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Total Phenolic Contents and Antioxidant Capacity of Aqueous Extract from *Pituranthos scoparius* (Coss. & Dur.) Growing in Algeria

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ABSTRACT

This study aims to estimate the total phenolics, flavonoids and tannins contents then to investigate both in vitro antioxidant capacity models of aqueous extract (AqE) from the stems of *Pituranthos scoparius*, growing in Algeria. Total polyphenol contents were determined using Folin Ciocalteu's reagent; flavonoids were quantified employing the $AlCl_3$ and method tannins using haemoglobin precipitation test. The in vitro antioxidant property was assessed by DPPH-scavenging radical and lipid peroxidation assays. The results revealed that aqueous extract presented a high total phenolic and tannins contents with values of 150.89 ± 0.68 mg GAE (gallic acid equivalent)/g and 71.24 ± 0.09 mg TAE (tannic acid equivalent)/g dry extract, respectively. This extract show an essential effect toward DPPH-scavenging assay and lipid peroxidation inhibition with 96.19 ± 0.00 µg/mL, 91.53 ± 0.98 %, respectively. This study indicates that the aqueous extract from *Pituranthos sciparius* has potent antioxidant effects and may prove to be of latent health benefit as well as supplementary sources for natural antioxidants drugs.

Keywords: *Pituranthos scoparius*, aqueous extract, antioxidant activity, phenolic compounds.

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INTRODUCTION

Recently, the exploration of natural antioxidant agents from plants is the important and the essential step in the evolution of effective alternative medications [1]. Therapeutic plants with pharmacological characteristics have been designated to be rich sources of components with the critical potential to prevent serious diseases[2]. In recent past, plants and its extracts have received an essential deal of attention worldwide given their potential biological importance .[3] The screening of extracts from plants has been of exceptional importance to scientists for the discovery of novel drugs efficient in the therapy of numerous diseases. These photochemical present essential antioxidant abilities that are linked with a lower incident and lower mortality rates of different human diseases.[4]

Several polyphenols compounds are generally obtained in plants and have gained much attention due to their antioxidant capabilities and free radical scavenging abilities, which probably have an interest in human health [5].

In this context, oxidative damage has been hypothesized to present a critical position in the evolution of a variety of human diseases [6]. It is believed that developed consumption of nutrition rich in native antioxidants agents is associated with lower risks of degenerative disorders; principally cancer and cardiovascular disorders [3], which conclude that oxidative damage, infections, and cancer are closely joined.

The genus *Pituranthos* of the family Apiaceae introduces more than 20 spices [7]. *Pituranthos scoparius*, regionally named "guezzah", belongs to Apiaceae family and it is an endemic plant of North Africa and is comprehensive use in Algeria, particularly in the high plateau of the Sahara [8]. Traditionally, this plant known to be used for the therapy of asthma and rheumatism, measles, firefighting indigestion, jaundice, digestive disorders postpartum care, sore stomach and abdomen [9].

Given the interest of *Pituranthos scoparius* in both phytochemical and pharmacological characteristics, the objectives of this study assess to identify the polyphenolic contents of the aqueous extract from stems of *Pituranthos scoparius* and evaluate the in vitro antioxidant activity using various assays. Furthermore, the topical anti-inflammatory effect of this plant extract was evaluated for the primitive time.

MATERIALS AND METHODS

Collection and identification of plant

The fresh stems from *Pituranthos scoparius* were collected from Setif (mountain djebel Zdimm) north-eastern part of Algeria, during the flowering stage (February 2017 and April 2017, respectively). The plant was identified and authenticated by Prof. Laouer H., a botanist at the Department of Biology and Ecology Vegetal, Sétif, Algeria. A voucher specimen (013/DBEV/UFA/18) was stored at the herbarium found at the Department of Biology, and Ecology Vegetal, Sétif, Algeria.

Chemicals and reagents

Chemicals such as quercetin, gallic acid, tannic acid, Folin-Ciocalteu, indometacin, croton oil and aluminum chloride ($AlCl_3$) were obtained from Sigma (Germany), whereas salts and solvents were purchased from Sigma Chemicals (Germany), Fluka and Prolab. These reagents were of analytical grade and were used as received without further purification.

Extraction procedure

The preparation of the plant extract was given out according to the method of Ferreira et al [10]. Aqueous extract (AqE) was prepared by boiling 100g of dried plant material in 1L of distilled water for 20 min. Then the solution was filtered through muslin cloth and centrifugation at 4000 rpm for 20 min. The dried extract thus obtained was screened for their pharmacological properties.

Determination of total phenolic and flavonoid contents

Total phenolic contents were assessed using the Folin-Ciocalteu's assay [11]. An aliquot of 100 µL of the extract was mixed with 500 µL of Folin-Ciocalteu's reagent (1:9 H_2O) for 4 min, followed by the addition of 400 µL of a 7.5% Na_2CO_3 solution. After 2h of incubation, the absorbance was measured at 765 nm. Polyphenols contents were expressed as µg gallic acid equivalent (GAE)/mg DW. In a similar fashion, the total flavonoids content was determined by the colorimetric method outlined by Bahorun et al [12]. According to this method, 500 µL of each sample was added to 500 µL solution of aluminum chloride (2%). After ten minutes of incubation, the absorbance of the mixture was measured at 430 nm. Total flavonoids were reported as µg of quercetin equivalent (QE)/mg DW.

Determination of tannins

We employed the procedure outline by Bate-Smith et al [13] to measure the precipitation of hemoglobin by tannins. Briefly, a 500 µl aliquot of different concentrations of extract was mixed with 500 µl of haemolyzed sheep blood (absorbance = 1.6). After 20 min of incubation at room temperature, this mixture was centrifuged for 10 min. Tannic acid (100–600 µg/mL) was also mixed with an identical volume of haemolyzed blood. Absorbance of the resulting supernatant was then measured at 576 nm, and the effectiveness of the precipitation of the solutions tested was expressed as µg tannic acid equivalent (TAE)/mg DW.

In vitro antioxidant activity

Radical-scavenging test using DPPH

DPPH scavenging capacity of the extract was estimated using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) activity by measuring the decrease in the DPPH maximum absorbance at 517 nm [14]. In this method, 50 µL of different concentrations of the extract was mixed with 1250 µL of DPPH solution (0.004%) in methanol. Absorbance of the sample was measured at 517 nm after a 30 min of incubation in the dark at room temperature; butylated hydroxytoluene (BHT) was employed as a positive control.

β-Carotene/linoleic acid assay

Inhibition of oxidative discoloration of β-carotene by the products of oxidation of linoleic acid can be used to determine the antioxidant capacity of the extract [15] according to the following procedure: An amount of 0.5 mg of β-carotene was dissolved in 1 mL of chloroform. To this solution, 25 µl of linoleic acid and 200 mg of Tween 40 were added. After evaporation of the chloroform by means of a rotary evaporator, 100 mL of distilled water saturated with O_2 was added, and the solution was vigorously shaken to form a stable emulsion. Then, 350 µL of the extract/standard (BHT) prepared at 2 mg/mL of concentration, then, was added to 2.5 mL of this mixture, followed by incubation for 48 h. Kinetics of discoloration of the reaction system in both presence and absence of the antioxidant was measured at 490 nm at intervals during 48 h (0, 1, 2, 3, 4, 6, 24 and 48) of incubation at room temperature and in the dark. Antioxidant activity was expressed as the percentage of inhibition of the extract, and was calculated as follows:

$I\ \% = (A_e/A_{BHT})\times 100$, where A_e: absorbance in the presence of AqE; A_{BHT}: Absorbance in the presence of BHT.

Statistical analysis

Results are represented as the mean ± standard deviation (SD) and all measurements were conducted in three determinations (n=3). The statistical interpretation was directed by the help of Student's t-test or by one-way analysis of variance (ANOVA) for significance with the aid of GraphPad Prism-5.03; differences were examined significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Total phenolics, flavonoids and tannins contents

Results revealed that the aqueous extract (AqE) was obtained in 9.62 ± 0.20 % yield, whereas the content of polyphenols, flavonoids, and tannins were 150.89 ± 0.68 mg GAE, 0.82 ± 0.39 mg QE, and 71.24 ± 0.09 mg TAE/g dry extract, respectively as shown in Table 1.

Table 1. Main constituent contents and extraction yield of AqE. Results are presented as mean ± SD (n = 3).

Extract	% yield (w/w)	Total phenolic content[(a)]	Total flavonoid content[(b)]	Tannin content[(c)]
AqE	9.62 ± 0.20	150.89 ± 0.68	0.82 ± 0.39	71.24 ± 0.09

AqE: Aqueous extract, [(a)]: µg GAE/mg, [(b)]: µg QE/mg and [(c)]: µg TAE/mg dry extract

In this study, the yield of extraction is in accord with Adida et al [13]. Whereas, the result revealed the presents a high amount of phenolic compounds in the AqE include polyphenol, flavonoids and tannins. These compounds may account for the high antioxidant activity [13].

Investigation of antioxidant activity

The EC_{50} values of DPPH, metal chelating and hydroxyl radical scavenging activities of the aqueous extract are presented in Table 2. Aqueous extract demonstrated scavenging activities against DPPH in a concentration-dependent manner with an EC_{50} value of 96.19 ± 0.00 µg/mL. In the β-carotene/linoleic acid assay, results showed that the extract displays high inhibition percentage with I % value of 91.53 ± 0.98 %. This suggests a significant antioxidant activity in lipid peroxidation assay of the AqE.

Table 2. Antioxidant capacities of aqueous extract (AqE).

		EC_{50} (µg/mL)
Extract/Standard	AA%	DPPH scavenging activity
AqE	91.53 ± 0.98**	96.19 ± 0.00**
BHT	99.13 ± 0.08	87.26 ± 0.001

** $p < 0.01$ compared to correspondent standards. AqE: Aqueous extract, DPPH: 2,2-diphenyl-1-picrylhydrazyl, AA: Antioxidant activity, BHT: butylated hydroxytoluene.

Various assays including DPPH-scavenging assay, lipid peroxidation, hydroxyl scavenging ability, iron-chelation and reducing power activities were employed to evaluate the in vitro antioxidant properties. Results revealed that the AqE presented a high scavenging activity against DPPH, chelating and inhibition the bleaching of β-carotene. This result suggests that AqE can attend as a free radical scavenger and it's obtained to inhibit the oxidation of β-carotene by compensating both the linoleate free and other liberal radicals generated in the reaction system.

CONCLUSION

This research highlights the total phenolic contents; antioxidant effects of *Pituranthos scoparius* stem extract from Algeria. The study data demonstrated that AqE had the highest total phenolic, flavonoid and tannins contents and exhibited significant antioxidant capacities using different assays. This may explain the medicinal use of this plant in folk medicine. These results suggest that AqE of *P. scoparius* might be promising for the treatment or prevention of many diseases associated with oxidative damage. However, more investigations are needed to establish the active constituents of this plant which are responsible for the antioxidant and anti-inflammatory activities.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

[1]: Singh M, Pandey N, Agnihotri V, Singh KK., Pandey A. Antioxidant, antimicrobial activity and bioactive compounds of Bergenia ciliata Sternb.: A valuable medicinal herb of Sikkim Himalaya. J Tradit Complement Med 2017; 7:152-157.

[2]: Cornara L, La Rocca A, Marsili S, Mariotti MG. Traditional uses of plants in the Eastern Riviera (Liguria, Italy). J. Ethnophrmacology 2009; 125:16-30.

[3]: Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatic. J Food Drug Anal 2014; 22:296-302.

[4]: Boutennoun H, Boussouf L., Rawashdeh A, Al-Qaoud K, Abdelhafez S, Kebieche M, Madani K. In vitro cytotoxic and antioxidant activities of phenolic components of Algerian *Achillea odorata* leaves. Arab J Chem 2017; 10:403-409.

[5]: Pereira DM, Patrícia V, Pereira JA, Andrade PB. Phenolics: From Chemistry to Biology. Molecules. 2009; 14:2202-2211.

[6]: Edziri H, Ammar S, Souad L., Mahjoub MA, Mastouri M, Aouni M, Mighri Z, Verschaeve L.. In vitro evaluation of antimicrobial and antioxidant activities of somen Tunisian vegetables. S Afr J Bot. 2012; 78:252-256.

[7]: Chahrazed E, Youcef M, Jean-Claude C, Gilles F, Pierre C, Samir B, Fadila B. Chemical Composition of the Essential Oil of Aerial Parts of *Pituranthos battandieri* Maire. IJPPR. 2016; 8(10):1731-1734.

[8]: Vérité P, Nacer A, Kabouche Z., Seguin E. Composition of seeds and stems essential oils of *Pituranthos scoparius* (Coss. & Dur.) Schinz. Flavour Frag. J. 2004; 19: 562-564.

[9]: Adida H, Benariba N, Bechiri A, Chekroun E, Djaziri R. Phytochemical study and evaluation of the antiradical activity of extracts of *Pituranthos scoparius*. Phytothérapie. 2015; 14: 207-212.

[10]: Ferreira A, Proença C, Serralheiro ML, and Araújo ME. The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. J. Ethnopharmacol. 2006; 108: 31-37.

[11]: Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 2007; 102: 771-776.

[12]: Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Gazin JC, inkas M, Uycky M, Gazin M. Oxigen species scavenging activity of phenolic extract from howthorn fresh plant organs and pharmaceutical preparation. Arzneim Forsch. 1996; 46: 1086-1094.

[13]: Bate-Smith EC. Haemanalysis of tannins, the concept of relative astringency. Phytochemistry. 1973; 12: 12-907.

[14]: Charef N, Sebti F, Arrar L, Djarmouni M, Boussoualim N, Baghiani A, Khennouf S, Ourari A, AlDamen MA, Mubarak MS, Peters DG. Synthesis, characterization, X-ray structures, and biological activity of some metal complexes of the Schiff base 2,2'-(((azanediylbis(propane 3,1diyl))bis(azanylylidene)) bis (methanylyli-dene)). Polyhedron. 2015; 85: 450-456.

[15]: Kartal N, Sokmen M, Tepe B, Daferera D, Polissiou M, Sokmen A. Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure. Food Chem. 2007; 100: 9-584.