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Evaluation of Antioxidant Activity and Polyphenols Content of the Hydro-methanolic Extract from *Saccocalyx satureioides* Coss and Dur

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ABSTRACT

This study aimed to estimate the total phenolic and flavonoid contents and to evaluate the antioxidant activity of the Hydro-methanolic from *Saccocalyx satureioides* Coss and Dur aerial part. Total polyphenols contents were determined using Folin-Ciocalteu's reagent. The flavonoids were estimated using the method of Aluminum chloride ($AlCl_3$). The antioxidant capacity was evaluated using two *in vitro* models (the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and reducing power). Total phenolic and flavonoid content in the extract were 171.34 ±1.43 mg Gallic acid equivalent/g of dry extract (GAE/g) and 18.6 ± 0.46 mg Quercetin equivalent / g of dry extract (QE/g), respectively. The methanol extract has an important capacity to scavenge the free radical DPPH with an IC_{50} of 0.03 ± 0. 0024 mg/ml. In addition, the plant extract exhibited high reducing power with an IC_{50} of 0.54 ± 0.00625 mg/ml. The results of the present study may prove that the medicinal plants are a good resource of natural antioxidants.

Keywords: Antioxidant activity, *Saccocalyx satureioides,* polyphenols, flavonoids.

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INTRODUCTION

Active oxygen and free radicals exist in human body in the form of superoxide anion (O2.-) hydrogen peroxideH_2O_2) and hydroxyl radical (OH.) and so on. As normal metabolic action going on in human body, active oxygen and free radicals are constantly formed. If they reach high levels, oxidative stress in human body would be created, which leads to a variety of biochemical and physiological lesions and often results in metabolic impairment and cell death [1].

Antioxidants are compounds that can delay or inhibit the oxidation of lipids and other molecules and by doing so inhibit the initiation and propagation of oxidative chain reactions. They act by one or more of the following mechanisms: reducing activity, free radical scavenging, potential complexation of pro-oxidant metals and quenching of singlet oxygen [2]. Plants develop different kind of antioxidants that aid in antioxidant defense system, protecting plants against damage caused by active O2 formed due to exposure to ultraviolet radiation. In the group of anti-oxidant and free radical scavenging agents, plants synthesize different compounds, principally phenolic derivatives, such as flavonoids, phenyl-propanoids, stilbenes and other [3]. The use of medicinal plants in health recovery has evolved over the times from the simplest forms of local treatment and up techno-logically sophisticated forms of industrial manufacturing currently used [4].

Saccocalyx satureioides Coss. &Du., an endemic species of Algeria, is a small aromatic shrub growing in Sahara septentrional and belonging to the Lamiaceae family [5]. This plant is 20–100 cm high. Its flowers can be white, pink or crimson [6]. It has attracted a great attention due to its traditional medicinal usage for gastric disorders and spasms [7]. The aim of the present study is to determine the polyphenolic content of the hydro-methanolic extract from *Saccocalyx satureioides* and to evaluate its antioxidant capacity using DPPH radical scavenging and reducing power assays.

MATHERIALS AND METHODS

Collection of plant and preparation of hydro-methanolic extract

The plant *Saccocalyx satureioides* Cossa nd Dur was harvested in June 2015, from Djelfa, located at an elevation of 3,734 1,138 m in the Ouled Naïl Range of North-Central Algeria. The plant was identified and authenticated by Prof. Laouer H., a botanist at the Department of Biology and Vegetal Ecology, University of Sétif, Algeria.

The collected plant was parched in dimness at room temperature. After drying, plant material was ground to a fine powder using electric grinder.100 grams of the plant powder was mixed with 1000 ml of methanol (85%) at room temperature for 3 days. The resultant suspension was filtered and concentered using evaporation at 45 C°, the extract was stored at 4 C° until use.

Determination of polyphenols and flavonoids content

Total phenolic determined using the Folin-Ciocalteu reagent [8]. A volume of 100 µl of each extract (or Gallic acid) was added to 500 µl of Folin-Ciocalteu reagent (diluted 10 times).After 4 min,400 µl of Na_2CO_3 (7,5%) solution was added. Then the final mixture was shaken and incubated in dark at room temperature for 90 min. The absorbance of all samples was measured at 760 nm. Total phenolics content of the plant extract was estimated using the calibration curve of Gallic acid. The results were expressed as mg of Gallic acid equivalent (GAE) per gram of dried plant extract.

Total flavonoids content was estimated using aluminum chloride assay [9]. 1ml of the extract or standard (Quercetin) was mixed with 1ml of solution (2%) of $AlCl_3$.The mixture was incubated for 10 min in dark at room temperature, and then the absorbance was read at 430nm against the blank. The flavonoid content was expressed as Quercetin equivalent per gram of dry plant extract weight (mg QE/g) using the calibration curve of Quercetin.

Estimation of *in vitro* antioxidant activities

DPPH radical scavenging assay

The DPPH assay was based on the measurement of altering the purple color of DPPH radical to yellow at 517 nm after reaction with antioxidant compound. The effect of antioxidants on DPPH radical was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [10].

The free radical-scavenging activity of the extract was estimated using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) by measuring the decrease of DPPH maximum absorbance at 517 nm [11].

In this test, 50 µl of different concentrations of the plant extract or standard was added to 1250 µl of DPPH (0.004% in methanol). The mixture was incubated at room temperature for 30 min, and then the absorbance was read at 517 nm. Vitamin C was used as standard.

% of Inhibition of free radical DPPH was calculated in the following way:

%inhibition = [(Absorbance of control-Absorbance of sample)/Absorbance of control] ×100

Where A control is the absorbance of the blank solution (containing all reagents except the test compound), and A sample is the absorbance in the presence of the test compound.

Reducing power assay

The reducing power of the Hydro-methanolic extract of the plant was estimated and the BHT was used as standard [12]. A volume of 0.1 ml of different concentrations of the plant extract or BHT was mixed with 0.1phosphate buffer (0.2 M, PH 6. 6) and potassium ferricyanide (1%). The mixture was incubated at 50 Cº for 20 min to reduce ferricyanide into ferrocyanide. After that, 0.25 ml of trichloroacetic acid (1%)was added to stop the reaction and the mixture was centrifuged at 3000 rpm for 10 min. A volume of 0.25 ml of the supernatant was mixed with 0.25 ml of distilled water and 0.5 ml FeCl3 (0.1%), then the absorbance was measured at 700 nm. BHT was used as a standard.

Statistical Analysis

The results were represented as the means ±standard deviation (SD) (n=3). All measurements were conducted in three determinations (n=3). The statistical interpretation was done by the help of Student's t-test for significance with the aid of Graph Pad Prism 7.00. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Total polyphenols and flavonoids content

Total phenolics and flavonoids contents the plant extract were 171.34± 1.43 mg Gallic acid equivalent/g of dry extract (GAE/g) and 18.6 ± 0.46mg Quercetin equivalent / g of dry extract (QE/g), respectively. The results are represented in table 1.

Table 1: Total polyphenols and flavonoids contents of *Saccocalyx satureioides* Coss and Dur Hydro-methanolic extract.

Extract	Total phenolics (mg GAE/g Dw)	Total flavonoids (mg QE/g DW)
HME	171.37 ± 1.43	18.6 ± 0.46

Results were presented as means ± standard deviation (SD), (n=3), HME: Hydro-methanolic extract.

Antioxidant activity estimation

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of the plant extract are presented in table 2. Hydro-methanolic extract showed high scavenging activity against DPPH (IC_{50}= 0.03003 ± 0.0024 mg/ml). This activity remains lower than Vitamin C as positive standard(IC_{50}= 0.002663 ± 0.0002 mg/ml).

Polyphenols possess ideal structural chemistry for free radical scavenging activity, and they have

Antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron [13]. The strong activity of the plant extract to scavenge the radical DPPH could be attributed to its richness in polyphenols (171.34± 1.43 mg Gallic acid equivalent/g of dry extract).

Table2: DPPH radical scavenging activity of *Saccocalyx satureioides* Hydro-methanolic extract.

Extract	HME(mg/ml)	Vit C (mg/ml)
IC_{50}	0.03003 ± 0.0024****	0.002663 ± 0.000209

Data were presented as means ± standard deviation (SD) of IC_{50}, (n=3), HME: Hydro-methanolic extract. **** **p**< 0.0001 compared to Vit C as standard.

Reducing power activity

The ferric reducing assay measures the ability of an antioxidant to reduce a reactive oxygen species. The results are represented in table 3. From these results, the reducing power (the effective concentration at which the absorbance was 0.5) of the plant extract (EC_{50} = 0.5435 ± 0.00625 mg/ml) was promising. These values are comparable to that of BHT as positive standard (0.3276 ± 0.00496 mg/ml).

Table 3: reducing power activity of *Saccocalyx satureioides* Hydro-methanolic extract.

Extract/standard	HME (mg/ml)	BHT (mg/ml)
IC_{50}	0.5435 ± 0.00625****	0.3276 ± 0.00496

Data were presented as means ± standard deviation (SD) of IC_{50}, (n=3), HME: Hydro-methanolic extract. **** p< 0.0001 compared to BHT standard.

CONCLUSION

The results of this study showed that the hydro-methanolic extract from *Saccocalyx satureioides* aerial parts is rich in polyphenols and has strong antioxidant capacity to scavenge free radicals. These results lead to think about the use of medicinal plants and by the correct way, especially in the field of medicine.

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