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Fractionation, Phytochemical Screening and Free Radical Scavenging Capacity of Different Sub-Fractions from *Pituranthos scoparius* Roots

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ABSTRACT

The purpose of this study was to prepare three sub-fractions from *Pituranthos scoparius* roots (PSR), characterize their phytochemicals contents and to investigate their free radical scavenging activity by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and hydroxyl scavenging activities. Tannins, flavonoids, steroids, and other bioactive compounds were found in the different sub-fractions. The Ethyle acetate extract (EAE) and chloroform extract (ChE) exhibited the highest antioxidant activity using ABTS (17.8 ± 0.87 µg/mL and 18.15 ± 0.68 µg/mL), respectively. Whereas, Crude extract (CrE) have been presented strong hydroxyl scavenging activity (14.9 ± 0.8 µg/mL). This study indicates that PSR extracts has potent free radical scavenging, and may prove to be of potential health benefit as well as additional resources for natural antioxidants.

Keywords: Medicinal plant; phytochemical screening; sub-fractions, free radical scavenging.

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INTRODUCTION

Antioxidants are substances that significantly delay or inhibit oxidation of an oxidizable substrate when present at low concentrations in comparison with those of the substrate [1]. Nowadays, the scientists have casted some toxicological doubts on synthetic antioxidants due to their adverse faction effects, and people are more concerned about food safety and quality [2].

Evidences showed that natural antioxidants deliver better effectiveness as compared to synthetic antioxidants. Natural products have always remained a profile source for the discovery of new drugs [3].

In this context, the present research aims to investigate one of the medicinal plants used for various therapeutic purposes in Algerian folk medicine, which is *Pituranthos scoparius*, commonly known as "Guezzah" [4]. This plant used for the treatment of asthma, measles, digestive disorders, jaundice and rheumatism. Preliminary phytochemical screening has indicated the presence of tannins, flavonoids, sterols and other phytochemical components in the roots [5]. However, few studies have investigated its free radical scavenging activities. Previous studies showed that the phytochemical analysis of ethyl acetate extract from *P. scoparius* roots revealed the isolation of two isocoumarins: 3-n-propyl-5-methoxy-6-hydroxy- isocoumarin and 3-n-propyl-5,7-dimethoxy-6-hydroxy isocoumarin [6]. In addition, Benalia et al. [7] have shown a high *in vitro* anti-urolithiatic effect of *P. scoparius* roots extracts.

The current study was undertaken to evaluate the *in vitro* antioxidant potential of Algerian *Pituranthos scoparius* root extracts (PSRE) by ABTS radical scavenging and hydroxyl scavenging activity. In addition, phytochemical of different extracts were also measured, to establish any relationship between the antioxidant activities and these compounds.

MATERIALS AND METHODS

Plant collection and identification

The Roots of *Pituranthos scoparius* were collected from the mountain Djebel Zdimm located about twenty kilometers south of Setif (Algeria) at an altitude of 1212 m above sea level. The plant was identified by Pr H. Laouer (Laboratory of Valorization of Natural Biological Resources, University of

Setif, Algeria) under voucher specimen (013/DBEV/UFA/18), then air-dried under shadow at room temperature to preserve their properties, then powdered and stocked in darkness until use.

Bioactivity-guided fractionation

The three sub-fractions of *Pituranthos scoparius* roots (PSR) were prepared according to [8] method, using solvents with different polarities. Dried plant material was macerated in methanol/water 85/15 (v/v), in a vegetal material/solvent ratio 1:10 (w/v) and the mixture was subjected to agitation during an overnight at 4°C with occasional shaking (**Figure 1**). All the solvents were eliminated by evaporation under reduced pressure.

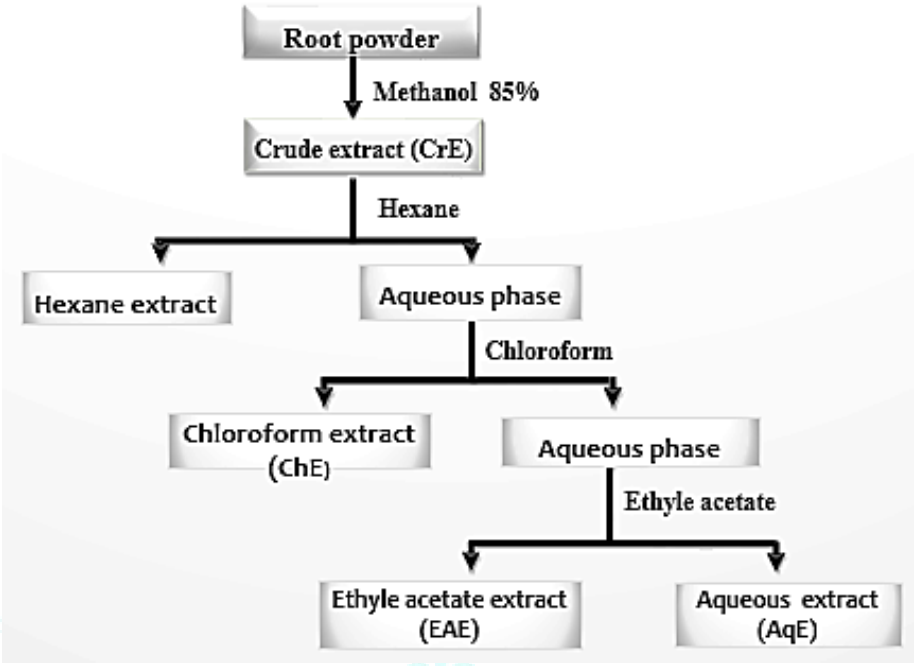

Figure 1: Steps for preparation of sub-fractions, where; crude extract (CrE), chloroform extract (ChE), ethyle acetate extract (EAE).

Qualitative detection of phytochemical constituents

Qualitative tests for the presence of different phytochemical compounds include: tannins, flavonoids, quinones, anthraquinones, saponins, steroids, glycosides, terpenoids and carbohydrates were carried out on the roots extracts using the procedures of [9].

Antioxidant capacity by ABTS radical assay

The colorimetric analysis of $ABTS^+$ radical scavenging assay was determined according to [10] method with slight modifications. The $ABTS^+$ solution was formed by the reaction of 7 mM of ABTS solution in 2.45 mM potassium persulfate. The mixture was saved in the dark at room temperature for 16 h before use. The solution was diluted with absolute ethanol and equilibrated at room temperature to give an absorbance of 0.7 at 734 nm. Then, 20 µL of the extract dilutions was mixed with 2 mL of $ABTS^+$ solution and kept for six min at room temperature. The absorbance was measured at 734 nm. The scavenging capability of $ABTS^+$ radical was calculated according to the following formula :

$$I\% = (A_{blank} - A_{test} / A_{blank}) \times 100.$$

Where A_{blank} is the absorbance of the solution except the test compound, and A_{test} is the absorbance of the tested compound.

Hydroxyl radical scavenging test

The hydroxyl radical scavenging ability was estimated using the spectrometric method [11]. Briefly, A mixture contained one mL of $FeSO_4$ (1.5 mM), 0.7 mL of H_2O_2 (6 mM) was mixed with varying concentrations of samples or ascorbic acid as a positive control. Then, 0.3 mL of sodium salicylate (20 mM) was added, the resulting mixture was incubated at 37°C for 20 min. After that, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as scavenging rate (Hydroxyl radical scavenging activity % or I %) by the following equation :

$$I\ (\%) = [1-(A_s-A_c)/ A_0] \times 100$$

Where A_0 was the absorbance of the control (without sample) and A_s was the absorbance in the presence of the sample, A_c was the absorbance without sodium salicylate.

Statistical analysis

Statistical analysis was performed by using the Graph Pad Prism (version 5.03 for Windows). In this study, statistical analysis was analyzed by one-way analysis of ANOVA. All determinations were carried in triplicate, and all results were estimated as the mean ± standard deviation (SD). Tests of significant differences were determined by multiple range tests at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening results of the PSR extracts are reported in table 1. The roots were observed to contain tannins, flavonoids, free quinones, steroids, keto compounds were detected in all extracts. However, anthraquinones, glycosides, saponins, terpenoids, reducing sugars were not

found in the all extracts. Phytochemical analyses carried out by [5, 12] on the roots part of *Pituranthos scoparius* extracts revealed the presence of reducing sugars, flavonoids, tannins and steroids. Moreover, terpenoids are also present in almost all the studied extracts. These differences may be related to different conditions of extraction, time of collection…etc.

Table 1: Phytochemical screening of different *P. scoparius* roots extracts

Phytochemical compounds	CrE	ChE	EAE
Tannins	+	+	+
Flavonoids	+	+	+
Quinones	+	+	+
Anthraquinones	-	-	-
Glycosides	-	-	-
Saponins	-	-	-
Steroids	+	+	+
Terpenoids	-	-	-
Reducing sugars *Fehling's solution's	-	-	-
*Seliwanoff's solution	+	+	+

+/-: Presence/absence of the compound

ABTS radical scavenging assay

The free radical scavenging activity of PSRE was also determined using ABTS radical. The results revealed that all extracts scavenged the ABTS cations with IC_{50} values varying from 17.8 to 51.47 µg/mL (Figure 2). These values are close to that of Vit C with an IC_{50} value 0.075 ± 0.001 µg/mL. As seen in Figure 2, the highest ABTS radical scavenging activity was exhibited with EAE and ChE followed by CrE with IC_{50} values of 17.8 ± 0.87 µg/mL, 18.15 ± 0.68 µg/mL and 51.47± 1.01 µg/mL, respectively.

The high ABTS radical scavenging ability of EAE and ChE can be attributed to the presence of phenolic compounds. The earlier studies reported that ABTS radical scavenging capacity of bioactive compounds depends on their molecular weight, structure and presence of aromatic groups [13].

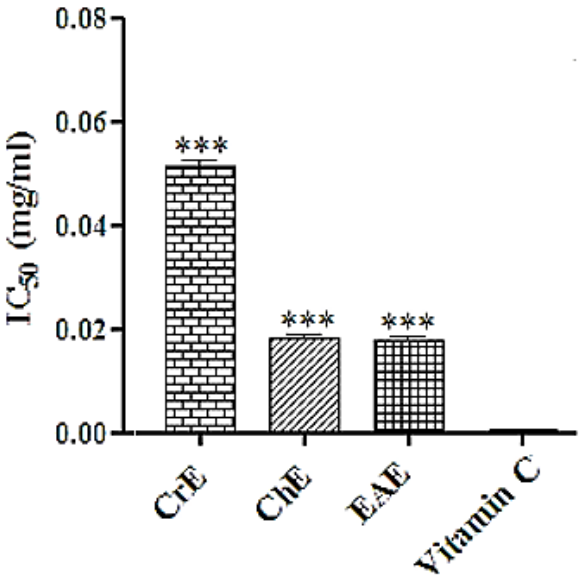

Figure 2: Free radical scavenging activity of different PSRE for ABTS assay. Data were presented as means ± SD (n=3). ***: $P < 0.001$ compared to Vitamin C as standard.

Hydroxyl radical scavenging activity

Hydroxyl radicals generated by the Fenton reaction could oxidize Fe^{2+} into Fe^{3+} which is reflected by the degree of decolorization of the reaction solution. In this assay, OH• radicals were generated using a system containing $FeSO_4$ and H_2O_2 and detected by their ability to hydroxylate salicylate. Vitamin C was used as a standard antioxidant for comparison (IC_{50} = 83.6 ± 1.4 µg/mL). The radical scavenging activity of PSRE decreased in the following order CrE (14.9 ± 0.8 µg/mL), ChE (290.3 ± 0.02 µg/mL) and then EAE (458.4 ± 0.61 µg/mL). The results are summarized in Figure 3.

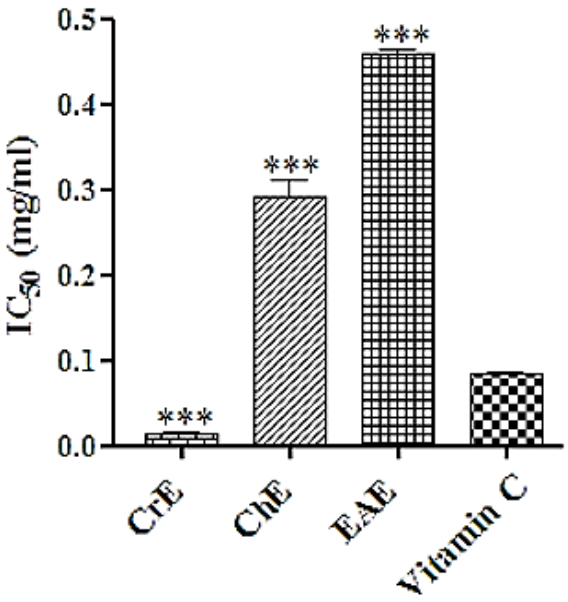

Figure 3: Hydroxyl radical scavenging activity of different *P. scoparius* L. extracts. Data were presented as means ± SD (n = 3). ***: $P < 0.001$ compared to standard.

Hydroxyl radical is a potent cytotoxic factor able to attack almost every molecule in the body resulting in peroxidation of cell membrane lipids and in the formation of malondialdehyde, which is mutagenic and carcinogenic [14]. Therefore, the scavenging of hydroxyl radical by extracts may provide significant protection to biomolecules by their ability to remove hydroxyl and superoxide free radicals due to inhibition of respective mechanisms involved in the formation of radicals [15].

The higher potency of the scavenging hydroxyl radicals may be attributable to the presence of the hydrogen donating ability phenolic compounds in the extracts; which is highly related to the presence of hydroxyl groups [16]. Furthermore, the potent radical scavenging effects of the extracts may be related to the expanded steric obstacle [17] in compounds contained in these extracts.

CONCLUSION

The findings of the present study indicate that *Pituranthos scoparius* could be a new source of natural antioxidant drugs. The data highlights the good free radical scavenging properties of different extracts from *Pituranthos scoparius* roots. This antioxidant potential is probably associated with the presence of various secondary metabolites which may have many benefits in treating oxidative stress-related diseases. These results lay the preparation for further studies on the molecular mechanisms underlying the

biological profile of these extracts, isolation and purification of more active principles in each extract as well as clarification of their mode of action. These *in vitro* results should be validated *in vivo* to develop a potent antioxidant agent from this plant.

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