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International Journal of Pharmaceutical Research&Allied Sciences, 2019, 8(4):1-5

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Phenolics Compounds and Biological Activity of Leaves of Anabasis Articulata, an Algerian Medicinal Plant

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ABSTRACT

Anabasis articulata is a Saharan plant used by Algerian traditional medicine practitioners for medicinal purposes. The objective of this study was to investigate the antioxidant properties of the extract and fractions of the leaves of A. articulata. The phenolic compounds were also quantified in this plant. Anabasis articulata was examined for antibacterial activities of its methanolic extract using disc diffusion method. The bacteria tested include Pseudomonas aeruginosa ATCC 14028, Salmonella typhimurium ATCC 9027 and Bacillus subtilis spizizenii ATCC 6633. The results showed that methanolic extract exhibits a higher level of phenolic compounds (230.000±0,415) as compared to all other extracts. The FRAP test revealed that the methanolic extract has a higher reducing power (0.1 mg / ml) compared to other extracts. IC50 (DPPH) was ranged from 3.200±0.088 to 4.900 ± 0.130. The compounds quantified by HPLC in the crude extract and fractions were quercetin and ascorbic acid. The results obtained indicated that A. articulata exhibits a good potential to prevent diseases and it might also be used as a natural preservative for food or cosmetic products.

Key words: *Plant, Anabasis articulata, polyphenolic, flavonoids, Antioxidant, antimicrobial activity.*

INTRODUCTION

"Ramet" is the Arabic name of the plant *Anabasis articulata*, belonging to the Chenopodiaceae family. This plant is widely used by the local population and traditional medicine practitioners to treat several diseases [1-4].
The phytochemical constituents of A. articulata, showed the presence of triterpenoid saponin glycosides and Phenolic compounds [5-8]. These secondary metabolisms are responsible for free radical scavenging and antioxidant activities (scavenging activity, and reducing power) of plants [9]. Oxidative stress is to be one of the mechanisms that correlate with oxidative damages caused by free radicals leading to chronic diseases like cancer, coronary heart diseases, diabetes and even aging [10].
The immuno-stimulant [11], cytotoxicity [12], antitumoral properties [13] and antimicrobial activities [14, 15] have been attributed to saponin. The present study was conducted to confirm the biological activity of *A. articulata* leaves previously reported by traditional medicine. Therefore, this study aimed to quantify total polyphenols and flavonoids in the methanolic extract, dichloromethane and ethyl acetate fractions from leaves

of *A. articulata*. Additionally, quercetine and ascorbic acids were quantified and characterized by high-performance liquid chromatography (HPLC). The antioxidant capacity was evaluated by two methods.

MATERIALS AND METHODS

Plant material

Voucher specimens were identified and authenticated with the assistance of one of the professors in Botanic Oran 1 University, Algeria (Prof. Hadjadj-Aouls, M.S).

The decoction of 150 g of the aerial parts was mixed with dichloromethane, methanol and ethyl acetate using a soxhlet **apparatus [16].**

Determination of Total Phenolics

Total phenolic contents were measured using Folin–Ciocalteu method [17]. Gallic acid was used as the control and the samples were read in triplicate at 765 nm in a spectrophotometer.

Total phenolic content was expressed in milligrams equivalents of gallic acid (GAE) per gram of each fraction. The equation obtained for the calibration curve of gallic acid was $y= 0.0082 x + 0.1391$, $R^2 = 0.9926$.

Determination of Total Flavonoids

Total flavonoids were determined according to a slightly modified colorimetric [18]. The samples were prepared at a concentration of 1.0 mg/mL in methanol. Briefly, each sample (01 ml) was added to AlCl3 (2%, w/v, 01 mL) and methanol (2.5 ml). The absorbance was calculated at 430 nm against the blank, after 10 mn of incubation. Rutin (standard) and samples were performed in triplicate. The flavonoid content was established in milligrams equivalents of quercetin per gram of each fraction. The equation of quercetin calibration was obtained $y= 0.0473x+ 0.0073$, $R^2 = 0.9961$

HPLC Quantitative Analysis of quercetine, Ascorbic acid HPLC

Quantitative analysis of quercetine, Ascorbic acid was performed with an HPLC (Agilent Technologies - 1260 infinity) UV-VIS detector. Reverse-phase chromatography analyses were carried out with C-18 column (4.6 mm × 125 mm) packed with 5 µm diameter particles, volume injection was 20 µL and the gradient elution was conducted according to the others method with minor modifications [19]. The mobile phase consisted of water containing 2.0% water, methanol, and acetic acid (50:48:2). The elution gradient was isocratic type. The UV absorption spectra of the standards and the samples were examined in the range of 200–400 nm. The standards (0.1–0.5 mg/ ml) were dissolved in methanol. Identification of the compounds was done by comparison of their UV absorption spectrum and retention's time with those of the standards.

For determination of total antioxidant activity and reducing power, various concentrations of the extracts (mg ml-1) in distilled water were mixed with 1% of solution (2.5 mL, K3 [Fe (CN)6]) and (2.5 ml, 0.2 M, pH 6.6) of phosphate buffer. The mixture was incubated at 50°C for 30 min. Trichloracetic acid (2.5 mL, 10%) was added to the mixture and centrifuged at 3000 rpm for 10 min. The higher layer of the solution (2.5 mL) was mixed with FeCl3 solution (0.5 ml, 0.1%) and water (2.5 mL). The absorbance was calculated at 700 nm and ascorbic acid and quercetin were used as the control [20].

Radical-Scavenging Activity—DPPH Assay

The antioxidant activity of the fractions and the crude extracts was evaluated by monitoring its ability in quenching the stable free radical DPPH **[21].** The DPPH quenching ability was articulated as IC50 (the extract concentration required to inhibit 50% of the DPPH in the assay medium). Different methanol dilutions of each sample at in the interval of 2–10 mg/ml were tested. A solution of DPPH (2.5 ml; 0.3 mM) in methanol was used as a negative control.

The absorption was calculated at 518 nm and. Ascorbic acid and quercetin were used as a positive control. The antioxidant activity was determined by the equation (1):

$$\% \text{ inhibition} = 100 - [(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control} \quad (1)$$

Abs_{sample} is absorbance of fraction;

Abs_{blank} is absorbance of fractions without adding the DPPH; and

$Abs_{control}$ is absorbance of the solution of negative control.

Disc diffusion method

Disc diffusion method was used to study the antimicrobial activity of the extract [22, 23]. The discs filter paper (6 mm diameter) was impregnated with 20 µl of the methanolic extract solution (12.5; 25; 50 mg/ml). Methanol was used as a negative control.

After incubation for 24 h at 37°C for bacteria plates, the diameter of the zone of growth inhibition was measured in millimeters. All experiments were performed in triplicates.

Statistical analysis

The correlation coefficient was determined using the Excel program and Origin 6. The experimental results were expressed as mean ± standard deviation (S.D).

RESULTS AND DISCUSSION

Total phenolic (TP), total flavonoids (TF) and Free Radical-Scavenging activities of DPPH (IC50) values are given in Table 1. The methanolic extract contains the highest content of polyphenols and flavonoids in this study 230.000±0.415 mg/g gallic acid equivalent (GAE) and 9.500±0.306 mg/g of quercetin equivalents (QE), respectively.

The total phenolic compounds were higher in dichloromethane extract (80.000±0.531 GAE/g) than in Ethyl acetate extract (60.000±0.917 GAE/g). The total flavonoids compounds was higher in dichloromethane extract (0.900±0.001mg RE/g) than in Ethyl acetate extract (0.150±0.049 mg RE/g). These results correlate with some studies such as [24] who determined that root and stems of *A. articulata* contained a high quantity of phenolics compounds.

In this Study, the methanolic extracts revealed the presence of high concentrations of phenolics. Various phytochemical components found in this species, among these compounds, the polyphenols can be mentioned which are known for their antioxidant activities [25]. In this regard, various studies have confirmed the assertion, showing that many flavonoids and polyphenols are significantly responsible for the total antioxidant capacity of medicinal plants [26].

Table1: Total polyphenols, total flavonoids and antioxidant capacity (IC50/DPPH) for methanolic extract and fractions of *A. articulata*

Extraits	Phénol TP±SD (µg AG/mg) d'extraits	flavonoïdes TP±SD (µg Q/mg) d'extraits	IC_{50}±SD (mg/ml) DPPH	IC_{50}±SD (mg/ml) FRAP
E.Dichloromethane	80.000±0,531	0.900±0,001	4.200±0.144	0.155±0.020
E.Methanolic	230.000±0,415	9.500±0,306	3.200±0.088	0.100±0.035
E.Ethylacetate	60.000±0,917	0.150±0,049	4.900±0.130	0.140±0.027
Quercetin	----------	----------	5.000±0.198	0.100±0.104
Acide Ascorbique	----------	----------	5.900±0.205	0.110±0.107

Values are expressed as mean ± standard deviation and are significantly different at ($p < 0.05$).

No significant change was observed in the IC50 values of the dichloromethane and ethyl acetate extract and those which showed the most excellent results in the antioxidant capacity measured by the DPPH test. The concentration IC50 of the methanolic extracts (EM = 3.200±0.088 mg / ml) exhibited a good activity of the trapping of the radical DPPH in contrast with the others crude extracts (ED) = 4.2 mg / ml and (EE) = 4.9 mg / ml.

This value remains more or less comparable to that of ascorbic acid (AAS = 5.9 mg/ml), and lower than those of quercetin (Q = 5 mg/ml). The FRAP iron reduction method indicated that the methanolic extract is a good antioxidant activity (0.100±0.035) and superior to the other extracts. This value is similar to quercetin (Q = 0.100±0.104 mg / ml).

This is in accord with other results, stating that these fractions are good sources of antioxidant compounds [27, 28]. The methanolic fraction showed a relatively high level of polyphenols, and the same antioxidant capacity using two methods. A positive correlation between phenolic compounds and the radical-scavenging activity is in good agreement with our results.

This can be explained by some factors, considreing the presence of different active compounds in the synergistic plant, the effects of different polyphenolic compounds, or the methods used for antioxidant reactions and the experimental conditions. The antimicrobial activities (Table 2) of *A. articulata* methanolic extract against microorganisms examined was quantitatively evaluated by the presence or absence of inhibition zones (zone diameters), and MIC (minimal inhibition concentration) value is deducted. The result showed that the methanolic extract at the concentration of 25 mg/ml exhibited a higher degree of antimicrobial activity against

Pseudomonas aeruginosa in contrary to 50 mg/ml concentration. This activity is probably the result of the synergetic effect. Unlike the absence of inhibition for *Bacillus subilis*, in the control (methanol), the colonies were developed normally.

Table 2. Antimicrobial activity of *Anabasis articulate* extracts

Concentration / Souche	C1 = 50 (mg/ml)	C2 = 25 (mg/ml)	C3 = 12.5 (mg/ml)	Témoin
Pseudomonas aeruginosa ATCC *14028*	10	**13**	12	6mm
Salmonella typhimurium ATCC *9027*	**10**	**10**	8	6mm
Bacillus subilis ATCC *6633*	6	6	6	6mm

In conclusion, a phenolic compound from *A. articulata* has strong antioxidant properties, which are useful as preventive agents against various diseases. A variety of biological activities has been described for ascorbic Acids. However, the most important activity is its antioxidant capacity, which is attributed to the tree hydroxyl groups present in the molecule as scavengers of radical species [29, 30]. The antioxidant capacity of these polyphenols is generally attributed to their hydroxyl groups.

CONCLUSION

In this study, the quantitative determination of phenolic compounds and antibacterial and the antioxidant activities of leaves extracts of A. *articulata* were evaluated. We showed that *A. articulata* has many biological activities, which can thus be correlated with the common use of this plant.

The results obtained may suggest that methanolic extracts possess compounds with pharmacological properties which can also be used as a natural preservative for food or cosmetic products.

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