenging Gram-negative pathogens. Given the ethnobotanical significance of *A. wilhelmsii* in Iran, particularly in Kashan city, its essential oil holds promise as a natural option for the treatment of various microbial infections. However, further studies are warranted to validate its beneficial properties and explore its potential therapeutic applications in clinical settings.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Author contributions

M.G. was the supervisor, designer of the hypotheses, and responsible for all the steps (laboratory, statisticalanalysis, data analysis, etc.) and wrote the text of the article. G.B. identified and confirmed the study plants, wrote part of the text and did the revision and formatting of the work. I.C., M.M. and M.L.M. wrote the text and did the revision and formatting of the work. Also M.L.M. interpretated part of data, substantively revised the text and edited English language.

Declarations

Competing interests

The authors declare no competing interests.

Ethical approval and consent to participate

All methods conducted comply with relevant institutional, national, and international guidelines and legislation.

Additional information

Correspondence and requests for materials should be addressed to M.G.