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Full Length Research Paper

Chemical composition and biological activities of a new essential oil chemotype of Tunisian *Artemisia herba alba* Asso

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The aim of the present study was to investigate the chemical composition, antioxidant, angiotensin I-converting enzyme (ACE) inhibitory, antibacterial and antifungal activities of the essential oil of *Artemisia herba alba* Asso (Aha), a traditional medicinal plant widely growing in Tunisia. The essential oil from the air dried leaves and flowers of Aha were extracted by hydrodistillation and analyzed by GC and GC/MS. More than fifty compounds, out of which 48 were identified. The main chemical class of the oil was represented by oxygenated monoterpenes (50.53%). These were represented by 21 derivatives, among which the *cis*-chrysantenyl acetate (10.60%), the sabinyl acetate (9.13%) and the α-thujone (8.73%) were the principal compounds. Oxygenated sesquiterpenes, particularly arbusculones were identified in the essential oil at relatively high rates. The Aha essential oil was found to have an interesting antioxidant activity as evaluated by the 2,2-diphenyl-1-picrylhydrazyl and the β-carotene bleaching methods. The Aha essential oil also exhibited an inhibitory activity towards the ACE. The antimicrobial activities of Aha essential oil was evaluated against six bacterial strains and three fungal strains by the agar diffusion method and by determining the inhibition zone. The inhibition zones were in the range of 8-51 mm. The essential oil exhibited a strong growth inhibitory activity on all the studied fungi. Our findings demonstrated that Aha growing wild in South-Western of Tunisia seems to be a new chemotype and its essential oil might be a natural potential source for food preservation and for further investigation by developing new bioactive substances.

Key words: *Artemisia herba alba*, essential oil, chemical composition, antioxidant activity, angiotensin I-converting enzyme inhibitory, antimicrobial activity.

INTRODUCTION

The genus *Artemisia* (Asteraceae family) includes a variable number of species (from 200 to over 400, depending on the authors) found throughout the northern half of the world (Marco and Barberá, 1990). *Artemisia herba-alba* Asso (Aha) is a shrub growing wild in semi-arid or arid areas all around the Mediterranean basin. This plant is widespread from Southeastern Spain to Turkmenistan and Uzbekistan and in Near East and North Africa (Quezel and Santa, 1962; Nabli, 1989).

The chemical composition of *A. herba-alba* essential oil has been studied previously. In fact, analysis of Aha essential oil samples from Morocco established the existence of at least seven chemotypes (Ouyahya et al., 1990; Lawrence, 1993). Essential oil composition of this specie collected from various Algerian areas was used to

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characterize a chemotype different from those found in Morocco (Lawrence, 1995; Vernin et al., 1995). Furthermore, other chemotypes were identified from Israel and the Sinai Desert (Segal et al., 1987) and Egypt (El-Sayed and Seida, 1990). In Tunisia, *A. herba-alba*, known as "Chih" grows wild in the mountains around Jebel Oust in El fahs until the South of the country (Nabli, 1989).

Essential oil composition of *A. herba-alba* growing wild in some locations of Tunisia (semi-arid and arid land of Tunisia) was also investigated (Boukrich et al., 2010). In this previous study, various compositions were presented, dominated either by a single component (α-thujone, camphor, chrysanthenone or *trans*-sabinyl acetate) or characterized by the occurrence, at appreciable contents, of two or more of these compounds (Boukrich et al., 2010).

A. herba-alba is widely used in the folk medicine. For instance, it is one of the most frequently used plants to treat diabetes, bronchitis, diarrhea, neuralgias and hypertension (Tahraoui et al., 2007). The antibacterial, antileshmanial, anthelmintic and antispasmodic activities of *A. herba-alba* essential oil from various chemotypes have been reported (Yashphie et al., 1987; Hatimi et al., 2001).

Free radical oxidation of the lipid components in food due to the chain reaction of lipid peroxidation is a major strategic problem for food industry. Due to undesirable effects of oxidized lipids, it is essential to decrease lipid peroxidation products in food (Karpiska et al., 2001). Lipid oxidation can be effectively prevented by using synthetic antioxidants such as butyl hydroxyanisole (BHA). However, chemical compounds use has begun to be restricted because of their induction of DNA damage and their toxicity (Sasaki et al., 2002). The elimination of chemically synthesized additives from food is a current demand worldwide. However, essential oils are of great interest both in the industry and scientific research because of their antioxidant and antimicrobial properties, which make them useful as natural additives in foods (Pattnaik et al., 1997). A new approach to protect food from oxidation and/or to prevent the proliferation of microorganisms is the use of essential oils as preservatives.

The angiotensin I-converting enzyme (ACE, EC 3.4.15.1) is a zinc metallopeptidase that can increase blood pressure by converting the inactive decapeptide angiotensin I to the potent vasoconstrictor angiotensin II (octapeptide) and in the degradation of a hypotensive peptide, bradykinin (Cushman et al.,1982). This enzyme plays an important physiological role in the blood pressure control. Consequently, inhibition of ACE activity is considered to be a useful therapeutic approach. Many studies have been carried out to attempt synthetic ACE inhibitors, which are currently used in the treatment of hypertensive patients, but these substances may provoke diverse undesirable side-effects (Kapel et al., 2006). Therefore, research to find safety and economical ACE inhibitors is necessary for high blood pressure treatment.

Aware of the high level of chemical polymorphism observed in *A. herba-alba* oils from Northern Africa to Middle East, studies on this species didn't cover the entire domain where this plant is growing wild, especially in the South-Western of Tunisia (Gafsa City). Therefore, the aim of the present work is to study deeply the chemical composition of Tunisian South-Western essential oils in order to search for a new chemotype evidencing an interesting essential oil composition, not yet reported in the literature.

To the best of our knowledge, there is no available report on antioxidant and antihypertensive activities of *A. herba-alba* Asso essential oil growing wild. The *in vitro* antioxidant, angiotensin I-converting enzyme inhibitory, antimicrobial activities of the essential oil were carried out.

MATERIALS AND METHODS

Chemicals

The angiotensin I-converting enzyme (ACE) from rabbit lung, the ACE synthetic substrate hippuryl-L-histidyl-L-leucine (HHL), 2,2-diphenyl-1-picrylhydrazyl (DPPH), butyl- hydroxyanisole (BHA), β-carotene, linoleic acid and Tween 40 were purchased from Sigma Chemical (St. Louis, USA). All culture media were from Bio-Rad (France), and the other chemicals used were from analytical grade.

Plant material

The aerial parts of *A. herba-alba* Asso were collected from Gafsa (Ayaycha mountain), a city in the South-Western of Tunisia (coordinates; UTM: latitude 34° 21' 05'' N, longitude 09° 23' 32'' E; Elevation: 192 m with an arid climate characterized by a mean rainfall of 150 mm/year) at the flowering stage (January 2009). After been harvested, the fresh vegetable matter was first weighted and then dried on the shadow, until weight stability (20 days). Then the leaves and flowers, were separated from stems.

Essential oil extraction

Air-dried leaves and flowers were submitted to hydrodistillation for 4 h, using a Clevenger type apparatus. The oil yield was expressed v/w vs. dry matter. The essential oil was dried over anhydrous sodium sulphate, filtered and stored in a sealed vial in the dark at +4 °C before analysis and bioassays tests.

Essential oil analysis

Gas chromatography (GC)

A Hewlett - Packard 5890 series II gas chromatograph equipped with HP-5MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm; Hewlett-Packard) and connected to a flame ionization detector (FID) was used. The column temperature was programmed at 50°C for 1 min, then 7°C/min to 250°C, and then left at 250°C for 5 min. The injection port temperature was 240°C and that of the detector 250°C, split: 1/60. The carrier gas was helium (99.995% purity) at a constant flow of 1.2 ml/min. The analysis was performed on 2 µl volume. Percentages of the constituents were calculated by electronic integration of FID peak areas, without the use of response factor correction. Retention indices (RI) were calculated for separate compounds relative to C_8-C_{17} n-alkanes

mixture (Aldrich Library of Chemicals Standards) (Kovàts, 1958).

Gas chromatography/Mass spectrometry (GC/MS)

The isolated volatile compounds were analysed by GC/MS, using a Hewlett-Packard 5890 series II gas chromatograph. The fused HP-5MS capillary column (the same as that used in the GC analysis) was coupled to a HP 5972A masse-selective detector (Hewlett-Packard, Palo Alto, CA, USA). The oven temperature was programmed at 50°C for 1 min, then 7°C/min to 250°C, and then left at 250°C for 5 min. The injector port temperature was held at 250°C, split: 1/100, the temperature of the detector was set at 280°C. The carrier gas was helium (99.995% purity); with a flow rate of 1.2 ml/min and the analysed sample volume was 2 µl. The mass spectrometer (MS) conditions were as follow: ionization voltage, 70 eV; ion source temperature, 150°C; electron ionization mass spectra were acquired over the mass range 50 - 550 m/z.

Identification of essential oil compounds

The essential oil compounds were identified by comparing the mass spectra data with spectra available from the Wiley 275 mass spectra libraries (software, D.03.00). Further identification confirmations were made referring to retention indices (RI) data generated from a series of known standards of n-alkanes mixture (C_8-C_{17}) (Kovàts, 1958) and to those previously reported in the literature (Adams, 2001; Asuming et al., 2005; Ferhat et al., 2007; Jalali-Heravi et al., 2006;Lopes-Lutz et al., 2008; Pourmortazavi et al., 2005; Salido et al., 2004; Saroglou et al., 2006; Vujisic et al., 2006; Vagionas et al., 2007).

Antioxidant activity

All determinations were performed in triplicate. Sample concentration providing 50% inhibition (IC50) was calculated from the graph plotting inhibition percentage against oil concentration.

Determination of antioxidant activity using β-carotene/linoleic acid assay

In this assay, oxidation of linoleic acid produces many oxidation products (lipid hydroperoxides, conjugated dienes, and volatile by-products) which attack the chromophore of β-carotene, resulting in bleaching of its characteristic color. However, the presence of antioxidant inhibits the β-carotene bleaching by the linoleic oxidation formed products. The ability of *A. herba-alba* essential oil to prevent the bleaching of β-carotene was carried out as described by Koleva et al. (2002). An emulsion of β-carotene/linoleic acid was prepared as follows: 0.5 mg of β-carotene in 1 ml of chloroform was mixed with 25 µl of linoleic acid and 200 µl of Tween 40. The chloroform was evaporated under vacuum using a rotary evaporator at 40°C. Then, 100 ml of distilled water was added and the resulting mixture was vigorously stirred. Aliquots (2.5 ml) of the β-carotene/linoleic acid emulsion (freshly prepared before each experiment) were transferred to test tubes containing different essential oil concentrations (0.1 to 1 mg/ml) and these were incubated for 2 h at 50°C. The same procedure was repeated with BHA used as positive standard. Finally, the absorbance of the mixtures was measured at 470 nm, and the relative antioxidant activity was calculated according to the formula:

$$\text{Antioxidant activity}(\%)=\left[1-\frac{A_0-A_t}{A_{00}-A_{0t}}\right]\times 100$$

where, A_0 was the absorbance at the initial incubation time with the essential oil; A_t was the absorbance after incubation with the essential oil; A_{00} was the absorbance at the initial incubation time without the essential oil and A_{0t} is the absorbance after incubation without the essential oil. Sample absorbances were read against a blank containing the β-carotene/linoleic acid emulsion.

Determination of DPPH radical - scavenging activity

The *A. herba-alba* essential oil antioxidant activity was measured in terms of hydrogen - donating or radical-scavenging ability, using the stable DPPH as reagent, according to the method described by Kirby and Schmidt (1997). A volume of 500 µl of diluted essential oil at different concentrations (0.1 to 1 mg/ml) was added to 375 µl of methanol and 125 µl of DPPH solution (0.2 mM in methanol) as free radical source. Absorbance was measured at 517 nm after 60 min of incubation time at room temperature and in the dark. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control and BHA was used as positive control. Scavenging activity was measured by monitoring the decrease in absorbance at 517 nm. In its radical form, DPPH has an absorption band at 517 nm which disappeared upon reduction by an antiradical compound. Low absorbance of the reaction mixture indicated high free radical-scavenging activity. The percentage inhibition of the DPPH radical (scavenging activity) was calculated according to the formula:

$$\text{Scavenging activity}\ (\%)=\left[\frac{\text{Ac - As}}{\text{Ac}}\right]\times 100$$

where, Ac was the control reaction absorbance and As was the Aha essential oil absorbance.

Determination of the angiotensin I-converting enzyme (ACE) inhibition activity

The ACE inhibition activity was assessed as reported by Nakamura et al. (1995). A volume of 80 µl containing different concentrations (50, 100 or 150 µg/ml) of *A. herba-alba* essential oil was added to 200 µl of 5 mM hippuryl-L-histidyl-L-leucine (HHL), and pre - incubated for 3 min at 37°C. *A. herba-alba* essential oil and HHL were prepared in 100 mM borate buffer, 300 mM NaCl, pH 8.3. Then, the reaction was initiated by adding 20 µl of 0.1 U/ml ACE dissolved in the same buffer and incubated for 30 min at 37°C. The enzyme reaction was stopped by the addition of 250 µl of 0.1 M HCl. Then, the released hippuric acid (HA) was extracted by 1.7 ml ethyl acetate and mixture was homogened using vortex for 15 s. One ml of the upper layer was transferred into a glass tube and evaporated at 90°C for 15 min. Finally, the released HA was redissolved in 1 ml of distilled water and the absorbance was measured at 228 nm using spectrophotometer (T70, UV/VIS spectrometer, PG Instruments Ltd, China). For each concentration, the average value from three determinations was used to calculate the ACE inhibition rate according to the following formula:

$$\text{ACE inhibition}(\%)=\left[\frac{\text{B - A}}{\text{B - C}}\right]\times 100$$

where A was the absorbance of HA generated in the presence of ACE inhibitor component; B was the absorbance of HA generated without ACE inhibitor and C was the absorbance of HA generated

without ACE (corresponding to HHL autolysis in the course of enzymatic assay). All determinations were performed in triplicate.

Antimicrobial activity

Microbial strains

Antibacterial activities of *A. herba-alba* essential oil were tested against 6 strains of bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883), *Bacillus cereus* (ATCC, 11778), *Enterococcus faecalis* (ATCC, 29212) and *Salmonella typhimurium* (NRRLB, 4420). Antifungal activities were tested using *Fusarium solani*, *Fusarium sp* and *Aspergillus oxysporum*. Microorganisms were obtained from the culture collection of the Centre of Biotechnology in Sfax, Tunisia).

Agar diffusion method

Antibacterial activities of *A. herba-alba* essential oil were assessed using the paper disk agar diffusion method according to Freney et al. (2002). Culture suspension (200 µl) of the tested bacteria (2 x 10^6 CFU/ml) was spread on the Mueller-Hinton broth medium. For fungal strains, spore suspension (200 µl) containing 10^8 spores/ml was spread on the potato dextrose agar medium. Then, absorbent disk (Whatman disk No. 3 of 6 mm diameter) containing 10 µl of essential oil were applied on the surface of the plate (90 mm) inoculated with different microbial strains. Negative control was prepared using a disk impregnated with sterile water. After that, the Petri dishes were kept for 1h at 4°C before incubation at 37°C for 24 h (bacteria strains) or at 30°C for 72 h (fungal strains). Finally, antimicrobial activity was evaluated by measuring the diameter (mm) of the growth inhibition zones including the 6 mm disk. Where there was no inhibition, the value 0 mm was assigned to the tested sample. The measurements of inhibition zones were carried out for three sample replications and values were the average of three replicates.

RESULTS AND DISCUSSION

Chemical composition of the *A. herba-alba* essential oil

A. herba-alba air dried leaves and flowers were subjected to hydrodistillation using a Clevenger apparatus and the yellow-colored essential oil obtained (yield 1.45% v/w) was analyzed by GC and GC/MS techniques. The percentages and the retention indices of the identified components are listed in Table 1 in the order of their elution on the HP-5MS column. From the data obtained, more than fifty compounds, out of which 48 were identified, accounting for 83.13% of the oil. *Cis*- chrysantenyl acetate (10.60%), sabinyl acetate (9.13%), α-thujone (8.73%), davana ether (6.16%) and chrysantenone (4.94%) were the most abundant components, constituting 39.56% of the total oil, followed by spathulenol (3.57%), *cis*-arbusculone (3.56%), *trans*-arbusculone (2.95%), 1,8-cineole (3.00%), isopinocarveol (2.62%) and β-thujone (2.57%). Others components were present in amounts less than 2.5%.

The chemical classes' distributions of *A. herba alba* essential oil could be separated into five classes (Table 1). These were monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and others. Monoterpenes represented about 55.8% of the total essential oil constituents. Oxygenated monoterpenes constituted the main chemical class of the oil (50.53%) and they were represented by 21 derivatives, with *cis*-chrysantenyl acetate (10.60%), sabinyl acetate (9.13%) and α-thujone (8.73%) as the principal components. Moreover, monoterpene hydrocarbons were represented by 8 compounds accounting for 5.27% of all the oil. Among these compounds, α-pinene (1.45%) and p-cymene (1.03%) were the most important. Sesquiterpenes constituted 18.7% of all the oil. Among them, hydrocarbons (3 compounds) represented only 3.06%, whereas oxygenated derivatives (8 compounds) reached 15.65% of all the oil. Among sesquiterpenes hydrocarbons, germacrene D (1.8%) and bicyclogermacrene (1.03%) were the main ones.

For oxygenated sesquiterpenes, the principal one was davana ether (6.16%). Thus, the isolated essential oil was characte-rized by large amounts of oxygenated monoterpenes, namely, *cis*-chrysantenyl acetate (10.60%), sabinyl acetate (9.13%), α-thujone (8.73%) and chrysantenone (4.94%), oxygenated sesquiterpenes (davana ether (6.16%) and spathulenol (3.57%)) and others oxygenated components, namely, *cis*-arbusculone (3.56%) and *trans*-arbusculone (2.95%). These arbusculones (*cis* and *trans*) were identified for the first time in the essential oil of *A. herba-alba* growing wild in Tunisia. A survey of the literature demonstrated that these arbusculones com-pounds have been detected only in the *A. herba alba* essential oil of Spanish origin at relatively low rates which did not exceed 1% (Salido et al., 2004). Among oxygenated sesquiterpene, spathulenol and davana ether were also found to be minor constituents in an oil of Spanish origin (Salido et al., 2004) but they were identified in the studied oil at a relatively high rates.

For further comparison, the composition of *A. herba alba* essential oil dominated by thujones was found in Morocco (Benjilali and Richard, 1980; Lamiri et al., 1997) and Algeria (Boutekedjiret et al., 1992; Lawrence, 1995; Vernin et al., 1995). Camphor-type oils were reported in Spain (Feuerstein et al., 1986), Morocco (Lamiri et al., 1997), Algeria (Boutekedjiret et al., 1992; Vernin et al., 1995) and Egypt (El-Sayed and Seida, 1990). Chrysanthenone was reported as an important component component in essential oil hydrodistillated from Spanish (Feuerstein et al., 1988), Moroccan (Benjilali and Richard, 1980; Lawrence, 1993) and Algerian species (Boutekedjiret et al., 1992; Lawrence, 1993; Vernin et al., 1995). *Cis*-chrysanthenyl acetate was found to be the major component in some oils from Morocco (Lawrence, 1993; Benjilali and Richard, 1980), Israel (Yashphie et al., 1987; Feuerstein et al., 1986) and Algeria

Table 1. Chemical Composition of *A. herba-alba* essential oil (Gafsa city, South-Western of Tunisia).

Compounds[a]	(%)[b]	RI[c]
α-Pinene	1.45	940
Camphene	0.57	954
2(5H)-Furanone, 5,5-dimethyl	0.24	960
Sabinene	0.37	979
β-Pinene	0.2	982
α-Terpinene	0.46	1022
p-Cymene	1.03	1031
1,8-Cineole	3	1037
cis-Arbusculone	3.56	1058
γ-terpinene	0.87	1064
trans-Artbusculone	2.95	1077
Terpinolene	0.32	1093
Linalool	0.28	1107
Filifolone	1.04	1109
α-Thujone	8.73	1114
β-Thujone	2.57	1124
Chrysanthenone	4.94	1132
Iso-Pinocarveol	2.62	1148
Camphor	1.42	1152
Unidentified	1.11	1170
Borneol	0.85	1176
4-Terpineol	1.93	1186
Myrtenal	0.27	1190
α-Terpineol	0.25	1200
cis-Piperitol	0.49	1203
Verbenone	0.41	1217
Nordavanone	0.3	1235
Methyl thymyl ether	0.19	1239
Cuminal	0.54	1248
Piperitone	0.25	1263
cis-Chrysantenyl acetate	10.6	1267
Bornyl acetate	0.37	1291
Sabinyl acetate	9.13	1298
Nor-chrysanthemic acid methyl ester	0.65	1359
Unidentified	2.2	1402
cis-Jasmone	0.68	1406
Davana furan	0.17	1419
β-Caryophyllene	0.23	1429
1-methoxy-4-nitro benzene	0.72	1463
Germacrene D	1.8	1491
Davana ether (isomer)	1.61	1496
Bicyclogermacrene	1.03	1507
Davana ether (isomer)	6.16	1517
Davana ether (isomer)	1.67	1535
Caryophyllene oxide	0.28	1565
Nerolidol	0.66	1570
Spathulenol	3.57	1593
Viridiflorol	1.05	1608
Unidentified	1.44	1637
Unidentified	1.56	1647
Unidentified	2.14	1653

Table 1. Contd.

α-Cadinol	0.65	1669
Unidentified	1.48	1683
Total	93.06	
Grouped components (%)		
Monoterpene hydrocarbons	5.27	
Oxygenated monoterpenes	50.53	
Sesquiterpenes hydrocarbons	3.06	
Oxygenated sesquiterpenes	15.65	
Others	8.62	
Unidentified	9.93	

[a]Compounds are listed in order of their elution from a HP 5MS column.
[b]Percentages obtained by FID peak-area normalization.
[c]Retention indices calculated against (C_8-C_{17}) *n*-alkanes on the HP 5MS column.

(Boutekedjiret et al., 1992). More recently, the chemical variability of *A. herba-alba* Asso, observed in Tunisian areas (North-Western, Center and South-Eastern of Tunisia) showed various compositions dominated either by a single component (α-thujone, camphor, chrysanthenone or *trans*-sabinyl acetate) or characterized by the occurrence, of two or more of these compounds at appreciable contents (Boukrich et al., 2009). By contrast, the present composition of the essential oil, with *cis*-chrysanthenyl acetate (10.60%) as the most abundant component, was considered as a new chemotype of *A. herba alba* growing wild in South-Western of Tunisia.

Antioxidant activity

To the best of our knowledge, there are no available reports on antioxidant activity of the essential oil from *A. herba-alba* growing wild. The potential antioxidant activity of the *A. herba alba* essential oil was determined on the basis of the inhibition of the oxidation of the coupled linoleic acid/β-carotene and scavenging activity of the free stable radical (DPPH).

In fact, the response of antioxidants depends on many factors and the antioxidant activity of a system can be better characterized by using different complementary assays based on different mechanisms (Moure et al., 2006).

β-carotene bleaching by linoleic acid assay

A. herba alba essential oil was efficient to inhibiting the oxidation of linoleic acid, which is an important issue in food processing and preservation (Figure 1). The antioxidant activity of *A. herba alba* essential oil increased with essential oil concentration. The IC50 was found to be around 0.2 mg/ml. Interestingly, *A. herba alba* essential oil antioxidant activity was more effective than that of four cultivated *A. herba alba* species. Indeed, these antioxidant activities at 2 mg/ml were lower than 12.5% (Mighri et al., 2009). Such weak antioxidant activity was related to the dominance of non-phenolic compounds in cultivated *A. herba alba* essential oils. Moreover, the studied *A. herba alba* essential oil antioxidant activity was also higher than those exhibited by other *Artemisia* species essential oils measured by the same test. In fact, antioxidant activity of essential oils at 1 mg/ml from *A. absinthium, A. biennis, A. cana, A. dracunculus, A. frigida, A. longifolia* and *A. ludoviciana* were lower than 22% (Lopez-Lutz et al., 2008).

DPPH free radical scavenging activity

Free radical scavenging activity of the *A. herba alba* essential oil, measured by DPPH assay are presented in Figure 2. These results showed that *A. herba alba* essential oil was able to reduce the stable free radical DPPH with an IC50 of 0.14 mg/ml, whereas that of the synthetic antioxidant BHA was 11 µg/ml. *A. herba alba* essential oil showed higher radical scavenging activity than those exhibited by other *Artemisia* species essential oils measured by the same test. In fact, DPPH radical scavenging activity of essential oils at 1mg/ml from *A. absinthium, A. biennis, A. cana, A. dracunculus, A. frigida, A. longifolia* and *A. ludoviciana* were lower than 15% (Lopez-Lutz et al., 2008).

Table 1 showed that essential oil of *A. herba alba* was markedly rich in oxygenated terpenes which may act as radical scavenging agents. It seems to be a general trend that the essential oils which contain oxygenated monoterpenes and/or sesquiterpenes have greater antioxidative properties (Tepe et al., 2004). Furthermore, some researchers showed that some essential oils poor in phenolic compounds also may have antioxidant potentials (El-Massry et al., 2002).

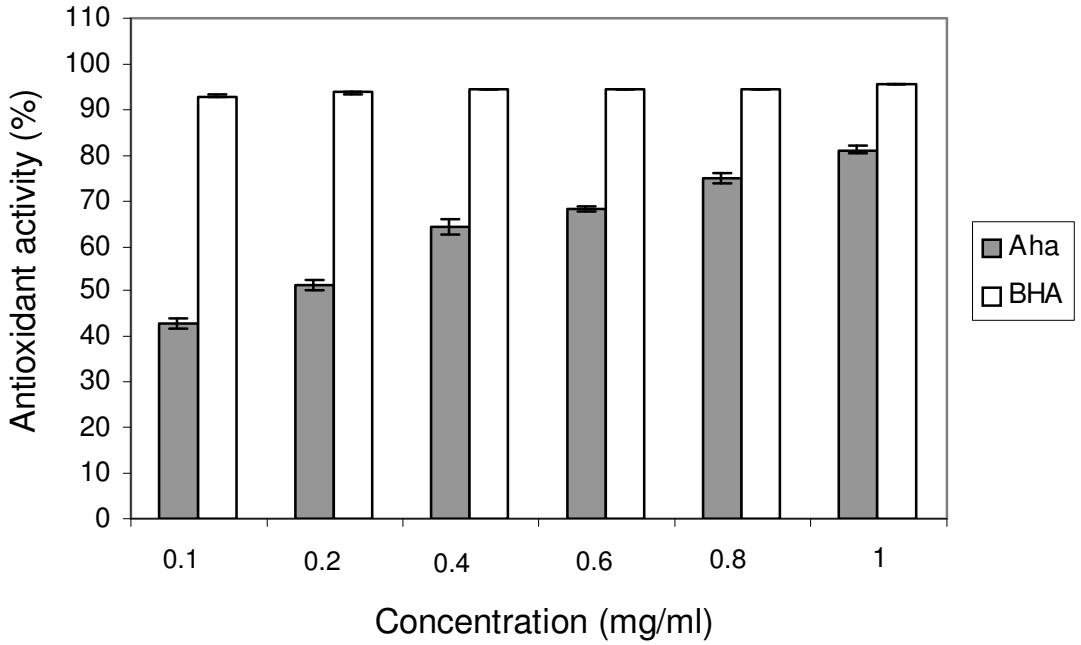

Figure 1. Antioxidant activities (%) of *A. herba alba* essential oil and BHA as positive control, measured by β-carotene bleaching assay.

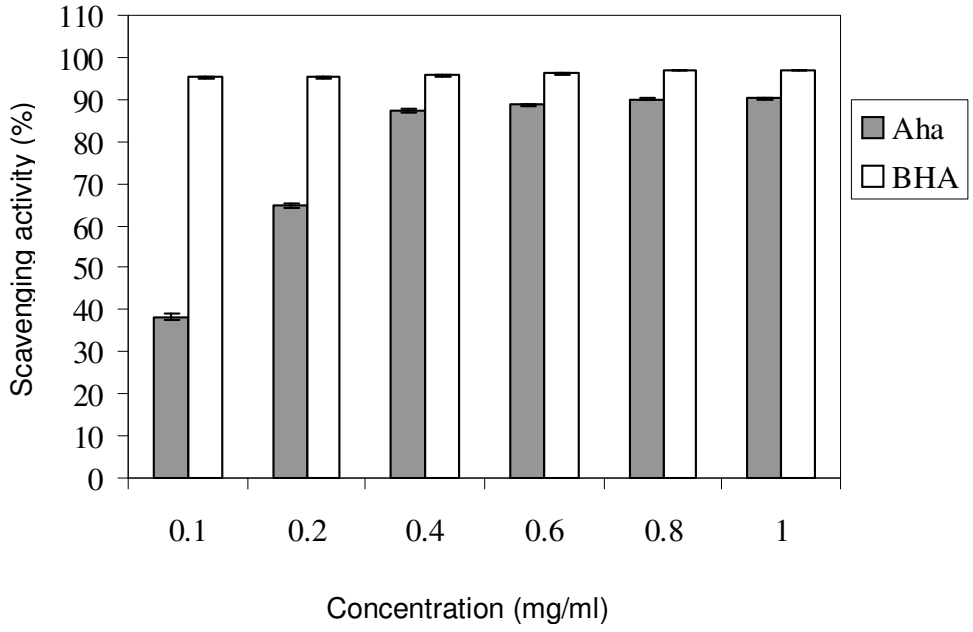

Figure 2. Free radical-scavenging capacities (%) of *A. herba alba* essential oil and BHA as positive control measured in DPPH assay.

ACE inhibitory activity

Because of the use of *A. herba-alba* growing wild in south Tunisia in folk medicine to treat hypertension (Tahraoui et al., 2007), the ACE inhibitory activity of *A. herba-alba* essential oil was investigated. Our results showed that 100 µg/ml of *A. herba-alba* essential oil exhibited *in vitro* ACE inhibitory activity of 25.4% (Figure 3). It was demonstrated that the ACE inhibition is considered to be a useful therapeutic approach in the treatment of high

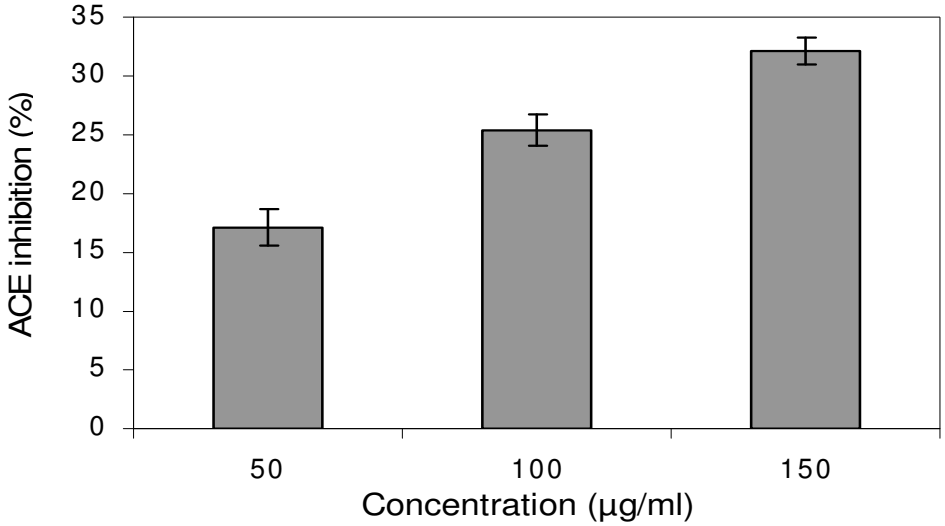

Figure 3. Angiotensin I-converting enzyme inhibitory effect of *A. herba alba* essential oil.

blood pressure. Since synthetic ACE inhibitors may cause adverse side-effects, plants essential oils could be used as natural and economical ACE inhibitors for hypertension prevention and remedy. Several chemical classes of ACE inhibitors compounds derived from plant extracts have been described such as tannins (Ottaviani et al., 2006), flavonoids (Loizzo et al., 2007) and peptides (Matsui et al., 2000). Furthermore, Castro Braga et al. (2000) reported a 20% ACE inhibition by the dichloromethane/methanol extract of *Cecropia glaziovii*. The flavonoids vitexin and isovitexin at 0.33 mg/ml isolated from the plants inhibited the ACE activity by 20% and 45%, respectively (Lacaille - Dubois et al., 2001). The antocyanins delphinidin- and cyaniding-3-*O*-sambubiosides were isolated from *Hibiscus sabdariffa* and they exhibited a 50% ACE inhibition at 84.5 and 68.4 µg/ml, respectively, (Ojeda et al., 2008). These compounds were suggested to inhibit the ACE activity by competing the enzyme active site. Some authors suggested that the inhibitory effect of flavonoids was due to the formation of chelate complexes with the zinc atom within the active site of zinc - dependant metallopeptidase (Chen et al., 1992).

Antimicrobial activity

The antimicrobial activities of *A. herba alba* essential oil was evaluated against six bacterial strains and three fungal strains. Table 2 showed that this oil had variable antimicrobial activity against all tested strains. The inhibition zones were in the range of 8-51 mm. Gram-positive bacteria were shown to be more sensitive to the *A. herba alba* essential oil. The most susceptible bacterium for *A. herba alba* essential oil was *B. cereus* (23 mm). The growth of Gram-negative bacteria (*K. pneumoniae, E. coli* and *S. typhimurium*) was partially inhibited. Furthermore, the oil was ineffective against *P. aeruginosa*. In fact, *P. aeruginosa* is known to have a high level of intrinsic resistance to many antimicrobials and antibiotics, due to a very restrictive outer membrane barrier (Mann et al., 2000). Moreover, most of the studies investigating the action of essential oils against food spoilage organisms agreed that, essential oils are slightly more active against Gram-positive than Gram-negative bacteria (Lambert et al., 2001). Under the same experimental conditions, 10 µl of *A. herba alba essential oil* had a strong inhibitory effect on the growth of all the studied fungi. *A. oxysporum* was the most sensitive fungal strain (51 mm).

The antimicrobial activity of *A. herba alba* essential oil would be related to its oxygenated monoterpenes components which constitute about 50.53% of the oil. Indeed, in essential oils, it was shown that monoterpenes hydrocarbons and oxygenated monoterpenes in essential

Table 2. Antimicrobial activity of *A. herba alba* essential oil.

Microorganism	Inhibition zone[a] (mm)
S. typhimurium	11.0±1.0
E. coli	11.3±0.6
K. pneumoniae	8.7±0.6
P. aeruginosa	No activity
E. faecalis	15.0±1.0
B. cereus	23.0±1.0
F. sp	27.0±1.6
F. solani	27.7±2.5
A. oxysporum	51.0±5.0

[a]Values represent averages ± standard deviations for triplicate experiments.

oils are able to destroy cellular integrity resulting in respiration inhibition and permeability alteration (Cox et al., 2000). However, it is difficult to attribute the activity of a complex mixture to a single or particular constituent. Major or trace compounds might give rise to the antimicrobial activity exhibited. In the oil, the possible compounds synergistic and antagonistic effects would play an important role in microbial inhibition and should also be taken into consideration.

Conclusion

This work allowed the identification of a new chemotype of *A. herba alba* Asso growing wild in South-Western of Tunisia and confirmed the tremendous chemical variability of the Tunisian *A. herba alba*. The arbusculones found at relatively high rates was described for the first time in the essential oil of Tunisian *A. herba alba* growing wild. Our results indicated also that this essential oil had multifunctional properties. The *A. herba alba* had a strong antifungal and antioxidant activities. Furthermore, the studied species had a higher antioxidant activity than the cultivated *A. herba alba* or other related *Artemisia* species. Interestingly, the *A. herba alba* essential oil was able to inhibit the angiotensin I-converting enzyme. These properties would be interesting from a pharmaceutical point of view because of antifungal, antioxidant and angiotensin I-converting inhibitory activities. Moreover, the *A. herba alba* oil has a characteristic flavour presenting strong sensory properties and thus could be also suitable for uses as antioxidant and flavouring agent in the food industry.

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