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Phytochemicals and antioxidant properties of extracts from the root and stems of *Anabasis articulata*

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Abstract

Anabasis articulata is a one of many Saharan plants, widely used in Algerian folk medicine for their medicinal properties. For the first time, antioxidant properties of stems and root extracts were investigated by different methods, e.g. Total Antioxidant Activity, Reducing Power, DPPH Radical Scavenging Activity and β-Carotene Bleaching Assay. The quantitative determination of total phenolic compounds, flavonoids, flavonols, condensed tannins and carotenoids was also reported. Results showed that stems exhibit a higher level of phenolic compounds (25.48 mg GAE/g DW) as compared to roots (19.85 mg/g DW). Total Antioxidant Activity in the two parts of the plant showed no significant difference. All extracts showed different levels of antioxidant properties in the test models used. Root crude extract showed the highest activity to reducing power with an IC_{50} of 0.36 mg mL^{-1}, to scavenge DPPH radical with an EC50 of 0.57 mg mL^{-1} and to inhibit the oxidation of β-carotene with an EC_{50} of 0.22 mg mL^{-1}.

Introduction

Many Saharan plant species have been used in folk medicine by the indigenous population against fever, diarrhea, diabetes, asthma, rheumatism and cancer therapies. Plants of the Chenopodiaceae family are used for their richness in bioactive substances. Anabasis genus grows in stony and sandy wadies, heavily browsed by camels and goats (Chopra, 1956). *Anabasis articulata*, locally named 'Ajrem', is a wild plant mainly found in the Algerian desert and used in folk medicine to treat diabetes, fever, headache and skin diseases, such as eczema (Hammiche and Maiza, 2006). It is taken orally after decoction in water as a single herb or with other medicinal plants. Other cholinergic properties have also been reported in this species (Tilyabaev and Abduvakhabov, 1998). Phytochemical constituents of *A. articulata* revealed the presence of saponins, among which triterpenoid saponin glycosides have been isolated and identified (Segal *et al*., 1969). It is recognized that saponins have strong biological properties such as the antidiabetic effect, cytotoxicity and antitumoral properties (Kambouche *et al.*, 2009). No scientific investigations concerning the antioxidant properties of the main families of secondary metabolites of *A. articulata* have been done so far.

The present study reports on the phytochemical analysis and antioxidant activities of various extracts of stem and root from *A. articulata.*

Materials and Methods

Plant material

The aerial part (stems, root) of *A. articulata* was collected from Bechar (Algeria) in May 2011. The plant materials were identified and authenticated by the Vegetable Ecological Laboratory and voucher specimens have been deposited at the Herbarium of the Department of Biology, Tlemcen University, Algeria. Plant samples were dried at room temperature and store for future use.

Extractions of chemical compounds from stems and root

Crude methanolic extracts

The stems and root of *A. articulata* (1 g) were powdered and extracted for 24 h with 20 mL of methanol at room temperature (Benhammou *et al.*, 2009). After filtration through Whatman No 0.45 µm, the resulting solutions were evaporated under vacuum at 60°C by Buchi Rotavapor R-200 to dryness. The residues were weighed and preserved for further use.

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Ethyl acetate and butanolic fractions

The sample of stem or root was directly extracted with methanol at room temperature (20 mL/24 hours). Then, the suspension was filtered and the solvent eliminated under vacuum. The residue was dissolved in 10 mL of boiling water, and then successively partitioned with 10 mL of ethyl acetate and 10 mL of n-butanol, respectively. After separation and evaporation, the organic phases were weighed and stored before use (Bekkara *et al.*, 1998).

Tannins

Tannins extraction from *A. articulata* was obtained according to the method of Zhang *et al.* (2008). The powder (5 g) of each part (stems, root) was extracted with 100 mL of acetone-water (70/30, v/v), and the mixture was stirred continuously for 72 h, at room temperature. Then, the mixture was filtered and evaporated under vacuum at 40°C to remove acetone. The remaining solution was washed with 30 mL of dichloromethane to remove lipid soluble substances. After the elimination of dichloromethane under reduced pressure, the aqueous phase was extracted with 30 mL of ethyl acetate. This process was repeated twice. Then, the organic phases (ethyl acetate) containing tannins were recovered and evaporated to dryness. The residues obtained were weighed and preserved until uses.

Total alkaloids

Alkaloid extracts were obtained by an acid/basic extraction, as described by Harborne (1998), and the stems and root of *A. articulata* were extracted using a soxhlet with 150 mL of absolute ethanol, during 5 h. The ethanolic extracts were then evaporated under vacuum at 40°C by Buchi Rotavapor R-200. The dry residues were taken up in 20 mL of chloroform and acidified to pH 3 with 5% HCl; they were let stand for 30 min at room temperature. The acid aqueous phases were extracted with 20 mL of chloroform, then basified to pH 9 with 5% $NaHCO_3$ solution, and let stand for 15 minutes at room temperature. The organic phases were evaporated and the dry residues, made of total alkaloids, were weighed then stored for future use.

Saponins

Saponins were extracted according to the method of Applebaum *et al.* (1969). Powdered stems and roots were delipided for 2 h by 150 mL of n-hexane. After elimination of the organic phases, the resulting delipidated stem or root samples were macerated in 50 mL of absolute ethanol under magnetic stirring at room temperature during 24 h. The ethanolic phases were evaporated at 40°C using the rotavapor. The dry residues were extracted by 50 mL of a mixture of distilled water/petroleum ether (v/v) and heated on water bath at 50°C for 30 min. The aqueous phases were mixed, and then treated by 5 mL of butanol during 30 min. The organic phases, evaporated at 40°C, were weighed and then stored for future use.

Determination of total phenolic content

The total phenolic content in stems and root methanolic extracts was determined by spectrometry using ''Folin-Ciocalteu'' reagent assay (Singleton and Rossi, 1965). A volume of 200 mL of the extract was mixed with 1 mL of Folin-Ciocalteu reagent diluted 10 times in water, and 0.8 mL of 7.5% sodium carbonate solution in a test tube. After 30 min of stirring, the absorbance was measured at 765 nm using a Jenway 6405 UV-vis spectrophotometer. Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Determination of total flavonoids

The total flavonoid content in stems and root methanolic extracts was determined by a colorimetric assay using a method described by Zhishen *et al.* (1999). Briefly, 500 µL of catechin standard solution with different concentrations or methanolic extracts was mixed with 1500 µL of distilled water in a test tube, followed by addition of 150 µL of a 5% (w/v) $NaNO_2$ solution, at time zero. After 5 min, 150 µL of $AlCl_3$ at 10% (m/v) was added. After 6 minutes of incubation, at room temperature, 500 µL of NaOH (1 M) were added. The mixture was homogenized immediately after the end of the addition. The absorbance of the solution was measured at 510 nm against the blank. The mean ± SD results for triplicate analyses were expressed as milligram catechin equivalents of dry weight (mg CE/ g DW).

Determination of total flavonols

The content of flavonols was determined by the method described by Kumaran *et al.* (2007). Aliquots (0.25 mL) of the methanolic solution of the extracts were mixed with 0.25 mL of $AlCl_3$ (2 mg mL^{-1}) and 1.5 mL of sodium acetate (50 mg mL^{-1}). The absorbance at 440 nm was recorded after 2.5 h. All determinations were carried out in triplicates. The content of flavonols was expressed as mg of quercetin equivalents per gram of dry weight (mg QE/ g DW).

Determination of total condensed tannins

Proanthocyanidins in stems and root methanolic

extracts were measured using the vanillin assay described by Julkunen-Titto (1985). To 50 µL of methanolic extract, 1500 µL of vanillin/methanol solution (4%, w/v) were added, and the solution was homogenized. Then, 750 µL of concentrated HCl were added and allowed to react at room temperature for 20 min. The absorbance at 550 nm was measured against the blank. The amount of total condensed tannins was expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/ g DW) from the calibration curve.

Determination of total carotenoids

Total carotenoids were extracted according to the method of Talcott and Howard (1999) using a β-carotene standard curve. Total carotenoids were expressed as β-carotene equivalents (mg βCE/g DM). Two grams of sample were homogenized with 20 mL of acetone/ethanol (1:1 v/v) and 200 mg/L of BHT. The samples were centrifuged at 15,000 rpm for 20 min. The supernatant was transferred into a 50 mL graduated cylinder and the solvent was added to a final volume of 50 mL. The total carotenoids content was determined by the spectrophotometric method at 470 nm.

Total antioxidant activity

The Total Antioxidant Capacity (TAC) of plant extracts was evaluated by the phosphomolybdenum method of Prieto *et al.* (1999). A 0.3 mL aliquot of extract was mixed with 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95°C for 90 min. Then, the samples were cooled down to room temperature and the mixture's overall absorbance was measured at 695 nm against a blank. The antioxidant activity of the samples was expressed as milligrams of ascorbic acid equivalent (mg AAE/ g DW) and gallic acid equivalent (mg GAE/ g DW) per gram of dry matter.

Determination of reducing power

The reducing power of the extract was determined according to the method of Oyaizu (1986). Various concentrations of the extracts (mg mL^{-1}) in distilled water were mixed with a phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% of potassium ferricyanide water solution (2.5 mL, $K_3[Fe(CN)_6]$). The mixture was incubated at 50°C for 20 min. Aliquots of trichloracetic acid (2.5 mL, 10%) were added to the mixture which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm. An increased absorbance of the reaction mixture was taken to mean an increased reducing power. Ascorbic acid was used as a positive control.

DPPH radical scavenging assay

The free radical scavenging activity was measured by a modified DPPH• assay (Sanchez-Moreno *et al*., 1998). Fifty microliters of various concentrations of the extracts in methanol were added to 1.950 mL of a 0.025 g/L methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = (A_{blanc} - As_{ample} / A_{blanc}) \times 100$$

where A_{blank} is the value of DPPH, A_{sample} is the value of the sample and DPPH.

The extract concentration providing 50% inhibition (EC_{50}) was calculated from the plotted graph of inhibition percentage against extract concentrations. The ascorbic acid methanolic solution was used as positive control.

β-*carotene-linoleic acid bleaching*

The antioxidant activity of methanolic extracts was evaluated using a β-carotene-linoleate model system, as described by Moure *et al.* (2000). Two milligrams of β-carotene were dissolved in 10 mL chloroform; and 1 mL of β-carotene solution was mixed with 20 µL of purified linoleic acid and 200 mg of Tween 40 emulsifier. Chloroform was removed in a rotary vacuum evaporator and the resulting mixture was immediately diluted with 100 mL of distilled water. To an aliquot of 4 mL of this emulsion, 200 µL of the methanolic solution of extracts or the reference antioxidants (Gallic acid and BHA) were added and mixed well. The absorbance at 470 nm, which was considered at t = 0 min, was immediately measured against a blank, consisting of the emulsion without β-carotene. The capped tubes were placed in a water bath at 50°C for a period of 2 h. Thereafter, the absorbance of each sample was measured at 470 nm (A_{120}). BHA was used for positive control. A negative control consisted of 200 µL methanol instead of methanolic extract or BHA. All tests were repeated twice. The antioxidant activity (AA) was calculated according to the following equation:

$$AA = [(A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)}] \times 100$$

Where $A_{A(120)}$ is the absorbance of the sample at t = 120 min; $A_{C(120)}$ the absorbance of the control at t = 120 min and $A_{C(0)}$ the absorbance of the control at t

= 0 min.

Statistical analysis

All evaluations of antioxidant activity were performed twice. The experimental data were expressed as means ± standard deviation (S.D). The correlation coefficient of antioxidant activity was determined using Excel programme and Origin 6.

Results and Discussion

Extract yields, total polyphenols, flavonoids, flavonols, condensed tannins and carotenoids contents

The phytochemical analysis of stems and root extracts of *A. articulata* showed the presence of different groups of secondary metabolites. Quantitative estimation of the percentage crude chemical constituents in *A. articulata* is summarized in Table 1. Percentage yield of alkaloids (5.95 ± 0.25%) and methanolic extract (5.47 ± 0.54%) for stems were the highest compared to the other phytochemical constituents. For the ethyl acetate fraction, tannins, butanolic fraction and saponins, the yields were the 1.86 ± 0.64, 0.96 ± 0.04, 0.8 ± 0.14 and 0.61 ± 0.00% respectively. These results correlate with some authors (Kambouche *et al*., 2009; Maatalah *et al*., 2012), who determined that stems of *A. articulata* were a good source of all investigated phytochemicals. Concerning the root part, the yield of methanolic extract (4 ± 0.04%) was dominated by the flavonoids in butanolic (3 ± 1.86%) and ethyl acetate fractions (1.31 ± 0.23%). The yields of tannins and saponins were 1.43 ± 0.34% and 0.39 ± 0.13%, respectively.

The amount of total phenolic compounds was higher in stems (25.48 ± 3.83 mg GAE/g DW) than in roots (19.85 ± 7.52 mg GAE/g DW). Compared to other species of Chenopodiaceae family, these values were higher than those reported elsewhere for *Atriplex halimus* (Benhammou *et al.*, 2009). The total flavonoid content of root methanolic extract (3.80 ± 0.06 mg CE/g DM) was higher than that of stem extract (3.08 ± 0.20 mg CE/g DM). On the others hand, the stem extract was rich in flavonols (1 ± 0.04 mg QE/ g DM) and condensed tannins (4.03 ± 0.34 mg CE/g DM), but there is no difference in the total carotenoids content between two parts of *A. articulata.*

Antioxidant activity of the extracts

To the best of our knowledge, there are no reports in the literature concerning the antioxidant activity of *A. articulata.* Bioactive compounds of *A. articulata* were subjected to screening for their possible antioxidant activities using the total antioxidant capacity, reducing power, DPPH radical scavenging and β-caroten/linoleic acid assay methods, at different concentrations.

Table 1. Total phenolics, flavonoids, flavonols, condensed tannins and carotenoids contents

Bioactive compounds	Yields (%)	
	Stems	Root
Methanolic extract	5.47 ± 0.54	4 ± 0.04
Ethyl acetate fraction	1.86 ± 0.64	1.31 ± 0.23
Butanolic fraction	0.8 ± 0.14	3 ± 1.86
Tannins	0.96 ± 0.04	1.43 ± 0.34
Saponins	0.61 ± 0.00	0.39 ± 0.13
Alkaloids	5.95 ± 0.25	
Total phenolics (mg GAE/g DM)	25.48 ± 3.83	19.85 ± 7.52
Total flavonoids (mg CE/g DM)	3.08 ± 0.20	3.80 ± 0.06
Total flavonols (mg QE/ g DM)	1 ± 0.04	0.57 ± 0.06
Total condensed tannins (mg CE/g DM)	4.03 ± 0.34	2.68 ± 0.13
Total carotenoids (mg βCE/ g DM)	0.27 ± 0.00	0.22 ± 0.00

Total antioxidant capacity

The antioxidant activity of *A. articulata* was expressed in gallic acid and ascorbic 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 with a maximal absorption at 695 nm. The study revealed that the antioxidant activity of stem extracts was similar to that of root extracts except for tannins and saponins (Table 2). The tannins root had a 2-fold higher antioxidant activity compared to that of tannins stems. Conversely, saponins stems showed more antioxidant activity than the saponins root. Moreover, alkaloids (2.86 ± 0.00 mg AAE/g DM) have stronger antioxidant activity compared to all other bioactive compounds. The high capacity in methanolic extracts might be attributed to the presence of phytochemicals such as phenolic compounds (Falleh *et al*., 2008), flavonoids and tannins presented in our previous results, with high contents. Recent studies have confirmed this assertion, showing that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits such as the red grape (Negro *et al.*, 2003), vegetables (Luo *et al*., 2002) and medicinal plants (Bourgou *et al*., 2008). In addition, the antioxidant capacity depends not only on the total phenol content but on the specific phenol composition as well. The total number of phenolic hydroxyl groups and their position on the aromatic core influence the antioxidant capacity. It is believed

Table 2. Total antioxidant capacity in *Anabasis articulata* stems and root extracts

Bioactive compounds	Stems		Root	
	TAC (mg AAE/g DM)	TAC (mg GAE/g DM)	TAC (mg AAE/g DM)	TAC (mg GAE/g DM)
Methanolic extract	9.76 ± 0.13	6.13 ± 0.08	10 ± 0.03	6.28 ± 0.02
Ethyl acetate fraction	1.28 ± 0.03	0.80 ± 0.02	1.18 ± 0.04	0.74 ± 0.02
Butanolic fraction	1.26 ± 0.05	0.79 ± 0.03	1.39 ± 0.09	0.87 ± 0.06
Tannins	1.45 ± 0.04	0.91 ± 0.02	2.50 ± 0	1.57 ± 0.00
Saponins	1.30 ± 0.01	0.81 ± 0.00	0.70 ± 0.02	0.44 ± 0.01
Alkaloids	2.86 ± 0.00	1.79 ± 0.00		

Table 3. Antioxidant capacities reducing power IC_{50} (mg mL^{-1}), DPPH EC_{50} (mg mL^{-1}) and β-carotene-linoleate system (mg mL^{-1}) of the different extracts of the stems and root of *Anabasis articulata*

	Reducing power IC_{50} (mg mL^{-1})		DPPH scavenging EC_{50} (mg mL^{-1})		β-carotene EC_{50} (mg mL^{-1})	
	Stems	Root	Stems	Root	Stems	Root
Methanolic extract	0.66 ± 0.00	0.36 ± 0.00	1.98 ± 0.15	0.57 ± 0.03	0.53 ± 0.00	0.22 ± 0.00
Ethyl acetate fraction	1.37 ± 0.00	1.26 ± 0.03	-	0.44 ± 0.01	0.50 ± 0.00	0.57 ± 0.00
Butanolic fraction	0.76 ± 0.00	0.57 ± 0.00	1.70 ± 0.02	1.76 ± 0.01	1.26 ± 0.04	1.88 ± 0.07
Tannins	1.20 ± 0.01	0.62 ± 0.00	0.53 ± 0.00	0.60 ± 0.00	0.50 ± 0.00	0.28 ± 0.01
Saponins	1.26 ± 0.03	0.89 ± 0.00	3.50 ± 0.04	0.62 ± 0.00	2.00 ± 0.19	0.82 ± 0.23
Alkaloids	0.52 ± 0.00		1.30 ± 0.02		1.67 ± 0.22	
Ascorbic acid	0.06 ± 0.00		0.12 ± 0.00			
Gallic acid					3.22 ± 0.02	
BHT					0.01 ± 0.00	

that the greater the number of ortho or para oriented phenolic hydroxyls, the higher the antioxidant activity (Frankel *et al*., 1995).

Reducing power

Fe (III) reduction is often used as an indicator of electron-donating activity. As shown in Table 2, the reducing power of all secondary metabolites in roots is stronger than that in stems extract, except for the ethyl acetate fraction in the two parts of the plant, which exhibited the same reducing effect. The IC_{50} values were 1.26 and 1.37 mg mL^{-1} in root and stems, respectively. The IC_{50} in root methanolic extract was (0.36 ± 0.00) mg mL^{-1}, indicating a good activity, but still lower than that of the ascorbic acid (0.06 ± 0.00 mg mL^{-1}). These results prove that the crude extract is rich in reductones, such as phenolic compounds which produce the total antioxidant activity, presented in Table 2. Therefore, the reducing power is a very important aspect for the estimation of the antioxidant activity (Ksouri *et al*., 2008).

DPPH radical scavenging activity

DPPH is usually used as a substrate to evaluate the antioxidative activity of antioxidants. It is a free radical and accepts an electron or a hydrogen radical to become a stable diamagnetic molecule (Soares *et al*., 1997). A lower value of EC_{50} indicates a higher antioxidant activity. The highest DPPH scavenging activity for root was found in ethyl acetate fraction with an EC_{50} of 0.44 mg mL^{-1}, followed by the methanolic extract with EC_{50} of 0.57 mg mL^{-1}, the tannins with EC_{50} of 0.60 mg mL^{-1} and saponins with EC_{50} of 0.62 mg mL^{-1} (Table 3).

However, the scavenging effect of the stem extracts was lower compared to those in roots, except for tannins which proved to be capable of donating hydrogen atoms to DPPH radicals, with EC_{50} value of 0.53 mg mL^{-1}. However, DPPH free radical scavenging of all the secondary metabolites tested was lower than that of ascorbic acid (0.12 mg mL^{-1}). The ability of all extracts to scavenge DPPH radicals, except for ethyl acetate fraction in stems of *A. articulata* in our study, do not correlate with the results of Shakeri *et al.* (2012), who showed no antioxidant activity of extracts from the aerial part of *A. Aphylla* using the DPPH method. On the contrary, Bouaziz *et al.* (2009) reported that extracts of the aerial part of *A. Oropediorum*, found in south Tunisia, exhibited a better scavenging efficiency toward DPPH radicals.

β-carotene bleaching assay

In the β-caroten/linoleic acid method, β-carotene undergoes rapid discoloration in the absence of an antioxidant. The presence of an antioxidant can hinder the extent of β-carotene destruction by neutralizing the linoleate free radical and any other

free radicals formed within the system (Kamath and Rajini, 2007). All the extracts were capable of inhibiting the bleaching of β-carotene by scavenging linoleate-derived free radicals and were more effective in comparison with gallic acid (3.22 mg mL^{-1}), but showed less inhibitory activity than BHT (0.01 mg mL^{-1}) (Table 3). Most effective were the methanolic extract (0.22 mg mL^{-1}) and the tannins (0.28 mg mL^{-1}) from the root, despite the effects of tannins and ethyl acetate fraction (0.50 mg mL^{-1}), methanolic extract of stems (0.53 mg mL^{-1}) and ethyl acetate fraction of root (0.57 mg mL^{-1}) which showed the same effects. The other extracts did not show any effect. In comparison with the work of Shakeri *et al*. (2012), *A. aphylla* showed greater capacity to inhibit linoleic acid oxidation, with an EC_{50} value of 3.12 mg mL^{-1} for ethyl acetate fraction, and EC_{50} between 6.25 and 12.5 mg mL^{-1} for methanolic extract.

Conclusion

In this study, the quantitative determination of phenolic compounds and the antioxidant properties of stems and root extracts of *A. articulata* were evaluated. The results obtained may suggest that methanolic extract possess compounds with antioxidant properties which can be used as natural preservative for food or cosmetic products. These activities were correlated with high level of total phenolic content, flavonoids and condensed tannins. Phytochemical analysis showed that the major chemical constituents of the extract were flavonoids, tannins, alkaloids and saponins. Likewise, these compounds may have potential use as antioxidative preservatives in emulsion-type systems because they are able to scavenge free radicals in a complex heterogenous medium.

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