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Research Article

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Antioxidant activity and determination of total phenolic compounds content of *Euphorbia regis-jubae (webb and berth)* from methanol and aqueous extracts

Fatima Azzahra LAHLOU*, Fouzia HMIMID, Mohammed LOUTFI and Noureddine BOURHIM
Department of biology, laboratory of Biochemistry and Molecular Biology Faculty of Sciences, University Hassan II-Casablanca, km 8 route d'El Jadida BP. 5366 Casablanca, Morocco
*Corresponding Author E-mail: lahloufz@hotmail.fr

ABSTRACT

The increasing interest in the biological activity of plant phenolics and flavonoids, outlined the necessity of determining their content. In this study, methanol and water were used for taking out the phenols from Euphorbia regis-jubae (webb and berth), an endemic Moroccan medicinal herb, also widely used as food for camels and goats. Moreover the effect of temperature with both solvents was investigated. Therefore the total polyphenols, flavonoids and proanthocyanidins content were determined. The antioxidative activity of crude methanolic and ethanolic extracts was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) assays. The results showed that this plant possesses a great potential of free radical scavenging activity; that has a linear relationship with an important amount of active compounds. It also revealed that the extraction was better when methanol and water were coupled with temperature.

Keywords: *polyphenols, flavonoids, proanthocyanidins, antioxidant, Euphorbia regis-jubae.*

INTRODUCTION

Plants are ancient source of bioactive compounds used as a source of remedies; that could be used to treat various ailments. The most important of these bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds.

It is reported that antioxidant effect of a plant is mainly due to phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes[15]. Antioxidant is defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate[6].

Polyphenols in vegetables, fruits, and teas can prevent degenerative diseases including cancers through antioxidative action. For example, in animal experiments, an oral dose of polyphenols suppressed the carcinogenesis of several carcinogens[20,21,22].

Flavonoids are a group of polyphenolic compounds widely distributed in the plant kingdom. Their structure, consisting of two hydroxy substituted aromatic rings joined by a three carbon link renders them hydrogen and electron donors. Structural variations within the rings divide the flavonoids into several families: flavonols, flavones, flavanones, flavanols and isoflavones. Flavonoids possess free radical-scavenging abilities; they have suppressive effects against cytotoxicity caused by those active oxygen species[12]. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups[18].

Proanthocyanidins are oligomers or polymers of polyhydroxy flavan-3-ol units, it is reported that anthocyanins and proanthocyanidins from blueberry may be active in protecting the integrity of the capillaries in rats exposed to oxygen toxicity[4].

Because very little is known about the antioxidant and phenolic content of *Euphorbia regis-jubae (webb and berth)* belonging to the *Euphorbiaceae* family. So the aim of this study is to determine the amount of phenolic compounds as well as the effect of solvents (methanol and water) and temperature on this content. Two methods namely FRAP and DPPH radical scavenging methods were used to find and correlate the antioxidant activity of the extracts.

MATERIEL AND METHODS

Plant material

The whole plant of *Euphorbia regis-jubae (webb and berth)* was collected from Sidi Ifni, Southern Anti-Atlas of Morocco and was authenticated by Prof. Laila RHAZI of the Department of Biology, Faculty of Sciences, University of Hassan II.

To avoid any contamination or dust, the plant's aerial parts were cleaned and spread to dry at room temperature in a clean room.

Preparation of plant extracts

Soxhlet extraction

Powdered sample of *Euphorbia regis-jubae (webb and berth)* was extracted with methanol using soxhlet system. Extraction was carried out for 16 h at 80°C. The extract was filtered then concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40°C.

Decoction

Powdered sample was mixed with distilled water in a round-bottom flask, linked to a column connected to a refrigerant. Then it was placed at 60°C for 1 hour. The decoction extract was filtered using gauze and Whatman No. 1 filter paper and concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40ºC.

Maceration

Plant material was allowed to stand for 24 hours under shaking at room temperature in methanol and water. After filtration and concentration as described above, methanol and aqueous filtrates were obtained.

Total flavonoid content

Total flavonoid content was determined using the method adapted by Arvouet-Grand *et al.*,[1]. 1 ml of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution. After 10 minutes, Absorption readings at 415 nm against a blank sample consisting of a 1 ml extract solution with 1 ml methanol or distilled water without $AlCl_3$ were assessed. The total flavonoid content was determined using a standard curve with quercetin or rutine (0 - 80 µg/ml), then expressed as mg of quercetin or rutin equivalents (QE or RE) / g of extract.

Total phenolic content

Total phenolics of various samples were determined by the method of Makkar *et al.*,[9]. : 0.1 ml of sample was combined with 2.8 ml of 10% Na_2CO_3 and 0.1 ml of 2N Folin-ciocalteu reagent. After 40 min absorbance at 725 nm was measured by UV-visible spectrophotometer (Thermo electron corporation, Biomate 3). Total phenolics were determined as milligrams of gallic acid and tannic acid equivalents per gram of sample (GAE mg/gE or TAE/gE) using a standard calibration curve between (0 to 100 µg/ml).

Reducing Power

The ability of the extracts to reduce Fe^{3+} was assayed by the method of Oyaizu[13]. Briefly, 1 ml of each extract were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% $K_3Fe(CN)_6$. After incubation at 50°C for 25 mn, 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 g for 10 min. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% aqueous $FeCl_3$. The absorbance was measured at 700 nm. The mean of absorbance values were plotted against concentration and a linear regression analysis was carried out. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid and trolox were used as positive control.

Proanthocyanidin Content

Proanthocyanidin content was estimated according to the procedure reported by Sun et *al*.,[18]. A volume of 1 ml solution was mixed with 3 ml of 4% vanillin/methanol solution and 1.5 ml hydrochloric acid and the mixture was allowed to stand for 15 min at room temperature. The absorbance at 500 nm was measured and the Proanthocyanidin content was expressed as mg catechin equivalents (mg CE/1g dry mass) using a catechin (0-80µg/ml) standard curve.

DPPH Radical Scavenging Activity

The free radical scavenging activities of the samples on the DPPH radical were measured using the method described by Brand-Williams *et al.,*[2]. A 0.1 ml of various concentrations of each extracts at different concentrations was added to 3.9 ml of DPPH solution (25 mg/l in methanolic solution). After the mixture was shaken and left at room temperature for 30 min, the absorbance at 517 nm was measured. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated from the results and used for comparison. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_1 - A_2)/A_1] \times 100$$

Where:

- A_1 = the absorbance of the control reaction,
- A_2 = the absorbance in the presence of the sample.

Ascorbic acid and Trolox were used as standards.

RESULTS AND DISCUSSION

Aqueous and methanol extracts were prepared to examine the total phenolic, flavonoid, proanthocyanidin conctent and antioxidant activity.

According to table 1, the total flavonoid content in the examined extracts ranged from 14.31±0.24 to 49.57±1.23 mg RE/g E and from 9.64±0.11to 33.40±0.83 mg QE/g E. the highest concentration was measured in methanolic (soxhlet), followed by decoction.

Table 1: Total flavonoid content of *Euphorbia regis-jubae (webb and berth)*

Extracts	MS	MM	D	AM
Total flavonoid content (mg QE/g E)	33.40±0.83	9.64±0.11	24.04±1.07	22.41±0.28
Total flavonoid content (mg RE/g E)	49.57±1.23	14.31±0.24	35.68±0.72	33.26±0.35

EQ: quercetin equivalents, RE: rutine equivalents, g E: g of extract, MS: methanolic hot Continuous Extraction (Soxhlet).
MM: methanol maceration, D: decoction, AM: aqueous maceration.

In table 2, total polyphenols content was high in decoction (37.12 ± 1.02 mg GAE/ gE /35.14±0.95 mg ATE/gE) and aqueous maceration (28.82±0.21 mg GAE/ gE/27.28±0.38 mg ATE/gE).

Table 2: Total phenolic content of *Euphorbia regis-jubae (webb and berth)*

Extracts/test	Total phenolic content	
	mg GAE/ gE	mg ATE/gE
MS	23.07±0.73	21.84±0.67
MM	22.15 ±0.26	20.97±0.18
D	37.12 ± 1.02	35.14±0.95
AM	28.82±0.21	27.28±0.38

EAG: gallic acid equivalents, ATE: tannic acid equivalents, gE: g of extract,
MS: methanolic Hot Continuous Extraction (Soxhlet). MM: methanol maceration, D: decoction, AM: aqueous maceration.

It was observed from table 3 that higher content of proanthocyanidin was gained with methanol (soxhlet) (8.19 ± 0.1 mgCE/gE) followed by decoction (4.33 ± 0,085 mgCE/gE). While maceration in methanol and water indicated lower concentrations.

Table 3: Total proanthocyanidin of *Euphorbia regis-jubae (webb and berth)*

Extracts	MS	MM	D	AM
Proanthocyanidin (mgCE/gE)	8.19 ± 0.1	3.42 ± 0.12	4.33 ± 0,085	2.76 ± 0.04

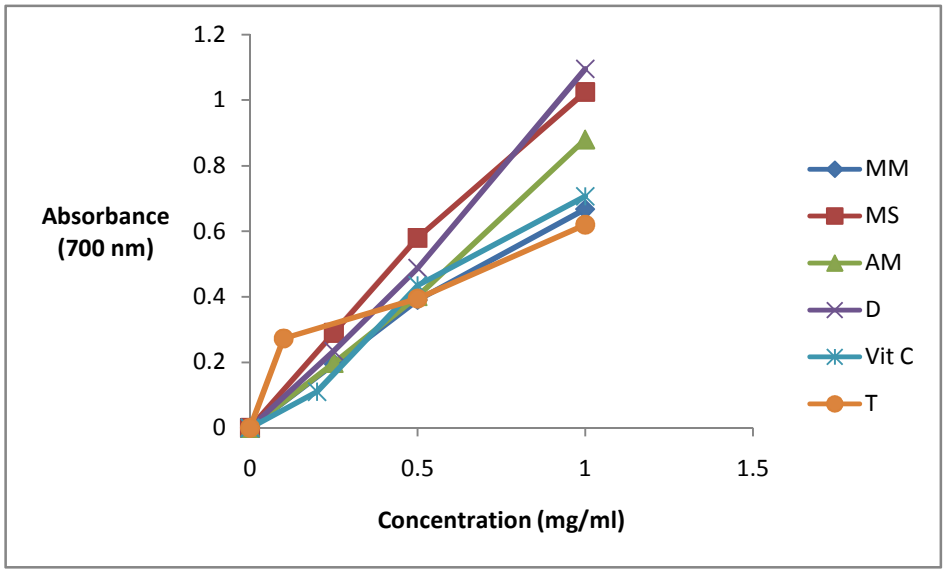
CE: cathechins equivalents, gE: g of extract, MS: methanolic Hot Continuous Extraction (Soxhlet).
MM: methanol maceration, D: decoction, AM: aqueous maceration.

These results also showed that *Euphorbia regis-jubae (webb and berth)* possess an important amount of flavonoids, followed by polyphenols then condensed tannins.

Extraction procedures play a decisive role for the qualitative and quantitative composition of the extracts. Hence, it is reported that the concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation[10]. This study revealed that methanol and water when combined to heat were more effective for phenolic compounds extraction. Factors that have been attributed to bringing variation include the method of extraction[12], mixture of different solvents[8] and use of different materials[7] among others.

The FRAP assay treats the antioxidants contained in the samples as reductants in a redox-linked colorimetric reaction and the value reflects the reducing power of the antioxidants. Thus, it has been used frequently in the assessment of antioxidant activity of various fruits and vegetables and some biological samples, though we understand that it has some limitations[4,6,15]. The reducing power of methanol (soxhlet) and decoction was more important than trolox and vitamin C (figure 1) indicating that *Euphorbia regis-jubae (webb and berth)* extracts have a great potential antioxidant activity.

Fig.1: Reducing power of different extracts of of *Euphorbia regis-jubae (webb and berth)*



MS: methanolic Hot Continuous Extraction (Soxhlet). MM: methanol maceration, D: decoction, AM: aqueous maceration, T: trolox, Vit C: Vitamin C.

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. It is the oldest indirect method for determining the antioxidant activity which is based on the ability of the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols[16]. Table 4 illustrates IC50 values; the concentration of antioxidant needed by 50% the initial concentration of DPPH radicals. A low IC50 value indicates strong antioxidant activity in a sample. The DPPH radical scavenging activity was found to be in the order: D> MS> Vit C> Trolox> AM> MM. this activity may be due to the presence of polyphenols, flavonoids and condensed tannins determined in *Euphorbia regis-jubae (webb and berth)*. Moreover the IC50 values obtained allowed us to categorize the antioxidant sources and the present results showed that methanolic and aqueous extracts when combined to heat are preferable sources for extracting antioxidant compounds as they showed high IC50 values.

Table 4: Antioxidant activity of *Euphorbia regis-jubae (webb and berth) using DPPH reagent*

Extracts and standards	MS	MM	D	AM	Trolox	Vitamin C
IC50 (mg/ml)	3.44	15.7	2.63	13.9	5	3.6

MS: methanolic Hot Continuous Extraction (Soxhlet). MM: methanol maceration, D: decoction, AM: aqueous maceration.

In conclusion, the results of this study showed that the highest antioxidant activity, total phenolic content of *Euphorbia regis-jubae (webb and berth)* were exhibited by methanol and aqueous extracts when they are combined to heat. From this sight, it can be concluded that inclusion of this plant in normal balanced diet could alleviate number of oxidative stress damages. Further studies of this plant species should be directed to carry out *in vivo* studies of its active components if they are to be used for medicinal purpose.

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