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Phenolic contents, *In Vitro* antioxidant, and *In Vivo* anti-inflammatory studies of aqueous extract from *Pituranthos scoparius* (Coss. & Dur.) growing in Algeria

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ABSTRACT

The present study aims to estimate the total phenolics, flavonoids and tannins contents then to investigate both in vitro antioxidant capacities models and topical anti-inflammatory activities 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 AlCl3 and method tannins using haemoglobin precipitation test. The in vitro antioxidant properties was assessed using different models, including DPPH-scavenging assay, lipid peroxidation, $OH^{\cdot}$ scavenging ability, iron chelation, and reducing power activities. In vivo, the topical anti-inflammatory effect of this extract was determined using croton oil-induced edema in mouse model. 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, lipid peroxidation inhibition and chelating effect, with 96.19 ± 0.00 µg/mL, 91.53 ± 0.98 % and 36.30 ± 0.00 µg/mL EC50, respectively. In a similar fashion, this extract exhibited an excellent topical anti-inflammatory activity value of 80.30 ± 5.91 % regardless of the dose of 2 mg/ear. This study indicates that the aqueous extract from Pituranthos scoparius has potent antioxidant and anti-inflammatory effects and may prove to be of latent health benefit as well as supplementary sources for natural antioxidants and anti-inflammatory drugs.

Keywords
Pituranthos scoparius,
Aqueous extract,
Anti-inflammatory activity,
Antioxidant activity,
Phenolic compounds

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INTRODUCTION

Recently, the exploration of natural antioxidant agents from medicinal plants is the important and the essential step in the evolution of effective alternative medications [1]. Therapeutic plants with pharmacological properties have been appointed to be rich sources of components with the critical potential to prevent severe 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

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discovery of novel drugs efficient in the therapy of numerous diseases. These phytochemicals present important antioxidant capabilities which are associated with a lower incident and lower mortality rates of various human disorders [4].

Various examinations pointed out those natural products in food present better effectiveness as compared to artificial antioxidants due to their lower volatilization, resistance and commonly important antioxidant potential [5, 6]. Several polyphenolic compounds are generally obtained in plants and have gained much attention due to their antioxidant capacities and free radical scavenging abilities, which probably have an interest in human health [7]. In addition, these compounds exert an effective capacity as anti-inflammatory agents, by blocking both two essential signaling pathways which serve the important function in the generation of various pro-inflammatory mediators [5].

In this context, oxidative damage has been hypothesized to present a critical position in the evolution of a variety of human diseases [8]. It is believed that developed consumption of nutrition rich in native antioxidants 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 [5].

The genus *Pituranthos* of the family Apiaceae includes more than 20 spices [9]. *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 [10]. A plant is widely used in food as a flavoring agent [11], but they are also traditionally known to be used for the therapy of asthma and rheumatism [12, 11, 13], measles, firefighting indigestion, jaundice, digestive disorders postpartum care, sore stomach and abdomen [13].

Many investigations indicated that the essential oils of *Pituranthos scoparius* have exhibited some biological activities such as antimicrobial [11, 14], antioxidant [13, 15], and antiurolithiatic [16]. Moreover, many researchers report that the essential oils of the genus *Pituranthos* display antimicrobial, antioxidant, antifungal, immunomodulatory and anticancer capacities [17-20]. In this respect, Dahia et al. [21], reported that the phytochemical profile of the aqueous and methanolic extracts of *P. scoparius* revealed a high wealth of flavonoid, glycosides, and cinnamic acids, which may demonstrate the effectiveness of this plant as a drug against rheumatism and fever, since these compounds possess anti-inflammatory properties [22, 23].

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 model assays. The choice of stems from aerial part is based on the traditional use of this plant in Setif region and as cited by several authors [13, 14]. 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, vitamin C, EDTA (Ethylene diamine tetra acetic acid), Folin-Ciocalteu, indomethacin, 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 [24]. 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 [25]. 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 [26]. 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 [27] 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

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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 [28]. 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. As a reference, 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 [29] 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.

$OH^{\cdot}$ radical scavenging test: Hydroxyl radical scavenging ability of the extract was evaluated via a spectrometric method as described by Ates and coworkers [30]. Briefly, a mixture containing 1000 µL of ferrous sulfate (0.015 M), 700 µL of hydrogen peroxide (0.006 M) was mixed with varying concentrations of extract or ascorbic acid as a positive control. Then, 300 µL of sodium salicylate (0.020 M) was added, followed by incubation for 20 min at 37°C. Absorbance of the obtained mixture was measured at 562 nm. Scavenging capacity of the extract was evaluated using the following equation:

$I\ (\%) = 100 \times [1-(A_e-A_c)/A_0]$. Where A_0: absorbance of control, A_e: absorbance in the presence of AqE, and A_c: absorbance in the absence of sodium salicylate reagent.

Iron-chelation test: Ferrous iron-chelation capacity of the extract was assessed using the Decker and Welch method [31]. According to this method, a mixture containing 250 µL of extract or EDTA as a positive control, 100 µL $FeCl_2$ (0.06 M in distilled water) and 900 µL methanol was made. The control included all reaction reagents except the test samples. This mixture was mixed well and approved to react for five minutes at room temperature, followed by the addition of 100 µL of ferrozine solution. Absorbance at 562 nm was measured after 10 min of incubation. The IC_{50} rate was determined as the efficient concentration of test sample which performs 50 % of the maximal chelating activity.

Reducing power assay: Aqueous extract capacity to reduce Fe^{+3} to Fe^{+2} ions was estimated following a procedure outlined by Chung et al [32]. According to this procedure, an aliquot of 400 µL of extract was mixed with an identical volume of both 200 mM phosphate buffer (pH = 6.6) and 10 ‰ potassium ferricyanide. This mixture was then incubated in a water bath at 50 °C for 20 min. The reaction was terminated by adding 400 µL of trichloroacetic acid (10%), and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (400 µL) was added to distilled water (400 µL) and 80 µL of 0.1% ferric chloride. The color intensity of the mixture was measured at 700 nm after ten minutes of incubation. In this context, a high absorbance of the solution means a high reducing power.

In vivo anti-inflammatory activity

Animals: Adult female mice (25–30 g) were purchased from Pasteur Institute of Algeria. These animals were kept in special cages underneath conventional laboratory limitations of 12:12 h light dark cycle and 25 ± 1°C for seven days before the experiments. They were given free access to water and standard diet. The guidance on the application and treatment of the animals adapted to internationally pleasant fashions mentioned in the Animals By-Laws N° 425–2008. The experimental animals were handled subsequent obtaining authorization from Institutional Ethic Committee, and the experiments were supported by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA).

Anti-inflammatory activity: Topical anti-inflammatory activity was assessed using croton oil-induced ear edema in mouse [33]. Mice were randomized into three groups of 6 mice for each. First, 15 µL of 50% acetone and water solution comprising 80 µg of croton oil was locally administered at the inner surface of the right ear for each mouse of the three groups used in this test. Simultaneously, the same volume at the concentration of 2 and 0.5 mg of the extract and indomethacin, respectively, were topically applied at the same local of the ear. Control group topically receive only the croton oil solution. The thickness of the ear was measured by digital caliper before treatment and 6 hours after the induction of inflammation. The difference in thickness before and after the application of croton oil was calculated. The inhibition percentage of ear edema was computed as follows:

Inhibition percentage $(\%) = 100 \times (B-A)/B$, where A is the difference of ear edema thickness in the treated group and B is the difference of ear edema thickness in the healthful group.

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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

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.

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 and chelating radical in a concentration-dependent manner with an EC_{50} value of 96.19 ± 0.00 µg/mL and 36.30 ± 0.00 µg/ml, respectively. Whereas, AqE present a moderate activity in hydroxyl radical-scavenging effect compared to the vitamin C. 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.

Hydroxyl scavenging activity of the extract was assessed using the hydroxyl radical assay. These radicals are detected by their ability to hydroxylate salicylate. From the EC_{50} (Table 2), the AqE showed a considerable activity with value of 526.34 ± 0.00 µg/mL likened to ascorbic acid. The reducing power demonstrated the capacity of the extract to reduce Fe^{3+} to Fe^{2+} form by donating an electron. In this assay, the AqE demonstrated the ability to reduce Fe^{3+} to Fe^{2+} form by donating an electron with increasing concentration. Table 2 showed that the AqE presented an EC_{50} = 131.26 ± 0.02 µg/mL witch obtained to have a considerable activity; but, it outlasted significantly ($P < 0.05$) low compared to Vitamin C. The current in vitro antioxidant study of aqueous extract from *Pituranthos scoparius* revealed promising antioxidant activity.

In vivo topical anti-inflammatory activity

Aqueous extract (AqE) was assayed for their topical anti-inflammatory activity using croton oil-induced ear edema assay, and their effect is showed in Figure 1. The results revealed that the AqE exhibited a high and significant inhibition of edema induced by croton oil in mice (at 6 h) with 80.30 ± 5.91 % regardless of the dose of 2 mg/ear. Whereas, the croton oil control represent an IC_{50} of 1.04 ± 0.59 % regardless of the dose of 80 µg/ear; where the inflammation is induced. As a reference, the non-steroidal anti-inflammatory drug indomethacin reduced less

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

Table 2. Antioxidant capacities of aqueous extract (AqE)

Extract/ Standards	AA%	EC_{50} (µg/mL) DPPH scavenging activity	Chelating activity	Hydroxyl scavenging activity	Reducing power activity
AqE	91.53 ± 0.98**	96.19 ± 0.00**	36.30 ± 0.00***	526.34 ± 0.00***	131.26 ± 0.00***
Vitamin C	-	-	-	83.65 ± 0.00	21.91 ± 0.48
BHT	99.13 ± 0.08	87.26 ± 0.001	-	-	-
EDTA	-	-	6.05 ± 0.00	-	-

** $P < 0.01$, *** $P < 0.001$ compared to correspondent standards. AqE: Aqueous extract, EDTA: Ethylenediaminetetraacetic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl, AA: Antioxidant activity, BHT: butylated hydroxytoluene.

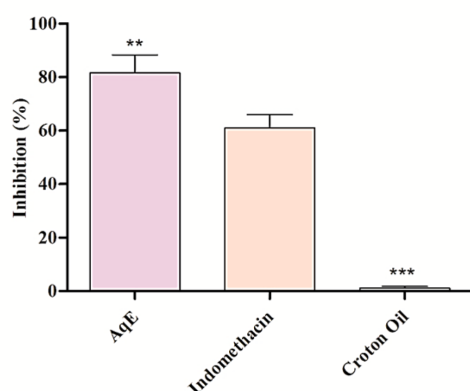

Figure 1. Inhibition percentage of the ear edema in mice after 6h. Data are presented as the mean ± SEM (n = 6), **$P < 0.01$, *** $P < 0.001$ compared with positive control (Indomethacin)

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edematous response by 61.02 ± 3.41 % at the dose of 0.5 mg/ear (Fig. 1).

DISCUSSION

Among the major problems concerning the uses of medicinal plants in traditional therapeutic preparations is the deficiency of experimental data. However, the screening of plant extracts has been of exceptional importance to scientists for the discovery of novel drugs efficient in the therapy of numerous diseases. In the scientific and therapeutic communities, antioxidants are considered to have the capability to scavenge free radicals and qualify oxidative damage [34]. 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] and anti-inflammatory properties [22, 23] observed for the polar extracts of these aromatic plants.

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 present 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. Ferrous chelating activity is based on the capability of the plant extract to chelate transition metals by joining them to ferrous (Fe^{2+}) ion catalyzing oxidation and inhibition the production of Fe^{2+}-ferrozine complex. The obtained results showed that the AqE had significant chelating activity, recommending that the extract presented a high efficient chelating ability by capturing the ferrous ion before it reacts to form a complex with ferrozine. Many reports highlight that high antioxidant activity could be associated with the presence of an amount of polyphenols compounds [36, 35].

Otherwise, lipid peroxidation and enormous biological damage could induce by the hydroxyl radical. It is an efficient cytotoxic factor and capable to attack the most molecules in the human body resulting in the formation of malondialdehyde, which is mutagenic and carcinogenic and the peroxidation of cell membrane lipids [37]. In the present study, AqE showed considerable antioxidant activity that it can mainly due to their interactions between different phenolic compounds. Therefore, the higher potency of the scavenging free radicals could be attributable to the appearance of the hydrogen donation capacity in the phenolic compounds of the extract; which is extremely linked to the presence of hydroxyl groups in the phenolic composites [38]. Therefore, the scavenging of hydroxyl radical by plant extract may exhibit significant protection of biomolecules against free radicals [39]. In addition, this extract showed a considerable reducing power designating that it can play a role as electron contributors and could transform the free radicals through more stable products by the reaction with them and then finish the free radical series reactions. In this fact, it is well known that especially free radicals play a major role in several inflammatory diseases. It is well documented that phenolic compounds and flavonoids might be responsible of antioxidant potential, which depends on their chemical structure and the distribution of hydroxyl groups [40]. This result corroborates with the previous study indicating that the aqueous extract from *Pituranthos scoparius* possesses high antioxidant activity [13]. A possible reason for antioxidant effects of *Pituranthos scoparius* could be the presence of several families of secondary metabolites, which can influence the activity of plant extracts by their synergistic and/or additive effects [41].

Similarly, the topical anti-inflammatory activity of AqE was further evaluated by the inhibition of croton oil induced ear edema in mouse model. Croton oil is a highly irritating agent that contains tetradecanoyl-phorbol acetate (TPA) which can stimulate an inflammatory response and then induce edema [42]. The molecular and cellular mechanism by which croton oil induces inflammation may be linked to the activation of numerous protein kinases C [43] by the secretion of high levels of intracellular factors such as calcium and diacylglycerol. The activation of the receptors coupled to a G protein resulting in the production of these factors. These receptors stimulated in inflammation, then implicated in pain. Diverse intracellular signal transduction pathways mediate by Protein kinase C (PKC) such as phospholipase A_2 associated with the releasing of arachidonic acid and eicosanoid production and influenced in the pathogenesis of inflammation.

The mechanism of action of indomethacin on inflammation is based on the inhibition of pro-inflammatory prostaglandin synthesis. Similarly, the topical treatment of the mice with the AqE cause significant inhibition of the development of edema. This inhibition may be due to the reduction of the release of PKC or the inhibition of its action and the extract may effectively suppress the exudative phase of acute inflammation and then pain. This result suggested that the AqE from stems of *P. scoparius* exhibited a potent antioxidant activity and inflammatory effect due to the diversity of their phenolic contents such as polyphenols, flavonoids and tannins. The antioxidant activity of AqE could have a significant effect on the prevention of inflammatory response. Griffiths et al. [5] reported that the development in oxidative damage inducing inflammation is a unifying explanation for the predisposing human to carcinogenesis, atherosclerosis and osteoporosis diseases. Furthermore, the influence of antioxidants and anti-inflammatory factors on cardiovascular disease, cancer and the interceptive mechanisms for the repression of propagation and infection.

CONCLUSION

This research highlights the total phenolic contents, antioxidant and anti-inflammatory effects of *Pituranthos*

scoparius stem extract from Algeria. The study data demonstrated that aqueous extract 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. In a similar fashion, findings from the topical anti-inflammatory effect reveal that the aqueous extract exhibit a significant reduction in croton oil induced edema in mouse. These results suggest that aqueous extract of *P. scoparius* might be promising for the treatment or prevention of many diseases associated with oxidative damage and inflammation. To the best of our information, this is the first investigation evaluating the topical anti-inflammatory activity of this species growing in Setif, Algeria. However, more investigations are needed to establish the active constituents of this plant which are responsible for the antioxidant and anti-inflammatory activities.

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CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article

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