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Antioxidant Activity and Phenolic Content of Artemisia Campestris from Two Regions of Algeria

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ABSTRACT

Artemisia campestris locally named "T'gouft" which belongs to family of Asteraceae, is known to have anti-inflammatory, antioxidant, antimicrobial and many other bioactivities. The objective of this study was to evaluate antioxidant activity of Artemisia campestris from two regions of Algeria (Bousaada and Oum El Bouaghi). A. campestris extracts were prepared with different polarity solvents (water and methanol) and analyzed using different tests in vitro in order to evaluate their antioxidant activity and phenolic content. Total phenolic, flavonoid and tanin contents were evaluated, and three tests were established to assess the antioxidant activity. Total phenolic of the samples was analyzed using Folin – Ciocalteu methods; it was expressed as mg gallic acid/g of extract, and the content varied from 7.47 to 88.61 mg/g of extract. Flavonoid of the samples was detected using aluminum chloride, expressed as mgquercetin/ g of extract, and the content varied from 12.91 to 33.14 mg QE/g extract. The antioxidant activity of A. campestris was evaluated using three complementary test systems: DPPH scavenging, FRAP andβ-carotene-linoleic acid respectively. The IC_{50} values for DPPH were ranged from 48 to 241 µg/ml. The EC_{50} values in FRAP assay were ranged from 266 to 871µg/ml while in β-carotene-linoleic acid assay, good inhibition was found in the methanolic extract.

Keywords: *Artemisia campestris, Antioxidant activity, IC50 and EC50.*

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1. INTRODUCTION

For a long time, medicinal plants have been used as remedies for human diseases because they contain hundreds, even thousands of active chemical compounds called secondary metabolites (Nostro et al., 2000). According to the World Health Organization(WHO), about 80% of world people are using unconventional medicines, in particular plants as a source, in their primary healthcare (Adamu et al, 2005). The importance of the use of medicinal plants is related to their content in secondary metabolites such as polyphenols, flavonoids and essential oils (Mohammedi & Atik, 2011).

Due to their richness by medicinal and endemic plants, Algerian flora plays an important role in supporting traditional medicine, which is widely practiced in the country (Marzouk et al., 2010). Several medicinal plants used in traditional medicine in Algeria constitute an inexhaustible source of bioactive substance endowed with many pharmacological properties (Beloued, 2005; Hammiche and Maiza, 2006). In the family of Astraceae (Compositae), Artemisiagenus has been considered as one of the most dispersed and important genera. It is a heterogeneous genus, consisting over 500 diverse species, which has a great importance in botany, pharmacy and food industry (Bora & Sharma, 2010).

Currently, several questions were raised about the safety of synthetic chemicals used in medicine or in the food industry; the development of microbial resistance to antibiotics and toxicity of synthetic antioxidants has led to search for natural substances with antioxidants and antimicrobial activities (Djeridane et al., 2006; Mothana and Lindequist, 2005).Several species of Artemisia have attracted researchers because of their content of chemical components among them Artemisia campestris, commonly known as "T'gouft", grows in sandy and desert grounds. The plant is widely used in traditional medicine specially as decoction for their antivenin, anti-inflammatory, antirheumatic and antimicrobial properties, for the treatment of digestive disorders, ulcers, burns, diarrhea and other diseases (Akrout, 2005; Dob et al., 2005; Ferchichi et al., 2006).

The aim of this study was to determine the antioxidant activity of different extracts (aqueous and methanolic) of Artemisia campestris by using three assays: DPPH radical-scavenging, β-carotene/linoleic acid bleaching, and ferric reducing/power assay.

2. MATERIALS AND METHODS

Plant Material

Artemisia campestris were collected in November 2015 from Boussaada (South east of Algeria) and Oum El Bouaghi (East of Algeria). Voucher specimens were deposited at the Herbarium of the Department of Biochemistry, Faculty of Nature and Life Sciences, Ferhat Abbas University Setif. After drying; the aerial part of the plant was crushed to obtain a powder.

Methanolic Extract Preparation

About 10g of the dried plant was extracted by maceration in 100 ml of methanol at ambient temperature for 24 h with continuous stirring, the process was repeated twice. The extracts were filtered and concentrated to dryness with a rotary evaporator at 45 °C (Khettaf et al., 2016).

Aqueous Extract

Following the method of Moualek et al. (2016), and Sefi et al. (2012) with modifications, the aqueous extract was prepared by decoction of 10g of the dried plant in 100 ml and set aside during a time of 15 min at ambient temperature, filtered with No. 1 Whatmann Millipore filter paper (0.45 µm), and concentrated to dryness with a rotary evaporator at 50 ± 1 ◦C to give solid residues.

Total Phenolic Content

The concentrations of total polyphenols contained in the extracts were calculated by using Folin-Ciocalteu method (Singleton et al., 1999) with slight modification. Gallic acid is the standard used to establish the calibration curve. The diluted solution of the extracts (200 µl) was blended with 1ml of Folin–Ciocalteu reagent (10%) for 5 min; and 800 µl of sodium bicarbonate solution (7.5 %) was added. The mixture was left to react for 2 hours at room temperature and the absorbance of each mixture was read at 765 nm. The amount of total polyphenols' indifferent extracts was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

Total Flavonoids Content

Flavonoids contained in the extracts were quantified and determined according to aluminum chloride colorimetric method of Bahorun et al. (1996). The calibration curve was made by using Quercetin. One milliliter of aluminum trichloridemethanolic solution (2%) was added to one milliliter of the sample solution. The mixture was left to react for 10 min at room temperature, and the absorbance of each mixture was read at 430 nm, total flavonoids were expressed as mg quercetin equivalent per gram of extract (mg QE/g extract).

Flavones and Flavonols Content

Flavones and flavonols were measured using a colorimetric assay developed by Kosalec et al. (2004). About 1ml of the extract solution was mixed with: 3ml of ethanol, 0.2 ml of of 10 % aluminium trichloride methanolic solution, 0.2 ml of sodium acetate and 2.8 ml of distilled water were added. Then, the obtained solution was incubated at room temperature for 30 min, at 415 nm the absorbance of the reaction mixture was assessed, and the findings were recorded as mg quercetin equivalent per gram of extract (mg QE/g extract).

Tanins Content

The content of tanins was measured based on vanillin assay described by Julkunen-Titto (1985). About 3ml of 4% methanol vanillin solution and 3 ml of concentrated HCl were added to 50µl of suitably diluted sample. The mixture was left to react for 15 min and the absorbance was measured at 500 nm. The amounts of tanins were expressed as mg catechin equivalent per gram of extract (mg CE/g extract). All samples were analyzed in triplicates.

Antioxidant Activity in vitro

DPPH Scavenging Activity

The capacity of A. campestris extracts to reduce the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was determined spectrophotometrically according to Ita and Eduok. (2017).

A volume of 1 ml of each extract at different concentrations was added to 1ml of DPPH solution (0.004 %) in methanol. After incubation for 30 min at room temperature, the absorbance of the reaction mixture was measured at 517 nm. But ylated hydroxanisole (BHA) was used as a standard antioxidant. The percentage of inhibition was calculated by using the following formula:

$$\% \text{ DPPH radical scavenging} = [A_{control} - A_{sample}] / A_{control} \times 100.$$

$A_{control}$: Absorbance of the control reaction (containing all reagents except the test sample).

A_{sample}: Absorbance of the tested sample.

Antioxidant activity of extracts was expressed as IC_{50}. All the operations were performed in triplicate.

The Ferric Reducing Antioxidant Power Assay (FRAP):

The antioxidant power by iron reduction, commonly known by FRAP of extracts was assessed by a modified method (Oliveira et al., 2007). A solution of 2.5 ml of extract was supplemented with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide solution [$K_3Fe(CN)_6$] and the resulted mixture was incubated for 20 min at 50 °C. Then 2.5 ml of trichloroacetic acid (10%) was added and centrifuged for 10 min at 3000 rpm.

A 2.5 ml aliquot of the supernatant from each sample mixture was combined with 2.5 ml of distilled water, and 0.5 ml of aqueous solution of $FeCl_3$(0.1%). The absorbance of the reaction medium was read at 700nm. Synthetic antioxidant (BHA) was used as a control. The effective concentration (EC_{50}) was used as an index to compare and express the reducing capacity of bioactive substances. All samples were analyzed in triplicates.

Bleaching Ability of β-carotene in Linoleic Acid System

The antioxidant activity of the plant extracts was also evaluated by using a β-carotene-linoleate model system according to the method of Shon et al., (2007). A solution of β-carotene /linoleic acid mixture was prepared by dissolving 2.0 mg of β-carotene in 1ml of chloroform, this solution was then pipetted into a round-bottomed flask and 25µl of linoleic acid and 200 mg of Tween 40 were added. The chloroform was evaporated under vacuum at 40°C, then the mixture was supplemented with 100 ml of distilled water saturated with oxygen, and the resulting mixture was vigorously stirred. Aliquots (2.5 ml) of β-carotene /linoleic acid emulsion were transferred to tubes containing 350 µl of each extract. The samples were then incubated in a water bath at 50 °C for two hours, the absorbance of each sample was read at 490 nm.

For synthetic antioxidant, the same procedure was applied, and butylated hydroxyanisol (BHA) was considered as a positive control. All samples were analyzed in triplicates.

Antioxidant activity in β-carotene bleaching model in percentage was assessed by the following equation **(**Bougatef et al., 2009**)**:

$$\textbf{Antioxidant activity\%} = 1 - \frac{(A_0 - A_t)\ \textbf{test}}{(A_0 - A_t)\ \textbf{control}} \times 100$$

A_0: Absorbance at time t = 0. A_t: Absorbance at time t =120min

Statistical Analysis:

The obtained results were calculated as mean ±standard deviation (SD), and statistical analyses were performed using Microsoft Excel stat (2009). To establish correlations between different parameters, simple linear regression analysis was used. Statistical significance was considered at $p < 0.05$.

3. RESULTS AND DISCUSSION

Medicinal plants are of a great importance for both individual and community health. The production of medicinal and aromatic plants as know is challenging, and involves a large variety of issues, including pharmacological, commercial agricultural and ecological domains (Vinha et al., 2012).

Extraction Yield

It is not easy to compare the results of this study with other studies' results, the performance is relative and depends on the method and conditions under which the extraction was performed. The extraction method also affects all the total content of phenols, flavonoids and biological activities for example antioxidant activity (Lee et al., 2003).

The isolation and quantification of antioxidant compounds were done frequently by using extraction with different solvents, the use of these later was explained by the difference in polarity of these compounds. For this reason, in this work, the extractions were carried out using two solvents: water and methanol (Soares et al., 2013).

As can be observed in (Figure 1), the highest yield was obtained with maceration using methanol as solvent (15.68 % and 6.36 % from extracts of Boussada and Oum El Bouaghi respectively), whereas the lowest yields were obtained with water (3.4% and 4.94 % respectively).

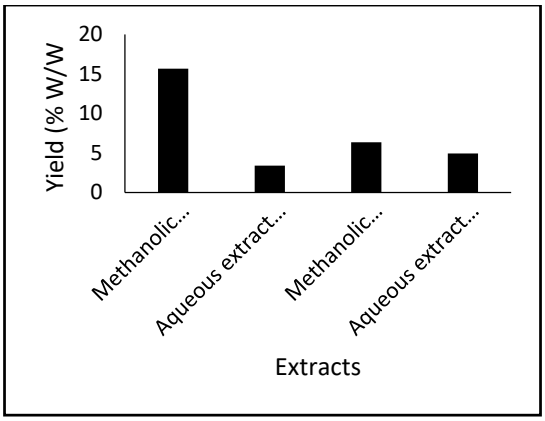


Figure 1: The variation of extraction yield

Total Phenolic Compounds

Lately, pharmacological properties of medicinal plants have attracted researchers (antioxidants and anti-inflammatories); these plants have been considered important because of containing the components like secondary metabolites (Złotek et al., 2016)

The contents of polyphenols, flavonoids, flavonols and tannins in methanolic and aqueous extract of Artemisia campestris are represented in Table 1.

Methanolic extracts showed the highest content of polyphenols, and aqueous extracts showed the lowest amount. This fact can be explained by a low degree of solubility of these compounds in water.

The amount of total phenolic compounds in A. campestris extract was higher compared to what was found by (Djeridane et al., 2006).

Total Flavonoid and Flavonols Contents

Total flavonoid and flavonols contents of extracts are shown in Table 1. Flavonoid and flavonol concentrations were reported as mg QE/g extract. In this study, it was noticed that methanolic extract contained the highest amount of flavonoids and flavonols, this can be explained by low solubility of these compounds in water (Khettaf et al., 2016).

Tanins Content:

Condensed tannins were determined using the method of Julkunen-Titto (1985), in this test a complex chromophore was formed, it had a maximum absorbance at 500 nm. A calibration range was established with catechin. The aqueous extract contained considerable amount of condensed tannins.

Table 1. Total phenolic, flavonoid, flavonols and tanins contents in A. campestris extracts

Plant material	Total phenolic (mg CAE/g extract)	Flavonoids (mg QE/g extract)	Flavonols (mg QE/g extract)	Tanins (mg CE/g extract)
Methanol extract (Boussaâda)	88,61 ± 0,22	12,91 ± 0,01	20,507 ± 0,01	36,88 ± 0,01
Aqueousextract (Boussaâda)	74,75 ± 0,01	31,84 ± 0,00	111,93 ± 0,02	35,29 ± 0,00
Methanol extract (Oum El Bouaghi)	82,84 ± 0,09	13,72 ± 0,00	18,37 ± 0,01	70,59 ± 0,00
Aqueousextract (Oum El Bouaghi))	87,35 ± 0,03	33,14 ± 0,01	217,75 ± 0,01	60,29 ± 0,01

Values expressed as mean ± standard deviation (n=3)

Antioxidant Activity

The antioxidant properties of medicinal plants must be evaluated by several methods because these plants contain complex phytochemicals. In this case, tests of the antioxidant activity whose mechanisms of action were different and complementary were necessary (Gioti et al., 2009).

In this work, three methods were used to determine antioxidant power of the different extracts (DPPH, FRAP and β-carotene/linoleic acid assay).

Djeridane et al. (2006) investigated the antioxidant activity of 11 medicinal Saharan plants used to treat gastric and inflammatory problems and they found that the majority of the tested plants possessed a good antioxidant power which was related to their content of phenolic compounds.

Scavenging Activity Against The diphenyl-picrylhydrazyl (DPPH) Radical

DPPH is nitrogen centered free radical having an odd electron which gives a strong absorption at 517 nm, the change of color from violet to yellow is due to the reduction of the radical DPPH in the presence of a radical scavenger (Cai et al., 2003).

The IC50 index is often used in experiments to determine the free radical scavenging capacity; it is defined as the concentration of the sample which entrains the scavenging of free radicals at 50% (Cuvelier, Richard, and Berset, 1992)

Free radicals scavenging of the extracts were comparable with those of the synthetic antioxidant butylated hydroxyl anisole (BHA), the values for IC_{50} of the extracts (Table 2) ranged from 48,42 µg/ml to 320,60 ± 22,58 µg/ml while IC_{50} of BHA was 15.18 µg/ml, Aqueous extracts of A. campestris from Oum El Bouaghi (48,42 µg.ml) showed better DPPH scavenging activities than A. campestris methanolic extracts. When compared to the synthetic antioxidant BHA, A. campestris methanolic and aqueous extracts exhibited lower antioxidant abilities to reduce DPPH radicals.

A previous study on the antioxidant capacity of methanolic extracts of A. campestrismeasured by DPPH showed an IC_{50} ranged between 8 and 20 µg/ml (Khettaf et al., 2016). For further comparison, Megdiche-Ksouri et al., (2015) found that the aqueous extract of the aerial part of the plant showed a value of IC_{50} equal to 27.5 µg/ml; in comparison with the standard antioxidant BHT (IC_{50} = 11.5 µg/ml) whose value was twice higher.

Table 2. Antioxidant activity of A. campestris extracts

Plant Extracts	DPPH IC50 (µg/mL)	Reducing power EC50 (µg/mL)	β-Carotene bleaching inhibition%
Methanolic extract (Boussaâda)	241,48 ± 61,86 **	455,2 ± 2,2 ***	85,18 ± 2,41^{ns}
Aqueousextract (Boussaâda)	320,60 ± 22,58 ***	871,28 ±49,96 ***	26,58 ± 3,34 ***
Methanolic extract (Oum El Bouaghi)	131,19 ± 18,81***	266,74 ± 16,51***	88,03 ± 0,58^{ns}
Aqueousextract (Oum El Bouaghi))	48,42 ± 13,19	414,52 ± 28,78 ***	52,65 ±3,50 ***
BHA	15,18 ±0,95	41,21 ± 0,78	89,43 ± 3,24

Comparison was realized against BHA, Values expressed are means ± S.D

* p< 0.05 significant difference, **p < 0.01 very significant difference, *** p<0.001 extremely significant difference. ns: non significance.

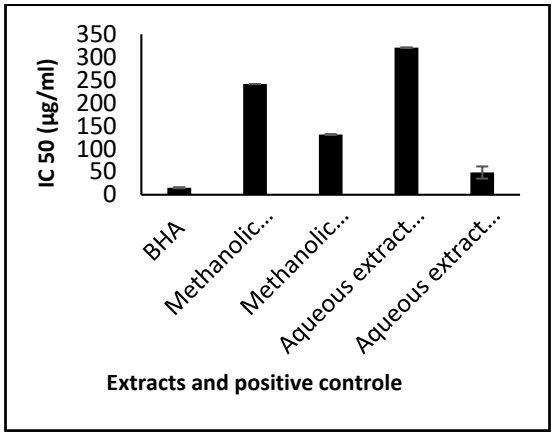


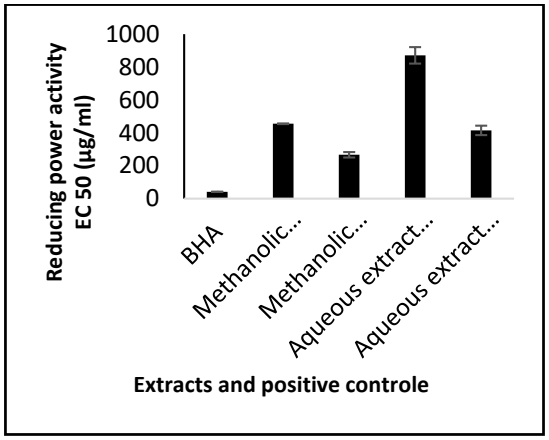
Figure 2: Free radical scavenging of A. campestris extracts and positive controls measured by DPPH assay

The Ferric Reducing Antioxidant Power Assay (FRAP):

In FRAP assay, in the potassium ferrocyanide complex and the presence of reductive agents, ferric iron is reduced to ferrous iron and causes the generation of a chromogenic complex (Kalita et al., 2013)

The reduction capacity of both extracts increased with increasing concentration, however, its reducing power was inferior to BHA.

The reduction capacity of methanolic extract of Artemisia campestrisfrom Oum El Bouaghi was the highest among the tested extracts with a EC_{50}= 266,74 µg/ml, followed by byaqueous extracts. The authors (Djeridane et al., 2006; Akrout et al., 2011) found that the antioxidant capacity of the extract of A. campestris was related to the content of this plant in phenolic compounds.



Data were presented as EC50 means ± SD (n = 3) compared to BHA as standard.

Figure 3: A comparison between different plant extracts in reducing power assay.

Inhibition of Linoleic Acid Peroxidation

According to several authors, the test of inhibition of the oxidation of linoleic acid coupled to β-carotene, looks very useful as a mimetic model of lipid peroxidation in biological membranes (Tepe et al., 2005).

In the β-carotene/linoleic acid assay, the methanolic extracts of A. campestris inhibited strongly in linoleic acid peroxidation with 84 % of inhibition. These results were comparable to those of the standard, BHA (89,43 %). The results of this study were in concordance with the study of Djidel and Khennouf, 2014, in which ethyl acetate extract produced 82% of bleaching inhibition, and chloroform extract also inhibited 79% of β -carotene bleaching.

Furthermore, the inhibition demonstrated by the aqueous extract was lower the standard antioxidant BHA.

Antiradical and antioxidant properties of the organic extract (methanolic extract) of Artemisia anomala were tested by Guangrong and collaborators (2008), by the DPPH test and technical discoloration β-carotene, this species showed higher values for the antiradical activity, in comparison with the synthetic antioxidant (BHT), and while testing β-carotene bleaching they found antioxidant activity value of 89%, which was the same the value which was found for the methanolic extracts in this search.

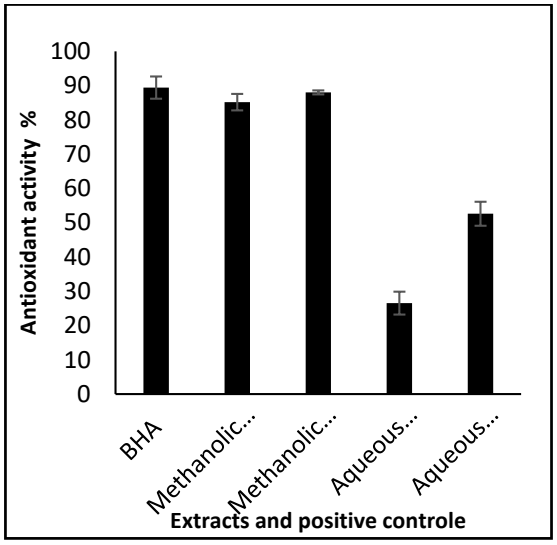


Figure 4: Antioxidant activity of A. campestris and positive control measured by β-carotene bleaching assay

4. CONCLUSION

For the extracts, the antioxidant capacity estimated by the DPPH method was very low. In the test of β-carotene bleaching, methanolic extracts showed excellent antioxidant activity, which can be explained by the fact that the chemical reaction in the technical DPPH involves the transfer of electrons from a donor (antioxidant) to the free radical DPPH and the reduction of the latter in DPPH-H.

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