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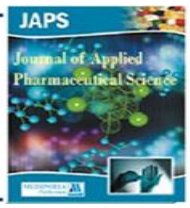

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Preliminary phytochemical screening and antioxidant activities of solvent extracts from *Daucus crinitus* Desf., from Algeria

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ABSTRACT

The presence of natural antioxidant in plants is well known. Plant phenolics constitute one of the major groups of components that act as primary antioxidant free radical terminators. This paper reports the antioxidative activity of methanolic and water extract of *Daucus crinitus* Desf. Phytochemical screening of the crude extracts of stems/leaves revealed the presence of different kind of chemical groups such as tannin, flavonoids, phenolic acids and coumarins. The amounts of total phenolics and flavonoids in the solvent extracts (methanol and water extract) were determined spectrometrically. From the analyses, methanolic extract had the highest total phenolic content (130.19 µg GA/mg extract) and antioxidant activity (89.82 %) using DPPH method. Increasing the concentration of the extracts resulted in increased ferric reducing antioxidant power for both extracts tested. Finally, a relationship was observed between the antioxidant activity potential and total phenolic and flavonoid levels of the extract.

Keywords: *Daucus crinitus* Desf., Solvent extract, Phytochemical screening, Antioxidant activity, DPPH, Reducing power.

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INTRODUCTION

In the last years, interest in medicinal plants as an alternative to synthetic drugs is more and more increasing, particularly against oxidative stress. Phenolic compounds are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step (Agraval, 1989). This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups (Havsteen, 2002). Polyphenolic compounds are also known for their ability to prevent fatty acids from oxidative decay (Fecka *et al*., 2007). The oxidation is caused by the rancidity of unpreserved aliments rich in unsaturated fatty acids (Li *et al*., 2008). Furthermore, many synthetic antioxidant components (BHA and BHT) have shown toxic and/or mutagenic effects; therefore, plant antioxidants are suggested as an interesting alternative. Numerous studies exhibited a strong relationship between total phenolic content and antioxidant activity in fruits, vegetables, and medicinal plants (Dorman *et al*., 2003; Velioglu *et al*., 1998). Flavonoid constituents possess a wide spectrum of chemical and biological activities, including radical scavenging properties (Shimoi *et al*., 1996). Indeed, Shimoi *et al* (1996) reported that plant flavonoids that show antioxidant activity in vitro also function as antioxidants in vivo. Malkowski (2006) showed the role of these compounds in the defense mechanism against oxidative stress from oxidizing agents and free radicals.

Several studies had been conducted to evaluate the correlation between phenolic compounds and antioxidant activity (Yen and Hsieh, 1998; Gülçin *et al.*, 2003). The antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, which are known to be potent as antioxidants (Wang *et al*., 1999). *Daucus crinitus* desf. is characterized by the presence of many subspecies that colonize the sands and cliffs (Quezel and Santa, 1963). In Algeria, this species is currently used in folk medicine as a tonic and against the coldness. A survey conducted by herbalists identified that, in folk medicine, a drink made from the roots of *D. crinitus* is used in decoction to expel the placenta after childbirth. However, all reports do not make reference to the antioxidative properties of *D. crinitus*. The aim of this work is to evaluate the antioxidative properties of the extracts of *D. crinitus.* Additionally, the total phenolic and flavonoid contents of methanolic and water extracts have been determined.

MATERIALS AND METHODS

Plant Material

The aerial parts (stems/leaves) from *D. crinitus* were collected on November 2010, in Bensekrane [260 m, 35°04'N 1°13'O] forests near Tlemcen, Algeria.

Preparation of the extracts

The air-dried sample (leaves/stems) (20 g) was extracted by using a Soxhlet extractor for 5 h, containing methanol under reflux conditions (250 mL). The residue was then extracted by boiling water (300 mL). Solvent was removed with a rotary evaporator to obtain the extract in the yield of 5.3% (w/w). The water extract was dried in a freeze-drier to obtain the extract in a yield of 9.8% (w/w).

Phytochemical prospecting.

The phytochemical tests to detect the presence of heterosides, saponins, tannins, flavonoids, steroids, triterpenes, coumarins, quinones, organic acids and alkaloids were performed according to the method described by Matos (1997). The tests were based on the visual observation of a change in color or formation of precipitate after the addition of specific reagents.

Determination of total phenolic contents

The concentration of phenolics in plant extracts was determined using spectrophotometric method (Slinkard and Singleton, 1977). Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanolic solution of extract, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL 7.5% $NaHCO_3$. Blank was concomitantly prepared**,** containing 0.5 mL methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45 °C for 45 min. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (µg GA/mg extract).

Determination of total flavonoids contents

Total flavonoid contents were determined using the Dowd method as adapted by Quettier *et al*., 2000. One milliliter of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of extracts (200 µg). The absorption at 430 nm was measured after 10 min against a blank sample consisting of 1 mL methanol without $AlCl_3$. The concentrations of flavonoid compounds expressed as µg quercetin equivalent per mg of extract were calculated according to the standard quercetin graph. All experiments were carried out in triplicate, and quercetin equivalent values were reported as X ± SD of triplicates.

ANTIOXIDANT ACTIVITY

Radical scavenging activity

The free radical-scavenging activities of essential oil and solvent extracts were measured using DPPH as described by Hatano *et al.*, 1988. Used as reagent, DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) obviously offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants. Fifty microliters of various concentrations of the solvents extracts were added to 5 mL of a 0.005% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition of the free radical, DPPH, in percent (I %) was calculated according to the formula:

$$\mathbf{I\ (\%) = 100.\ (A_0 - A_s)/A_0}$$

where A_0 is the absorbance of the control (containing all reagents except the test compound), and As is the absorbance of the tested sample. The actual decrease in absorbance induced by the tested sample (change of color from deep-violet to light yellow) was compared to that of the positive control ascorbic acid. The IC_{50} value represented the concentration of extract that causes 50% inhibition was determined. Experiments were carried out in triplicate and the mean value was recorded.

Reducing power

The reducing power of roots and stems/leaves was determined as per the reported method of Oyaizu (1986). Different concentrations of extract (100–1000 µg/mL) in 1ml of methanol were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was

mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm and compared with standards. Increased absorbance of the reaction mixture indicated increased reducing power.

RESULTS AND DISCUSSION

Phytochemical prospecting

With the increase of various diseases such as cancer, cardiovascular diseases atherosclerosis and inflammatory injuries, alternative natural products of plants could be of interest. Some plant extracts and phytochemicals are known to have antioxidant properties, which could be of great importance in the therapeutic treatments. In the last years, various studies have been conducted in different countries, demonstrating the efficacy of this type of treatment (Capecka *et al*., 2005). Table 1 shows the presence of various compounds such as tannins, flavonoids, phenolic acids and coumarins. Through phytochemical prospecting of the extracts, it was possible to determine the presence of diverse classes of secondary metabolites that show a wide variety of biological activities. polyphenols are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Barreiros *et al*., 2006; Okuda *et al*., 1989). Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 (Li *et al*., 2003), and this property may explain the mechanisms of antioxidative action of *D. crinitus*.

Table. 1: Phytochemical prospection of extracts of *D. crinitus*.

Extracts	Metabolites					
	1	2	3	4	5	6
Methanol						
Stems/leaves	+	+	+	+	-	-
Water						
Stems/leaves	+	+	+	-	-	-

1: tannins; 2: flavononols; 3: phenolic acids; 4: coumarins; 5: saponins; 6: alkaloids; +: presence; _: absence.

Assays of total phenolics and flavonoids from solvent extracts

The amounts of total phenolics in the extracts were determined spectrometrically according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalent. The amounts of total phenols found in the plant extracts are shown in Table 2. The results showed that the methanolic extract has higher total phenol components than the water extract. The total phenolics and contents of the methanolic and water extracts of *D. crinitus* were 130.19 ± 5 and 89.80 ± 3 µg GA/mg extract respectively. Similarly, the methanolic extract was found to be richer in flavonoids (86.72 ± 4 µg GA/mg extract) than the water extract (49.77 ± 2 µg GA/mg extract).

Table. 2: Total phenol and flavonoid contents of *D. crinitus* solvent extracts.

Solvent extract	Total polyphenol content (µg GA/mg extract)	Total flavonoid content (µg quercetin/mg extract)
Methanolic extract of stems/leaves	130.19 ± 5	86.72 ± 4
Water extract of stems/leaves	89.80 ± 3	49.77 ± 2

Values expressed are means ± SD of three parallel measurements.

ANTIOXIDANT PROPERTIES

Radical scavenging activity

The antioxidant activity of the extracts was determined by the DPPH test system. Table 3 demonstrates DPPH scavenging activity, expressed in percentage, caused by different concentrations of solvent extracts from *D. crinitus*. The weakest radical scavenging activity (20.54%) was exhibited by the water extract of 0.01 mg/mL, whereas the strongest activity (89.82%) was exhibited by the methanolic extract at a concentration of 0.2 mg/mL. The next highest activity (80.56%) was for the water extract at a concentration of 1.4 mg/mL. At shown in Table 2, the antioxidant activity of extracts increased with an increase in their concentrations. At higher concentrations, the antioxidant activity of extracts was closer to the scavenging effect of ascorbic acid. For instance, at 0.08 mg/mL, the scavenging activity of ascorbic acid was around 97.84%, and a methanolic extract solution of 0.2 mg/mL had a scavenging activity of 89.82%. The same value was obtained for the water extract at a concentration of 1.4 mg/mL. Therefore, DPPH scavenging activity is usually presented by the IC_{50} value. Concentrations of the antioxidant providing 50% inhibition of DPPH in the test solution (IC_{50}) were calculated and presented in Table 2. The methanolic extract of *D. crinitus* had the highest radical scavenging activity with the lowest IC_{50} value of 0.068 mg/mL. This was higher than the water extract with an IC_{50} value of 0.64 mg/mL.

Table. 3: DPPH radical-scavenging of solvent extracts from *D. crinitus* at different concentrations.

Sample	Antioxidant activities					
Water	Extract concentration (mg/mL)	0.01	0.02	0.7	1.4	
	Scavenging effect on DPPH (%)	20.54	26.47	53.80	80.56	
	DPPH IC_{50} (µg/mL)					0.64
Methanol	Extract concentration (mg/mL)	0.04	0.06	0.08	0.2	
	Scavenging effect on DPPH (%)	35.08	46.00	57.74	89.82	
	DPPH IC_{50} (mg/mL)					0.068
Ascorbic acid	Extract concentration (mg/mL)	0.04	0.05	0.06	0.08	
	Scavenging effect on DPPH (%)	39.40	51.03	68.57	97.84	
	DPPH IC_{50} (mg/mL)					0.048

Reducing power

Figure 1 indicates the values of the Antioxidant activity of the methanolic and water extracts. The measurement of the Antioxidant activity of the ferric ions by the extracts of *D. crinitus* was evaluated in ascorbic equivalent of Acid used to establish a curve of reference. Fig. 1 depicts the reducing power of the extracts from *D. crinitus*. Both extracts showed the presence the reductive effects, which increased with an increase in concentration. However, the methanolic extract was more potent on reducing power compared to water extract. Actually, reducing

power is a very important aspect for the estimation of the antioxidant activity (Ksouri *et al*., 2008). Therefore, the antioxidant activity of plant extracts might be due to the reduction of superoxide anion, inactivation of free radicals or complexion with metal ions or combination of the three. This good antioxidant activity is attributed to the presence of natural antioxidants such as phenolic compounds in *D. crinitus*. However, it is extremely important to point out that there is a positive correlation between the antioxidant activity potential and the amount of phenolic compounds in the extracts. Moreover, as reported in literature data (Bellakhdar, 1997), the antioxidant activity of extracts could be attributed to its relatively high content of the phenolic compounds.

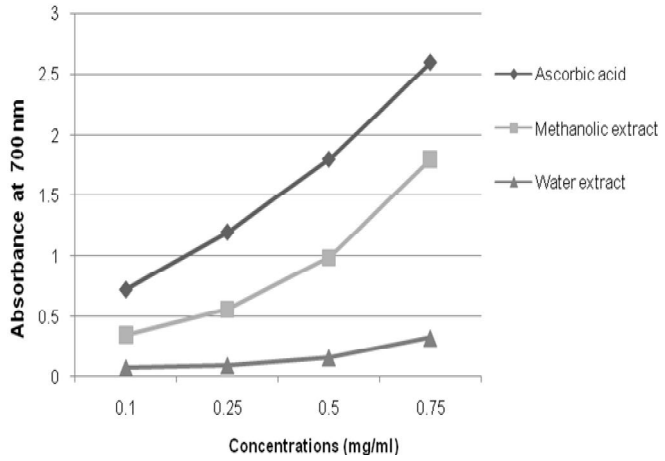

Fig. 1: Reducing power of methanolic and water extract of *D. crinitus*

CONCLUSIONS

This study affirms the in vitro antioxidant potential of solvent extracts of *D. crinitus*, with results comparable to those of the standard compounds such as gallic acid and can therefore be proposed as new potential sources of natural additives for the food and/or pharmaceutical industries. However, the components responsible for the antioxidant activities of the extracts were not identified and further work should be conducted to isolate and identify these bioactive compounds.

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