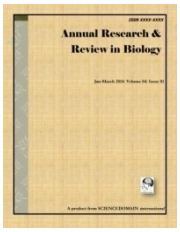

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Radical Scavenging, Reducing Power, Lipid Peroxidation Inhibition and Chelating Properties of Extracts from *Artemisia campestris* L. Aerial Parts

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Authors' contributions

This work was carried out in collaboration between all authors. Author SK designed the study, wrote the protocol and wrote the first draft of the manuscript. Author SD managed the analyses of the study, performed the statistical analysis, and performed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In this study, we estimated the antioxidant activity of various extracts prepared from *Artemisia campestris* L. aerial parts used in Algeria to treat gastro-intestinal disorders. The determination of polyphenols and flavonoids contents showed that the ethyl acetate extract (EAE) is rich in phenolic compounds with 481.25±0.026 mg gallic acid equivalent/g dry weight, while the chloroform extract (CHE) had the highest content of flavonoid with 34.37±0.056 mg quercetin equivalent/g dry weight. The evaluation of DPPH scavenging activity of extracts confirmed that EAE is the most active extract with IC_{50} of 0.0058 mg/ml. In addition, EAE showed the most scavenging activity against hydroxyl radical generated in the H_2O_2/Fe^{+2} system with IC_{50} of 0.17 mg/ml which is comparable to the activity of the standard antioxidant ascorbic acid (0.15 mg/ml). Ferrous ion chelating capacity assay showed that aqueous extract (AQE) was the most active with 0.11 mg/ml. The inhibition of linoleic acid/ß-carotene coupled oxidation was estimated by the ß- carotene bleaching assay, which showed a highest relative antioxidant activity for the crude extract (CE) (82.72% of inhibition). In conclusion, the present study showed that EAE of *A. campestris* L. is rich in phenolics and flavonoids and has a considerable antioxidant activity.

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Keywords: Artemisia campestris L; polyphenols; antioxidant activity; reducing power; scavenging activity; metal chelating.

1. INTRODUCTION

The reactive oxygen species (ROS) are generated in the human body as a result of normal endogenous metabolic process. The ROS generated are normally detoxified by the antioxidants present in the body such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. These free radicals cause oxidative damage to many biomolecules like proteins, lipids and DNA [1]. This oxidative damage is a major factor implicated in many chronic diseases such as cancer, diabetes, hypertension and other cardiovascular disorders [2]. The interest in finding antioxidants from natural sources is growing nowadays. These antioxidants scavenge free radicals and can help prevent many of these diseases. The antioxidants may mediate their effect by directly reacting with ROS, quenching them and/or chelating the catalytic metal ions [3]. Several synthetic antioxidants were commercially available but were unsafe. Natural antioxidants, especially phenolics and flavonoids, were safe and also bioactive.

A. campestris (Asteraceae) is a perennial scarcely aromatic herb or small shrub. It is widespread in the south of Algeria. The aerial parts of this plant are used in folk medicine in Algeria for the treatment of gastrointestinal disorders, ulcer, and diarrhea. The preparation of leaves and flowers of *A. campestris* (infusion, maceration and decoction) have been recommended in Tunisian folk medicine for their antivenin [4], anti-inflammatory, antirheumatic and antimicrobial activities [5]. The hypoglycemic and hypolipidemic effects of *A. campestris* leaf extracts in rat pancreatic tissue has been reported and the effect was connected with antioxidative potential of the plant [6]. The flavonoid profile of *A*. *campestris* is quite complex consisting of flavones, flavonols, flavanones, dihydroflavanols and their methyl ethers [7].

The aim of this study was to determine polyphenol contents and the antioxidant activity of extracts from the aerial parts of *A. campestris* L. growing in Algeria.

2. MATERIALS AND METHODS

2.1 Chemicals

Linoleic acid, b-carotene, butylated hydroxytoluene (BHT), were purchased from Fluka Chemical Co. (Buchs, Switzerland). 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), ethylenediamine tetraacetic acid (EDTA), gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Potassium ferricyanide, trichloroacetic acid (TCA), ferrous and ferric chloride were obtained from Merck. All other reagents were of analytical grade.

2.2 Plant Material

The aerial parts of *A. campestris* L. were collected from Bousaada southern Algeria. This plant was identified by Pr. Laouar H from the laboratory of Botanical Sciences, Faculty of Natural and Life Sciences, University Ferhat Abbas, Setif 1, Algeria. A voucher specimen was kept at this Laboratory.

2.3 Preparation of *A. campestris* Aerial Parts Extracts

The extraction of phenolic compounds was conducted as reported by Markham [8]. Aerial parts of *A. campestris* (100 g) were powdered, mixed with one liter of methanol-water solution (85: 15 v/v) and kept at room temperature for 3 days. It was then filtered and the solvent was evaporated to get crude extract (CE). The aqueous solution was washed with hexane several times until a clear upper layer of hexane was obtained. The lower layer was then extracted successively with chloroform and ethyl acetate to obtain 3 fractions; chloroform (CHE), ethyl acetate (EAE) and aqueous extracts (AQE). Each fraction was stored at -20ºC until use.

2.4 Estimation of Total Phenolic Content

Total phenolic compounds in *A. campestris* extracts were determined by the Folin–Ciocalteu reagent, using the method of Li et al. [9] with slight modifications. 0.1 ml of samples or standard was mixed with 0.5 ml of Folin-Ciocalteau reagent (diluted 10 times). After 4 min, 0.4 ml of 7.5 % sodium carbonate (Na_2CO_3) solution was added. After the incubation for 1 h and 30 min in the dark at room temperature, the absorbance was measured at 760 nm using a spectrophotometer and the results are expressed in mg of gallic acid equivalent per gram dry weight of plant (GEA).

2.5. Estimation of Flavonoid Content

Total flavonoid content in each extract was determined using the aluminum chloride colorimetric method [10]. Briefly, 1 ml of diluted sample was mixed with 1 ml of 2% aluminum chloride in methanol. After incubation at room temperature for 10 min, the absorbance of the reaction mixture was measured at 430 nm and the flavonoid content was expressed in mg Quercetin equivalent per gram of dry weight (QE).

2.6 DPPH Radical Scavenging Assay

Free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured using the method used by Burits and Bucar [11] with slight modifications. 50 µl of different dilutions of the extracts were added to 1250 µl of 4% solution DPPH dissolved in methanol. After 30 min at room temperature, the absorbance was measured at 517 nm. Rutin was used as positive standard. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = $(A_C – A_S / A_C) \times 100$.

A_C: is the absorbance without extract.
A_S: is the absorbance in the presence of sample.

2.7 Hydroxyl Radical-scavenging by Phenanthroline-Fe (II) Oxidation Assay

Fenton reaction is a key reaction in the organism which produces hydroxyl radicals. This radical can attack aromatic compound (salicylic acid) hydroxylation into hydroxylated product (2,3-dihydroxyl benzoic acid), which can be detected colorimetriclly. The adding of antioxidants can reduce hydroxyl radicals and hydroxylation so that the efficiency of scavenging hydroxyl radical can be determined. The ability of the hydroxyl radical-scavenging was carried out as described by Li et al [12]. 600 µL of (5 mM) phenanthroline, 600 µL (5mM) FeSO4, 600 µl of EDTA (15 Mm), 400 µl phosphate buffer (0.2 M, pH= 7.4)

and 800 µl (0.01%) H_2O_2 were added into 600 µl of extract. After 1 h of incubation at 37°C, the absorbance at 536 nm was recorded.

2.8 Reducing Power

The ability to reduce ferric ions was measured using the method described by [13]. 0.1 ml of different concentrations of each extract, was mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 min. After, 0. 25 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction and centrifuged at 3000 rpm for 10 min. The supernatant (0.25 ml) of the solution was added to distilled water (0.25ml) and 0.1% ferric chloride (0.5 ml). Absorbance was measured to determine the amount of ferric ferrocyanide (Prussian blue) formed at 700 nm against a blank in spectrophotometer.

2.9 Beta Carotene/Linoleic Acid Assay

The ability of extracts to prevent bleaching of b-carotene was assessed as described by [14]. A stock solution of b-carotene/linoleic acid was prepared by dissolving 0.5 mg of b-carotene in 1 ml of chloroform, 25 µl of linoleic acid and 200 mg of Tween 40. The chloroform was completely evaporated under vacuum in a rotatory evaporator at 40ºC, then 100 ml of distilled water were added and the resulting mixture was vigorously stirred. The emulsion obtained was freshly prepared before each experiment. Aliquots (2.5 ml) of the b-carotene/ linoleic acid emulsion were transferred to test tubes containing different concentrations. The absorbance of each sample was measured at 470 nm. BHT was used as a positive standard. The antioxidant activity was calculated as following equation:

$$AA\% = Abs_{sample}/Abs_{BHT} * 100.$$

2.10 Chelating Activity on Fe^{2+}

The iron (II)-chelating ability of the extract was assessed by the method of [15]. In brief, to a 0.25 ml aliquot of dissolved extract was added to 0.05 ml (0.6 mM) aqueous $FeCl_2 - 4H_2O$ and 0.45 ml Methanol. After 5 min, the reaction was initiated by the addition of 0. 05 mL (5 mM) ferrozine solution. After 10 min, the absorbance at 562 nm was recorded. The control contained all the reagents except the extract or positive control. EDTA was used as a positive control. The percentage of inhibition of ferrozine-Fe2+ complex formation was calculated using the formula:

$$\text{Chelating activity } \% = ((A_{control} - A_{sample})/A_{control})*100$$

EC50 values were calculated by linear regression analysis; linearity range between antioxidant concentration and chelating activity.

2.11 Statistical Analysis

Experimental results recorded were means±standard deviation (SD) of triple determinations. The data were analyzed by one-way analysis of variance (ANOVA). Tests of significant differences were determined by tukey multiple range tests at p=.05.

3. RESULTS AND DISCUSSION

3.1 Total Phenol and Flavonoid Contents of the Extracts

The results showed that *A. campestris* fractions contained phenolic compounds in the following order: EAE> CHE> CE> AQE, as shown in Table 1. Flavonoid quantification using aluminum chloride is a suitable method. Aluminum chloride forms stable complexes with these compounds [16]. The total flavonoid contents of different *A. campestris* fractions were reported as mg Quercetin equivalent per g of dried extract. The flavonoid contents of the *A. campestris* fractions have the following order: CHE >EAE > CE >AQE Table 1. Based on these data, it can be concluded that the EAE extract of *A. campestris* possesses high content of phenolic and the CHE contains high content of flavonoids.

Table 1. Phenolics and flavonoid content of *Artemisia campestris* L.

Extracts	Total phenolic content mg Gallic acid equivalent/g of dry plant	Total flavonoid content mg Quercetin equivalent/g of dry plant
CE	143.4±0.033	17.75±0.001
CHE	169.2±0.08	34.37±0.056
EAE	481.25±0.026	22.11±0.013
AQE	65.5±0.0035	8.56±0.0005

Results are expressed as means ± standard deviation (n = 3). CE : crude extract, CHE : chloroform extract, EAE : ethyl acetate extract, AQE : aqueous extract

Djeridane et al. [17] determined the content of polyphenols in 11 Algerian medicinal plants. They reported that the content of polyphenols in 70% ethanolic extract of *A. campestris* was 20.38 mg EAG/g, this value is lower than the value reported in this study. This can be attributed to the method of extraction and the type of solvent.

In another study, Djeridane et al. [18] evaluated the polyphenol contents in 80% ethanolic extract of *A. campestris*, the value found was 103.4 mg mg gallic acid equivalent. This is relatively similar to that found in our study using 85% methanolic extract (143.4±0.033 mg gallic acid equivalent/g of dry plant).

3.2 DPPH Radical Scavenging

DPPH is relatively stable free radical that easily accepts an electron or hydrogen atom to become a stable molecule [19]. The amount of each extract needed for 50% inhibition (IC_{50}) is presented in Fig. 1. IC_{50} of the standard compound, rutin was 0.004 mg/ml. The highest radical scavenging activity was presented by EAE with IC_{50}=0.0058 mg/ ml which is lower than that of standards (p=0.5). The radical scavenging activity in the plant extracts decreased in the following order: EAE > CHE> CE > AQE.

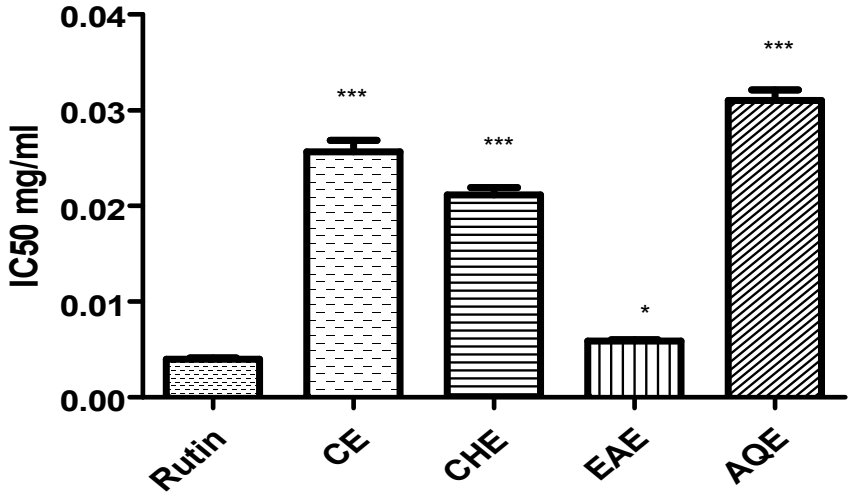

Fig. 1. IC_{50} values of plant extracts for free radical scavenging activity by DPPH method. Lower IC_{50} value indicates higher antioxidant activity. CE: crude extract, CHE: chloroform extract, EAE: ethyl acetate extract, AQE: aqueous extract

The result of the present study showed that the EAE, which contain highest amount of phenolic compounds, exhibited the greatest antioxidant activity with 0.0058 mg/ml. The high scavenging activity of EAE may be due to hydroxyl groups existing in the phenolic compounds that can provide the necessary component as a radical scavenger. Many studies confirmed that the antioxidant properties of these molecules arise from their high reactivity as electron donors, and from the ability of the polyphenol to stabilize the unpaired electron [20].

Several studies on *A. campestris* demonstrated that ethyl acetate fraction showed to be active as DPPH radical scavenger. This fraction is rich in flavonoids with antioxidant properties (Nikolova et al., [21]. Serteser et al.,[22] estimated the antioxidant activity of methanolic extract (50%) of *A. campestris*, they found that IC50 was 2.467 mg/ml, this value show that its activity is lower than of methanolic extract (85%) of our study (0.025mg/ml). This can be not attributed to the percentage of water in alcoholic solvent. The radical scavenging and antioxidant activity results for these plants show differences from the earlier reported results above. However these contradictory results are most likely due to differences in methodology and experimental conditions used in the different studies and differences in the method of sample extraction can results in a wide variation in the antioxidant activity of the extract.

3.3 Hydroxyl radical-scavenging by phenanthroline-Fe (II) oxidation assay

The hydroxyl radical is the most reactive of the reactive oxygen species, and it induces severe damage in adjacent biomolecules [23]. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins [24].

In the phenanthroline- Fe (II) assay, hydroxyl radicals produced in H_2O_2/Fe^{2+} system can oxidize phenanthrolene-Fe^{2+} into phenanthrolene-Fe^{3+}, and the absorption was measured at 536 nm. All extracts of *A. campestris* and vitamin C exhibited scavenging activities against

hydroxyl radicals Fig. 2. The radical scavenging activity for *A. campestris* areal part extracts decreased in the following order EAE> CHE> AQE > CE respectively.

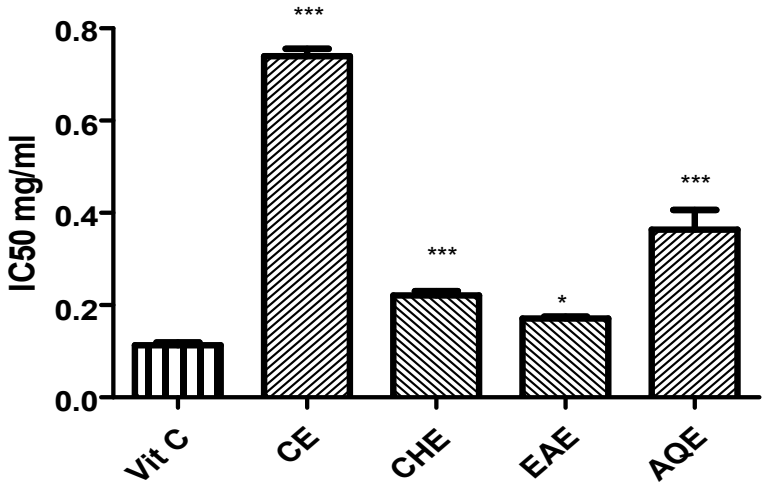

Fig. 2. IC_{50} values of plant extracts for hydroxyl radical scavenging activity by phenantroline method. Lower. CE : crude extract, CHE : chloroform extract, EAE : ethyl acetate extract, AQE : aqueous extract, VIT C : vitamin C

EAE and CHE showed a highest antiradical effect with 0.17 and 0.22 mg/ml respectively, these results are similar to those of DPPH. The results of previous studies with different antioxidant assay on *A. campestris* ethanol-water (50%) extract suggest that polyphenol content should be considered as an important feature of *A. campestris*, as some of its effects, such as antioxidant activity, could be attributed to the presence of these constituents [25].

3.4 Reducing Power

Fe^{+3} reduction is often used as an indicator of electron- donating activity, which is an important mechanism of phenolic antioxidant action, and can be strongly correlated with other antioxidant properties [26]. There are dose-response curves for the reducing powers of the extracts from *A. campestris* Fig. 3. The reducing power of the CE and AQE extracts increased from 0.078±0.01 and 0.137±0.006 at 0.07 mg/ml to 1.36±0.024 and 0.888±0.021 at 0.14 mg/ml respectively. The reducing power of EAE extract increased from 0.256±0.029 at 0.004 mg/ml to 1.95±0.042 at 0.045 mg/ml. The reducing power of CHE extract increased from 0.15±0.027 at 0.008 mg/ml to 1.63±0.027 at 0.17 mg/ml. BHT was used as standard. All extracts showed degree of electron donation capacity in a concentration-dependent manner but the capacities were inferior to that of BHT (1.16±0.003 at 0.025 mg/ml).

A relationship between Fe^{3+} reducing activity and total phenol content has been reported in the literature [27], however the correlation may not be always linear as compared to other methods [28]. Phenolic compounds have been reported to be significantly associated with the antioxidant activity of plant and food extracts mainly because of their redox properties,

allowing them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and hydroxyl radical quenchers [29].

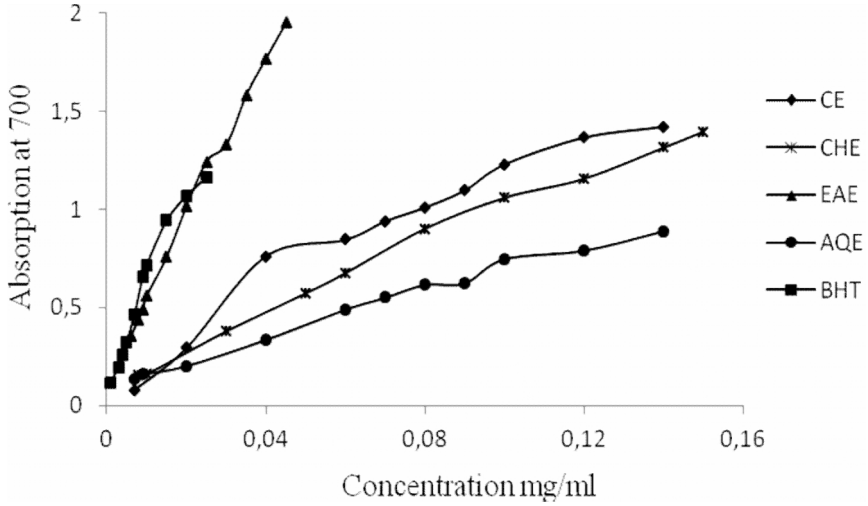

Fig. 3. Antioxidant activity of *A. campestris* extracts expressed as reducing power. CE : crude extract, CHE: chloroform extract, EAE : ethyl acetate extract, AQE : aqueous extract.

3.5 B-carotene/Linoleic Acid Assay

This method is based on the loss of the orange color of B-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The ability of extracts to inhibit the lipid peroxidation and to be evaluated by B-carotene bleaching test showed that the peroxidation of lipids was effectively inhibited by *A. campestris* extracts. The results show the antioxidant activity of the extracts and BHT as measured by the bleaching of the b-carotene/linoleic acid system Fig. 4. In this system, CE and EAE exhibited a highest inhibition activity with values of 82 and 79 % ($P<.001$). This activity may be attributed primarily to the high content of phenolic components of the *A*. *campestris*. Other studies realized by [25] showed that ethanolic extract of this plant possessed a low inhibition of b-carotene oxidation (34%). This difference may be related to the type of solvent used in the extraction of antioxidant molecules.

3.6 Metal Chelating Activity

Ferrozine can quantitatively form complexes with Fe^{2+}. However, in the presence of chelating agents, the complex formation is disrupted with the result that the violet color of the complex is decreased. Measurement of color reduction, therefore, allows the estimation of the chelating activity of chelator. The transition metal ion Fe^{2+} possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively nonreactive radicals [30]. All extracts showed chelating activity of ferrous ion before ferrozine in dose dependent Fig. 5.

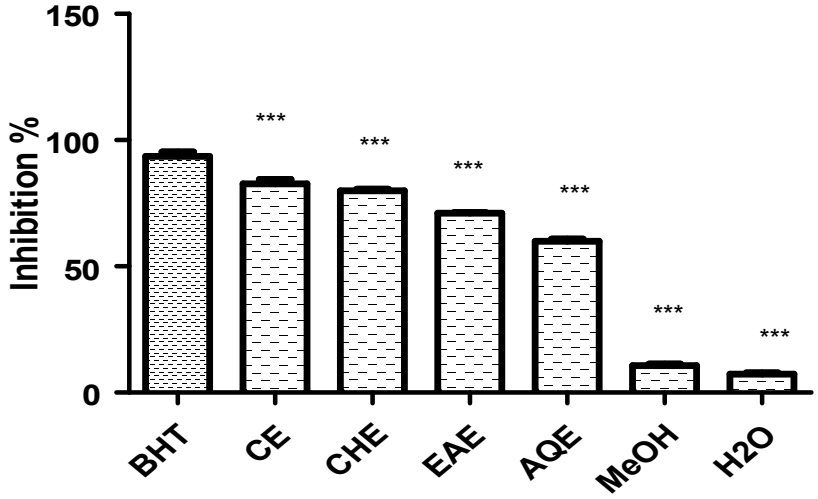

Fig. 4. Antioxidant activities of *A. campestris* extracts measured by b-carotene bleaching method at 24 hours. CE: crude extract, CHE: chloroform extract, EAE: ethyl acetate extract, AQE: aqueous extract. Values are means±SD (n = 3)

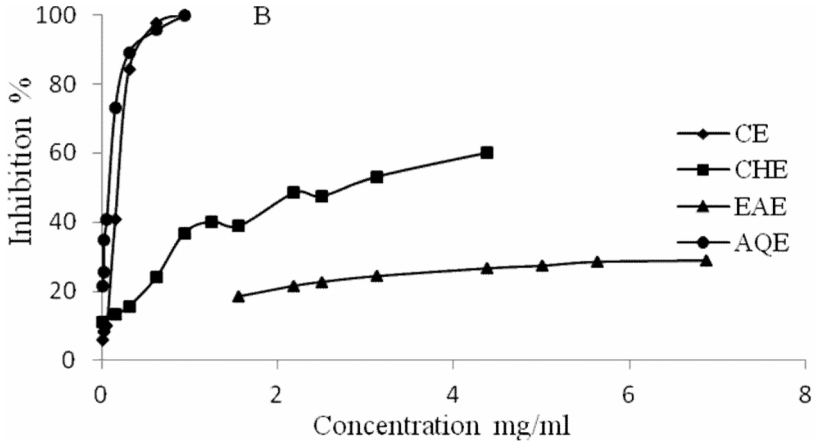

Fig. 5. Chelating activity of the extracts from *A. spinosa* leaves. EDTA was used as the positive control. CE: crude extract, CHE: chloroform extract, EAE: ethyl acetate extract, AQE: aqueous extract. Values are means ± SD (n = 3)

EAE posses a high content of polyphenols but it showed a lowest chelating activity. This may be related by other molecules witch chelate the ions of Fe^{+2}. Similar study established that ethyl acetate extract of *Smilax excelsa* L. had a weaker activity among other extracts with 16% at 1.2 mg/ml [31].

4. CONCLUSION

This study demonstrated that EAE of *A. campestris* aerial part contains high levels of phenolic compounds and was capable of inhibiting lipid peroxidation, directly quenching free radicals to terminate the radical chain reaction and acts as reducing agents. Phenolic compounds present in this plant are probably responsible for its antioxidant potential. These results explain the pharmacological properties of *A. campestris* and gave a scientific base of the use of this plant in the Algerian traditional medicine.

COMPETING INTERESTS

Authors declare that no competing interests exist with other people or organizations that could inappropriately influence our work.

REFERENCES

1. Farber JL. Mechanisms of cell injury by activated oxygen. Environ. Health Perspect. 1994;102:17-24.
2. Giasson BI, Ischiropoulos H, Lee VM Y, Trojanowski JQ. The relationship between oxidative/nitrosative stress and pathological inclusions in Alzheimer's and Parkinson's diseases. Free Radical Biol Med. 2002;32:1264-1275.
3. Robak J, Marcinkiewicz E. Scavenging of reactive oxygen species as the mechanism of drug action. Pol J Pharmacol. 1995;47:89-98.
4. Memmi A, Sansa G, Rjeibi I, El Ayeb M, Srairi-Abid N, Bellasfer Z, Fekhih A. Use of medicinal plants against scorpionic and ophidian venoms. Arch Inst Pasteur Tunis. 2007;84:49-55.
5. Behmanesh B, Heshmati GH, Mazandarani M, Ghaemi EA. Chemical composition and anti bacterial activity from essential oil of Artemisia sieberi Besser sieberi in North of Iran. Asian J Plant Sci. 2007;6(3):562-564.
6. Sefi M, Fetoui H, Makni M, Zeghal N. Mitigating effects of antioxidant properties of *Artemisia campestris* leaf extract on hyperlipidemia, advanced glycation end products and oxidative stress in alloxan-induced diabetic rats. Food Chem Toxicol. 2010;48(7):1986-1993.
7. Wollenweber E, Maan K, Valant-Vetschera K. "External flavonoid aglycones in Artemisia and some further Anthemideae (Asteraceae)". Fitoterapia. 1989;60(5):460-463.
8. Markham KR. Techniques of flavonoid identification. Academic Press London. 1982;1-133.
9. Li WD, Wei CL, White PJ, Beta T. High-amylose corn exhibits better antioxidant activity than typical and waxy genotypes. J Agric Food Chem. 2007;55:291-298.
10. Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Gazin JC, Pinkas M, Luycky M, Gazin M. Oxygen species scavenging activity of phenolic extract from Hawthorn fresh plant organs and pharmaceutical preparation. Arzneim Forsch/Drug Res. 1996;1-6.
11. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother. Res. 2000;14:323-328.

12. Li YH, jiang D, Zhang T, Mu WM, Liu J. Antioxidant and free radical scavenging activity of chicktea protein hydrolysate (CPH). Food Chem. 2008;106:444-450.
13. Chung YC, Chen SJ, Hsu CK, Chang CT, Chou ST. Studies on the antioxidative activity of *Graptopetalum paraguayense* E. Walther. Food Chem. 2005;91:419-424.
14. Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal. 2002;13:8-17.
15. Decker EA, Welch B. Role of ferritin as lipid oxidation catalyst in muscle food. J Agric Food Chem. 1990;36:674-677.
16. Chang CC, Yang MH, Wen HM, Chern JCh. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178-182.
17. Scholer P. Natural antioxidants exploited commercially, In B.J.F Hudson, Food antioxidants. London: Elsevier. 1990;99-170.
18. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997;2:152-159.
19. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. J Food Chem. 2006;97:654-660.
20. Djeridane A, Yousfi M, Najemi B, Vidal N, Lesgards JF, Stocker P. Screening of some Algerian medicinal plants for the phenolic Compouds and their antioxidant activity. Eur Food Res Techenol. 2007;224:801-809.
21. Nikolova M, Gussev Ch, Nguyen T. Evaluation of the antioxidant action and flavonoid composition of Artemisia species extracts. Biotechnol Biotechnolo EQ. 2010;24(2):101-103.
22. Serteser A, Kargıoglu M, Gök V, Bagcı Y, Musa Özcan M, Arslan D. Antioxidant properties of some plants growing wild in Turkey. GRASAS Y ACEITES. 2009;60(2):147-154.
23. Gutteridge MC. Reactivity of hydroxyl and hydroxyl-like radicals discriminated by release of thiobarbituric acid reactive material from deoxy sugars, nucleosides and benzoate. Biochem J. 1984;224:761-767.
24. Sakanaka S, Tachibana Y, Okada, Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem. 2005;89:569-575.
25. Akrout A, Gonzalez LA, El Jani HJ, Madrid PC. Antioxidant and antitumor activities of *Artemisia campestris* and Thymelaea hirsuta from southern of Tunisia. J Food Chem Tox. 2011;49:342-347.
26. Dorman HJD, Peltoketo A, Hiltunen R and Tikkanen MJ. Characterization of the antioxidant properties of de odourised aqueous extracts from selected Lamiaceae herbs. Food Chem. 2003;83:255-262.
27. Jayaprakasha GK, Singh, RP, Sakariah KK. Antioxidant activity of Grape seed (*Vitis vinefera*) extracts on peroxidation models *In vitro*. Food Chem. 2001;73:285-290.
28. Yildirim A, Mavi A, Oktay AA, Algur OF, Bilaloglu V. Comparison of antioxidant and antimicrobial activity of tilia (Tilia argenta Desf. Ex. D.C.), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis* L.) extracts. J Agric Food Chem. 2000;48:5030-5034.
29. Gupta S, Prakash J. Studies on Indian green leafy vegetables for their antioxidant activity. Plant Food Hum Nutr. 2009;64:39-45.

30. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause or consequence. Lancet. 1994;344:721-724.
31. Ozsoy N, Can A, Yanardag R, Akev N. Antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chem. 2008;110:571-583.

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