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Original Article

Evaluation of antioxidant activity of hydromethanolic extracts of some medicinal species from South Algeria

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Abstract

Background: Phytochemicals are found in abundance at different levels in many medicinal plants. It is important to measure the total phenolic compounds correctly in such medicinal plants, the better to assess their antioxidant capacity.
Methods: Our study sought to evaluate the total phenolic, flavonoid, and tannin contents of three Algerian medicinal plants: *Echium pycnanthum* Pomel, *Haloxylon articulatum* Boiss, and *Solenostemma oleifolium* Bull. & Bruce. We employed six different testing methods to help ascertain whether these compounds have an antioxidant capacity, including total antioxidant capacity, 1,1-diphenyl-2-picrylhydrazyl$^{\bullet}$, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid$^{\bullet+}$, β-carotene, reducing, and chelating assays.
Results: Our study showed that these medicinal plants exhibited significantly different total polyphenol contents varying from 27.3 ± 2.1 mg to 120.3 ± 5.6 mg gallic acid equivalents/g dry weight. The phenolic content in *H. articulatum* was superior to those in *E. pycnanthum* and *S. oleifolium*. The same tendency was observed for the relative amounts of flavonoids and condensed tannins in the three medicinal plants. The antioxidant activities varied greatly among the different plants used in this study. Indeed, *H. articulatum* shoots exhibited the strongest antioxidant activity, with the lowest IC_{50} (6.3 ± 0.25 μg/mL) and EC_{50} (0.21 ± 0.01 mg/mL) values for 1,1-diphenyl-2-picrylhydrazyl$^{\bullet}$ and iron reducing tests, respectively. In addition, the superiority of this plant was more marked as compared to positive controls. Strong and positive correlations were found between phenolic classes and antioxidant activities with a correlation coefficient reaching $R \geq 0.99$.
Conclusion: This investigation confirmed that several medicinal herbs from South Algeria possess high *in vitro* antioxidant potency. Overall, our results can be considered very promising in the continuing effort to utilize plant species successfully for medicinal purposes in humans, providing further justification for the hypothesis that phenolic compounds in these plants can provide substantial antioxidant activity.

Keywords: antioxidant activity; correlation coefficients; extracts; medicinal plants; percentage inhibition; quantification of phenolics

1. Introduction

There is ample evidence that reactive oxygen species (ROS) generated in the human body can cause oxidative damage associated with many degenerative diseases such as atherosclerosis, coronary heart diseases, aging, and cancer. It is well understood that ROS such as superoxide radical, hydroxyl radical, peroxyl radical, and nitric oxide radical attack biological molecules such as lipids, proteins, enzymes, DNA, and RNA, leading to cell or tissue injury. ROS can induce peroxidation of lipids generating secondary oxidants such as heptanol and hexanal, which contributes to oxidative rancidity, deteriorating the flavor of food. These not only cause a loss in food quality but are also believed to be associated with carcinogenesis, mutagenesis, arthritis, diabetes, inflammation, cancer, and genotoxicity. To overcome these problems a wide range of synthetic antioxidants [butylated hydroxytoluene

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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(BHT), butylated hydroxyanisole (BHA), propyl gallate, and butylated hydroquinone] have been used as food preservatives. However, these synthetic antioxidants can have side effects including liver damage and are suspected of being mutagenic and neurotoxic. Due to this troubling correlation, there is currently an upsurge of interest in phytochemicals as potential new sources of natural antioxidants. The goal is to use them in foods and pharmaceutical preparations to replace synthetic antioxidants.[1–4] Most antioxidants isolated from plants are polyphenols. However, reliable scientific information on the antioxidant properties of various wild plants is still rather scarce, particularly for those plants that are less widely used due to their endemic nature.

Haloxylon articulatum Boiss (family Chenopodiaceae) is distributed primarily in desert and semidesert areas in soils containing high levels of salt. It is frequently used in traditional medicine, and is reportedly used for diabetes, with antiseptic and anti-inflammatory characteristics.[5] *Solenostemma oleifolium* Bull. & Bruce (family Asclepiadaceae) is distributed especially in the Algerian Sahara. It is frequently used in traditional medicine and has been reported to be rich in steroidal glycosides, whose leaves are commonly used as a purgative, antipyretic, expectorant, and antispasmodic.[6] *Echium pycnanthum* Pomel (family Boraginaceae) is a wild plant endemic to the arid desert, well-recognized among traditional healers, and sometimes used to treat hepatitis. Those segments of the plant most sought-after for medicinal purposes are its mostly red roots, which are sold in markets.[7] The goal of this study was to use six testing methods to determine the total phenolic content in and evaluate antioxidant activity of the three Algerian medicinal plants. To the best of our knowledge, no existing reports exist providing this information, despite a thorough search of the literature. It would appear that our study is the first to provide data on the antioxidant effect of the phenolic extract of these plants.

2. Methods

2.1. Preparation of plant extracts

The plants we intended to test were collected from several Algerian regions in March, 2012 and April, 2012. Specifically, aerial parts of *H. articulatum* were obtained from Bechar (31°37′N, 2°13′W), aerial parts of *S. oleifolium* were obtained from Tamanrasset (22°47′N, 5°31′E), and the roots of *E. pycnanthum* were obtained from Naâma (33°16′N, 00°19′W). Thereafter, the gathered plant sections were identified in the Laboratory of Natural Products, Department of Biology, University of Tlemcen, Algeria, and voucher specimens were deposited at the herbarium of the laboratory. The plants were dried at room temperature for 2 weeks, and extracts were obtained by magnetic stirring for 24 hours: a mixture of 2 g of the plant powder with 25 mL of hydromethanol (80:20). The extracts were evaporated at 45°C under reduced pressure, redissolved in methanol at a concentration of 10 mg/mL, and stored at 4°C for further use.

2.2. Quantification of phenolic classes

2.2.1. Total phenol quantification

Total phenolic content of the plant extracts was determined using Folin–Ciocalteu reagent.[8] An aliquot (150 μL) of 100 μg/mL plant extract was added to 0.5 mL of distilled water and 125 μL of the Folin–Ciocalteu reagent. The mixture was then shaken and allowed to stand for 6 minutes, prior to adding 1.25 mL of Na_2CO_3 (7%). The solution was then adjusted with distilled water to reach a final volume of 3 mL and mixed thoroughly, and then held in darkness for 90 minutes at ambient temperature. After incubation, the absorbance at 760 nm was recorded. Total phenolic content of plant parts was calculated as mg of gallic acid equivalents/g dry weight (mg GAE/g DW), using a gallic acid calibration curve (range, 0–400 μg/mL).

2.2.2. Flavonoid quantification

Total flavonoid content was measured using a colorimetric assay.[8] To achieve this, an aliquot (150 μL) of 100 μg/mL plant extract or standard solution of catechin was added to 75 μL of $NaNO_2$ solution (7%), and mixed for 6 minutes, prior to adding 0.15 mL $AlCl_3$ (10%). After 5 minutes, 0.5 mL of 1 M NaOH solution was added. The final volume was adjusted to 2.5 mL, thoroughly mixed, and the absorbance of the mixture was determined at 510 nm. Total flavonoid content was calculated as mg catechin equivalent/g dry weight (mg CE/g DW), using a catechin calibration curve (range, 0–400 μg/mL).

2.2.3. Tannin quantification

The tannin content was measured using a colorimetric assay.[9] An aliquot (50 μL) of concentrations (100 μg/mL) of plant extract or standard solution of catechin was mixed with 3 mL of 4% vanillin–methanol solution and 1.5 mL of concentrated hydrochloric acid, and the mixture was allowed to stand for 15 minutes. Absorbance was read at 510 nm against the blank (water). Tannin content was calculated as mg CE/g DW, using a catechin calibration curve (range, 0–400 μg/mL).

2.3. Determination of antioxidant activities

2.3.1. Antioxidant capacity

This assay is based on the reduction of molybdenum (VI) to molybdenum (V) by the plant extracts, which produces a green phosphomolybdenum (V) complex under acidic conditions.[10] An aliquot (0.1 mL) of concentrations (100 μg/mL) of plant extract was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95°C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as GAE/g DW. The calibration curve of gallic acid range was 0–400 μg/mL.

2.3.2. 1,1-diphenyl-2-picrylhydrazyl assay

The ability of the corresponding extracts to donate hydrogen atoms or electrons was measured from the bleaching of purple-colored methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method described by Masuda et al.[11] Various concentrations (50 μL, 1−100 μg/mL) of plant extracts were added to 1950 μL of 6.34×10^{-5} M DPPH radical solution in methanol. The mixture was shaken vigorously and allowed to stand for 30 minutes in the dark. The absorbance of the resulting solution was measured at 517 nm and butylated hydroxytoluene (BHT) was used as a positive control. Inhibition of DPPH radical was calculated by the following formula (1):

$$\text{DPPH scavenging effect}(\%) = [A_0 - A_1/A_0] \times 100 \quad (1)$$

where A_0 and A_1 are the absorbance at 30 minutes of the control and the sample, respectively. The antiradical activity was expressed as IC_{50} (μg/mL), the extract dose required to cause a 50% decrease of the absorbance at 517 nm. A lower IC_{50} value corresponds to a higher antioxidant activity.

2.3.3. 2,2′-azino-bis-3-ethyl benzthiazoline-6-sulfonic acid assay. The 2,2′-azino-bis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS) radical-scavenging activity of extracts was determined according to Re et al.[12] The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 5 mL of 14 mM $ABTS^{\bullet+}$ solution and 5 mL of 4.9 mM potassium persulfate solution, and stored in the dark at room temperature for 16 hours. Prior to use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. In a final volume of 1 mL, the reaction mixture comprised 950 μL of $ABTS^{\bullet+}$ solution and 50 μL of the plant extract at various concentrations (20−200 μg/mL). The reaction mixture was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after a minimum of 6 minutes. Similarly, the reaction mixture of the standard group was obtained by mixing 950 μL of $ABTS^{\bullet+}$ solution and 50 μL of BHT and Trolox. As for the antiradical activity, $ABTS^{\bullet+}$ scavenging ability was expressed as IC_{50} (μg/mL). The inhibition percentage of $ABTS^{\bullet+}$ radical was calculated using the following formula (2):

$$ABTS^{\bullet+}\text{scavenging effect}(\%) = [A_0 - A_1/A_0] \times 100 \quad (2)$$

where A_0 and A_1 have the same meaning as in Equation (1).

2.3.4. β-carotene bleaching test. To undertake this bleaching test, modification of the method described by Koleva et al[13] was employed. Beta-carotene (2 mg) was dissolved in 20 mL chloroform. Four milliliters of this solution were combined with linoleic acid (40 mg) and Tween 40 (400 mg). The chloroform was evaporated under vacuum at 40 °C and 100 mL of oxygenated ultrapure water was added, and the emulsion was vigorously shaken. Sample extract and reference compound (BHA) were prepared in methanol. An aliquot (150 μL) of the β-carotene/linoleic acid emulsion was distributed in each of the wells of 96-well microtiter plates and 10 μL of various concentrations (20−200 μg/mL) of phenolic extract was added. The microtiter plates were incubated at 50°C for 120 minutes, and the absorbance was measured using a model EAR 400 microtiter reader (Labsystems Multiskan MS) at 470 nm. Readings of all samples were performed immediately (t = 0 min) and after 120 minutes of incubation. The antioxidant activity of the extracts was evaluated in term of β-carotene bleaching using the following formula (3):

$$\beta - \text{carotene bleaching inhibition}(\%) = [S - C_{120}/C_0 - C_{120}] \times 100 \quad (3)$$

where C_0 and C_{120} are the absorbance values of the control at 0 minutes and 120 minutes, respectively, and S is the sample absorbance at 120 minutes. The results were expressed as IC_{50} values (μg/mL).

2.3.5. Chelating effect on ferrous ions. The ferrous ion chelating activity of extracts was assessed as described by Zhao et al.[14] One milliliter of various concentrations (1−8 mg/mL) of phenolic extract was added to 50 μL of $FeCl_2$ ($4H_2O$) solution (2 mM) and left to incubate at room temperature for 5 minutes. Then, the reaction was initiated by adding 0.1 mL of ferrozine (5 mM), and the mixture was adjusted to 3 mL with distilled water, shaken vigorously, and then left standing at room temperature for 10 minutes. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine−Fe^{2+} complex formation was calculated using the formula given below (4):

$$\text{Metal chelating effect}(\%) = [A_0 - A_1/A_0] \times 100 \quad (4)$$

where A_0 and A_1 have the same meaning as in Equation (1). Results were expressed as IC_{50} (mg/mL).

2.3.6. Iron reducing power. The capacity of plant extracts to reduce Fe^{3+} was assessed by the method of Oyaizu.[15] One milliliter of various concentrations (0.1−3 mg/mL) of plant extract was mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 minutes. After that, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 650*g* for 10 minutes. The upper layer fraction (2.5 mL) was combined with 2.5 mL of distilled water and 0.5 mL of ferric chloride and then thoroughly mixed. The absorbance was measured at 700 nm and ascorbic acid was used as a positive control. The EC_{50} value (mg/mL) is the effective concentration giving an absorbance of 0.5 for reducing power and was obtained from linear regression analysis. In our case, a higher absorbance indicated a higher reducing power.

2.4. Statistical analysis

Values shown in tables are the means ± standard deviations of three parallel measurements. The IC_{50} values were calculated from linear regression analysis, and the correlation coefficients

Table 1
Total antioxidant capacity, phenol content, flavonoid, and tannin contents in plant extracts.

	Polyphenol content (mg of GAE/g DW)	Flavonoid content (mg of CE/g DW)	Tannin content (mg of CE/g DW)	Total antioxidant capacity (mg of GAE/g DW)
Haloxylon articulatum	120.32 ± 5.6	84.74 ± 4.3	9.98 ± 1.6	128.79 ± 7.3
Solenostemma oleifolium	38.78 ± 2.2	7.15 ± 1.2	3.33 ± 0.5	52.31 ± 3.2
Echium pycnanthum	27.31 ± 2.1	16.26 ± 1.4	2.08 ± 0.2	40.07 ± 2.8

CE = catechin equivalents; DW = dry weight; GAE = gallic acid equivalents.

Table 2
Correlation coefficients (*R*) for relationships between assays.

	TAC	$IC_{50/DPPH}$	$IC_{50/ABTS}$	$IC_{50/\beta\text{-carotene}}$	$IC_{50/iron\ chelation}$	$EC_{50/iron\ reducing\ power}$
Polyphenols	0.99	0.722	0.458	0.882	0.845	0.71
Flavonoids	0.97	0.856	0.642	0.964	0.941	0.846
Tannins	0.99	0.70	0.427	0.866	0.826	0.684

ABTS = 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonique acid; DPPH = 2,2-diphényl picrylhydrazyl; EC_{50} = effective concentration at which the absorbance was 0.5; IC_{50} = inhibition concentration 50%; TAC = total antioxidant capacity.

between total phenolics and the methods of antioxidant activity were demonstrated using Microsoft Excel (2010).

3. Results

3.1. Total phenolic, flavonoid, and condensed tannin contents

Total phenolic content (TPC) has been reported to be directly associated with antioxidant activity. These compounds are known as powerful chain-breaking antioxidants.[16,17] Our results demonstrate that the phenolic extracts from plants exhibit large variations in TPC levels (Table 1). The highest TPC was measured in *H. rticulatum* (120.32 ± 5.6 mg GAE/g DW), followed by *S. oleifolium* (38.78 ± 2.2 mg GAE/g DW) and *E. pycnanthum* roots (27.31 ± 2.1 mg GAE/g DW). Flavonoids are natural phenolic compounds. Many studies have demonstrated a positive correlation between flavonoid amounts and antioxidant activity.[18] The results of the colorimetric analysis are given in Table 1. Regarding TPC levels, significant differences were observed for total flavonoid contents in different plants. The flavonoid content of *H. articulatum* (84.74 ± 4.3 mg CE/g DW) was higher than the flavonoid content of *S. oleifolium* and *E. pycnanthum* roots (7.15 ± 1.2 and 16.26 ± 1.4 mg CE/g DW, respectively). Condensed tannins are a class of phenolic compounds consisting of oligomers and polymers of the flavan-3-olmonomer units. It has been reported that they possess various biological activities, such as antioxidant activity.[19] The *H. articulatum* extract contained the highest tannin concentration (9.98 ± 1.6 mg CE/g DW), followed by *S. oleifolium* and *E. pycnanthum* roots (3.33 ± 0.5 mg CE/g DW and 2.08 ± 0.2 mg CE/g DW, respectively).

3.2. Antioxidant activity of phenolic extracts

3.2.1. Total antioxidant capacity

The global antioxidant activity of extracts was expressed as the number of gallic acid equivalents. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex. The antioxidant activity of *H. articulatum* extract was 3.2 fold higher than that of *E. pycnanthum* root extract and 2.4 fold higher than that of the *S. oleifolium* extract (Table 1). The relationship between total polyphenol content, flavonoids, condensed tannins, and total antioxidant capacity of phenolic extracts of different plants shows a significant relationship with coefficient correlation $R \geq 0.97$ (Table 2).

3.2.2. DPPH radical-scavenging activity

DPPH$^{\bullet}$ is a free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule.[20] The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants. The scavenging effect of phenolic extracts and standard on the DPPH radical expressed as IC_{50} values was in the following order: *H. articulatum* (6.32 ± 0.25 μg/mL), BHT (10.5 ± 0.40 μg/mL), *E. pycnanthum* root (30.50 ± 2.20 μg/mL), and *S. oleifolium* (69.37 ± 4.55 μg/mL; Table 3). The results of the correlation between DPPH IC_{50} and total phenols, flavonoids, and condensed tannins showed that they were significantly correlated ($R \geq 0.70$). This allows us to infer that the capacity of the trapped radical DPPH is mainly due to the 72.2% of polyphenols, 85.6% of flavonoids, and 70 % of condensed tannins.

3.2.3. ABTS radical-scavenging activity

The reduction capability of ABTS radical was determined by the decrease in absorbance induced by plant antioxidants. The scavenging effect of extracts and standards on the ABTS radical expressed as IC_{50} values was in the following order: *H. articulatum* (40.94 ± 3.30 μg/mL), Trolox (58.14 ± 2.50 μg/mL), BHT (73.1 ± 1.70 μg/mL), *E. pycnanthum* root (80.40 ± 7.50 μg/mL) and *S. oleifolium* (579.66 ± 12.5 μg/mL; Table 3). A correlation is established between ABTS IC_{50} and the content of flavonoids ($R = 0.642$), and a low correlation with polyphenols ($R = 0.458$) and with condensed

Table 3
Antioxidant activities, expressed as μg/mL, on DPPH, ABTS, β-carotene, and in mg/mL, on chelating and reducing power, for the hydromethanolic extracts of plants and standards.

	$IC_{50/DPPH}$ (μg/mL)	$EC_{50/iron\ reducing\ power}$ (mg/mL)	$IC_{50/ABTS}$ (μg/mL)	$IC_{50/iron\ chelation}$ (mg/mL)	$IC_{50/\beta\text{-carotene}}$ (μg/mL)
Haloxylon articulatum	6.32 ± 0.25	0.21 ± 0.01	40.94 ± 3.30	4.21 ± 0.30	14.66 ± 1.55
Echium pycnanthum	30.50 ± 2.20	1.04 ± 0.04	80.40 ± 7.50	5.32 ± 0.45	77.29 ± 5.35
Solenostemma oleifolium	69.37 ± 4.55	2.44 ± 0.05	579.66 ± 12.5	6.18 ± 0.20	114.20 ± 9.40
BHT	10.50 ± 0.40	0.13 ± 0.00	73.1 ± 1.70	ND	ND
BHA	ND	ND	ND	ND	48 ± 0.90
Trolox	ND	ND	58.14 ± 2.50	ND	ND
EDTA	ND	ND	ND	0.00465 ± 0.0003	ND

ABTS = 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonique acid; BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; DPPH = 2,2-diphényl picrylhydrazyl; EC_{50} = effective concentration at which the absorbance was 0.5; IC_{50} = inhibition concentration 50%; ND = not determined.

tannins ($R = 0.427$; Table 2). This allows us to infer that the capacity of the trapped radical ABTS is mainly due to the 64.2% of flavonoids.

3.2.4. β-carotene bleaching test

In this model, β-carotene undergoes rapid discoloration in the absence of an antioxidant. The presence of antioxidants such as phenolics can hinder the extent of β-carotene destruction by neutralizing the linoleate free radical and any other free radicals formed within the system.[21]

Table 3 depicts the inhibition of β-carotene bleaching by the phenolic extracts of plants, and by the positive control (BHA). In terms of β-carotene bleaching effect, those samples exhibited the following order: *H. articulatum* > BHA > *E. pycnanthum* root > *S. oleifolium*.

H. articulatum extract exhibited an interesting antioxidant activity (IC_{50} = 14.66 ± 1.55 μg/mL) compared to BHA (IC_{50} = 48 ± 0.90 μg/mL). The results of the relationship between inhibition of bleaching of β-carotene and the phenolic compounds contents of different phenolic extracts show a correlation between total polyphenol content, flavonoids, and condensed tannins with values of $R \geq 0.866$ (Table 2). This allows us to deduce that the inhibition of β-carotene bleaching capacity is due to the 88.2% of polyphenols, 96.4% of flavonoids, and 86.6 % of condensed tannins.

3.2.5. Iron (II) chelation

Although iron is essential for oxygen transport, respiration, and enzyme activity, it is a reactive metal that catalyses oxidative damage in living tissues and cells.[22] Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted resulting in a decrease of the blue color of the complex. Results indicate that phenolic extracts interfered with the formation of ferrous and ferrozine complex (Table 3). However, those extracts exhibited the following order: EDTA > *H. articulatum* > *E. pycnanthum* root > *S. oleifolium*.

H. articulatum extract exhibited a significantly higher antioxidant activity (IC_{50} = 4.21 ± .30 mg/mL) compared to other phenolic extracts (IC_{50} between 5.32 ± 0.45 mg/mL and 6.18 ± 0.20 mg/mL), but none of the extracts appeared to be better chelators of iron (II) ions than the positive control EDTA (IC_{50} = 0.00465 ± 0.0003 mg/mL). These results suggested that our extracts are very weak ferrous chelators. The results have shown a correlation between the levels of total polyphenols, flavonoids, and condensed tannins with values of $R \geq 0.826$ (Table 2). This enables us to deduce that this capacity is due to the 84.5% of polyphenols, 94.1% of flavonoids, and 82.6 % of condensed tannins.

3.2.6. Iron reducing power

Another reaction pathway in electron donation is the reduction of an oxidized antioxidant molecule to regenerate the active reduced antioxidant. Reducing power is a very important aspect for the estimation of the antioxidant activity.[16] As shown in Table 3, the reducing power, expressed as EC_{50}, of *H. articulatum* extract (0.21 ± 0.01 mg/mL) was clearly more important than that of *E. pycnanthum* root and *S. oleifolium* (1.04 ± 0.04 mg/mL and 2.44 ± 0.05 mg/mL, respectively). This capacity is attributed to the presence of natural antioxidants such as phenolic compounds in the plants.[23] However, the BHT concentration (0.13 mg/mL) required to reduce the ferric iron was lower than the other phenolic extracts, indicating superior activity. The results show a correlation between the levels of total polyphenols, flavonoids, and condensed tannins with values of $R \geq 0.684$ (Table 2).

4. Discussion

Our results are a contribution to the valorization of some medicinal plants from southern Algeria, which have never been published. Therefore, this investigation can be evaluated as the first report about their antioxidant properties in respect to polyphenol content. It is extremely important to point out that there was a positive correlation between antioxidant potential and phenolic content estimated by the assays. The high content of total phenols in extracts and synergistic interactions might explain the strong antioxidant properties of these plants. Such a concept has been proven by several works.[3,16,18] Comparing the obtained results with the previously published data of *H. articulatum*, we have found that the total phenolic and flavonoid contents are comparable with studies of Alghazeer et al.[24] (119.76 mg GEA/g DW and 81.07 ± 6.14 mg routine/g DW, respectively). Recent studies have shown that storage time,[25] extrinsic,[16] genetic,[26] and physiological factors[27] have a strong influence on the content of phenols, which makes comparison difficult. Recent studies have shown that many flavonoids contribute significantly to

the total antioxidant activity of many fruits such as the red grape,[28] vegetables,[29] and medicinal plants.[30,31] Phenolic compounds of the *H. articulatum* extract were probably involved in their antiradical activity. In this way, Alghazeer et al[24] showed that *H. articulatum* are involved in several biological processes, including antioxidant activities (DPPH/ IC_{50} = 8 μg/mL). The experimental data reveal that *H. articulatum* extract is likely to have a stronger effect of scavenging free radical than positive control (BHT). It has been found that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids, and tannins reduce and decolorize DPPH due to their hydrogen-donating capacity.[32] As shown for DPPH scavenging, these data indicate the higher capacity of *H. articulatum* extract to quench $ABTS^{\bullet+}$ as compared to the other extracts and to the synthetic antioxidant (BHT and Trolox). According to Oszmianski et al,[33] the antioxidant activities against $ABTS^{\bullet+}$ or $DPPH^{\bullet}$ were correlated with the concentration, chemical structures, and polymerization degrees of organ antioxidants. In fact, numerous studies have indicated that plant extracts rich in phenolic compounds are capable of complexing with and stabilizing transition metal ions, rendering them unable to participate in metal-catalyzed initiation and hydroperoxide decomposition reactions.[31]

Additional biological testing will be necessary to ascertain new and beneficial activities of these plants. Consequently, phytochemical investigations should be planned to identify and characterize active principles, and assess toxicity by laboratory assays.

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