J. Appl. Environ. Biol. Sci., 6(5)59-65, 2016
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ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Antioxidant Activity of Essential Oil of *Artemisia herba alba*

Nebia Bouzidi*[1], Khalladi Mederbal[2] and Bachir Raho G[3]

*[1]University of Mascara. Faculty of Nature and life. Laboratory of research on Geo-environment and Development. Mascara 29000. Algeria. Tel 0791182816
[2]University of Tiaret. Algeria
[3]University of Mascara. Algeria

Received: January 13, 2016
Accepted: March 14, 2016

ABSTRACT

Synthetic antioxidants are widely used in the food industry because of their ability to prevent deterioration of foods and prolong their shelf life, but a good number of them will be considered harmful to health and have carcinogenic risks. Particular interest was manifested in recent years to essential oils that are an effective alternative to chemical preservatives. *Artemisia herba alba* is characterized by its wealth in essential oil of different composition which led to the definition of several chemotypes; its high feed value and a very important ecological role against erosion and desertification.

The objectives of this study were to evaluate the antioxidant activity of essential oils of the aerial part of *Artemisia herba alba*. The essential oil was isolated by hydrodistillation using a Clevenger apparatus, and the identification and quantification of constituents, through GC/MS analysis. Antioxidant effectiveness was examined by the radical scavenging method (DPPH) and determination of ferric reducing antioxidant power (FRAP). The yield obtained of essential oil is 0.932%. This oil has a density and a refractive index of the order of 0.912 and 1.4811 respectively. In chromatographic analysis, the majority constituents found in the essential oil of *Artemisia herba alba* were camphor (29.8%), 1,2,5,5-tetramethyl-1,3-Cyclopentadiene (15.6 %), Chrysanthenone (8.2%), eucalyptol (6.5%), Arthole (4.5%). The results of the antioxidant activity obtained in vitro with both methods showed that essential oil from *A. herba alba* possess an interesting antioxidant effect. The results suggest that *A. herba alba* oil has promising use as a natural source of antioxidants that can be a valid alternative to replace chemicals.

KEYWORDS: Antioxidant, chemical composition, Camphor, CG/SM, white wormwood

INTRODUCTION

Given the risks related to chemical additives in terms of public health and dizzying demand for foods with less synthetic chemicals, increased search for substitutes natural agents such additives to improve food safety is one priorities researchers.

Aromatic plants have been used since ancient times as well as in therapy in preserving and flavoring food, but only in the last decade scientific research has focused its interest on their essential oils and natural extracts as sources of antimicrobial compounds and antioxidants [1, 2, 3, 4].

A large number of medicinal plants have been reported to exhibit antioxidant activity, including *Ocimum sanctum*, *Piper cubeba* Linn., *Allium sativum* Linn., *Terminalia bellerica*, *Camellia sinensis* Linn., *Zingiber officinale* Roscoe and several Indian and Chinese plants [5]. Many studies report the results of using plant-derived compounds as natural antioxidants in food products. These results led the researchers to conclude that some plant compounds could be considered as proper alternatives to synthetic antioxidants [6]. Steppe zones occupied a large area of Algeria, which is characterized by its appreciable biodiversity [7]. Nowadays, many researchers from different fields namely pharmacology and nutrition are interested in the flora of the high Algerian steppe plains, to develop, expand and valorize this floristic heritage which is dominated by three plant species namely alfa (*Stipa tenacissima*) Esparto (*Lygeum spartum* L.) and sagebrush (*Artemisia herba alba*. Asso)[8, 9]. *Artemisia herba-alba* 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 [10]. *Artemisia herba alba,* or the white wormwood designated Arabic as the "Chih" of the Asteraceae family, usually grows in small clumps sizes. It is a plant for different uses. *A. herba-alba* has traditionally been used in the treatment of diabetes, bronchitis, diarrhea, neuralgias and hypertension [11]. The plant is reported to possess hypoglycemic [12], anticancer [13, 14], anti-angiogenic [15], insectisidal [16], hypotensive and diuretic [17], anti-inflammatory [14, 18], antimicrobial activity [19, 20], and many other biological activities. Thus, the aims of the present study are to investigate the chemical composition and to determine the antioxidant activity of the essential oil of *A. herba-alba*.

Corresponding author: Nebia Bouzidi, University of Mascara. Faculty of Nature and life. **La**boratory of research on Geo-environment and Development. Mascara 29000. Algeria. Tel 0791182816 lina_kholoud@yahoo.fr

MATERIEL AND METHODS

Plant Material and essential oil extraction

The aerial part of the plant was collected in Sidi Ahmed station (Saida province), situated in the Tell steppe interface area in North West of Algeria. The samples were submitted to hydrodistillation for 3 h. The essential oil was dried over anhydrous sodium sulphate and stored in a sealed vial in the dark at +4 °C before analysis tests.

Qualitative analysis of the essential oil of *A. herba alba*

Physical analysis of essential oil

Determination of the relative density [21]

Determination of the refractive index [21]

Gas chromatography/Mass spectrometry (GC/MS) analysis of Essential oil

The compounds of essential oil of *A. herba alba* were analysed by GC/MS, using a Hewlett- Packard (HP) chromatograph (Agilent Technologies) MDS 5973 coupled to a 6890 plus mass spectrometer, equipped with fused-silica capillary column HP-5MS (30m; 0.25mm i.d.; film thickness 0.25µm). The oven temperature was programmed from 60°C for 1 min, to 280°C at the rate of 2°C/min, and then left at 280°C for 10 min. The injector port temperature was held at 250°C, split: 1/20, the temperature of the detector was set at 280°C. The carrier gas was helium with a flow rate of 0.5 ml/min and the analysed sample volume was 2 µl. The mass spectrometer (MS) conditions were as follow: scan, Interface temperature: 280°C, Ionization type: electronic, Intensity of the filament: 70ev, Type of mass analyzer: Quadrupole, Source temperature: 230°C, Empty: 65m torr.

Determination of Antioxidant Activity

Measure of the Antioxidant activity by DPPH free radical scavenging test [22]

The antioxidant capacity of the essential oil of *Artemisia herba alba* was tested by the method which uses the DPPH• like a relatively stable free radical. In this test, the DPPH• of purple color is reduced in a yellow compound, the diphenylpicrylhydrazine, whose intensity of the color is inversely proportional to the reducing capacity of antioxidants present in the medium. The reaction is carried out in a total volume of 2 ml containing 0,4 ml of DPPH• (0,5 mM) solubilized in ethanol.

For the test, the sample was prepared by dissolution in absolute methanol. We prepare a solution in absolute methanol (500µg/ml). From this solution, different dilutions are made to have various concentrations about microgram per ml (31.25, 62.5, 125, 250 and 500 µg/ml). These same concentrations were prepared with the BHT to be useful as a positive control. a white control is also carries out with absolute ethanol only. For each concentration, the test is repeated three times. The samples are then left in dark for 30 minutes, and discoloration compared to the negative witness containing only the solution of DPPH• is measured at 517 nm. The antioxidant activity is estimated according to the following equation:

$$AA\ \% = ([Abs\ control - Abs\ test]/Abs\ control) \times 100$$

AA : antioxidant activity, Abs : absorbance at 517 nm.

Determination of Ferric Reducing Antioxidant Power (FRAP Assay)

The antioxidant capacity was determined following the procedure described by Oyaisu [23]. one millilitre of sample solution was added to 2,5ml of phosphate buffer (0,2 M, pH 6,6) and 2.5 ml of hexacyanoferrate of potassium [$K_3Fe(CN)_6$] (10 g/l), and warmed in water bath at 50°C for 20 min. Then 2.5ml of trichloracetic acid (100 g/l) was added to the mixture and centrifuged at 3000 turns/min. during 10 minutes. Finally, 2,5ml of the supernatant were mixed with distilled water (2,5 ml) and 0.5 ml of ferric chloride [$FeCl_3$] (1g/l). The absorbance was measured in a spectrophotometer at 700 nm. A control without sample is prepared under the same conditions and ascorbic acid was used as positive control.

RESULTS AND DISCUSSIONS

Evaluation of the quality of the essential oil of *Artemisia herba alba*

Artemisia herba-alba dried aerial part were subjected to hydrodistillation and the yellow-colored essential oil obtained was analyzed by GC/MS techniques.

The yield of essential oil is of the order of 0.932%. Comparable yields are obtained in samples of white wormwood different regions in Algeria [24, 25]. The yield and quality of essential oils of species of Artemisia genus are influenced by soil pH [26]. The soils of the study area are characterized by a sandy-loam texture, alkaline pH and low chemical quality [27]. The refractive index and density are 1.4811 and 0.912 respectively. Chemical composition of the essential oils of the aerial parts from *A. herba-alba* are reported in table1. 31 components were identified, in which camphor (29.8%), 1, 2, 5, 5-tetramethyl- 1, 3-Cyclopentadiene (15.6 %), Chrysanthenone (8.2%), eucalyptol (6.5%), Arthole (4.5%) were the most abundant components. The minor

compounds detected in *A. herba-alba* oils were thujone (1,08% - 1, 40%), camphene (0.67%), eugenol (0.47%),....

Camphor has been found as major compounds in the present study which is in agreement with several research [28, 29, 30]. In other studies different dominated compounds were found like thujone, Chrysanthenone and Cis-chrysanthenyl acetate.

Differences in chemical composition of essential oils extracted from a species often occur, since the production of secondary metabolites, including essential oils, is strongly influenced by the environment in which the producing organism is inserted, and the factors responsible for such variations are, seasonality, age and plant development, as well as the different plant organs, temperature, nutrients, altitude, and attack of pathogens and herbivores [31, 32, 33]. According Rota et al.[34], the biological activity of essential oils depends on their chemical composition.

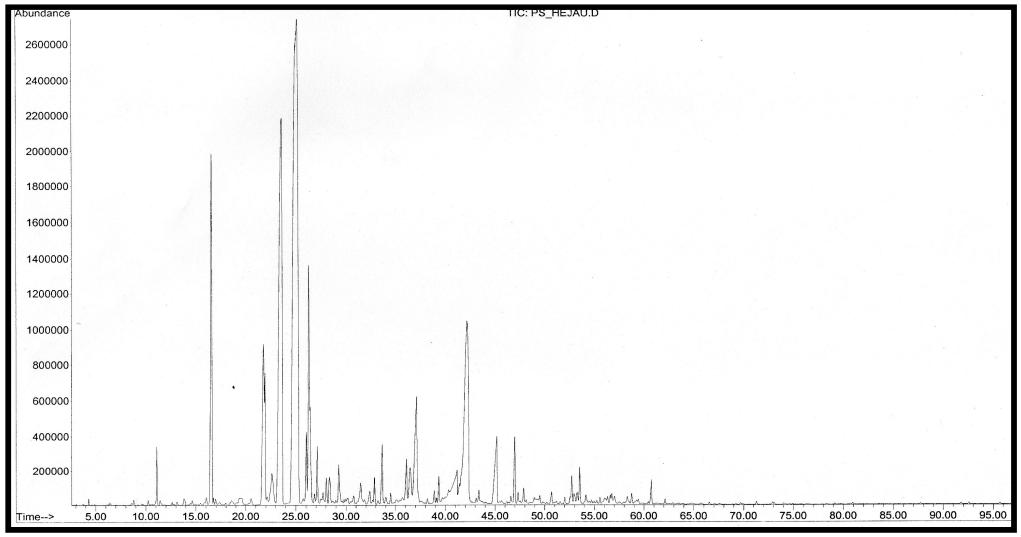


FIGURE 1: GC-MS Chromatogram of essential oil of *Artemisia herba alba*

TABLE 1. Essential oil of *Artemisia herba alba*

N°	Compounds	R.T (min)	%
1	Camphene	11.102	0.663
2	**Eucalyptol**	16.595	6.513
3	Bicyclo [3.2.0] hept-2-ene, 2-methyl	21.806	4.311
4	Thujone (alpha)-	21.942	1.409
5	Thujone (beta)-	22.623	1.089
6	**1,3-Cyclopentadiene, 1,2,5,5- tetramethyl-**	23.634	15.582
7	1,6-Dimethylhepta-1,3,5-triene	23.672	2.333
8	**Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1R)-**	25.218	29.816
9	Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1S)-	25.245	4.184
10	2(10)-Pinen-3-one-	26.066	1.097
11	Arthole	26.315	4.526
12	Borneol	26.439	1.105
13	Terpinen-4-ol	27.148	0.812
14	3-Carene	28.050	0.360
15	Bicyclo [3.1.1] hept-2-ene-2-carboxaldehyde,6,6- dimethyl-	28.342	0.578

16	Verbenone	29.256	0.755
17	Octadiyne	31.467	0.450
18	Bicyclo [3.1.1] hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	32.873	0.327
19	2-Cyclohexen-1-one, 3,5,5-trimethyl-	33.657	1.074
20	1,5-Hexadiene, 2,5-dimethyl-3-methylene-	36.106	0.765
21	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	36.462	1.093
22	1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl	37.138	3.803
23	Eugenol	39.355	0.377
24	Pipéritenone	41.204	1.063
25	**Chrysanthenone**	42.198	8.216
26	2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	42.247	2.659
27	1-Penten-3-one, 2-methyl-	45.193	2.381
28	Germacrène D	46.988	1.032
29	(-)-Spathulenol	47.875	0.221
30	g-Gurjunene	52.724	0.517
31	Hepatonic acid, phenyl ester	53.524	0.507
Total			99.61

Antioxidant activity

The antioxidant activity of essential oil of *A. herba alba* was examined by comparing it to the activity of known antioxidants such as BHT and ascorbic acid by the following two in vitro assays; inhibition of DPPH radical and the ferric reducing antioxidant power (FRAP). All results are reported in Figure 2, 3. These results showed that A. herba alba essential oil was able to reduce the stable free radical DPPH with an IC50 of 2.66 µg/ml, whereas that of the synthetic antioxidant BHA was 1.66 µg/ml. A. herba alba essential oil was found to be less active than BHA since their IC50 values were found to be higher. But with careful and cautious consideration, it is interesting that BHT is a pure chemical substance, while the essential oil of A. herba alba used consists of several natural active substances or a few of them must have this antioxidant capacity. If we consider that it is the camphor which has antioxidant power in our essential oil (because of its ketone structure), and it is the majority compound with 29%, we can recalculate the IC50 should be 29% of the value of 2.66 g / ml, and therefore clutched 0.77 mcg / ml, so this is a value that attributes to our essential oil, which is organic and natural, anti-radical power stronger than BHT itself. This result still encourages us to give more importance to natural substances in the field of additives.

Khlifi et al. [14] reported that the extract of *Artemisia herba alba* showed strong antioxidant activity but with an IC50 much higher than ours which is 20.64 mg / l (equivalent to 20.64 µg / ml).

The anti-radical activity of the essential oil of sagebrush could be attributed to its high content of oxygenated monoterpenes [35., 36, 37, 38]. However, camphor, predominant in the essential oil of *Artemisia herba alba* in the region of Sidi Ahmed (29%), has a considerable antioxidant power [39, 40].

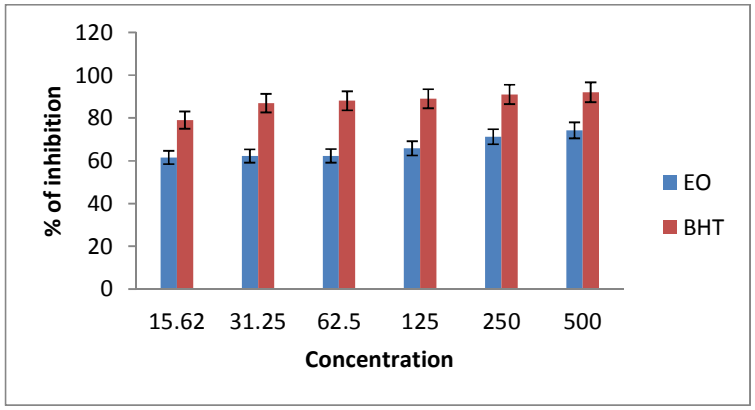
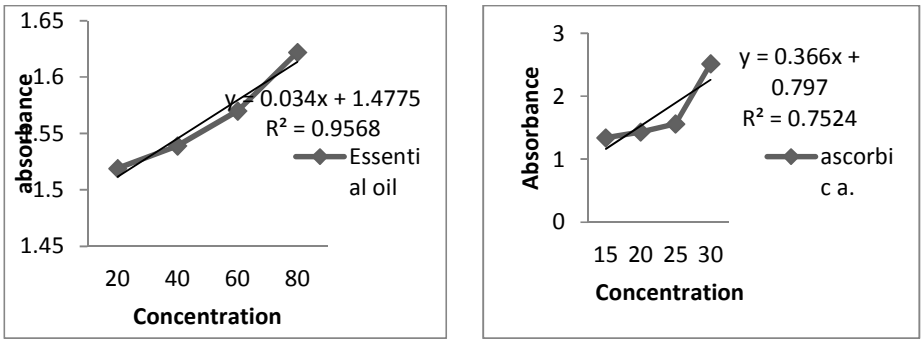


Figure 2: Test DPPH• histogram, expressed as a percentage inhibition, illustrating the antioxidant activity of BHT and essential oil according to their concentrations.

The same remark was showed by FRAP test which has indicated a little antioxidant activity in comparison the A. herba alba essential oil to ascorbic acid. In this test, the result (Figure 3) revealed that a good linearity to the essential oil (R2 = 0.956) than the positive control (R2 = 0.752). This may indicate that the reducing effect of the oil of *A. herba alba* is more correlated with its concentration, its effect is direct and does not exhibit phase affected by the mass effect



(a) **(b)**

Figure 3: reducing power of essential oil (a) and of the ascorbic acid (b) by the method of FRAP

The finding that the results of the DPPH and FRAP assays for plant extracts were highly correlated agrees with the work of others, and is consistent with the view that the two assays share a similar mechanistic basis, viz. transfer of electrons from the antioxidant to reduce an oxidant [41]. In the present study, the antioxidant activity of essential oil could be attributed to the presence of camphor, eugenol, α -thujene, pinen. [35, 42, 43, 44]. Many previous studies reported that the antioxidant capacity of plant extracts could be attributed to the total phenolic content [45, 46, 47].

To conclude, we can say that our results show a significant antioxidant capacity of the essential oil of sagebrush Algerian Northwest.

Conclusion

In the present study, results from antioxidant activities reflected by the DPPH and FRAP assay have demonstrated that the *Artemisia herba-alba* essential oil possesses a important antioxidant activity. Thus, Artemisia herba-alba may therefore be a good candidate for functional foods as well as plant-based pharmaceutical products.

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