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.

**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. 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.

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.

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. 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. In addition, Benalia et al. 7 have shown a high *in vitro* anti-urolithiatic effect of *P. scoparius* roots extract.

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 a 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.

![Diagram of sub-fractions preparation](image)

**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:

\[ I(\%) = \frac{(A_{blank} - A_{test})}{A_{blank}} \times 100 \]

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 FeSO₄ (1.5 mM), 0.7 mL of H₂O₂ (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:

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

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 and carbohydrates were detected in all extracts. However, anthraquinones, glycosides, saponins, terpenoids, reducing sugars were not
found in 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’s* | +   | +   | +   |

+/−: 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 IC₅₀ values varying from 17.8 to 51.47 µg/mL (Figure 2). These values are close to that of Vit C with an IC₅₀ value of 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 IC₅₀ 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.

**Concentration of IC₅₀ (µg/ml)**

**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 Fe²⁺ into Fe³⁺ which is reflected by the degree of decolorization of the reaction solution. In this assay, OH radicals were generated using a system containing FeSO₄ and H₂O₂ and detected by their ability to hydroxylate salicylate. Vitamin C was used as a standard antioxidant for comparison (IC₅₀ = 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.

**Figure 4:** Hydroxyl radical scavenging activity of different *P. scoparius* L. extracts. Data were presented as means ± SD (n = 3). ***: P < 0.001 compared to Vitamin C as standard.

**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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