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Research Article

ANTIOXIDANT ACTIVITY AND QUANTIFICATION OF PHENOLIC COMPOUNDS OF EUPHORBIA ECHINUS

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ABSTRACT

Objective: Plant phenolic metabolites are gaining interest due to their potential to improve human health. One of the most important properties of these secondary metabolites is their antioxidant capacity. In this present study, we aimed to evaluate total phenolics, flavonoids, proanthocyanidins content and antioxidant activity of *Euphorbia echinus*. Moreover the effect of solvent (methanol and water) and temperature was investigated.

Methods: *Euphorbia echinus* was used as a plant material which was subjected to a decoction, maceration (with both water and methanol) and soxhlet extraction. Ferric reducing/antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging tests were investigated to evaluate antioxidant activity. Folin-Ciocalteu reagent serves to determine total phenolic content, aluminium trichloride method for flavonoids and a mixture of vanillin and hydrochloric acid for proanthocyanidins; employing colorimetric method.

Results: This study revealed that methanol when combined with temperature was the best solvent to take out polyphenols (21.53±1.03 mgGAE/gE) and proanthocyanidins (13.04±0.68 mgCE/gE), followed by decoction which was more efficient for flavonoids (14.31±1.02 mgRE/gE). Therefore *Euphorbia echinus* seems to exhibit a high radical scavenging activity; showing a low IC_{50} which is approximately similar to IC_{50} of vitamin C and trolox. In addition *Euphorbia echinus* methanolic extract showed a reducing power greater than standards. These capacities have a linear relationship with the larger quantity of antioxidant compounds.

Conclusion: The results of the present study suggest that *Euphorbia echinus* contained potential antioxidant bioactive compounds that could serve as therapeutic agent in preventing diseases caused due to oxidative stress.

Keywords: *Euphorbia echinus*, Antioxidant activity, Polyphenols, Flavonoids, Proanthocyanidins, DPPH, FRAP.

INTRODUCTION

Euphorbia echinus belongs to the family *Euphorbiaceae*. It comprises 280 genera with the largest genus *Euphorbia* having about 1600 species. They have characteristic milky latex [1]. *Euphorbia echinus* is alternatively known by its Moroccan name as "Daghmous". *This plant* is widely used by the local population to dissolve cysts. Moreover it has been used as traditional Moroccan medicines to treat various diseases. It might possess laxative, anti-inflammatory, hypoglycemic as well as anti-tumor activity.

Plant secondary metabolites are phytochemicals with antioxidant properties, which play a very important role in the body defense system against reactive oxygen species (ROS), which are associated with the development of many chronic and degenerative diseases [2-3]. In fact several polycyclic diterpenoids have been isolated from the genus *Euphorbia* [4]. In addition phenolic compounds were isolated as Methyl gallate from *Euphorbia teheranica* [5-6], also flavonoid glycosides and hydrolysable tannins were identified in *Euphorbia aucherii* [7].

Many polyphenolic compounds have been found to have a much stronger antioxidant activity than vitamins C, E and β-carotene within the same food [8].

Phenolics are characterized by at least one aromatic ring possessing one or more hydroxyl groups. They are divided into several groups: anthocyanins, flavonoids, catechins, phenolic acids, stilbenes, coumarins and isoflavones. Most of them arise from a common origin: the amino acids phenylalanine or tyrosine. After deamination of these amino acids they enter the phenylpropanoid pathway.

Flavonoids are one of the most diverse and widespread group of natural compounds, their basic structure is derived from a diphenylpropane system C6-C3-C6. They can be divided into six subclasses: flavanols, flavones, flavanones, flavonols, isoflavones, and anthocyanidins. The anti-oxidant activity of these compounds is due to the presence of these phenolic hydroxyl groups, which create their electron-donating property against free radicals [9].

Proanthocyanidins or condensed tannins are phenolic compounds that are highly bioavailable and provide a significantly greater protection against damage from oxidative stress than vitamin C, vitamin E, and β-carotene [10]. The beneficial effects of proanthocyanidins on human health have been attributed mainly to their strong free radical-scavenging and antioxidant activities [11].

The aim of this study is to quantify total phenolics, flavonoids and proanthocyanidins content. Therefore DPPH (2, 2-dipheny-l-picrylhydrazyl) and FRAP (ferric reducing antioxidant capacity) tests were carried out to evaluate the antioxidant capacity of *Euphorbia echinus*. Moreover it investigated the influence of temperature using 2 extracting solvents: methanol and water; in order to evaluate their efficacy to extract phytochemicals.

MATERIELS AND METHODS

Plant material

The whole plant of *Euphorbia echinus* was collected from Sidi Ifni, Southern
Anti-Atlas of Morocco and was authenticated by Prof. Leila EL GHAZI of the Department of Biology, Faculty of Sciences, University of Hassan II, Casablanca.

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 echinus* 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 then concentrated by Rotavapor-R20 (Heidolph Bioblock Scientific) at 40ºC.

Maceration

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

Total phenolic content

Total phenolics of various samples were determined by the Folin-Ciocalteu method [12].

0.1 ml of sample was combined with 2.8 ml of Na_2CO_3 (10%) 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).

Total flavonoid content

Total flavonoid content was determined using aluminium trichloride method [13] .1 ml of aluminium trichloride ($AlCl_3$ 2%) 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 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.

Proanthocyanidin Content

Pro anthocyanidin content was estimated according to the procedure reported by [14]. A volume of 1 ml solution was mixed with 3 ml of 4% vanillin/methanol solution and 1.5 ml hydrochloric acid then the mixture was allowed to stand for 15 min at room temperature. The absorbance at 500 nm was measured and the Pro anthocyanidins was expressed as mg catechin equivalents (mgCE/g dry mass) using a catechin standard curve (0-80µg/ml).

Reducing Power

The ability of the extracts to reduce Fe^{3+} was assayed by the method of [15]. 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.

DPPH Radical Scavenging Activity

The free radical scavenging activities of the samples on the DPPH radical were measured using the method described by [16]. 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:

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

The amount of total phenolics in *Euphorbia echinus* ranged from 21.53±1.03 mgGAE/gE or 20.38±0.76 mgATE/gE to 2.46±0.11 mgGAE/gE or 2.33±0.09 mgATE/gE (table 1). Total polyphenol content, as determined by the Folin-Ciocalteu method, was higher in methanolic extract when it's combined with temperature (80°c), followed by the decoction (60°C).

While extraction at room temperature with both methanol and water offered the lowest content. Plant phenolics are the widest spread secondary metabolite in plant kingdom; they are known as powerful chain breaking antioxidants and act as free radical scavengers [17]

Table 1: Total phenolic content in various extracts of *Euphorbia echinus*

Extracts/test	Total phenolic content	
	mgGAE/ gE	**mgATE/gE**
MS	21.53±1.03	20.38±0.76
MM	6.92 ±0.26	6.55±0.45
D	14.56 ±0.95	13.78±0.71
AM	2.46±0.11	2.33±0.09

Note: 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.

Table 2: Total flavonoid content in various extracts of *Euphorbia echinus*

Extracts/test	Total flavonoid content	
	mgQE/gE	m RE/gE
MS	8.29±0.37	12.31±0.47
MM	4.1 ±0.26	6.1±0.13
D	9.64 ± 0,89	14.31±1.02
AM	6.73±0.12	10 ±0.33

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

As it's resumed in table 2, higher content of flavonoid was achived with, decoction: 9.64±0.89 mgQE/gE or 14.31±1.02 mgRE/gE, followed by methanol (soxhlet): 8.29±0.37 mgQE/gE or 12.31±0.47 mgRE/gE. However maceration with methanol and water showed the lowest concentrations.

Flavonoids serve as health promoting compound as a results of its presence as anion radicals [18]. It has been acknowledged that flavonoids show antioxidant action on human health trough scavenging or chelating process [19, 20]. According to table 3, methanolic extract (soxhlet) showed the highest total proanthocyanins content (13.04±0.68 mgCE/gE) prepared from

euphorbia echinus. Decotion and methanolic maceration showed approximately the same concentration (≈ 11mgCE/gE). Therefore the aqueous maceration revealed the lowest amount (8.23±0.41 mgCE/gE).

Pro anthocyanidins are a type of bioflavonoid that has been shown to have very potent antioxidant activity. In fact several studies have reported on the antioxidant and antiradical activity of these ubiquitous compounds [21, 22].

Table 3: Total proanthocyanidin in various extracts of *Euphorbia echinus*

Extracts	MS	MM	D	AM
Proanthocyanidin (mgCE/gE)	13.04 ±0.68	10.57± 0.31	11.71±0.85	8.23 ± 0.41

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

A comparison of *Euphorbia echinus* to three *Euphorbia* species phytochemicals content, leads to the fact that *Euphorbia echinus* possesses a higher amount of antioxidant compounds.

For instance total flavnoids content of *Euphorbia helioscopia* in methanolic extract are respectively *1.98±0.004 and 5.32*±0.002 mgQE/g of dry weight in stem and leaves [23]. *Euphorbia tirucalli* showed 11.9 ± 1.2 µgGAE of dry weight [24]. Determination of proanthocyanidin content in *Euphorbia neriifolia* evaluated a concentration of 3.96±0.06 mg ER/gE [25]. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Increasing absorbance indicates an increase in reductive ability. Figure 1 shows that there was increase in reducing power of different plant extracts as the extract concentration increases. Moreover MS showed a higher reductive capacity than standards: trolox and Vitamin C at the same concentration (1 mg/ml). Decoction antioxidant capacity was similar to Trolox. The reducing capacity of an extract is an indicator of antioxidant activity; the results found supposed that *Euphobia echinus* is an important source of antioxidants.

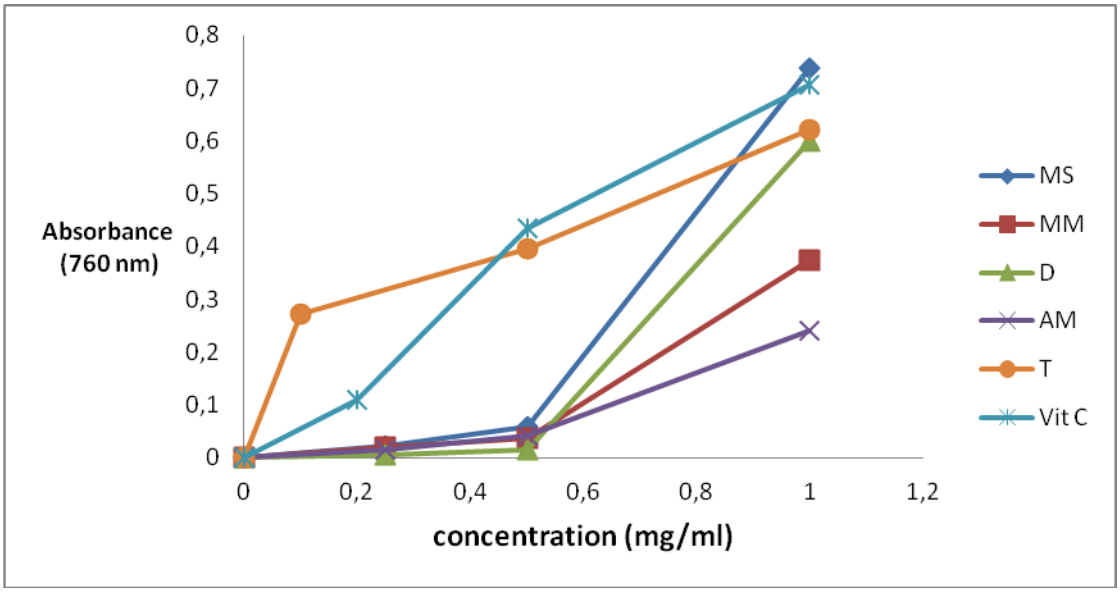

Fig. 1: Reducing power in different extracts of of *Euphorbia echinus* MS= methanolic Hot Continuous Extraction (Soxhlet). MM= methanol maceration, D= decoction, AM= aqueous maceration, T= trolox, Vit C= Vitamin C.

Free radical scavenging effects results were defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% in 30 minutes (IC_{50}). Lowest IC_{50} values indicated that the highest antioxidant capacity. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (DPPH solution, initially purple in color, changed to yellow). DPPH method allows estimation of hydrogen radical donating ability of the extract [26]. This model represents the situation in metabolic system where an antioxidant will stabilise a free radical by reacting with the hydrogen radical. After 30 min of incubation, all extracts showed an increasing antioxidant activity while the concentrations of tested extracts increased. Nevertheless MS and decoction showed the lowest IC_{50} compared to methanol and aqueous maceration (table 4).

Table 4: IC_{50} of DPPH scavenging capacities in various extracts of *Euphorbia echinus*

Extracts and standards	MS	MM	D	AM	Trolox	Vitamin C
IC_{50} (mg/ml)	4.57	21.9	5,9	28.6	5	3.6

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

The present study showed that *Euphorbia echinus* have exerted a high antioxidant power with both assays (FRAP and DPPH); which might be correlated to the high amounts of total phenolics, flavonoids and proanthocyanidins found in this plant. Since phenolics and flavonoids compounds have been recognized as one of the most important groups of secondary metabolites with significant antioxidant and chelating properties [27, 28]. It also revealed that both methanol and water when coupled to temperature are efficient solvents for extracting antioxidants.

CONCLUSION

In conclusion, this study suggested that *Euphorbia echinus* could be a potential source of natural antioxidants, thus theses phytochemicals couldd serve as therapeutic agents or prevention of the progress of ageing associated with oxidative stress, degenerative diseases such as cancer and various other human ailments.

It is interesting to note that the temperature has an important impact on extracting antioxidants. However, further investigations regarding more biological activities of the plant need to be conducted. An elucidation of antioxidant activity in vivo must be investigated, in addition to an identification of these phytochemicals.

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