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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OILS OF ARTEMISIA HERBA-ALBA GROWN WILD IN EL-BAYADH REGION (NORTHWESTERN ALGERIA)

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Abstract

In this study we investigated the chemical composition and antibacterial activity of the essential oils of *Artemisia herba-alba* (*A. herba-alb*) collected in El-Bayadh province, Algeria. The essential oils (EOs) were obtained using steam distillation method and analyzed using a gas chromatograph coupled with mass spectrometry (GC-MS). The in vitro antimicrobial activity against five pathogenic bacteria was evaluated. The minimum inhibitory concentrations (MIC) were also determined using the macrodilution technique. The obtained essential oil yield was 0.81%, and GC-MS analyses resulted in the identification of 43 compounds, making up 96.09% of the total EO composition. Oxygenated monoterpenes were the major class of compounds (51.6%). The main compounds were camphor (33.98%), chrysanthenone (13.7%), camphene (8.35%), dovanone (6.53%), 1,8-Cineol (5.38%), borneol (4.22%), filifolone (2.55%), and pinocarvone (2.24%). The essential oils of *A. herba-alb* exhibited promising antibacterial effects against tested bacteria, with inhibitory diameter values values ranging from 12 to 19 mm, and MIC values ranging from 0.312 to 5 µl/ml. These results suggest their significant potential use as control agents against a wide spectrum of pathogens.

Keywords: antibacterial activity, *artemisia herba-alba*, chemical composition, essential oils, monoterpenes

INTRODUCTION

For centuries and across continents, plants have historically served as sources of pharmacologically active compounds, offering potential therapeutic agents for the alleviation and prophylaxis of various maladies. The success of phytotherapy is primarily explained by the level of technical and scientific mastery that has been achieved in this field. Agronomy, chemistry and pharmacology, through progress, have allowed the development of safer, more adapted and more effective therapeutic and galenic forms (Chabrier, 2010). The World Health Organization estimated that 40% of current pharmaceuticals are based on natural products, with many groundbreaking drugs originating from traditional medicine.

Essential oils (EOs) are natural complex mixtures of volatile compounds usually obtained by hydrodistillation and characterized by a strong odor (Caputo *et al*., 2022). The EOs from aromatic plants have been used in folk medicine since ancient times. They exhibit a multidirectional action mode and a variety of biological activities (Sun *et al*., 2022). The antimicrobial activity of EOs can be attributed to the contained monoterpenes that act by destroying the microbial cytoplasmic membrane due to their lipophilic nature (Cristiani *et al*., 2007). Moreover, herb EOs exert their diverse biological activities by acting on various pathways using different chemical components (Sun *et al*., 2022). Currently, they maintain attractive characteristics for medicine, aromatherapy, microbiology, agriculture, livestock, and the food industry (Pezantes-Orellana *et al*., 2024). Furthermore, EOs are used in agriculture as soil correctors due to their targeted action and biodegradable nature (Benomari *et al*., 2018). Most plants contain essential oils, albeit typically in small quantities. Only plants called "aromatic" produce them in sufficient quantity (Lardry and Haberkorn, 2007). There are at least 150 types of EOs marketed on the international market (Kusuma and Mahfud, 2017). The chemical composition of plant essential oils is influenced by both biotic and abiotic factors (Farias *et al*., 2023; Boaro *et al*., 2019).

Artemisia herba-alba is a medicinal and aromatic plant abundantly found on the highlands of Algeria, in semi-arid regions (Dahmani-Hamzaoui and Baaliouamer, 2010). White Mugwort, also known as desert wormwood in Europe and “Shih” in Algeria, is a steppe plant that grows in arid or semi-arid lands of North Africa, the Middle East, and Spain (Bezza *et al*., 2010). White Mugwort belongs to the genus *Artemisia*, which includes more than 500 species (Jung *et al.*, 2007).

The essential oils of *A.herba-alba*, notably rich in non-phenolic compounds, are highly interesting from a pharmaceutical perspective due to their antimicrobial properties (Al-Khazraji *et al*., 1993; Mighri *et al*., 2010). *Artemisia* has been widely used to treat gastric disorders, and for its anthelmintic and antispasmodic effects; it also exhibits a highly prized vermifuge character for livestock (Bezza *et*

al., 2010). In addition, the infusion made with this herb is used as analgesic, antimicrobial, antidiabetic, and coagulant agent (Aziz *et al*., 2018).

Hence, the present paper aims to determine the chemical composition of the essential oils extracted from *A. herba-alba*, and to assess its antibacterial potential against some bacterial strains belonging to different genera.

MATERIALS AND METHODS

Study Area and Plant Material

The sector of Brezina (33° 5' 59" North, 1° 15' 25" East) is located in the province of El-Bayadh situated within the steppe area of North Africa at an altitude of 1310 m and covers an area of 463.5 km². The region is characterized by a hot humid subtropical climate without a dry season according to the Köppen-Geiger classification and showed relatively abundant populations of *Stipa tenacissima*, *Lygeum spartium*, and *Artemisia herba alba* (Salemkour *et al*., 2022). The average yearly temperatures and total precipitation amounts are 15.3°C and 287.2 mm (www.fr.climate-data.org).

The plant material used for this study is locally known as "Shih" (Fig.1), and it was chosen based on its uses in local traditional medicine. The aerial parts of *A. herba-alba* were harvested at full bloom during March 2022, and then air-dried in a shady place at room temperature ranging between 25-30 °C for about 12-15 days.

Isolation of essential oils

A sample of 500 g of dried *A. herba-alba* was subjected to steam distillation in a vaporizer for 2 hours with 2500 ml of distilled water according to Duru *et al*. (2003). The obtained essential oils were dried with anhydrous sodium sulfate (Na_2SO_4). The essential oils were then stored in darkness at 4°C until further analysis (Gardeli *et al*., 2008). The essential oils yield was calculated using the following equation (Kusuma and Mahfud, 2017).

$$Y = \frac{V}{W} \times 100$$

With: Y represents the yield of essential oils (% w/w), V is the weight of extracted oil (g), and W is the weight of the dried plant material (g).

Figure 1. *Artemisia herba-alba* from El-Bayadh region (Dif *et al*., 2016).

Gas Chromatography-Mass Spectrometry (GC–MS) analysis

The analysis of the essential oils was conducted using a Shimadzu GC-2010 chromatograph connected to a mass spectrometer MS-QP2010 SE at the Unit of Research in Organic Chemistry and Macromolecules (URCOM), University of Le Havre, France.

The essential oils were initially diluted at 1/100 (v/v) in ethanol 96°. Compounds separation was performed on a ZB-5MS capillary column (5% phenyl, 95% dimethyl siloxane, 30 m × 0.25 mm, 0.25 μm film thickness). Helium (He) was used as the carrier gas at a flow rate of 1 ml/min. The injection volume was 1 μl of an ethanol-oil solution injected in split mode (split ratio 1/50). The column was initially programmed at 50°C for 3 minutes, gradually increased to 270°C with a heating ramp of 5°C/min, and then held for 15 minutes. The mass spectrometer operated in electronic impact mode with an ionization energy of 70 eV, and the analyser scanned in the range of 35-300 m/z. The components of the oils were identified by co-injection with standards (where possible) and confirmed using the NIST (National Institute of Standards and Technology) GC-MS Library V.2.0. The relative concentration of each compound in the essential oils was expressed as a percentage by normalizing the peak area (Babushok *et al*., 2011).

Antibacterial activity of essential oils

The essential oils of *A. herba-alba* were screened against five microbial species relatively resistant to commonly used therapeutic antibiotics, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Staphylococcus aureus,* and *Enterococcus faecalis*. These microbial agents were isolated from patient's stool samples at medical analysis laboratories in Mostaganem province, Algeria. All microorganisms were identified by studying their cellular morphology and biochemical tests using the API system (bio Mérieux Marcy-l'Étoile, France).

The aromatogram is a laboratory test that allows phytotherapists to analyse *in vitro* the antimicrobial activity of essential oils and to more precisely select the essential oils most capable of suppressing or destroying targeted germs (El Amri *et al*., 2014). Two types of aromatogram, in solid or liquid medium, can be utilized. However, in current practice, the aromatogram in solid medium is the simplest and most easily reproducible (Pibiri, 2005) . The antimicrobial activity of essential oils was determined by the agar diffusion method (Hazzit *et al*., 2009). Petri dishes (90 mm) were prepared by pouring 20 ml of Mueller Hinton Agar (MHA) medium and allowed to solidify and dry for 30 minutes. The McFarland density of the bacterial culture was adjusted in normal saline solution (0.9%, v/v) using a densitometer to reach a final concentration of approximately 10^6 CFU/ml for each tested microorganism individually (Mohapatra *et al*., 2011). Then, 0.1 ml of the standardized inoculum suspension (0.5 McF~10^6 CFU/ml) was poured and evenly spread, then allowed to dry for 5 minutes.

To prepare the stock solution of the sample, a volume of pure essential oils was dissolved at 10% (v/v) in dimethyl sulfoxide (DMSO) (Sigma Aldrich-Química, S.A.). Then, sterile filter paper disks with a diameter of 6 mm (Filter LAB ANOIA, Barcelona, Spain) were impregnated with 5 µl of the stock solution using a micropipette. The plates were left for 15 minutes at room temperature to allow the diffusion of the essential oils, then incubated at 37°C for 24 hours. A negative control was performed by depositing 5 µl of DMSO on disks placed on a previously inoculated medium.

The sensitivity of the tested germs to the essential oils is classified based on the diameters of inhibition zones (Ponce *et al*., 2003) as follows: Ø < 8 mm: Resistant; 9 mm < Ø < 14 mm: Sensitive; 15 mm < Ø < 19 mm: Very sensitive; Ø > 20 mm: Extremely sensitive. The minimum inhibitory concentration (MIC) corresponds to the lowest concentration of essential oils capable of inhibiting microbial growth. This technique involves inoculating a range of decreasing concentrations of essential oils using a standardized inoculum (Guinoiseau, 2010).

The determination of MIC was performed using the macrodilution method in liquid medium. Essential oils are first prepared by emulsification in DMSO solution (10%) to disperse the compounds and improve their contact with the germs tested. Dilutions obtained through successive geometric progression with a ratio of 2 result in the following dilutions : 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, and 1/128. The range of final concentrations of essential oils used ranges from 0.0781 to 10 µl/ml (v/v). 0.2 ml of each of the essential oil solutions is introduced into a test tube of the experimental series containing 1.8 ml of a bacterial suspension prepared in liquid Mueller Hinton media, with turbidity adjusted the required concentration of 10^6 Colony Forming Units (CFU)/ml (Abdelli, 2017).

The results are read by observing the change in turbidity in the tubes after incubation and comparing them with controls. The MIC corresponds to the lowest concentration of EO that inhibited microbial growth (absence of cloudiness) (Oussou *et al.*, 2004, Razzouk *et al.*, 2022).

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RESULTS AND DISCUSSION

Chemical composition of essential oils

The average yield of essential oil obtained in the current study was 0.81%. A similar result was reported by Lakehal *et al.* (2017). However, the essential oil content obtained in this study is lower compared to 0.94% observed in Biskra (Bezza *et al*., 2010), but higher than 0.3 and 0.7% observed by Touil and Benrebiha (2014) in Djelfa province (Algeria). The oil yields recorded in the present study were within the ranges reported in the literature (0.1–4.9%) (Belhattab *et al*., 2014).
The chemical constituents of the essential oils of *A. herba-alba* are listed in order of elution on the ZB-5MS capillary column (Fig. 2).

The chromatographic analysis of *A. herba-alba* essential oils led to the identification of a total of 43 compounds contributing to 96.09% of the oil (Table I). The major classes of compounds included oxygenated monoterpenes (51.46%), monoterpene hydrocarbons (11.84%) and sesquiterpene hydrocarbons (4.49%), while the oxygenated sesquiterpene fraction was 0.59%. The data found are in agreement withwith previous studies (Haouari and Ferchichi, 2009; Belhattab *et al*., 2014). The major compound in the oil of *A. herba-alba* was Camphor (33.98%).This compound was also significantly abundant in samples collected from other regions of Algeria (Belhattab *et al*., 2014, Lakehal et al, 2016; Dahmani-Hamzaoui and Baaliouamer, 2010), Morocco (Ghanmi *et al*., 2010, Paolini *et al*., 2010, Aziz *et al*., 2012, Amkiss *et al.*, 2021), Tunisia (Haouari and Ferchichi, 2009),, and Egypt (Sallam *et al*., 2011).
The other major constituents of the essential oils were chrysanthenone (13.7%), camphene (8.35%), dovanone (6.53%), 1,8-Cineol (5.38%), borneol (4.22%), filifolone (2.55%), and pinocarvone (2.24%). The variation observed in the oil composition from plants cultivated in different countries or within different areas of the same country highlights the presence of numerous chemotypes associated with the plant (Naser Al-Wahaibi *et al*., 2020). Regular monoterpenes such as α-thujone, β-thujone, and germacrene D (4.8%),were predominant in Tunisia (Younsi *et al*., 2016; Bellili *et al*., 2017). Similarly, cis-thujone (25.5%), trans-Thujone (17.7%), vanillyl alcohol, and nor-davanone, were reported as the principle constituent compounds in Morocco Amor *et al.* (2019).

Likewise, chrysanthenone, cis-chrysanthenyl acetate, and cis-thujone (13.6%) from libya (Janackovic *et al*., 2015), chrysanthenone , followed by camphor, verbenone, tridecane, and borneol from Morocco (Fadli *et al*., 2016), *α*-Chrysanthenone, camphor,*β*-thujone, thujone, 1,8-cineole, and piperitenone from East Algeria (Rekkab *et al*., 2016), piperitone, and (*E*)-ethylcinnamate, (*Z*)-ethylcinnamate, thymol, isophrone from Saudi Arabia (Naser Al-Wahaibi *et al.*, 2020).

Based on various findings, essential oils extracted from *A.herba-alba* predominantly consist of monoterpenes, albeit with variations in proportions. These differences are likely attributed to geo-climatic conditions and temporal factors, such as time of harvest. The chemical composition of essential oils can also vary among genotypes within the same speciesand between different plant parts of the same species (Razzouk *et al*., 2022). Other factors that can affect the chemical composition include climate, altitude, hygrometry, soil quality, and extraction method (Cunha *et al.*, 2013) and nutrients (Djenane *et al.*, 2011; Tefiani *et al.*, 2015; Benomari *et al*, 2023). Furthermore, the yield and composition of the oils were correlated with herbivores, and fungal pathogen attacks, especially during the rainy month (Hassiotis *et al.*, 2010).

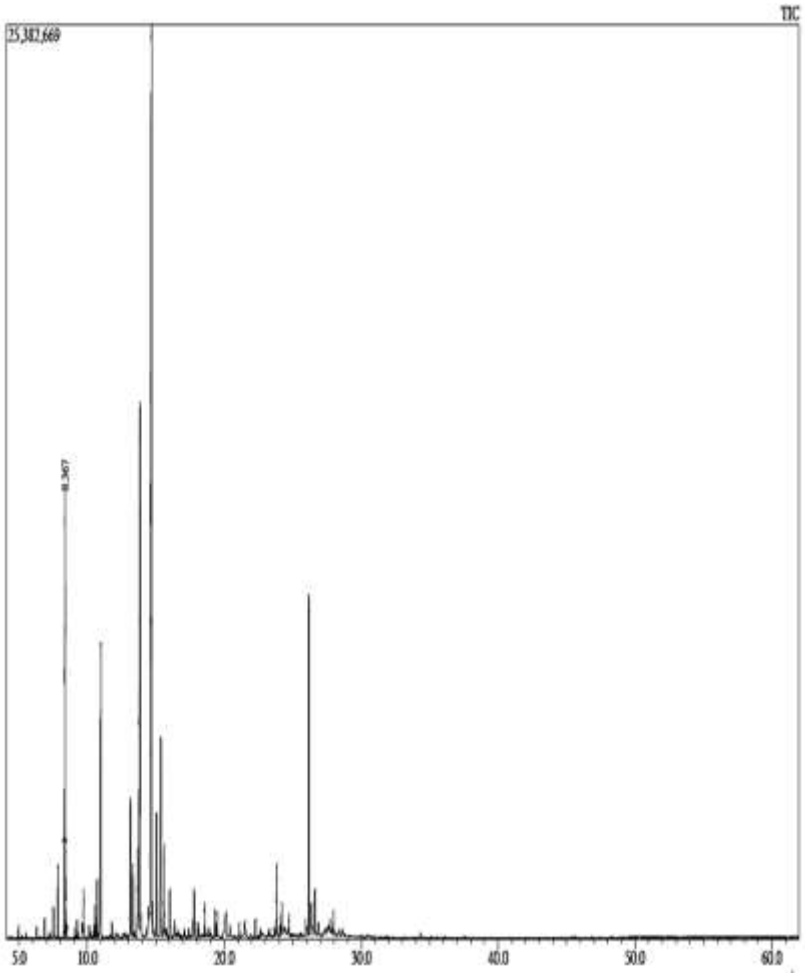

Figure 2. Chromatogram of the essential oils of *A.herba-alba* from El-Bayadh region

Antibacterial activity

The use of biologically active molecules isolated from plant species aims to eradicate pathogenic microorganisms. The *in vitro* antibacterial activity of *A. herba-alba* essential oils was evaluated qualitatively and quantitatively by the presence or absence of inhibition zones and the determination of minimum inhibitory concentrations (MIC).

The essential oils of *A. herba-alba* exhibited significant inhibitory activity against the tested bacteria, with the average diameter of the inhibition zone ranging from 12 to 19 mm. As also indicated in the literature (Mighri *et al*., 2010; Bertella *et al*., 2018)., the antimicrobial activity of *A. herba-alba* essential oils was tested using the diffusion method, and the results showed that they had a strong antimicrobial activity.

Table II shows that the essential oils of *A. herba-alba* exhibited a high antibacterial activity against Gram-positive bacteria compared to Gram-negative bacteria. According to the spectrum indicated above (Ponce *et al*., 2003), the inhibition zones obtained allowed the classification of bacteria based on their sensitivity to the tested essential oils. The inhibition diameters obtained for *Enterococcus faecalis* and *Staphylococcus aureus* were 16, and 19 mm, respectively (Fig. 3). Therefore, these bacterial strains were considered highly sensitive to the essential oils.

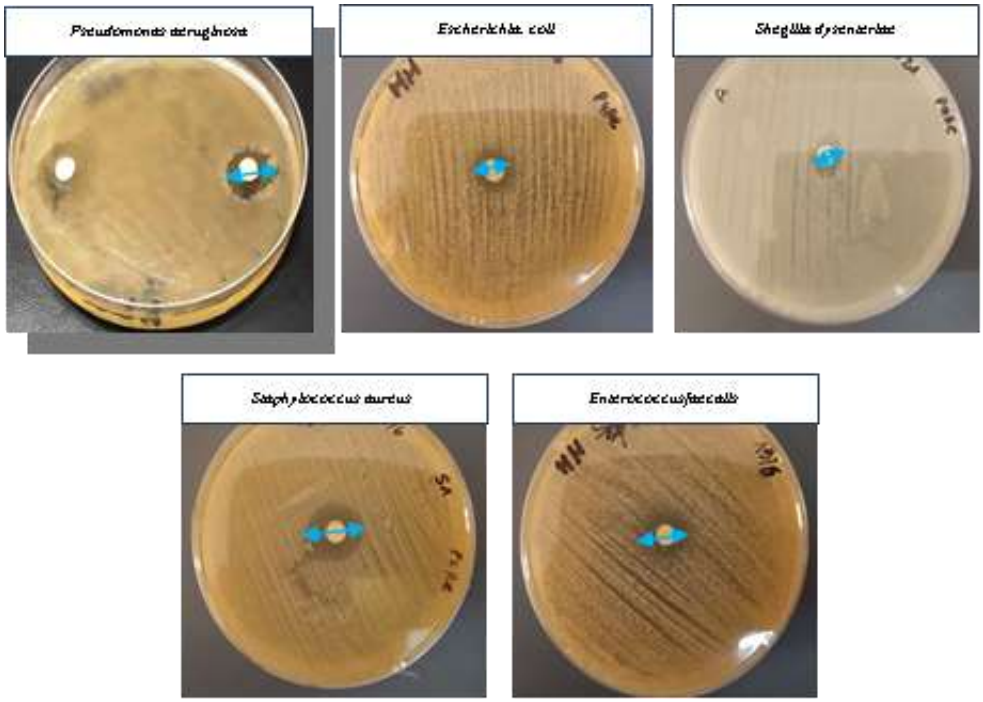

Figure 3. The Aromatogram of *A. herba-alba* essential oils against the tested bacteria.

Additionally, *Pseudomonas aeruginosa* showed the highest sensitivity to *A. herba-alba* EOs (12.00 mm diameter of inhibition) compared to *Escherichia coli and Shigella dysenteriae* with inhibition zone diameters of 12 and 13 mm, respectively. Our findings are consistent with those of Imelouane *et al*. (2010) and Heleili *et al.* (2018) who reported inhibition diameters of 12 and 12.52 mm against *E. coli* ATCC 35218 and *Pseudomonas aeruginosa strains,* respectively.

Table 1. Chemical composition of essential oils isolated from *Artemisia herba-alba* from El-Bayadh region.

No.	Compound[a]	RT (min)	RI	Area	%
1	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	4.96	841	717697	0.19
2	2,6-dimethyl heptadiene	6.30	884	671368	0.18
3	Santolinatriene	6.87	902	1288682	0.34
4	Tricyclene	7.49	922	1802654	0.47
5	α-Pinene	7.83	933	4975251	1.3
6	Camphene	8.36	949	31973939	8.35
7	Thujadienne	8.47	953	742860	0.19
8	β-Pinene	9.22	977	1072225	0.28
9	β-Myrcene / TE 3,6-dimethylheptatrienne	9.59	989	1862060	0.49
10	Benzene 1,2,4-trimethyl	9.71	992	3038053	1.33
11	α-Terpinene	10.46	1016	558451	0.15
12	o-Cymene	10.70	1024	3746375	0.98
13	1,8-Cineol	10.97	1032	20600018	5.38
14	ɣ-Terpinene	11.77	1058	1129168	0.29
15	Filifolone	13.11	1100	9752749	2.55
16	β-Thujone	13.31	1106	4841799	1.26
17	α-Thujone	13.67	1118	7604589	1.98
18	Chrysanthenone	13.83	1123	52505436	13.7
19	2-p-Menthen-1-ol	13.89	1125	1367691	0.36
20	Trans pinocarveol	14.44	1143	2769728	0.72
21	Camphor	14.67	1151	130243196	33.98
22	Mentha-2,8-dien-1-ol	14.73	1153	1951643	0.51
23	Pinocarvone	15.03	1162	8568676	2.24
24	Borneol	15.35	1173	16186921	4.22
25	Terpinen-4-ol	15.58	1180	5831528	1.52
26	α-Terpinol	16.01	1194	4182694	1.09
27	D-Verbenone	16.365	1206	1135978	0.3
28	trans Piperitone	16.42	1208	569457	0.15
29	5,7-Dimethyloctahydrocoumarin	17.7	1252	1224235	0.32
30	cis Chrysanthenyl acetate	17.78	1255	3063201	0.8
31	(S,E)-2,5-Dimethyl-4-vinylhexa-2,5-dien-1-yl acetate	18.015	1263	843844	0.22
32	Isopiperitenone	18.13	1267	878438	0.23
33	Bornyl acetate	18.575	1282	2232981	0.58
34	α-Copaene	21.09	1375	929355	0.24
35	Caryophyllene	22.26	1420	1173417	0.31
36	Germacrene D	23.825	1481	4729133	1.23
37	Bicyclogermacrene	24.19	1495	1897699	0.5
38	β-Cadienne	24.715	1517	1076565	0.28
39	5,6-dimethyl-2-heptanone	25.96	1568	807982	0.21
40	Dovanone	26.17	1576	25003989	6.53
41	Globulol	26.605	1594	3576294	0.93
42	Tiglate de bornyl	26.88	1606	1215180	0.32
43	α-Cadinol	27.715	1640	542835	0.59
	Total identified				96.09
	Monoterpenes hydrocarbons	.			11.84
	Oxygenated monoterpenes				51.6
	Sesquiterpenes hydrocarbons				4.49

	Oxygenated sesquiterpenes				0.59
	Ketones				26.23
	Esters				1.34
	Others				3.91

The MIC values of *A.herba-alba* essential oils are shown in Table III. The *A. herba-alba* EO exhibited strong inhibitory activity against all tested strains. The MIC values ranged from 0.31 to 10 µl/ml (v/v) depending on the strain tested. The results aligned with previous investigations (Imelouane *et al*., 2010; Sbayou *et al*., 2014). In the study, the lower MIC value (0.312 µl/ml) was obtained with *Staphylococcus aureus*. According to Rios and Recio (2005), extracts or oils from plant species with MIC values below 100 µg/ml are considered promising as potential antimicrobial agents. The differences in the MIC values of bacteria may be related to the differential susceptibility of the bacterial cell wall, which serves as the functional barrier against minor differences present in the outer membrane in the composition of the cell wall (Zhao *et al*., 2001). Gram-negative bacteria are surrounded by a thin cell wall of peptidoglycan, itself surrounded by an outer membrane containing lipopolysaccharide, which creates a barrier against hydrophobic compounds such as those found in essential oils. In contrast, Gram-positive bacteria do not have an outer membrane but are surrounded by layers of peptidoglycan several times thicker than those found in Gram-negative bacteria (Silhavy *et al*., 2010). Previous studies have demonstrated that the majority of EOs tested for their antibacterial properties have a more pronounced effect against Gram-positive bacteria. The resistance of Gram-negative bacteria is attributed to their hydrophilic outer membrane, which can block the penetration of hydrophobic compounds into the target cell membrane (Wan, 1998).

Table II. Results of the aromatogram of *A. herba-alba* essential oils against the studied bacteria.

	Diameters of inhibition zones in mm				
	P. aeruginosa	***S. dysenteriae***	***E.coli***	***S. aureus***	***E. faecalis***
Cefalexin	20	/	20	26	22
Cefotaxime	/	*26*	24	*6*	*6*
Chloramphenicol	/	/	12	24	8
Ciprofloxacin	30	12	30	/	/
Colistin	/	/	10	/	/
Doxycycline	10	/	/	/	/
Imipeneme	/	30	/	/	/
Levofloxacin	30	/	/	/	/
Oxacillin	/	/	/	6	6
Piperacillin	10	6	/	/	6
Sulfamethoxazole	/	/	6	/	26
Tetracyclin	8	/	/	18	16
Ticarcillin	6	/	6	/	6
Ticarcillin + clavulanic acid	6	6	/	/	/
A herba- alba EOs	14	13	12	19	16

In the current study, the potent antimicrobial activity of *A. herba-alba* could be related to the presence of considerable amount of oxygenated monoterpenes, particularly the high percentages of camphor, chrysanthenone, and 1,8-Cineole (Mighri *et al*., 2010). Several studies reported that the biological activity of EOs are due to the synergy between the major compounds rather than a single one (Ghalem and Mohamed, 2009; Razzouk *et al*., 2022). Moreover, the relative action of thujones and eucalyptol (or 1,8-cineole) has been associated with their low water solubility and the ability to form hydrogen bonds, which limits their entry into Gram-negative bacteria that possess ineffective hydrophobic pathways in the outer membrane (Younsi *et al*., 2016).

Table III. Results of the MIC of the essential oils of *A. herba-alba* with respect to the microbial strains studied.

Bacteria	Concentration of *A.herba-alba* EOs in µl/ml (v/v)								Witness
	10	5	2.5	1.25	0.625	0.312	0.156	0.078	0
Escherichia coli	-	-	-	+	+	+	+	+	+
Shigella dysenteriae	-	-	+	+	+	+	+	+	+
Pseudomonas aeruginosa	-	-	-	-	-	+	+	+	+
Staphylococcus aureus	-	-	-	-	-	-	+	+	+
Enterococcus faecalis	-	-	-	-	-	+	+	+	+
+ Presence of growth, - Lack of growth									

CONCLUSION

The analysis of the essentiel oil of *A. herba-alba* grown wild in the arid region of El-Bayadh revealed the predominance of oxygenated monoterpenes with the abundance of some interesting bioactive compounds such as camphor, chrysanthenone, borneol, and pinocarvone. The antibacterial activity of *A. herba-alba* oil demonstrated that the EO exhibited a high degree of inhibitory activity against various pathogenic bacteria pathogens, especially *Staphylococcus aureus*. Thus, *A. herba-alba* can be considered as good candidateas a natural antibacterial agents for industrial applications such as pharmacy, cosmetics, and food preservation. Further toxicological and clinical research are crucial to ensure the safety of the oil and to confirm whether the observed effects are suitable for practical applications.

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Authors' Contribution

AA and LD contributed to the conception and design of the study. AA, KB, SB, DEB, and IS performed the analysis. AA and LD wrote the original draft. RD supervised the project and revised the manuscript. All authors read and approved the submitted version.

Conflict of Interest

The authors declare that they have no conflict of interests.

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