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Original Research Article

Antioxidant Properties and Mineral Contents of Different Solvent Extracts of Some Medicinal Plants Cultivated in Algeria

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ABSTRACT

The current study aims to evaluate the antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) as well as the mineral contents of the aqueous, ethanol, and acetone extracts of three Algerian plants; *Haloxylon scoparium*, *Artemisia campestris* L., and *Juniperus phoenicea* L. The phytochemical screening of the extracts was done using standard methods. The total phenolic and flavonoid contents were evaluated using the Folin-Ciocalteu's and Aluminium chloride colorimetric methods, respectively. The antioxidant activity was evaluated using the DPPH, ABTS, TAC, and CUPRAC assays. The mineral content was evaluated using ICP-AES. Phytochemical investigation revealed phenols, flavonoids and tannins as the predominant phytochemicals in the plants. The plant extracts showed various degrees of antioxidant activity. The ethanol extract of *Juniperus phoenicea* exhibited the highest antioxidant activity in both the DPPH and ABTS assays with EC_{50} values of 39.74 and 13.42 µg/mL, respectively. While the ethanol and acetone extracts of *Haloxylon scoparium* showed the highest antioxidant activity in the TAC and CUPRAC, respectively with EC_{50} values of 101.65 and 263 µg/mL, respectively. The TPC of the extracts ranged from 2.38 to 58.03 mg GAE/g DW, while the TFC ranged from 1.03 to 47.59 mg RE/g DW. The mineral content analysis reveals the presence of vital elements like Ca, Mg, Sr, Mn and Ni in the plants. These plants are considered promising natural sources of antioxidants and nutritional supplements that can be utilized in pharmaceutical formulations.

***Keywords*:** Algerian plants, Antioxidant, Total phenolic content, Total flavonoid content Mineral content.

Introduction

The Algerian environment is rich in plants with great medicinal and economic importance. Some of these plant species have been explored for their biological activities as well as their chemical composition, while some others still need further exploration.[1-3]

Artemisia campestris L., is an aromatic herb that belongs to the family Asteraceae. It is distributed in different regions of southern Algeria where it is locally called "dgouft". The plant has been widely used in folk medicine to treat many ailments. The plant extracts have been shown to have strong antioxidant activity, which has been related to the presence of several phenolic compounds.[1] *Juniperus phoenicea* L., is an evergreen tree that grows in North African countries, including Algeria. The plant has been used in folk medicine to treat many health disorders such as hypoglycemia and diarrhea.[2]

Haloxylon scoparium Pomel, is a flowering plant belonging to the family Chenopodiaceae. It is widely distributed in the semi-arid regions of North Africa and the Middle East. Traditionally, the plant has been used to treat several ailments and health related challenges.[4-6]

Free radicals are highly reactive chemical species with harmful effects resulting from their interaction with vital biomolecules such as lipids and nucleic acids.

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The excessive production of free radicals leads to their accumulation in the body and the occurrence of a phenomenon known as oxidative stress. It is important to note that natural antioxidants play a vital role in mitigating the harmful effects of such reactive species.[7-14] Therefore, the present study aims to evaluate the antioxidant activity, total phenolic and flavonoid contents, and mineral content of the aqueous, ethanol and acetone extracts of three Algerian medicinal plants namely; *Haloxylon scoparium*, *Artemisia campestris* (L.), and *Juniperus phoenicea* (L.).

Materials and Methods

Chemicals and reagents

Folin-Ciocalteu's reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, ascorbic acid, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) were obtained from Sigma (St Louis, MO, USA). Sodium carbonate, gallic acid, aluminum chloride, rutin, potassium persulphate ($K_2S_2O_8$), sulphuric acid, sodium phosphate, ammonium molybdate, ethanol, and acetone were obtained from Sigma-Aldrich (Munich, Germany).

Plant materials

The aerial parts of the study plants including *Artemisia campestris*, *Haloxylon scoparium*, and *Juniperus phoenicea* were collected from their natural habitats in Laghouat region, Algeria (Latitude: 33°47'59'', Longitude: 2°51'54'', Altitude: 764 m), between the months of March and April, 2021. The voucher specimens were deposited at the Laboratory of Process Engineering, University of Laghouat, with the numbers LGP Ac/03/21, LGP Hc/04/21 and LGP Jp/04/21 for *Artemisia campestris*, *Haloxylon scoparium*, and *Juniperus phoenicea*, respectively. The plants were reduced to a fine powder using electric mill.

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Preparation of extracts
Aerial parts of *A. campestris*, *H. scoparium*, and *J. phoenicea* were extracted separately using three organic solvents on the basis of the polarity of the solvents (Acetone, Ethanol and Aqueous). Five grams of each plant material were macerated individually in 100 mL of water, ethanol and acetone in a conical flask. The conical flask containing the mixture was kept on a mechanical shaker (Vibratory Sieve Shaker ANALYSETTE 3 PRO, FRITSCH, Idar-Oberstein, Germany) for 24 h to obtain the aqueous extract (AE), ethanol extract (EE), and acetone extract (ACE) of the various plants. The extracts were filtered through Whatman filter paper (150 mm). The extracts were concentrated using Rotavapour (Buchi, R-300) at 40°C. The dried extracts were recovered and stored in a refrigerator for further use.

Phytochemical screening
The phytochemical tests to detect the presence of compounds such as phenolic, flavonoids, tannins, saponins, cardiac glycosides, sterols and triterpenes, anthocyanins and reducing sugars were performed according to the method described by Ibrahim and Ghareeb (2020).[15]

Determination of total phenol content (TPC)
The total phenolic content was assessed using Folin-Ciocalteu's reagent as previously described by Habib *et al*. (2022).[16] Briefly, 200 µL of the extract was mixed with 1 mL of Folin-Ciocalteu's reagent and 800 µL of sodium carbonate (7.5%), the reaction mixture was shaken and allowed to stand for 30 min. The absorbance was measured at 750 nm. A standard calibration curve was prepared using gallic acid monohydrate (10-100 µg/mL). The total phenol content was estimated from the standard curve. The total phenol content was expressed as milligrams of gallic acid equivalent per gram dry weight (DW) of the extract (mg GAE/g DW). All measurements were carried out in triplicates.

Determination of total flavonoid content (TFC)
Total flavonoid content was determined by aluminum chloride method. Herein, 1 mL of the extract was mixed with 1 mL of 2% $AlCl_3$, and incubated at room temperature for 15 min. The absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer (Spectronic 601, Milton Roy, USA). All determinations were carried out in triplicates. Standard calibration curve was prepared using rutin at a concentration range of 1-10 µg/mL. The total flavonoid content was expressed milligrams of rutin equivalent per gram dry weight (DW) of the extract (mg RE/g DW).[17]

Determination of antioxidant activity by DPPH radical scavenging assay
The free radical scavenging activity of the solvent extracts using the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical was determined by the method described by Boulanouar *et al.* (2013).[18] DPPH solution (0.004%), plant extracts, and standard (Ascorbic acid) solution were prepared in methanol. Plant extracts and standard (Ascorbic acid) solution were prepared in different concentrations (10, 20, 40, 60, 80, and 100 µg/mL). The standard solution or plant extracts solution (1.9 mL for each) of the different concentrations were taken in different test tubes and then 0.1 mL of DPPH (0.004%) solution was added and kept in the dark for 30 min. Afterwards the absorbance was recorded at 517 nm using a spectrophotometer. The decrease in absorbance of the DPPH radical which was caused by the antioxidants components of the extracts was visually noticeable as a colour change from purple to yellow. The percentage radical scavenging activity was calculated using the formula below (equation 1).

$$\text{DPPH scavenging activity (\%)} = \left[\frac{A_0 - A_{01}}{A_0}\right] \times 100$$

Eqn. 1
Where; A_0 is the absorbance of the control reaction
A_1 is the absorbance in the presence of the sample of the extracts or standard.
Determination of antioxidant activity by ABTS radical scavenging assay
The scavenging activity of the extracts against ABTS radical was determined by following the method described by Boulanouar *et al.* (2013).[18] Briefly, the stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate ($K_2S_2O_8$) in equal volumes were allowed to stand in the dark for 12-16 h at room temperature. Prior to the assay, ABTS solution was diluted in ethanol to give an absorbance of 0.700 ± 0.02 at 734 nm, then, 2 mL of the resulting solution was allowed to react with 200 µL of the plant extracts at different concentrations, afterwards the reaction mixture was vortexed (IKA, Deutschland, Germany) and after 30 min, the absorbance was measured at 734 nm using a spectrophotometer. The same procedure was applicable to the ascorbic acid standard at various concentrations (1-100 µg/mL). The amount of sample necessary to decrease the absorbance of ABTS by 50% (IC_{50}) was calculated graphically.

Determination of total antioxidant capacity (TAC) by phosphomolybdenum assay
Basically, the assay involves the reduction of Mo (VI) to Mo (V) by the test sample and subsequent formation of a green coloured complex at acidic pH. The phosphomolybdenum assay was determined by the method of Rezzoug *et al.* (2019).[19] Briefly, 0.3 mL of each sample at different concentrations was combined with 3 mL of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath (Memmert WTB24, Aeussere Rittersbacher Strasse, Germany) at 95°C for 90 min, and then cooled to room temperature. The absorbance of each solution was then measured at 695 nm. Ascorbic acid was used as the standard.

Determination of cupric reducing antioxidant capacity (CUPRAC)
Cupric reducing antioxidant capacity (CUPRAC) was used to evaluate the antioxidant capacity of the plant extracts according to the method described by Apak *et al*. (2008).[20] Briefly, 0.5 mL 10 mM copper dichloride hydrate was mixed with 0.5 mL of 7.5 mM neocuproine, 1 mL of 1 M ammonium acetate buffer (pH 7), 0.2 mL of the test sample and 1 mL of distilled water. The mixture was vortexed for 10 s and allowed to stand for 30 min. The absorbance was measured at 450 nm against a reagent blank. Ascorbic acid was used as the standard.

Determination of mineral contents
The pulverized plant samples (0.5 gram each) were taken into a burning cup and then incinerated in an oven (Memmert UN55, Aeussere Rittersbacher Strasse, Germany) at 450°C for approximately 6 h. The ashed residue was extracted with 15 mL pure nitric acid (HNO_3), then, transferred to a volumetric flask, and was diluted to 50 mL with distilled water. The concentrations of minerals were determined with an Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Working conditions of the ICP-AES were (Instrument: ICP-AES (Avio 200); RF Power: 1500 W; Injector: 1.2 mm ceramic; Plasma Gas Flow rate (Ar): 08 L/min; Auxiliary gas flow rate (Ar): 0.7 L/min; Nebulizer Gas Flow rate (Ar): 0.5 L/min; Torch Position: -3 and Read Delay: 30 sec.

Statistical analysis
Data were expressed as the mean ± standard deviation of three independent experiments. Correlation coefficients and EC_{50} values were obtained from linear regression analysis in the Microsoft Excel® 2016.

Results and Discussion

Phytochemical constituents of the plant extracts
In the present study, three plant extracts were examined for the presence or absence of some phytochemicals. The results showed a noticeable difference in the phytochemical composition of the plant extracts. Phenolic compounds, flavanoids and tannins were predominant in the extracts, while anthocyanins were absent in most of the extracts (Table 1).

Total phenolic content (TPC) and total flavonoid content (TFC) of the extracts
The TPC and TFC of the different solvent extracts of the plants are presented in Table 2. The results revealed that the aqueous extract of *H. scoparium* had the highest TPC value of 58.03 mg GAE/g DW followed

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by the acetone and ethanol extracts of *A. campestris* with TPC values of 57.96 and 57.08 mg GAE/g DW, respectively. On the other hand, the acetone extract of *A. campestris* had the highest TFC value of 47.59 mg RE/g DW followed by the acetone extract of *J. phoenicea* and ethanol extracts of *A. campestris* with TFC values of 14.92 mg RE/g DW and 10.05 mg RE/g DW, respectively. Previous studies have shown that the 80% aqueous methanol extract of Algerian *A. campestris* has TPC value of 212.87 mg GAE/g dry extract and TFC value of 75.96 mg RE/g dry extract.[21]

Antioxidant activity of the plant extracts

The antioxidant activity of the different solvent extracts of the three plants is presented in Table 3. In the DPPH free radical scavenging assay, among the various extracts, the most potent free radical scavenging activity was exhibited by the ethanol extract of *J. phoenicea* with EC_{50} value of 39.74 µg/mL followed by the ethanol extract of *A. campestris* with EC_{50} value of 104.14 µg/mL, the acetone extract of *J. phoenicea* with EC_{50} value of 127.32 µg/mL and the aqueous extract of *H. scoparium* with EC_{50} value of 133.14 µg/mL (Table 3). While in the ABTS radical scavenging assay, the most potent extract was the ethanol extract of *J. phoenicea* with EC_{50} value of 13.42 µg/mL followed by the acetone extract of *A. campestris* with EC_{50} value of 20.45 µg/mL and the aqueous extract of *H. scoparium* with EC_{50} value of 25.11 µg/mL (Table 3). In the phosphomolybdenum assay for the determination of the total antioxidant capacity (TAC), the ethanol extract of *H. scoparium* showed the highest TAC with EC_{50} value of 101.65 µg/mL followed by the ethanol extract of *A. campestris* with EC_{50} value of 119.44 µg/mL, and the ethanol extract of *J. phoenicea* with EC_{50} value of 160.45 µg/mL (Table 3). Furthermore, the highest CUPRAC activity was exhibited by the acetone extract of *H. scoparium* with EC_{50} value of 263 µg/mL, while the acetone extract of *A. campestris* had the lowest CUPRAC activity with EC_{50} value of 2434 µg/mL (Figure 1). In all the assays, the standard (Ascorbic acid) had the lowest EC_{50} values, indicating that ascorbic acid has higher antioxidant activity than all the extracts of the different plants. The EC_{50} values for ascorbic acid include 3.42 µg/mL, 2.15 µg/mL, 10.65 µg/mL, and 183.4 µg/mL for DPPH radical scavenging activity, ABTS radical scavenging activity, total antioxidant capacity (TAC), and CUPRAC activity, respectively. Previous studies have shown that the investigated plants had promising antioxidant activity, for example the hydroalcoholic extract of Algerian *A. campestris* showed oxygen radical antioxidant capacity (ORAC) of 120.5 µmol TEAC/g DW).[1] Also, the 80% aqueous methanol extract of Algerian *A. campestris* showed free radical scavenging activity with IC_{50} values of 20.2 and 9.50 µg/mL in DPPH and ABTS assays, respectively.[21] The acetone water extract of Moroccan *Haloxylon scoparium* aerial part showed free radical scavenging activity against DPPH radical with IC_{50} value of 550.82 µg/mL and FRAP value of 681 µg/mL.[22] The methanol, ethanol and acetone extracts of *Juniperus phoenicea* leaves at 500 µg/mL showed free radical scavenging activity against DPPH radical with percentage inhibition of 92%, 95% and 96%, respectively.[23]

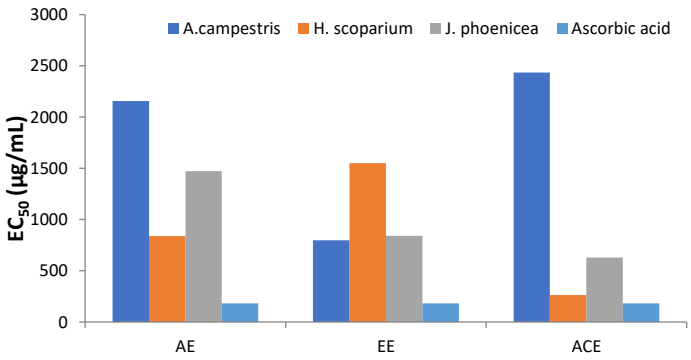

Figure 1: Cupric reducing antioxidant capacity (CUPRAC) of the aqueous, ethanol and acetone extracts of *A. campestris*, *H. scoparium* and *J. phoenicea*
AE: Aqueous extract, EE: Ethanol extract, ACE: Acetone extract.

Table 1: Phytochemical constituents of the aqueous, ethanol and acetone extracts of *A. campestris*, *H. scoparium* and *J. phoenicea*

Phytochemicals	Inference								
	A. campestris			*H. scoparium*			*J. phoenicea*		
	AE	EE	ACE	AE	EE	ACE	AE	EE	ACE
Phenols	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Saponins	+	-	-	+	-	-	+	-	-
Cardiac glycosides	+	+	+	+	-	-	+	+	+
Sterols and Triterpenes	-	-	+	+	-	+	-	+	+
Anthocyanins	-	-	-	-	-	-	+	-	-
Reducing sugars	+	+	+	+	-	-	+	+	+

+ Present; - absent. The determination was done in triplicates.
AE: Aqueous extract, EE: Ethanol extract, ACE: Acetone extract.

Table 2: Total phenolic and flavonoid contents of the aqueous, ethanol and acetone extracts of *A. campestris*, *H. scoparium* and *J. phoenicea*

Extract/ Plant	Total phenolic content (mg GAE/g DW)			Total flavonoid content (mg RE/g DW)		
	A. campestris	*H. scoparium*	*J. phoenicea*	*A. campestris*	*H. scoparium*	*J. phoenicea*
AE	4.30 ± 0.09	58.03 ± 4.69	42.75 ± 4.61	1.03 ± 0.13	3.21 ± 0.3	2.09 ± 0.35
EE	57.08 ± 2.12	11.68 ± 1.15	53.51 ± 1.34	10.05 ± 0.15	5.91 ± 0.05	8.43 ± 1.26
ACE	57.96±2.87	3.12 ± 0.31	2.38 ± 0.13	47.59 ± 1.87	6.17 ± 0.63	14.92 ± 0.42

Values are presented as mean ± SD. AE: Aqueous extract, EE: Ethanol extract, ACE: Acetone extract.

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Table 3: Antioxidant activity of the aqueous, ethanol and acetone extracts of *A. campestris*, *H. scoparium* and *J. phoenicea*

Extracts	EC_{50} (µg/mL)		
	DPPH Radical Scavenging Activity	ABTS Radical Scavenging Activity	Total Antioxidant Capacity (TAC)
A. campestris			
AE	374.25 ± 6.12	540.18 ± 183.25	193.65 ± 4.12
EE	104.14 ± 10.35	54.35 ± 1.12	119.44 ± 10.28
ACE	926.12 ± 38.85	20.45 ± 3.02	219.75 ± 8.55
H. scoparium			
AE	133.14±2.3	25.11 ± 2.04	392.66 ± 10.12
EE	716.20±40.05	499.16 ± 10.55	101.65 ± 20.75
ACE	NA	1032.66 ± 30.12	2280.40 ± 30.20
J. phoenicea			
AE	2152.11 ± 94.35	98.22 ± 1.04	197.65 ± 3.24
EE	39.74 ± 1.04	13.42 ± 0.25	160.45 ± 6.18
ACE	127.32 ± 30.12	255.88 ± 40.12	1315.86 ± 30.14
Ascorbic acid	3.42 ± 0.62	2.15 ± 0.14	10.65 ± 0.34

Values are presented as mean ± SD. AE: Aqueous extract, EE: Ethanol extract, ACE: Acetone extract, NA: Not active

Table 4: Mineral contents of *A. campestris*, *H. scoparium* and *J. phoenicea*

Plant	Mineral Composition (mg/kg DW)								
	Ca	Cu	Mg	Mn	Zn	Ni	Pb	Mo	Sr
A. campestris	92870	86	95824	386	30	328	72	18	842
H. scoparium	71362	144	23376	330	184	176	120	22	526
J. phoenicea	88572	104	13578	134	116	270	146	20	808

Mineral contents of the plants

The mineral contents of the three plants are presented in Table 4. *A. campestris* has the highest Ca, Mg, Mn, Ni and Sr contents with values of 92870, 95824, 386, 328, and 842 mg/kg DW, respectively, while the highest Cu, Zn and Mo contents were observed in *H. scoparium* with values of 144, 184, and 22 mg/kg DW, respectively. Moreover, the highest Pb content was recorded in *J. phoenicea*. The current findings are in agreement with the findings from a previous study, where the hydro-methanol extract of Algerian *A. campestris* aerial parts were found to contain high amounts of minerals like Ca (10.53 mg/kg DW), Cd (0.0988 mg/kg DW), Co (1.28 mg/kg DW), Cr (1.083 mg/kg DW), Fe (1181.8 mg/kg DW), Li (46.6 mg/kg DW), Mg (990.7 mg/kg DW), Mn (41.06 mg/kg DW), Mo (0.29 mg/kg DW), Ni (2.75 mg/kg DW), Pb (0.68 mg/kg DW), Sr (23.93 mg/kg DW), Ti (20.38 mg/kg DW), Zn (47.07 mg/kg DW), Na (298.41 mg/kg DW), and Cu (70.22 mg/kg DW).[24]

Conclusion

The current study investigated the antioxidant, total phenolic and total flavonoid contents as well as the mineral contents of the aqueous, ethanol, and acetone extracts of the Algerian plants; *H. scoparium*, *A. campestris* (L.), and *J. phoenicea* (L.). The free radical scavenging activity (EC_{50} values) ranged from 39.74 to 2152.11 µg/mL and from 13.42 to 1032.66 µg/mL, for DPPH and ABTS assays, respectively. While the EC_{50} values for the total antioxidant capacity (TAC) of the plant extracts ranged from 101.65 to 2280.40 µg/mL. The plants also have high contents of minerals like calcium, magnesium, copper, zinc, and manganese. These findings suggest that the plant species (*H. scoparium*, *A. campestris*, and *J. phoenicea*) are promising sources of natural antioxidant agents as well as sources of nutritional supplements due to their high content of essential elements.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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