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*Full Length Research Paper*

# Chemical constituents and antioxidant activity of the essential oil from aerial parts of *Artemisia herba-alba* grown in Tunisian semi-arid region

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**Essential oils and their components are becoming increasingly popular as naturally occurring antioxidant agents. In this work, the composition of essential oil in *Artemisia herba-alba* from southwest Tunisia, obtained by hydrodistillation was determined by GC/MS. Eighteen compounds were identified with the main constituents namely, α-thujone (24.88%), germacrene D (14.48%), camphor (10.81%), 1,8-cineole (8.91%) and β-thujone (8.32%). The oil was screened for its antioxidant activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, *β*-carotene bleaching and reducing power assays. The essential oil of *A. herba-alba* exhibited a good antioxidant activity with all assays with dose dependent manner and can be attributed to its presence in the oil.**

**Key words:** *Artemisia herba alba*, essential oil, chemical composition, antioxidant activity.

## INTRODUCTION

Natural products and naturally derived components from plants have been applied in controlling toxicity, preventing food spoilage and deterioration and also for extending shelf life of foods. In recent years, increasing attention has been paid to the exploration of naturally-occurring antioxidants and antimicrobials because of the growing consumer demand for food products free from synthetic chemical additives. In this area, the Mediterranean region has attracted special interest because of its remarkable diversity and it constitutes a reservoir for the production of medicinal plants.

*Artemisia herba-alba* is a greenish-silver perennial herb, grows 20 to 40 cm in height and belongs to the daisy family Asteraceae. The vegetative growth of this plant takes place in the autumn; the flowering starts from September to December and basically develops at the end of the summer with many basal, erect and leafy stems covered by woolly hairs. The genus may be divided into sections *Artemisia* and *Dracunculus* (Tutin et al., 1976), the former including the species *A. herba-alba Asso*. which is a medicinal and strongly aromatic dwarf shrub that grows wild in arid areas of the Mediterranean basin extending into northwestern Himalayas. In Tunisia, this plant commonly known as the white wormwood in arbaic as "Chih" and in France as "Armoise balnche" (Segal et al., 1987), is one of five spontaneous *Artemisia* species that were identified (Nabli, 1989) and was used as aromatisant for tea. In folk medicine, was known for its therapeutic and medicinal properties, was used for treatment of colds, coughing, intestinal disturbances, as antidiabetic agent (Bailey and Danin, 1981; Jouad et al.,

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**Abbreviations: DPPH,** 2,2-Diphenyl-1-picrylhydrazyl; **GC/MS,** gas chromatography/mass spectrometry.

2001), for bronchitis, diarrhea, neuralgias and hypertension (Tantaoui-Elaraki et al., 1994). Many researchers have reported various biological and/or pharmacological activities of *A. herba-alba* essential oil as an antibacterial (bacteria and fungi), antileshmanial, anthelmintic and antispasmodic agent (Yashphe et al., 1987).

Considerable work on the composition of the essential oils of *A. herba-alba is* reported in literature. In Spain (Feuerstein et al., 1988; Salido et al., 2004), the most abundant skeletons were monoterpene hydrocarbons and oxygenated monoterpenes, with large amounts of sesquiterpenes. The major constituent found were camphor, 1,8-cineole, *p*-cymene and davanone. In Jordan, regular monoterpenes were predominant and the principal components were α- and β-thujones, classifying the plant as being a thujone chemotype (Hudaib and Aburjai, 2006). For Egyptian chemotype, artemisia ketone was reported as the principal component (El-Sayedand and Seida, 1990), while a French type (Hurabielle et al., 1981) was found to predominate in 1,8-cineole, camphor and chrysanthenone. Essential oil from Israel and Sinai (Feuerstein et al., 1986) showed two types of oil, cineol-thujane bornane type and the pinane type with monoterpene skeletons. For Algerian oil, camphor, α- and β-thujones, 1,8-cineole and chrysanthenyl derivatives were the major components (Vernin and Merad, 1994; Vernin and Parkanyi, 2001). In Tunisian oil, oxygenated monoterpenes were found to be the major components of *A. herba-alba* oil extracted from aerial parts of plants originated from arid regions (Akrout, 2004; Neffati et al., 2008, Mighri et al., 2010). In some studies, the main components were cineole, thujones, chrysanthenone, camphor, borneol, chrysanthenyl acetate, sabinyl acetate, davana ethers and davanone. Monoterpenes, sesquiterpenes are found as the major components (Haouari and Ferchichi, 2009). In Morocco, 16 chemotypes of *A. herba-alba* essential oil were found and the oil was characterized by the presence of ketones such as α- and β-thujones and camphor (Cohen et al., 1972; Benjilali and Richard, 1980; Ouyahya, 1990).

In recent years, there has been increasing attention in the search of natural antioxidants from plants because they can protect the human body from the attack of free radicals, and retard the progress of many chronic diseases as well as retarding the lipid-oxidative rancidity in foods. Many medicinal plants contain large amounts of antioxidant compounds, which could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders. The appearance of foods is one of the major determinants of its appeal to consumers, consequently, lipid oxidation is one of the main factor that determine food quality loss and shelf-life reduction. Therefore, delaying lipid oxidation is highly relevant to food processors. In fact, oxidative processes in food products lead to the degradation of lipids and proteins which, in turn, contribute to the deterioration in flavour, texture and colour of the products. In this context, plant-exhibit numerous biological activities and some of them can be employed as food additives and can be evaluated for their antioxidant properties as food preservatives (Peschel et al., 2006).

In this work, we studied the chemical composition and the antioxidant activity of *A. herba-alba* essential oils collected in the southwest of mountainous region from Tunisia, where people frequently use this plant in traditional medicine.

## MATERIALS AND METHODS

### Chemicals, reagents and plant material

Chemicals and reagents were supported by Prolabo (Paris, France) and Pharmacia (Uppsala, Swedeen). Plant materials (aerial parts) of *A. herba-alba* were collected from the local area of the mount from Bir Elhfay, in the government of Sidi Bouzid, Tunisia, in February to March 2009.

### Distillation of essential oil

The dried aerial parts were ground prior to the operation and then 300 g of ground *A. herba-alba* were submitted to water distillation for 4 h using a Clevenger apparatus. The distilled essential oils were dried over anhydrous sodium sulfate, filtered and stored at +4°C.

### GC/MS analysis conditions

The essential oil was analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 250 and 280°C, respectively. The column temperature was programmed from 35 to 250°C at a rate of 5°C/min, with the lower and upper temperatures being held for 3 and 10 min, respectively. The flow rate of the carrier gas (helium) was 1.0 ml/min. A sample of 1.0 µl was injected, using split mode (split ratio, 1:100). All quantifications were carried out using a built-in data-handling programme provided by the manufacturer of the gas chromatograph. The composition was reported as a relative percentage of the total peak area. The identification of the essential oil constituents was based on a comparison of their retention times to *n*-alkanes, compared to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their mass spectra, compared to the Wiley version 7.0 library.

### Antioxidant activity

### DPPH radical scavenging assay

The ability of *A. herba-alba* essential oil to scavenge free radicals was assayed with the use of a synthetic free radical compound, 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method employed by Bersuder et al. (1998). Briefly, a volume of 500 µl of each sample was mixed with 500 µl of ethanol and 125 µl (0.02%, w/v) of DPPH in 99.5% ethanol. The mixture was shaken vigorously and incubated in the dark. After 60 min, the absorbance was measured at 517 nm using a spectrophotometer. The DPPH radical-scavenging activity was calculated as follows:

$$\text{Radical-scavenging activity} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where $A_{blank}$ and $A_{sample}$ are the absorbance of the control (blank) and the sample, respectively. The $IC_{50}$ value was defined as the amount of antioxidant necessary to inhibit DPPH radical formation by 50%. The synthetic antioxidant reagent, butylated hydroxytoluene (BHT) was used as a positive control. The values are presented as the means of triplicate analysis.

#### *β-carotene* bleaching assay

The antioxidant assay using the β-carotene bleaching was determined according to the protocol previously described (Koleva et al., 2002). β-Carotene (0.5 mg) was dissolved in 1 ml of chloroform and mixed with 25 µl of linoleic acid and 200 µl of Tween 40. The chloroform was evaporated under vacuum at 40°C, then, 100 ml of distilled water was added and the resulting mixture was vigorously stirred. About 2.5 ml of the obtained emulsion was transferred into different tubes containing 500 µl of essential oil dissolved in absolute ethanol at different final concentrations (5 to 70 µg/ml). The tubes were immediately incubated at 50°C for 120 min and the absorbance was measured at 470 nm before and after heat treatment. A control blank containing 0.5 ml of ethanol instead of the sample test was carried out in parallel. Synthetic antioxidant BHT was used as positive control and all tests were carried out in triplicate.

#### Reducing power antioxidant

The ability of oil to reduce iron (III) was determined according to the Yildirim's method (Yildirim et al., 2000) with some modifications. An aliquot of 500 µl of each sample at different final concentrations was dissolved in ethanol and mixed with 1.25 ml of reagent of 0.2 M phosphate buffer (pH 6.6) and 1.25 ml of 1% potassium ferracyanide.

The mixture was incubated 30 min at 50°C, followed by addition of 1.25 ml of 10% (w/v) trichloroacetic acid. The mixture was then centrifuged at 1500 g for 10 min. Finally, 1.25 ml of the supernatant solution was mixed with 1.25 ml of distilled water and 250 µl of 0.1% (w/v) ferric chloride. After 10 min, the absorbance was measured at 700 nm spectrophotometerically. Increased absorbance of the reaction mixture indicated increased reducing power. Synthetic antioxidant BHT was used as positive control and all tests were carried out in triplicate.

## RESULTS AND DISCUSSION

### Chemical composition

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in discovering the actual value of folkloric remedies. In this work, the composition of *A. herba-alha* oil has been thoroughly investigated, and the diversity in oil composition from plants grown in different countries and even those from different localities in the same country have led to the many oil-dependent chemotypes assigned to the plant. Hydrodistillation of the aerial parts of these *A. herba-alba* samples yielded yellowish liquid oils. The percentages of the identified components are listed in Table 1 in the order of their elution from a low-polar HP-5MS column. This study allowed the identification of eighteen compounds accounting for 100% of the oil. The major compounds, which were identified by GC–MS, were α-thujone (24.88%), germacrene D (14.48%), camphor (10.81%), 1,8-cineole (8.91%) and β-thujone (8.32%). Other representative compounds were identified as (+,-)-lepidozene (5.66%), sabinyl acetate (5.26%), chrysanthenone (4.73%), chrysanthenyl acetate (3.29%), borneol (3.05%) and α-pinene (2.81%).

With respect to the terpenoid compounds, the oil was predominately composed of monoterpene, with 70.28% of oxygenated monoterpene and 4.54% of hydrocarbons monoterpene in comparison with the sequiterpenes. The main constituent of oxygenated monoterpene were α-thujone (24.88 %), camphor (10.81%), 1,8-cineole (8.91%), β-thujone (8.32%), sabinyl acetate (5.26%), chrysanthenone (4.73 %), chrysanthenyl acetate (3.29%) and borneol (3.05%) followed by other components at a percentage less than 3%. The monoterpene hydrocarbons contain α-pinene (2.81%) and camphene (1.71%). Sesquiterpenes constituted 24.05% of the total oil composition with 23.15% of hydrocarbons derivatives and 0.9% oxygenated derivatives. Among sesquiterpenes hydrocarbons, germacrene D (14.48%) and (+,-)-lepidozène (5.66%) are the most abundant components.

As shown in this study, this oil has an original composition and no similarity pattern to those published for other geographical Tunisian regions with α-thujone (24.88 %) and germacrene D (14.48%) as the most abundant components, was considered as a new chemotype of *A. herba alba* growing wild in Tunisia. This composition is different from that described by other authors on *A. herba-alba* oils, where β-thujone, α-thujone, α-thujone/β-thujone and 1,8-cineole/camphor/α-thujone/β-thujone were respectively, the major components of the oil types cultivated plant in southern Tunisia. *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 in the essential oil composition from the aerial parts of *A. herba-alba* cultivated in the south-western of Tunisia.From other countries, 1,8-cinéole, cis-chrysanthenol (Salido et al., 2004), davanone (Benjilali et al., 1982; Salido et al., 2004), chrysanthénone (Hurabielle et al., 1982) and cis**-**chrysanthenyl acetate (Fleisher et al., 2002) are the most abundant constituents. We attributed the great variability and the diversity observed in the chemical composition of this essential oil to the geographical location, ecological conditions (Santos-Gomes and Fernandes-Ferreira, 2001), genetic factors (Skoula et al., 1999), geology, part of the plant and the method used to obtain the essential oil. In fact, these factors influence the plant's biosynthetic pathways and, consequently, the relative proportion of the main characteristic compounds (Chryssavgi et al., 2008).

### Antioxidant activity

Antioxidants retard oxidation and are sometimes added

**Table 1.** Chemical composition of the essential oil of *A. herba-alba*.

| S/N | Compound | Percentage (%) |
|---|---|---|
| 1 | α- Pinene | 2.81 |
| 2 | Camphene | 1.73 |
| 3 | 1,8-Cineole | 8.91 |
| 4 | α-Thujone | 24.88 |
| 5 | β–Thujone | 8.32 |
| 6 | Chrysanthenone | 4.73 |
| 7 | Camphor | 10.81 |
| 8 | 1-Nitro-cyclohex-1-ene | 1.13 |
| 9 | Borneol | 3.05 |
| 10 | Chrysanthenyl acetate | 3.29 |
| 11 | Bornyl acetate | 1.03 |
| 12 | Sabinyl acetate | 5.26 |
| 13 | α-Copaene | 0.85 |
| 14 | Trans-caryophyllene | 0,87 |
| 15 | Germacrene-D | 14.48 |
| 16 | (+,-)-Lepidozene | 5.66 |
| 17 | δ-Cadinene | 1.29 |
| 18 | γ-Epoxy-elemene | 0.90 |
| | | 100 |
| | Monoterpenes | 74.82 |
| | *Hydrocarbon monoterpenes* | 4.54 |
| | *Oxygeneited monoterpenes* | 70.28 |
| | Sesquiterpenes | 24.05 |
| | *Hydrocarbon sesquiterpenes* | 23.15 |
| | *Oxygeneited sesquiterpenes* | 0.9 |

to meat and poultry products to prevent or slow oxidative rancidity of fats that cause browning and deterioration. The antioxidant potential of plant products can be evaluated using numerous assays, because it is very difficult to assess the antioxidant activity of a product on the basis of a single method. A single method will provide basic information about antioxidant properties, but a combination of methods describes the antioxidant properties of the sample in more detail. In this work, three methods were used to evaluate the antioxidant of *A. herba-alba* essential oil activities and the results were compared with the synthetic antioxidant BHT which is an efficient synthetic antioxidant agent in food: scavenging of DPPH free radicals, *β*-carotene bleaching test and reducing power assay.

## DPPH radical-scavenging activity

In our study, the antioxidant activity of the investigated essential oils was evaluated *in vitro* by the DPPH-radical scavenging assay. In this assay, the ability of the essential oil to act as the donor of hydrogen atoms or electrons in transformation of DPPH into its reduced form, DPPH-H (yellow-colored diphenyl-picrylhydrazine) was measured spectrophotometrically by the decrease in absorption at 517 nm. The method is based on the reduction of DPPH in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form, DPPH-H (Singleton et al., 1999) in the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

The results reported in Figure 1 demonstrate the DPPH radical scavenging activities (% inhibition), caused by different concentration of *A. herba-alba* essential oil, which increased with increasing its concentration. At a concentration lower than 242 µg/ml, the antioxidant activity of the oil is lower than the positive control BHT, however for a concentration higher than 242 µg/ml, the oil exhibited greatest inhibitory activity reaching as high as 87.82%. The IC50 values (the concentration reducing 50% of DPPH) obtained for scavenging activities on DPPH radical, were evaluated. The lower the IC50 value,

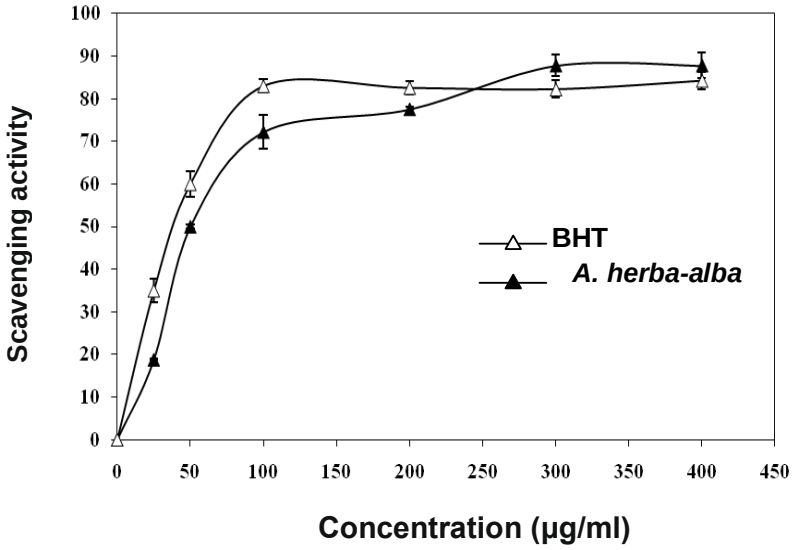


**Figure 1.** DPPH radical scavenging activity of *A. herba-alba* essential oil and the synthetic antioxidant BHT in different concentrations. Scavenging activity was measured using the DPPH radical assay. Each points is a mean from triplicate measurement.

the greater the free radical-scavenging activity. Comparison of the DPPH scavenging activity of the investigated essential oil (50.00 µg/ml) and those expressed by BHT (37.80 µg/ml) showed that the oil possessed weaker antioxidant effects than BHT. From the results reported in Table 1, *A. herba-alba* essential oil contain higher amount of oxygenated monoterpenens, which promotes its property of being a radical scavenging agents.

### *β*-Carotene bleaching method

This test measures the potential of the plant to inhibit conjugated diene hydroperoxide formation from linoleic acid oxidation. The antioxidant capacity was evaluated by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide arising from linoleic acid oxidation which results in the discolouration of *β*-carotene. This method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion.

The ability of essential oil to inhibit the lipid peroxidation and to be evaluated by *β*-carotene bleaching test, showed that the peroxidation of lipids was effectively inhibited by *A. herba-alba* essential oil. The results depicted in Figure 2 shows the antioxidant activity of the essential oil and BHT as measured by the bleaching of the β-carotene–linoleate system. In this system, the linoleic acid inhibition activity is somewhat lower than that of BHT which was used as positive control (72.21 ± 1.00% for oil *vs.* 77.50 ± 2.00% for BHT, respectively at 70 µg/ml). These values revealed that the antioxidant activity of *A. herba-alba* essential oil was still less active than BHT. This activity may be attributed primarily to the less content of phenolic components of the *A. herba-alba* essential oil.

### Reducing power antioxidant

Determination of ferric reducing antioxidant power is a simple direct test for measuring antioxidant capacity. This assay is often used to evaluate the ability of natural antioxidant to donate electron (Yildirim et al., 2000; Dorman et al., 2003) by measuring the potential of the plant to inhibit conjugated diene hydroperoxide formation from linoleic acid oxidation. The presence of reductants (antioxidants) in the tested samples would result in $Fe^{3+}$ reduction or reduction of the ferricyanide complex to the ferrous ion ($Fe^{2+}$). The resultant $Fe^{2+}$ can be monitored by UV/VIS monitoring of Prussian blue, which absorbs at 700 nm. The reducing power increased with increasing essential oil concentration. The values of the reducing power (absorbance at 700 nm) at different essential oil concentrations (5 to 70 µg/ml) of *A. herba-alba* are shown in Figure 3 and were found to be 0.250 ± 0.021, 0580 ± 0.040 and 0.925 ± 0.032, respectively at 10, 30 and 70 µg/ml. This activity was somewhat lower than those obtained for the positive control, BHT at the same concentration (0.390 ± 0.020, 0.760 ± 0.005 and 1.051 ± 0.010, respectively).

It should be noted that more explanation of the different three antioxidant methods, used in this paper, confirm the

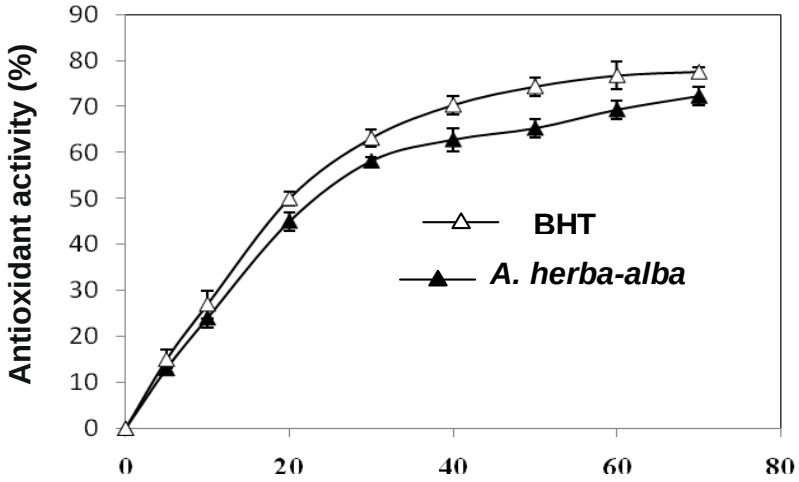


**Figure 2.** Antioxidant activity of *A. herba-alba* essential oil and the synthetic antioxidant BHT determined by *β*-carotene bleaching test in different concentrations.

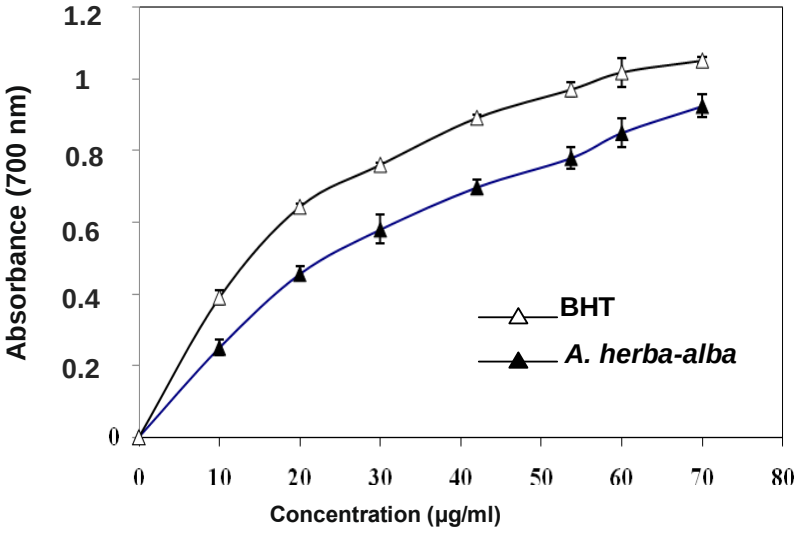


**Figure 3**. Reducing power of *A. herba-alba* and the synthetic antioxidant BHT in different concentrations.

antioxidant activity of oil. Each test has a basis for its usage. The most active composition of GC-MS results is the non-phenolic compound, which showed an anti-oxidant activity that is explained by its mechanism.

As shown in the three different assays, the *A. herba-alba* essential oil exhibited a higher antiradical and linoleic acid inhibition activities, attributed to the presence of oxygenated monoterpenes in higher amount and to the mixture of mono- and sesquiterpene hydrocarbons (Bozin et al., 2007). On the other hand, a less effective anti-oxidant activity was shown when compared to the synthetic antioxidant (BHT). We explained this by the dominance of non-phenolic compounds in the *A. herba-alba* essential oil. The same trends was found and confirmed by other studies (Lopez-Lutz et al., 2008).

## Conclusion

In this study, we confirmed the identification of new chemical composition of *A. herba-alba* essential oil. When our results were compared with the literature, the chemical composition and content of the investigated essential oil showed significant differences with the abundance, for the first time, of germacrene D in higher amount. From the obtained results, it is obvious that the

chemical composition of the essential oil has an important impact on the antioxidant activity of the oil, in relation to the presence of some substances such as α-thujone, camphor, 1,8-cineole, β-thujone and borneol. This shows that antioxidative activities of this plant /essential oil studied may be a potential source of natural antioxidants in foods in order to find possible alternative to synthetic antioxidant, and the pharmaceutical industry for the prevention and the treatment of various human diseases.

**REFERENCES**


Akrout A (2004). Essential oil study of some pastoral plants from Matmata (south Tunisia) (in French). Cah. Options. Med. 62: 289-292.

Bailey C, Danin A (1981). Bedouin plant utilization in Sinai and Negev. Econ. Bot. 35: 145-162.

Benjilali B, Richard H, (1980). Study of some populations of *Artemisia herba alba* from Morocco. Riv. Ital. Essen. 62: 69-74.

Benjilali B, Sarris J, Richard H (1982). Nouveaux chémotypes d'Artemisia blanche du Maroc. *Artemisia herba alba*. Sciences. Aliment. 2: 515-527.

Bersuder P, Hole M, Smith G (1998). Antioxidants from a heated histidineglucose model system I: Investigation of the antioxidant role of histidine and isolation of antioxidants by high performance liquid chromatography. J. Am. Oil Chem. Soc. 75: 181-187.

Bozin B, Mimica-Dukic N, Samojlik I, Jovin E (2007). Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus offi cinalis* L. and *Salvia offi cinalis* L., Lamiaceae) essential oils. J. Agric. Food Chem. 55(19): 7879-7885.

Chryssavgi G, Vassiliki P, Athanasios M, Kibouris T, Michael K (2008). Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: evaluation of antioxidant capacity of methanolic extracts. Food Chem. 107: 1120-1130.

Cohen A, Lavergne JP, Leblanc A, Viallefont P (1972). Constituents of *Artemisia Herba Alba* (typica). I. Essential oils. Bull. Soc. Sci. Nat. Phys. Maroc. 52: 1-19.

Dorman HJD, Peltoket A, Hiltunen R, Tikkanen MJ (2003). Characterisation of the antioxidant properties of deodourised aqueous extracts from selected *Lamiaceae* Herbs. Food Chem. 83: 255-262.

El-Sayedand AM, Seida AA (1990). Comparative study of the major constituents of the essential oils of wild and cultivated Egyptian *Artemisia herba alba* with those of plants produced abroad. Bull. Fac Pharm. (Cairo Univ.). 28: 57-58.

Feuerstein I, Danin A, Segal R (1988). Constitution of the essential oil from an *Artemisia herba-alba* population of Spain. Phytochem. 27: 433-434.

Feuerstein I, Mueller D, Hobert K, Danin A, Segal R (1986). The constitution of essential oils from *Artemisia herba alba* populations of Israel and Sinai. Phytochem. 25: 2343-2347.

Fleisher Z, Fleisher A, Nachbar R (2002). Chemovariation of *Artemisia herba alba* Asso. Aromatic plants of the Holy Land and the Sinai. Part XVI. J. Essen. Oil Res. 14: 156-160.

Haouari M, Ferchichi A (2009). Essential oil composition of *Artemisia herba-alba* from southern Tunisia. Molecules. 14: 1585-1594.

Hudaib M, Aburjai T (2006). Composition of the Essential Oil from *Artemisia herba-alba* Grown in Jordan. J. Essen. Oil Res. 18: 301-304.

Hurabielle M, Malsot M, Paris M (1981). Chemical study of two oils from wormwood: *Artemisia herba-alba* Asso and *Artemisia vulgaris Linnaeus* of chemicotaxonomic interest. Riv. Ital. EPPOS. 63: 296-299.

Jouad H, Haloui M, Rhiouani H, Hilaly JE, Eddouks M (2001). Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez–Boulemane). J. Ethnopharmacol. 77: 175-182.

Koleva I, van Beek TA, Linssen JPH, de Groot A, Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 13: 8–17.

Lopez-Lutz D, Alviano DS, Alviano CS, Kolodziejczyk PP (2008). Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochem. 69: 1732-1738.

Mighri H, Hajlaoui H, Akrout A, Najjaa H, Neffati M (2010). Antimicrobial and antioxidant activities of *Artemisia herba-alba* essential oil cultivated in Tunisian arid zone. C. R. Chimie. 13: 380-386.

Nabli MA (1989). Elements of botany and phyto-ecology (in French). Faculty of Sciences of Tunis; Official printing office of the Tunisian republic: Tunisia.

Neffati A, Skandrani I, Sghaier MB, Bouhlel I, Kilani S, Ghedira K, Neffati M, Cherif I, Hammami M, Chekir-Ghedira L (2008). Chemical composition, mutagenic and antimutagenic activities of essential oils from (Tunisian) *Artemisia campestris* and *Artemisia herba-alba*. J. Essen. Oil Res. 20: 471-477.

Ouyahya A, Negre R, Viano J, Lozano YF, Gaydou EM (1990). Essential oils from Moroccan Artemisia negrei, A. mesatlantica and *A. herba alba*. Lebensm. Wiss. Technol. 23: 528-530.

Peschel W, Sanchez-Rabaneda F, Dieckmann W Plescher A, Gartzia I, Jimenez D, Lamuela-Raventós R, Buxaderas S, Codina C (2006). An industrial approach in the search of natural antioxidants from vegetable and fruits wastes. Food Chem. 97:137-150.

Salido S ,Valenzuela LR, Altarejos J, Nogueras M, Sánchez A, Cano E (2004). Composition and infraspecific variability of *Artemisia herba-alba* from southern Spain. Biochemistry. Systems Ecology, 32, 265-277.

Santos-Gomes PC, Fernandes-Ferreira M (2001). Organ- and season dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. J. Agric. Food Chem. 49: 2908-2916.

Segal R, Feuerstein I, Danin A (1987). Chemotypes of Artemisia herba-alba in Israel based on their sesquiterpene lactone and essential oil constitution. Phytochem. 15(4): 411-416.

Singleton VL, Orthofer Lamuela-Ravent R (1999). Analysis of total and other oxidation substrates and antioxidants by means of olin-Ciocalteru reagent. Methods Enzymol., 299: 152-178.

Skoula M, El Hilali I, Makris AM (1999). Evaluation of the genetic diversity of *Salvia fruticosa* Mill. clones using RAPD markers and comparison with the essential oil profiles. Biochem. Syst. Ecol. 27: 559-568.

Tantaoui-Elaraki A, Errifi A (1994). Antifungal activity of essential oils when associated with sodium chloride or fatty acids. Gras. Aceit. (Seville). 45: 363-369.

Tutin TG, Persson K, Gutermann W (1976). Artemisia L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea, vol. 4. Cambridge University Press, Cambridge, pp. 178–186.

Vernin G, Merad LO (1994). Mass spectra and Kovats indexes of some new cis-chrysanthenyl esters found in the essential oil of *Artemisia herba alba* from Algeria. J. Essen. oil Res. 6: 437-448.

Vernin G, Parkanyi C (2001). GC/MS analysis of *Artemisia herba alba* Asso from Algeria: Nonpolar and polar extracts. Riv. Ital. EPPOS. 32: 3-16.

Yashphe J, Feuerstein I, Barel S, Segal R (1987). The antibacterial and antispasmodic activity of *Artemisia hertoa alba* Asso. II. Examination of essential oils from various chemotypes. Int. J. Crude Drug Res. 25: 89-96.

Yildirim A, Mavi A, Oktay M, Kara AA, Algur ÖF, Bilaloglu V (2000). Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argentea* Desf ExDC), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis*) extracts. J. Agric. Food Chem. 48: 5030–5034.