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Research Article

Total Phenolic Contents, DPPH Radical Scavenging and β-Carotene Bleaching Activities of Ethyl Acetate Extract from Saccocalyx satureioides Coss and Dur

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ABSTRACT

Saccocalyx satureioides Coss and Dur, an endemic species of Algeria, has attracted a great attention due to its traditional medicinal usage for gastric disorders and spasms. The purpose of this study was to estimate the total phenolics and flavonoids content, and the *in vitro* antioxidant capacity of the ethyl acetate extract (EAE) from Saccocalyx satureioides Coss and Dur aerial part. Folin-Ciocalteu's reagent and Aluminum chloride ($AlCl_3$) were used to quantify the total polyphenols and flavonoids content, respectively. However, DPPH (1,1-diphenyl-2-picrylhydrazyl) and β-carotene bleaching method were applied to assess the in vitro antioxidant activity. Total phenolic and flavonoid content in the extract were 317.55 ±1.38 mg gallic acid equivalent/g of dry extract (GAE/g) and 40.38 ± 0.88 mg quercetin equivalent / g of dry extract (QE/g), respectively. The EAE extract has an important capacity to scavenge the free radical DPPH with an IC_{50} of 0.02±0.00048mg/ml. In addition, it was powerfully able to inhibit the lipid peroxidation with a percentage of 71.59± 0.53%. The results of the present study may prove that the medicinal plants are a good resource of natural antioxidants.

INTRODUCTION

Oxidative stress was defined as the lack of balance between the occurrence of reactive oxygen/nitrogen species (ROS/RNS) and the organism's capacity to counteract their action by the antioxidative protection systems [1]. Free radicals are molecules produced when our body breaks down food. They can also be produced by environmental exposures like tobacco smoke and radiation. Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases [2].

Antioxidants that can neutralize free radicals may be used to protect the human body from diseases and retard lipid rancidity in foods. Many synthetic antioxidant components, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have shown toxic and/or mutagenic effects, which have shifted the attention to naturally occurring antioxidants [3].

For many years, herbal medicines have been used and are still used in developing countries as the primary source of medical treatment. Plants have been used in medicine for their natural antiseptic properties. Thus, research has developed into investigating the potential properties and uses of terrestrial plant extracts for the preparation of potential nanomaterial based drugs for diseases including cancer [4]. Plant secondary metabolites such as polyphenols, play an important role in the defense against free radicals. Medicinal plant parts (roots, leaves, stems, flowers and fruits) are commonly rich in phenolic compounds, such as flavonoids, tannins, stilbenes, coumarins and lignans. The antioxidant properties of polyphenols are due to their redox properties, which allow them to act as reducing agents,

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hydrogen donators, metal chelators and single oxygen quenchers [5].

Saccocalyx satureioides Coss et Durieu is a small shrub belonging to the Lamiaceae family, locally its popular name is zaâter, a common appellative for oregano and thyme in all North African regions [6]. In folk medicine, the aerial parts are commonly used in decoction for treatment of gastric disorders and spasms [7]. The aim of this study is to quantify the polyphenolic and flavonoids content of the ethyl acetate extract from Saccocalyx satureioides coss and dur aerial part and evaluate *in vitro* its antioxidant capacity using DPPH radical scavengingand β-carotene bleaching assays.

MATHERIALS AND METHODES

Plant Material

The plant Saccocalyx Satureioides Coss and Dur was harvested in June 2015, from Djelfa, located at an elevation of 3,734 feet 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 Setif, Algeria.

Preparation of the Ethyl Acetate Extract

The collected plant was parched in dimness at room temperature. After drying, plant material was ground to a fine powder using electric grinder. 100 g rams of the plant powder was mixed with 1000 ml of methanol (85%) for 3 days. After filtration and concentration, the resulting suspension was splitted by successive washing with different solvents of increasing polarity (hexane, chloroform and ethyl acetate). All ethyl acetate fractions (EAE) were combined and concentrated in vacuum and further used for antioxidant screening as previously described by [8].

Polyphenols Content Determination

Total phenolics were determined using the Folin-Ciocalteure agent [9]. 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 phenolic content of the extracts 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.

Flavonoids Content Determination

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

Estimation of *In Vitro* Antioxidant Activities

DPPH Radical Scavenging Assay

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]. The free radical scavenging activity was measured as previously described by [12]. Briefly, 50 µl of different concentrations of sample or standard was mixed with 1250 µl of DPPH (0.004%). 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:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

β-Carotene Linoleic Acid Bleaching Activity

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated dienehydroperoxides arising from linoleic acid oxidation [13].

A stock solution of *β*-carotene-linoleic acid mixture was prepared as follows:
0.5 mg β-carotene was dissolved in 1 ml of chloroform and then 25 µl linoleic acid and 200 mg Tween 40 were added. The chloroform was completely evaporated using a rota vapor, and then 100ml of oxygenated distilled water was added (Bubbled with oxygen for 30 min with a flow rate of 100ml/min). A volume of 2.5 ml of this emulsion was added to 350 µl of the EAE or to the standard synthetic antioxidant BHT, the blank was prepared with distilled water or solvent. The absorbance was recorded after 0, 1, 2, 4, 6 and 24 hours at 490 nm.

Antioxidant activity was expressed as the percentage of inhibition of the extract, and was measured as follows:

$$AA\% = \frac{\text{ABS test}}{\text{ABS BHT}} \times 100$$

AA%: Percentage of the antioxidant activity.
ABS test: Absorbance in the presence of the test (extract).
ABS BHT: Absorbance in the presence of positive control (BHT).

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

Total Polyphenols and Flavonoids Content

The results (Table 1) showed that the *Saccocalyx satureioides* ethyl acetate extract (EAE) was rich in polyphenols and flavonoids 317.55±1.38mg gallic acid equivalent/g of dry extract (GAE/g) and 40.38±0.88mg quercetin equivalent / g of dry extract (QE/g), respectively.

Table 1: Total polyphenols and flavonoids contents of *Saccocalyx satureioides* ethyl acetate extract

Extract	Total phenolics (mg GAE/g Dw)	Total flavonoids (mg QE/g DW)
EAE	317.55 ±1.38	40.38 ± 0.88

Results were presented as means ±standard deviation (SD), (n=3), EAE: ethylacetate extract.

In Vitro Antioxidant Activities

DPPH Radical Scavenging Activity

The results of DPPH radical scavenging ability of the EAE extract (Table 2) showed that the plant extract exhibited a strong and significant antioxidant activity on DPPH radical with an IC_{50} value of 0.02±0.00048mg/ml.

Table 2: DPPH radical scavenging of *Saccocalyx satureioides* Coss and Dur ethyl acetate extract.

Extract/standard	EAE (mg/ml)	Vit c (mg/ml)
IC_{50}	0.02±0.00048****	0.002 ± 0.0002

EAE: ethyl acetate extract, Vit C: vitamin C, Data were presented as means ± standard deviation (SD) of IC_{50}, (n=3), **** p< 0.0001 compared to Vit C as standard.

β-Carotene / Linoleic Acid Bleaching Assay

As seen in the Table 3, ethyl acetate extract of the plant displayed high inhibition percentage of 71.59±0.53% compared to BHT (92.22±0.76) as drug reference. This suggests a significant antioxidant activity of *Saccocalyx satureioides* ethyl acetate extract.

Table 3: Antioxidant activity of *Saccocalyx satureioides* ethyl acetate extract using β-carotene /linoleic acid bleaching assay

Extract/standard	EAE	BHT
AA %	71.59± 0.53****	92.22± 0.76

EAE: ethyl acetate extract, BHT: butylatedhydroxytoluene, Data were presented as means ± standard deviation (SD) of IC_{50}, (n=3), **** p< 0.0001 compared to BHT as standard.

DISCUSSION

The results of our study showed that the *Saccocalyx satureioides* ethyl acetate extract (EAE) was rich and a good source of polyphenol and flavonoid compounds. The present results are in accordance with those of [14]. The DPPH-scavenging radicals and ß- Carotene / linoleic acid bleaching assay estimated the antioxidant ability of EAE. The effect of antioxidants on DPPH radical scavenging was conceived to be due to their proton-donating ability. In DPPH test, the antioxidants were able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine [8]. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present) [15]. The plant extract of the present study showed high scavenging activity against DPPH (IC_{50}= 0.02±0.00048mg/ml). These results are in line with those of [16] with an IC_{50} of 20.81±3.52µg/ml). This extract (EAE) also presented a good scavenging capacity against DPPH comparing to the hydro-methanolic extract (HME) (0.03003±0.0024) mentioned previously [17].

β-Carotene bleaching essay was used to test the ability of the plant extract to inhibit lipid peroxidation. β-carotene in the absence of the antioxidant undergoes a rapid decolorization since the free linoleic acid radical attacks the β-carotene, which loses the double bonds and, consequently, its orange color. The presence of a phenolic antioxidant can hinder the extent of β-carotene destruction by "neutralizing" the linoleate free radical (utilizing its redox

potential) and any other free radicals formed within the system [18]. EAE inhibited the oxidation of ß- carotene in important level compared to the standard positive BHT. These results could be attributed to the richness of the EAE in polyphenols and flavonoids. In fact, several studies evaluated the relationships between antioxidant activity of plant products and their phenolic content. Some authors found a correlation between the phenolic content and the antioxidant activity [19].

CONCLUSION

The ethyl acetate extract of *Saccocalyx satureioides* aerial parts is rich in polyphenols. The plant extract has significant antioxidant activity to scavenge free radicals. The results may explain the medicinal use of this plant in folk medicine. However, more research needed to identify the active principals that are responsible for the antioxidant activities.

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