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Antimicrobial and antioxidant activities of *Artemisia herba-alba* essential oil cultivated in Tunisian arid zone

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ABSTRACT

This study was conceived to examine the antimicrobial and antioxidant activities of four essential oil types extracted by hydrodistillation from the aerial parts of *Artemisia herba-alba* cultivated in southern Tunisia. The chemical composition was investigated using both capillary GC and GC/MS techniques. β-thujone, α-thujone, α-thujone/β-thujone and 1,8-cineole/camphor/α-thujone/β-thujone were respectively, the major components of these oil types. The antimicrobial activity of different oils was tested using the diffusion method and by determining the inhibition zone. The results showed that all examined oil types had great potential of antimicrobial activity against strains. In addition, antioxidant capacity was assessed by different in vitro tests and weak activity was found for these *A. herba-alba* oils.

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RÉSUMÉ

Cette étude a été conçue pour examiner les activités antioxydantes et antimicrobiennes de quatre types d'huiles essentielles extraites par hydrodistillation de la partie aérienne d'*Artemisia herba-alba* cultivée dans le sud de la Tunisie. La composition chimique a été étudiée en utilisant les techniques CPG et CPG/SM. β-thujone, α-thujone, α-thujone/β-thujone et 1,8-cinéole/camphre/α-thujone/β-thujone sont respectivement, les composés majeurs de ces types d'huiles. L'activité antimicrobienne des différentes huiles a été testée par la méthode de diffusion et de détermination de la zone d'inhibition. Les résultats ont montré que tous les types d'huiles examinés ont une importante activité antimicrobienne vis-à-vis les souches testées. En addition, la capacité antioxydante évaluée *in vitro* par différents tests a montré une faible activité pour ces huiles d'*A. herba-alba*.

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1. Introduction

Artemisia herba-alba (*Asteraceae*) is a greenish-silver perennial dwarf shrub growing in arid and semi-arid climates. It is characteristic of the steppes and deserts of the Middle East (Egypt, desert of Israel and Sinai), North Africa (Tunisia, Morocco and Algeria), Spain, extending into Northwestern Himalayas [1]. In Tunisia, *A. herba-alba* is found from the mountains around Jebel Oust (Fahs) until the South of the country [2]. This plant is widely used in the traditional medicine to treat diabetes, bronchitis, diarrhea, hypertension and neuralgias [3–5]. The essential oil of this

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species was known for its therapeutic disinfectant, anthelminthic and antispasmodic virtues [6]. Numerous studies in the literature have reported the composition of *A. herba-alba* essential oil from different parts of the world and confirmed the many oil-dependent chemotypes assigned to the plant. According to the reviews of Lawrence and Salido et al. [7,8], two types of oils could be distinguished: those whose composition is dominated by a major compound (camphor, β or α-thujone, chrysanthenone, chrysanthenyl acetate, or davanone) and those characterized by the codominance of two or more of these compounds. In Tunisia (semi-arid and arid land), various compositions were observed, dominated either by a single component (α-thujone, β-thujone, 1,8-cineole, camphor, chrysanthenone or *trans*-sabinyl acetate) or characterized by the occurrence, at appreciable content, of two or more of these compounds [9–11].

Many reports ascribed various biological and/or pharmacological activities to particular components of the *A. herba-alba* essential oil and found that variations in oil composition were usually associated with substantial changes in activity, in particular the antimicrobial activity [12,13]. The antibacterial and the antispasmodic activity of *A. herba-alba* essential oil from various chemotypes has been examined [14]. The exhibited oil has also been reported to have an antileishmaniose activity [6].

This study deals with the valorization of medicinal and aromatic plants of the Tunisian flora, in order to find new bioactive natural products. Information concerning in vitro antioxidant activities of the essential oil from the *A. herba-alba* has not been reported earlier. The aim of this work is to provide more information on the chemical composition of the essential oil obtained from aerial part of *A. herba-alba* originated from southern Tunisia and investigate their antimicrobial and antioxidant activities.

2. Materials and methods

2.1. Chemicals and spectrophotometric measurements

DPPH and chemical standards were purchased from Sigma Aldrich Co. (St. Louis, USA). All other chemicals and reagents used were of analytical grade and were purchased from Carlo Erba Reagenti Spa (Starda Rivoltana, Italy), Merck (Darmstadt, Germany) and Kemika (Zagreb, Croatia). Spectrophotometric measurements were performed by UV-VIS spectrophotometer (Uv mini-1240) model SHIMADZU.

2.2. Plant material

Aerial parts of *A. herba-alba* were collected at the flowering stage in November 2007 from cultivated plants at the Institut des régions arides (Médenine, southern Tunisia). After drying, the big stems were discarded and the rest of samples (leaves and flowers) were used for the extraction of essential oil with a modified Clevenger-type apparatus for 4 h. The oil was collected, dried by anhydrous sodium sulfate and stored at 0 °C in tight vials until analysis.

2.3. GC and GC-MS identification

GC analysis was carried out using an Agilent 6890N Network GC system gas chromatograph fitted with flame ionization detector (FID) and an electronic integrator, using capillary columns (30 m × 0.32 mm i.d., film thickness 0.25 μm) HP-5 (5% phenyl methyl siloxane) and HP-INNOW.ax (polyethylene glycol) (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 50–280 °C at 7 °C/min; injector temperature: 220 °C; detector temperature: 240 °C; carrier gas: nitrogen (1.0 mL/min); sample manually injected: 0.2 μL. Retention indices (RI_s) were determined with C_6–C_{22} alkane standards as reference. Relative amount of individual components are based on peak areas obtained without FID response factor correction.

Essential oil constituents were also analyzed by GC-MS using the Agilent 6890N Network GC system combined with Agilent 5975 B Inert MSD detector (quadrupole) with electron impact ionization (70 eV). A HP-5-MS (5% phenyl methyl siloxane) column (30 m × 0.25 mm i.d., film thickness 0.25 μm) was used. The column temperature was programmed to rise from 50–280 °C at rate at 7 °C/min. The carrier gas was helium adjusted to a linear velocity of 34 cm/s. Scan time and mass range were 2.2 s and 50–550 m/z, respectively. Samples (0.1 μL) were injected with a split ratio of 1:100.

Identification of the components was based: (i) on comparison of their GC RI_s on apolar and polar column. RI_s were calculated with the help of a series of linear alkanes C_6–C_{22} on apolar and polar columns (HP-5 and HP-INNOW.ax); (ii) by comparison of their recorded mass spectra with those of a computer library (Wiley 275 library and NIST98 database/ChemStation data system) provided by the instrument software and MS literature data [15,16]; (iii) identities of some other components were further confirmed by co-injection of pure standards available in the laboratory under the same GC/MS conditions as above.

2.4. Antioxidant activity

2.4.1. Free radical scavenging ability by the use of a stable DPPH radical (2,2-diphenyl-l-1-picrylhydrazil)

Antiradical activity was evaluated by measuring the scavenging activity of *A. herba-alba* essential oils on the 2,2-diphenyl-l-1-picrylhydrazil (DPPH) radical, using the method described by Braca et al. [17] with slight modifications. The diluted essential oil solutions (0.5, 1, 2, 5, 10 and 20 mg/mL) were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 2 to 20 mg/mL. We prepared 0.004% DPPH in methanol. Then 1 mL of this solution was mixed with 1 mL of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using spectrophotometer against methanol. The blank was used as 1 mL of methanol with 1 mL of DPPH solution (0.004%).

All determinations were performed in triplicate. The optical density was recorded and percent of inhibition (*PI*) was calculated as follows: $PI\% = [(A_0 - A_t)/A_0] \times 100$, where

A_0 is optical density of the blank and A_t is optical density in the presence of essential oil extract.

2.4.2. Free radical scavenging ability by the use of a stable ABTS radical cation (2,2′ azinobis-3-ethylbenzthiazoline-6-sulphonic acid)

The free radical scavenging activity of the examined essential oils was determined by the method of ABTS radical cation decolorization assay described by Re et al. [18] with a minor modification. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{*+}$) was produced by reacting ABTS solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the study of essential oil samples, the $ABTS^{*+}$ solution was diluted with methanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 23 ± 2 °C. Reagent blank reading was taken (A_0). After addition of 1 mL of diluted $ABTS^{*+}$ solution ($A_{734\ nm} = 0.70 \pm 0.02$) to 20 mg of essential oil, the absorbance reading was taken 7 min after initial mixing (A_t). Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times. The *PI* of absorbance at 734 nm was calculated using above formula and decrease of the absorbance between A_0 and A_t. The free radical scavenging capacity of the *A. herba-alba* essential oil samples, calculated as percentage inhibition of $ABTS^{*+}$, was determined against a Trolox standard curve (0, 200, 400, 600, 800 and 1000 μmol/L). The activity was expressed in terms of μmol Trolox equivalent/g.

2.4.3. Bleachability of β-carotene in linoleic acid system

The antioxidant activity of the examined essential oils was evaluated using a β-carotene-linoleate model system following the method described by Wanasundara et al. [19] with slight modifications. A solution of β-carotene was prepared by dissolving 2.0 mg of β-carotene in 10 mL of chloroform. One millilitre of this solution was then pipetted into a round-bottom flask and 20 μL of purified linoleic acid and 200 mg of Tween 40 emulsifier were added. After the chloroform was rotary evaporated at 40 °C under vacuum, 50 mL of oxygenated water were added to the flask under vigorous shaking. Aliquots (5 mL) of this emulsion were transferred into a series of tubes containing 500 μL of each essential oil (2 mg/mL) or 2 mg BHA for comparative purposes. As soon as the emulsion was added to each tube, the zero time absorbance was read at 470 nm. Subsequent absorbance readings were recorded at 120 min by keeping the sample in a water bath at 50 °C until the visual color of β-carotene in the control sample disappeared. Antioxidant activities (Inhibition %) of the samples were calculated using the following equation:

$$\text{Inhibition}\,(\%) = \left(\frac{A_{\beta-\text{carotene after 2h assay}}}{A_{\text{initial}\,\beta-\text{carotene}}}\right) \times 100$$

where $A_{\beta\text{-carotene after 2 h assay}}$ is the absorbance of β-carotene after 2 h assay remaining in the samples and $A_{\text{initial}\ \beta\text{-carotene}}$ is the absorbance of β-carotene at the beginning of the experiments. All tests were carried out in triplicate.

2.5. Antmicrobial activity

2.5.1. Bacterial strains

For the determination of antibacterial activity of *A. herba-alba* essential oils, standard and isolated strains of the following Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NCIMB 8166; Gram-negative bacteria: *Escherichia coli* ATCC 35218, *Salmonella typhimirium* NRLB 4420, *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC29212 and yeasts: *Candida albicans*, *Candida glabrata*, *Candida sake* and *Candida tropicalis*, were used. The microorganisms were obtained from the culture collection of the "Laboratoire d'analyses, traitement et valorisation des polluants de l'environnement et des produits", Faculty of Pharmacy of Monastir, Tunisia.

2.5.2. Screening for antibacterial activity

Antimicrobial activity was tested by the agar-well diffusion method [20]. All bacterial cultures were first grown on MHI plates at 37 °C for 18–24 h prior to inoculation onto the nutrient agar. One or several colonies of similar morphology of the respective bacteria were transferred into API Suspension medium (*biomérieux*) and adjusted to 0.5 McFarland turbidity standard with a densimat (*bioMérieux*).

The inoculums of the respective bacteria were streaked onto MHI agar plates using a sterile swab. A sterile filter disc (diameter 6 mm, Whatman paper No. 3) was placed. The disc was impregnated by the tested essential oils (10 μL/disc). The treated Petri dishes were placed at 4 °C for 1–2 h and then incubated at 37 °C for 18–24 h. Antimicrobial activity was evaluated by measuring the zone of growth inhibition around the discs after 24 h of incubation at 37 °C. The standard discs (6 mm diameter) of the antibiotics Gentamycin (10 μg) were served as positive antibacterial control.

The diameter of the zones of inhibition around each of the discs was taken as measure of the antimicrobial activity. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

2.5.3. Screening for antifungal activity

The human pathogenic yeast used in this study was isolated from patients suffering from condidosis. These strains were isolated on Sabouraud chloramphenicol agar plates and identified with Api ID 32 C test strips (*biomérieux*) according to the Manufacturer's recommendations.

For screening the antifungal activity of *A. herba-alba* essential oils, the agar-disc diffusion method was used [21]. All *Candida* strains were first grown on a Sabouraud chloramphenical plate at 30 °C for 18–24 h prior to inoculation onto the nutrient agar. Several colonies of similar morphology of the clinical yeast were transferred into Api suspension medium (bioMérieux, Marcy l'Etoile, France) and adjusted to 2 McFarland turbidity standard with a densimat. The inoculum of the respective yeast was streaked onto Sabouraud chloramphenicol agar plates at 30 °C using a sterile swab and then dried. A sterile filter disc, diameter 6 mm (Whatman paper No. 3) was placed in

the plate at room temperature for 15 min. Ten microlitres of the essential oil were dropped on each paper disc (10 μL/disc). The treated Petri dishes were placed at 4 °C for 12 h and then incubated at 37 °C for 18–24 h. The antifungal activity was evaluated by measuring the diameter of the growth inhibition zone around the discs.

The susceptibility of the standard drug Amphoterecin B was determined using a disc paper containing 20 μg of Amphetorecin B. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

2.5.4. Micro-well determination of MIC and MBC

The minimal inhibition concentration (MIC) and the minimal bactericidal concentration (MBC) values were determined for all bacterial strains used in this study as described by Gulluce et al. [22]. The inoculums of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Each essential oil sample that was dissolved in 10% dimethylsulfoxide (DMSO), was first diluted to the highest concentration 10 mg/mL to be tested, and then serial two-fold dilutions were made in a concentration range from 0.009–10 mg/mL for oils in 5 mL sterile test tubes containing nutrient broth.

The 96-well plates were prepared by dispensing into each well 95 μL of nutrient broth and 5 μL of the inoculum. A 100 μL aliquot from the stock solutions of each essential oil was added into the first wells. Then, 100 μL from the serial dilutions were transferred into eleven consecutive wells. The last well containing 195 μL of nutrient broth without essential oil and 5 μL of the inoculum on each strip was used as the negative control. The final volume in each well was 200 μL. The plates were incubated at 37 °C for 18–24 h. The essential oil tested in this study was screened two times against each organism. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms. The MBC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no development of turbidity and without visible growth.

2.6. Statistical analysis

All data of antioxidant and antibacterial activities were expressed as mean ± SD. One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to determine significant differences ($P < 0.05$) between the means. The analyses were carried out using SPSS package software (Version 11.5).

3. Results and discussion

3.1. Chemical composition

Seventeen major volatile compounds accounting for > 68% of different oil types distilled from *A. herba-alba* plant species, collected from cultivated plant in southern Tunisia, are presented in Table 1.

Oil type I was characterized by high β-thujone content (58.0%). β-thujone has been reported as the major constituent of the *A. herba-alba* essential oil originating from Morroco [23], Algeria [1,24] and Israel [25]. Other *Artemsia* species with high β-thujone content such as *A. absinthium* oils extracted from plants grown in USA has been reported [26].

Oil type II was characterized by high α-thujone content (49.3%). β-thujone (15.0%) was the other component followed by *trans*-sabinyl acetate (7.6%), 1,8-cineole (4.5%) and camphor (3.9%). Like β-thujone, this α-thujone oil has being described in oils from Tunisia [9], Morocco [23], Algeria [1] and Israel [25].

β-thujone and α-thujone in oil type III were the main components presented with equivalent contents (24.3 and

Table 1
Artemisia herba-alba essential oils composition.

Compounds	Identification methods			Oil types			
	RI^a_{ap}	RI^a_p	Others	β-Thujone	α-Thujone	Thujones (α + β)	1-8,Cineole/Camphor/ Thujones (α + β)
α-Pinene	951	1024	A,B	1.1	0.8	1.4	2.2
Camphene	974	1056	A,B	1.6	1.0	1.1	1.4
Sabinene	990	1126	A	0.3	0.5	0.4	1.5
α-Terpinene	1018	1186	A,B	0.8	0.9	1.0	1.2
p-Cymene	1027	1261	A	1.0	0.9	1.8	1.7
1-8,Cineole	1035	1202	A,B	5.0	4.5	12.3	18.4
γ-Terpinene	1060	1261	A,B	0.6	0.6	0.8	2.0
α-Thujone	1111	1423	A,B	5.5	49.3	24.1	10.7
β-Thujone	1129	1443	A,B	58.0	15.0	24.3	14.1
Chrysanthenone	1143	1499	A	2.1	1.4	1.8	1.4
Camphor	1155	1510	A,B	5.4	3.9	7.5	10.8
trans-Pinocarveol	1168	1653	A	1.7	1.4	2.2	3.5
Borneol	1182	1709	A,B	1.7	1.4	2.6	2.5
Bornyl acetate	1294	1609	A,B	0.5	0.3	0.6	1.2
trans-Sabinyl acetate	1295	1830	A	3.4	7.6	3.8	7.8
trans-Jasmone	1396	1938	A	0.2	0.3	0.2	1.2
Germacrene D	1489	1718	A	0.8	0.8	1.1	1.3

RI^a_{ap} Retention indices relative to C_6–C_{22} n-alkanes calculated on apolar HP-5 capillary column.
RI^a_p Retention indices relative to C_6–C_{22} n-alkanes calculated on polar HP-INNOWax capillary column.
A: GC-MS; B: co-injection with authentic standard.

Table 2
Antioxidant capacity of *Artemisia herba-alba* essential oils.

	Artemisia herba-alba essential oils				Synthetic antioxidant		
	Type I	Type II	Type III	Type IV	Ascorbic acid	Rutin	BHA
DPPH (IC_{50} μg/mL DPPH solution)	8,552 ± 79[a]	17,961 ± 232[b]	8,236 ± 281[a]	18,036 ± 342[b]	6.93 ± 0.28	7.73 ± 0.11	6.20 ± 0.21
ABTS^{*+} (μmol Trolox Equivalent/g)	27.6 ± 1.3[a]	44.6 ± 1.3[b]	43.7 ± 1.0[b]	29.6 ± 0.9[a]	545 ± 3	1538 ± 44	-
Inhibition in linoleicacid system (%)	12.5 ± 2.1[b]	7.4 ± 2.2[a]	5.0 ± 0.3[a]	6.0 ± 0.5[a]	23.1 ± 2.7	54.1 ± 0.3	89.2 ± 0.5

Oil types: I: β-Thujone; II: α-Thujone; III: Thujones (α and β); IV: 1,8-Cineole, camphor and thujones (α and β).
Values are means ± standard deviation of three separate experiments.
Different letters in superscript indicate significant differences within oil types.

24.1%, respectively). This oil type was especially marked by the high 1,8-cineole content (12.3%) in addition to relatively high content of borneol (2.6%) and *trans*-pinocarveol (2.2%) compared to oil types I and II. β-thujone and α-thujone have been found to be the major constituents in some *A. herba-alba* oils from Tunisia [10,11] and Morocco [27].

The fourth oil type is not described in the literature, because this is the first time that such codominance of four main components of 1,8-cineole (18.4%), β-thujone (14.1%), camphor (10.8%) and α-thujone (10.7%) has been reported in *A. herba-alba* oils. Another marked differences between this oil type and the others cited in this study, were the relatively high contents of *trans*-pinocarveol (3.5%), α-pinene (2.2%) and γ-terpinene (2.0%). High contents of 1,8-cineole (21.5–27.6%) and camphor (15.9–37.3%) were found in oils of many species of *Artemisia* genus such as: *A. cana*, *A. frigida*, *A. longifolia* and *A. ludoviciana* [28].

3.2. Antioxidant activity

As shown in Table 2, *A. herba-alba* essential oils exhibited weak antioxidant abilities for preventing the linoleic acid oxidation and to reduce DPPH radicals and stable ABTS^{*+}.

In different test systems, when compared to ascorbic acid, BHA or rutin, all oil types were clearly less effective than these synthetic antioxidant agents. The dominance of non-phenolic compounds in the *A. herba-alba* oils examined may be related to this weak activity. Similar results were found with essential oils of other *Artemisia* species such as *A. absinthium*, *A. biennis*, *A. cana*, *A. dracunculus*, *A. frigida*, *A. longifolia* and *A. ludoviciana*, which are dominated by non-phenolic components [28].

3.3. Antimicrobial activity

The antimicrobial activities of *A. herba-alba* oils originating from southern Tunisia were evaluated by a paper disc diffusion method against tested bacteria and fungi. The results showed that the essential oils were active against the microorganisms assayed. Related to the inhibition of growth, significant differences were detected among these cited oil types, since all of them showed an interesting activity for all tested strains (Table 3). All bacteria strains showed less susceptibility to the *β* and *α*-thujone-rich oils of types I and II. Oil type III (with equilibrate amounts of thujones (α and β)) was the most active against *B. cereus* and *S. auerus* with the largest inhibition zone (22.7 ± 1.2 and 22.3 ± 1.2 mm, respectively). Oil type IV (marked by codominance of four main compo-

Table 3
Antibacterial and antifungal activity of *Artemisia herba-alba* essential oils against human pathogenic bacterial and yeast strains.

Microorganisms	Inhibition zone diameter (mm ± SD)				
	Type I	Type II	Type III	Type IV	Antibiotic
Gram-positive bacteria					Gen
S. aureus ATCC25923	17.7 ± 0.6[b]	13.3 ± 1.2[a]	22.3 ± 1.2[c]	13.7 ± 1.5[a]	32.7 ± 0.6[d]
M. luteus NCIMB 8166	14.3 ± 0.6[b]	10.0 ± 0.0[a]	14.3 ± 0.6[b]	14.3 ± 0.6[b]	27.7 ± 1.5[c]
B. cereus ATCC 11778	12.3 ± 0.6[a]	14.7 ± 0.6[ab]	22.7 ± 1.2[c]	12.3 ± 0.6[a]	24.0 ± 1.0[c]
E. faecalis ATCC29212	14.0 ± 0.6[a]	16.0 ± 1.0[ab]	16.7 ± 1.2[ab]	18.0 ± 1.7[b]	26.0 ± 1.0[c]
Gram-negative bacteria					
E. coli ATCC 35218	11.7 ± 1.5[a]	12.0 ± 0.0[a]	12.7 ± 1.5[a]	20.3 ± 1.0[b]	27.3 ± 0.6[c]
S. typhimirium NRLB 4420	12.3 ± 0.6[a]	13.0 ± 1.7[a]	17.3 ± 1.2[b]	12.3 ± 0.6[a]	21.0 ± 1.0[c]
Yeast strains					Am B
Candida albicans	14.3 ± 0.4[ab]	13.3 ± 0.6[a]	16.0 ± 0.0[b]	19.0 ± 1.0[c]	23.0 ± 1.0[d]
Candida glabrata	12.7 ± 0.9[ab]	10.7 ± 0.6[a]	14.0 ± 0.0[b]	18.7 ± 0.6	20.3 ± 0.6[d]
Candida sake	14.3 ± 0.4[a]	13.3 ± 0.6[a]	15.7 ± 0.4[b]	17.5 ± 1.0[c]	19.1 ± 0.6[c]
Candida tropicalis	13.3 ± 0.4[a]	15.7 ± 0.6[b]	16.3 ± 0.4[b]	22.3 ± 0.6[c]	24.0 ± 0.6[d]

SD: standard deviation; Am B: Amphotericin B (20 μg/disc); Gen: Gentamycin (10 μg/disc).
Oil types: I: β-Thujone; II: α-Thujone; III: Thujones (α and β), IV) 1,8-Cineole, camphor and thujones (α and β).
Values are means ± standard deviation of three separate experiments.
Different letters in superscript indicate significant differences within oil types and antibiotic.

Table 4
The MIC and MBC values (mg/mL) of the *Artemisia herba-alba* essential oils against 6 human pathogen bacteria strains tested in microdilution assay.

Bacteria species	Type I		Type II		Type III		Type IV		Antibiotic(Gent.)
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MBC (μg/ml)
S. aureus ATCC25923	0.625	5	0.625	2.5	0.156	1.25	0.156	1.25	15.62
M. luteus NCIMB 8166	1.25	5	0.625	2.5	0.156	>0.625	0.078	0.625	15.62
E. coli ATCC 35218	0.312	2.5	0.312	2.5	0.312	1.25	0.039	>0.625	>3.90
S. typhimirium NRLB 4420	0.312	1.25	0.156	1.25	0.078	0.625	0.039	0.625	15.62
B. cereus ATCC 11778	0.625	2.5	0.312	2.5	0.078	0.312	0.039	0.312	7.81
E. faecalis ATCC29212	0.156	2.5	0.312	1.25	0.039	>0.625	0.078	>0.312	7.81

Oil types: I: β-Thujone; II: α-Thujone; III: Thujones (α and β), IV) 1,8-Cineole, camphor and thujones (α and β).

nents 1,8-cineole, camphor, β-thujone and α-thujone) was the most active against *E. coli* and *S. typhimirium*.

As shown in Table 4, more precise data on the antibacterial properties were obtained through the determination of MICs and MBCs values. In fact, against all assayed bacteria, the major effectiveness was achieved by the essential oils from type IV with MIC ranged from 0.039 to 0.156 mg/mL, followed by type III (MIC ranged from 0.039 to 0.312 mg/mL) and types I and II (MIC ranged from 0.156 to 0.625 mg/mL). Types III and IV showed the lowest MBC of 0.312 mg/mL for *B. cereus* and the highest MBC of 1.250 mg/mL for *S. aureus* and *E. coli*.

All *A. herba-alba* oils exhibited an interesting antifungal activity against all human pathogenic yeast used in this study. The fourth oil type was the most active against the four *Candida* strains assayed. In fact, the antimicrobial activity of 10 μL of oil was comparable to antibiotic (20 μg/disc) for *C. sake* and *C. tropicalis*.

From our results, the variation in quantities of the main components e.g. camphor and 1,8-cineole, might be responsible for the different antimicrobial activity. Camphor was revealed to inhibit the growth of bacteria and fungi [29]. The percentage of camphor in the fourth oil type (10.8%) was higher than other oil types, which might be the potential reason that all tested microorganism are more sensitive to the oil. These results are in agreement with those reported in the literature for other *A. herba-alba* oil rich in camphor (14.2%) that showed a very strong action versus *S. aureus*, *C. tropicalis* and *C. albicans* [12]. Other *Artemisia* oils rich in camphor and 1,8-cineole were previously demonstrated to have potent antimicrobial activities in vitro [30].

4. Conclusion

The *A. herba-alba* essential oil marked by codominance of four main components such 1,8-cineole, camphor and thujones (α and β) is not described in the literature and seems characteristic of south Tunisia *A. herba-alba* oil. Markedly rich in non-phenolic compounds, *A. herba-alba* oils exhibited weak antioxidant abilities but are quite interesting from a pharmaceutical standpoint because of their antimicrobial properties. Indeed, the agar diffusion method indicated a strong activity of *A. herba-alba* essential oils against pathogenic microorganisms (fungi and bacteria species). In the same way the values of the MIC obtained are very significant. Given the commercial value of essential oils from *Artemisia*, these data suggest that *A. herba-alba* may be used as a source of commercialized essential oil in Tunisia and as to enhance the food safety as this oil has an antibacterial and antifungal activities.

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