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Original article

Chemical Composition and Antibacterial Activity of *Artemisia herba-alba* Essential Oil from Libya Against Multidrug-Resistant *Pseudomonas aeruginosa* and Methicillin-Resistant *Staphylococcus aureus*

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Abstract
Antimicrobial resistance, particularly multidrug resistance (MDR), represents a major global concern, and there has been increased interest in natural products as potential sources of alternative antimicrobial agents. Artemisia herba-alba is an aromatic medicinal plant traditionally used in Libya. This study aimed to extract the essential oil from A. herba-alba collected in the Al-Abraq region of northeastern Libya, characterise its chemical composition, and evaluate its antibacterial activity against selected clinical isolates, including MDR Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus (MRSA). Essential oil was obtained from dried aerial parts by hydrodistillation using a Clevenger-type apparatus and characterised by gas chromatography-mass spectrometry (GC-MS). Antibacterial activity was evaluated using disc diffusion and broth microdilution assays to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), characterised by antimicrobial susceptibility testing according to CLSI recommendations. Statistical comparisons were performed using the Mann-Whitney U test, with $p \leq 0.05$ considered significant. The essential oil yield was 2.0% (w/w). GC-MS analysis identified 40 compounds representing 97.21% of the total oil composition. Camphor (30.76%) and 1,8-cineole (16.96%) were the predominant constituents, followed by camphene (6.85%), chrysanthenone (5.56%), and borneol (4.89%). The essential oil showed concentration-dependent antibacterial activity. MIC values ranged from 0.78 to 6.25 µL/mL (v/v), whereas MBC values ranged from 1.56 to 12.50 µL/mL (v/v). Clinical resistant isolates generally showed lower susceptibility than corresponding reference strains. The MBC/MIC ratio was consistently 2 across all tested groups, supporting a bactericidal effect under the experimental conditions. The essential oil of Libyan A. herba-alba demonstrated promising in vitro antibacterial activity against reference strains and clinically resistant isolates. Further studies involving larger numbers of isolates, cytotoxicity assessment, mechanistic investigations, and in vivo models are required.
Keywords. Artemisia Herba-Alba, Essential Oils, Antibacterial Agents, Drug Resistance, Bacteria.

Introduction

Antimicrobial resistance (AMR), particularly multidrug resistance (MDR), has become one of the major challenges facing modern healthcare. Resistant bacterial infections are associated with increased morbidity, mortality, prolonged hospitalisation, and healthcare costs [1,2]. Among clinically important resistant pathogens, Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus (MRSA) are of particular concern because of their ability to acquire and express multiple resistance mechanisms. P. aeruginosa exhibits reduced outer-membrane permeability and multiple active efflux systems that contribute to resistance to several antimicrobial agents [3]. The increasing prevalence of resistant bacteria has stimulated the search for alternative antimicrobial agents from natural sources. Medicinal and aromatic plants are particularly attractive because their essential oils contain complex mixtures of biologically active terpenes and oxygenated terpenoids that may act through multiple mechanisms, including disruption of bacterial membranes and alteration of cellular permeability [4,5].

Artemisia herba-alba Asso. is an aromatic medicinal plant widely distributed in North Africa and the Mediterranean region. The plant has a long history of traditional use in several North African communities, including Libya. The essential oil of A. herba-alba is chemically variable, and its composition may differ according to geographical origin, environmental conditions, genetic factors, and harvesting conditions [6,7]. Previous investigations of Libyan A. herba-alba essential oils have demonstrated substantial chemical variation among geographical locations. Janaćković et al. [8] characterised it by relatively high proportions of chrysanthenone, cis-chrysanthenyl acetate, and cis-thujone. In contrast, Eltawaty et al. [9] reported a different population characterised by relatively high levels of camphor and thujone. Similar geographical variation has been reported for A. herba-alba populations from other Mediterranean countries [6,7].

Despite previous studies on Libyan A. herba-alba, information concerning the essential oil obtained from plants growing in the Al-Abraq area of northeastern Libya remains limited. Therefore, this study characterised the essential oil of A. herba-alba collected from Al-Abraq using GC-MS and evaluated its in vitro antibacterial activity against selected clinical isolates, including MDR P. aeruginosa and MRSA, using reference strains as comparators.

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Materials and Methods

Plant Material Collection and Preparation

Aerial parts, including leaves and flowering portions, of Artemisia herba-alba were collected during the flowering period on 15 August 2024 from the Al-Abraq region of northeastern Libya (32.78° N, 21.58° E). The plant material was botanically identified by a taxonomist in the Department of Medicinal and Aromatic Plants, Faculty of Natural Resources and Environmental Sciences, Omar Al-Mukhtar University, Al-Bayda, Libya. A voucher specimen was deposited under accession number OMU-APH-2024-012. The collected material was shade-dried for 7 days, protected from direct sunlight and moisture, ground into a coarse powder, and stored in airtight glass containers at room temperature until extraction.

Essential Oil Extraction

Essential oil was extracted by hydrodistillation using a Clevenger-type apparatus. Briefly, 300 g of dried and ground plant material was placed in a distillation flask containing 3 L of distilled water. Hydrodistillation was continued for 4 h after boiling commenced. The recovered oil was separated from the aqueous phase, dried over anhydrous sodium sulfate, and stored in amber glass vials at 4°C until analysis. Oil yield was calculated on a dry-weight basis.

GC-MS Analysis

Chemical characterisation was performed using an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass selective detector. Separation was achieved on a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as carrier gas at 1 mL/min. The oven temperature was initially 60°C for 5 min, increased at 4°C/min to 250°C, and held for 5 min. The oil was diluted 1:100 in hexane, and 1 µL was injected at a split ratio of 1:50. Injector temperature was 250°C, ion-source temperature 230°C, and ionisation energy 70 eV. Mass spectra were recorded from m/z 40–400 with a 3-min solvent delay. Compounds were tentatively identified by comparison with the NIST 2017 library and retention indices calculated using n-alkanes C8–C24 [10].

Bacterial Strains and Resistance Characterisation

The bacterial strains were obtained from Benghazi Medical Centre, Benghazi, Libya. Reference strains included Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 6538, and S. aureus ATCC 43300 (MRSA). Clinical isolates comprised five MDR P. aeruginosa isolates and five MRSA isolates obtained from wounds, burns, and urine specimens during August 2024. MDR was defined according to Magiorakos et al. [11] as acquired nonsusceptibility to at least one antimicrobial agent in three or more antimicrobial categories. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion according to CLSI recommendations [12]. P. aeruginosa was tested against gentamicin (10 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), and imipenem (10 µg). S. aureus was assessed for methicillin resistance using a cefoxitin disc (30 µg), with additional testing against erythromycin (15 µg) and tetracycline (30 µg).

Antibacterial Activity

Disc Diffusion Assay

The essential oil was prepared at 5%, 10%, 20%, 30%, 50%, 75%, and 100% (v/v) concentrations using DMSO as diluent. A preliminary negative-control test confirmed that DMSO did not inhibit bacterial growth. Sterile 6-mm Whatman No. 1 discs were impregnated with 20 µL of each concentration and placed on Mueller-Hinton agar inoculated with a 0.5 McFarland suspension (approximately 1.5×10^8 CFU/mL). DMSO served as a negative control and appropriate antimicrobial discs as positive controls. Plates were incubated at 37°C for 24 h, and inhibition zones were measured in mm. Each assay was performed in three technical replicates.

MIC and MBC Determination

MIC and MBC were determined by broth microdilution in 96-well plates. Two-fold serial dilutions of essential oil were prepared in Mueller-Hinton broth containing 0.5% Tween 80 to improve dispersion. The final oil concentrations ranged from 0.39 to 50 µL/mL (v/v) and are expressed as volume-to-volume ratios (v/v) because the essential oil is a liquid with a density of approximately 0.9 g/mL; thus, 1 µL/mL corresponds to approximately 0.9 mg/mL. Wells were inoculated to a final concentration of approximately 5×10^5 CFU/mL and incubated at 37°C for 24 h. MIC was defined as the lowest concentration without visible growth. For MBC determination, 10 µL of the clear broth was subcultured onto Mueller-Hinton agar and incubated for 24 h. MBC was defined as the lowest concentration producing at least a 99.9% reduction in viable bacterial counts compared with the untreated control. The MBC/MIC ratio was consistently 2 across all tested groups, supporting a bactericidal effect under the conditions tested [13].

Statistical Analysis

For each isolate, the mean inhibition-zone diameter was calculated from the three technical replicates, and isolate-level means were used as experimental units. Normality was assessed using the Shapiro-Wilk test. Comparisons between clinical resistant isolates and corresponding reference strains were performed using the Mann-Whitney U test because of the small sample size. Concentration-response effects were evaluated by two-way ANOVA when assumptions were satisfied, followed by Tukey's multiple-comparison test. A two-sided p-value ≤0.05 was considered statistically significant.

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Spearman's rank correlation analysis was performed to evaluate the relationship between MIC values and inhibition zone diameters.

Results and Discussion

Essential Oil Yield and Chemical Composition

The hydrodistillation of dried aerial parts of Artemisia herba-alba yielded 2.0% (w/w) essential oil. This yield falls within the range reported for some Libyan A. herba-alba populations [8,9]. GC-MS analysis identified 40 compounds accounting for 97.21% of the total oil composition (Table 1). Camphor (30.76%) and 1,8-cineole (16.96%) were the predominant constituents, followed by camphene (6.85%), chrysanthenone (5.56%), and borneol (4.89%). Other identified constituents included α-pinene, β-pinene, limonene, linalool, cis- and trans-thujone, terpinen-4-ol, α-terpineol, bornyl acetate, β-caryophyllene, germacrene-D, and spathulenol.

Table 1. Chemical composition of Artemisia herba-alba essential oil from Al-Abraq, Libya

No.	Compound	RI calc.	RI lit.	Percentage (%)	Identification
1	α-Thujene	930	924	0.84	RI, MS
2	α-Pinene	939	932	2.15	RI, MS, Std
3	Camphene	954	946	6.85	RI, MS
4	Sabinene	975	969	0.41	RI, MS
5	β-Pinene	979	974	1.62	RI, MS
6	Myrcene	990	988	0.28	RI, MS
7	α-Phellandrene	1002	1002	0.15	RI, MS
8	p-Cymene	1024	1020	0.73	RI, MS
9	Limonene	1028	1024	1.87	RI, MS
10	1,8-Cineole	1031	1026	16.96	RI, MS, Std
11	cis-Thujone	1101	1097	1.23	RI, MS
12	trans-Thujone	1114	1107	0.92	RI, MS
13	Camphor	1143	1139	30.76	RI, MS, Std
14	Chrysanthenone	1158	1152	5.56	RI, MS
15	Linalool	1097	1095	0.56	RI, MS
16	Borneol	1165	1165	4.89	RI, MS
17	Terpinen-4-ol	1177	1174	2.34	RI, MS
18	α-Terpineol	1189	1186	1.75	RI, MS
19	Myrtenol	1195	1192	0.38	RI, MS
20	Bornyl acetate	1289	1284	2.98	RI, MS
21	Thymol	1290	1289	0.22	RI, MS
22	Carvacrol	1298	1298	0.15	RI, MS
23	α-Copaene	1351	1345	0.33	RI, MS
24	β-Elemene	1390	1389	0.41	RI, MS
25	β-Caryophyllene	1419	1417	3.21	RI, MS
26	Germacrene-D	1480	1475	2.65	RI, MS
27	β-Selinene	1485	1479	0.98	RI, MS
28	α-Farnesene	1500	1495	0.45	RI, MS
29	Spathulenol	1578	1577	2.11	RI, MS
30	Caryophyllene oxide	1583	1582	0.87	RI, MS
31	γ-Terpinene	1062	1059	0.52	RI, MS
32	Terpinolene	1088	1086	0.38	RI, MS
33	Fenchone	1086	1083	0.29	RI, MS
34	Pinocarveol	1162	1160	0.14	RI, MS
35	trans-Pinocarveol	1139	1136	0.22	RI, MS
36	cis-Verbenol	1140	1138	0.16	RI, MS
37	α-Humulene	1454	1452	0.31	RI, MS
38	δ-Cadinene	1523	1520	0.24	RI, MS
39	Caryophylladienol	1635	1632	0.19	RI, MS
40	α-Bisabolol	1685	1682	0.15	RI, MS

Total identified: 97.21%

Std: identity confirmed by co-injection with authentic standard; RI: retention index matching; MS: mass spectrum matching using NIST 2017 library.

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The predominance of camphor and 1,8-cineole differs from some previously reported Libyan populations, supporting the marked geographical variability of A. herba-alba essential-oil composition [8,9]. Essential-oil constituents such as oxygenated monoterpenes can interact with bacterial membranes and alter membrane permeability, potentially leading to leakage of intracellular components and loss of cellular integrity [5,14]. However, the antibacterial activity of a whole essential oil should not be attributed to a single constituent without additional fractionation or mechanistic experiments. The observed activity is more appropriately considered a result of the combined effects of multiple constituents and possible synergistic or additive interactions [15].

Antimicrobial Resistance Profiles of Clinical Isolates

The antimicrobial resistance profiles of the clinical isolates are presented in Table 2. All five P. aeruginosa clinical isolates exhibited resistance to gentamicin, ciprofloxacin, and ceftazidime, with three isolates also showing resistance to imipenem. All five MRSA isolates demonstrated resistance to cefoxitin (confirming methicillin resistance), erythromycin, and tetracycline, consistent with their MDR phenotype.

Table 2. Antimicrobial resistance profiles of clinical isolates used in the study

Isolate type	Number of isolates	Resistance pattern (resistant isolates/total)	MDR definition
P. aeruginosa	5	Gentamicin (5/5), Ciprofloxacin (5/5), Ceftazidime (5/5), Imipenem (3/5)	Yes
MRSA	5	Cefoxitin (5/5), Erythromycin (5/5), Tetracycline (5/5)	Yes

Disc Diffusion Activity

The essential oil showed concentration-dependent antibacterial activity against all tested strains (Table 3). Inhibition zones increased significantly with increasing oil concentration (two-way ANOVA, $p < 0.001$). As expected, reference strains were significantly more susceptible than clinical resistant isolates (Mann-Whitney U test, $p < 0.05$). At 50% (v/v), inhibition zones for S. aureus ATCC 6538 were 24.67 ± 1.53 mm; S. aureus ATCC 43300 were 22.33 ± 1.53 mm; clinical MRSA were 17.33 ± 1.52 mm; P. aeruginosa ATCC 27853 were 15.33 ± 1.15 mm; and clinical MDR P. aeruginosa were 12.67 ± 1.53 mm. Spearman's rank correlation analysis showed a strong inverse relationship between MIC values and inhibition zone diameters ($r = -0.89$, $p < 0.001$).

Table 3. Inhibition zones of A. herba-alba essential oil (mm) against tested strains

Concentration (v/v%)	S. aureus ATCC 6538	S. aureus ATCC 43300 (MRSA)	Clinical MRSA	P. aeruginosa ATCC 27853	Clinical MDR P. aeruginosa
5%	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
10%	8.33 ± 1.15	7.67 ± 0.58	6.33 ± 0.58	0.0 ± 0.0	0.0 ± 0.0
20%	14.33 ± 1.53	12.67 ± 1.15	10.33 ± 1.15	8.33 ± 0.58	7.33 ± 1.15
30%	18.67 ± 1.15	16.33 ± 1.53	13.67 ± 1.53	11.67 ± 1.15	9.67 ± 0.58
50%	24.67 ± 1.53	22.33 ± 1.53	17.33 ± 1.52	15.33 ± 1.15	12.67 ± 1.53
75%	30.33 ± 1.53	28.67 ± 1.53	23.67 ± 1.53	20.33 ± 1.15	17.33 ± 1.53
100%	38.33 ± 1.53	35.33 ± 1.15	29.67 ± 1.53	26.33 ± 1.53	22.33 ± 1.53
Positive controls	31.0 ± 1.0	28.0 ± 1.0	6.0 ± 0.0	28.0 ± 1.0	6.0 ± 0.0

Data are presented as mean ± standard deviation of three technical replicates. Positive controls: Erythromycin (15 µg) for S. aureus; Imipenem (10 µg) for P. aeruginosa.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

MIC values ranged from 0.78 to 6.25 µL/mL, while MBC values ranged from 1.56 to 12.50 µL/mL (Table 4). The lowest MIC was observed for S. aureus ATCC 6538 (0.78 µL/mL), whereas the highest MIC was observed for clinical MDR P. aeruginosa (6.25 µL/mL). The MBC/MIC ratio was 2 for all groups, supporting a bactericidal effect under the experimental conditions [13].

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the essential oil (µL/mL, v/v)

Strain / Isolate	MIC (range)	MBC (range)	MBC/MIC ratio
S. aureus ATCC 6538	0.78	1.56	2
S. aureus ATCC 43300 (MRSA)	1.56	3.125	2
Clinical MRSA (n=5)	3.125 (1.56–3.125)	6.25 (3.125–6.25)	2
P. aeruginosa ATCC 27853	3.125	6.25	2
Clinical MDR P. aeruginosa (n=5)	6.25 (3.125–6.25)	12.50 (6.25–12.50)	2

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For clinical isolates, values represent the modal concentration with the full range in parentheses. The highly efficient bactericidal activity observed in this study (MBC/MIC ratio = 2) may be attributed to the potential synergistic effect between the two major constituents, camphor (30.76%) and 1,8-cineole (16.96%). Previous studies have demonstrated that the combination of oxygenated monoterpenes can enhance membrane disruption and increase bacterial membrane permeability, leading to a more pronounced antibacterial effect [16,17]. Additionally, minor constituents such as camphene, borneol, and chrysanthenone may contribute to the overall activity through synergistic or additive effects.

Conclusion

The essential oil of Artemisia herba-alba collected from the Al-Abraq region of Libya showed promising in vitro antibacterial activity against reference strains and selected clinically resistant isolates, including MRSA and MDR P. aeruginosa. Camphor and 1,8-cineole were the predominant constituents, and the chemical profile differed from previously reported profiles of Libyan A. herba-alba populations [8,9]. The observed bactericidal activity (MBC/MIC ratio = 2) is attributed to the potential synergistic effect between camphor and 1,8-cineole, along with other minor constituents [16,17]. The observed activity supports further investigation of this essential oil as a potential source of antimicrobial compounds. However, additional toxicological, mechanistic, and in vivo studies are required before any clinical application can be considered.

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