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Antioxidant activities, phenolic, flavonoid and tannin contents of endemic *Zygophyllum Cornutum Coss.* from Algerian Sahara

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ABSTRACT

Zygophyllum cornutum Coss. is an endemic plant growing in the northern Sahara of Algeria. It is used in traditional medicine against diabetes, hypertension and dermatitis. The antioxidant activities of the crude hydro-alcoholic extract and the organic fractions of Z. cornutum were investigated by DPPH test, ferric reducing activity and phosphomolybdenum assay. Also the content of phenols, flavonoids and tannins was estimated by spectrophotometric methods. The best DPPH scavenging activity was found in water fraction followed by chloroform fraction (IC_{50}= 25 and 38.5 µg/ml, respectively), more effective than BHT. For the ferric reducing activity and phosphomolybdenum assay, the best activity was found in water and butanol fractions, the results were better than BHA and BHT. The content in phenolics, flavonoids and tannins may be responsible for the good activities of the plant Z. cornutum.

Keywords: *Zygophyllum cornutum Coss.*, Phenol, Flavonoid, Tannin, DPPH, Ferric reducing activity, Total antioxidant activity.

INTRODUCTION

The importance of medicinal plants has increased recently with the aim to find drugs against diabetes, hypertension and cancer as well as finding new molecules that possess antioxidant activities for using them in agri-food sector, pharmaceutics and cosmetic industries. The synthetic antioxidants have shown toxic effects on health, for that reason many studies have occurred to find new natural antioxidants as an alternative [1].

Zygophyllum are shrubby plants with leaves in two leaflets belongs to the family Zygophyllaceae [2]. The plants of this genus have been the object of several studies where they could confirm their biological activities [3].

Zygophyllum cornutum is an endemic xerophyte plant characterized by its dilated fruits on top in a free portion of carpels recurved into hooks as long as the welded portion [2]. In Algeria, *Z. cornutum* is used for the treatment of dermatitis, diabetes, hypertension, rheumatism, gout and asthma as other *zygophyllum* species [4, 5].

Many studies were confirmed the antidiabetic, antihypercholestolemic, anti-inflammatory and antidiarrhoeal activities of *Z. album* and *Z. gaetulum* [6-12]. While only hypoglycemic activity of *Z. cornutum* was confirmed in a preliminary investigation [13, 14].

The aim of our study was to evaluate the antioxidant properties of the crude extract of *Z. cornutum* and its factions and also to determine their content of phenolic, flavonoid and tannin compounds. Which, to the best of our knowledge, have not yet been reported.

MATERIALS AND METHODS

2. 1. Chemicals and reagents

All solvents were analytic grade purity purchased from Biochem. sodium carbonate, Folin-Ciocalteu reagent, aluminum chloride, phosphate buffer, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ammonium molybdate, gallic acid, quercetin, catechin, ascorbic acid, BHA and BHT were obtained from Sigma-Aldrich and Biochem.

2. 2. Plant material

The aerial parts of *Z. cornutum* were collected in the month of April 2013 from Biskra, southeast of Algeria (northern Sahara). The identification was done on the basis of Quezel and Santa [2] by Doctor Halis Youcef researcher in Touggourt's Scientific and Technical Research Centre for Arid Areas.

2. 3. Preparation of the extract

The plant was air-dried in shadow. Defatted aerial parts of *Z. cornutum (*100 g) were macerated at room temperature with EtOH–H_2O (70:30, v/v) for 24 h, two times. After filtration, the filtrate was evaporated till dryness, recovered with distilled water and partitioned successively using chloroform, ethyl acetate and n-butanol. The extracts, also the remaining water fraction, were concentrated under reduced pressure to calculate the yield and then re-dissolved with minimum of ethanol or water and kept at 4C°. We have obtained: crude extract (CE), chloroform fraction (CF), ethyl acetate fraction (EF), butanol fraction (BF) and water fraction (WF).

2. 4. Total phenolic content TPC

The total phenolic content in the crude extract and the fractions of *Z. cornutum* was estimated by using Folin-Ciocalteu reagent [15].Briefly; 0.1 ml of the extract was mixed with 0.5 ml of a (10%) Folin-Ciocalteu reagent .After 5 min, 2.0 ml of (20%) sodium carbonate were added, the mixture was shaken and reacted for 30 min at room temperature in the dark. The absorbance was measured at 760 nm and the results were expressed as mg gallic acid equivalent per gram of plant dry weight (mg GAE/g).

2. 5. Total flavonoid content TFC

The total flavonoid content in the crude extract and the fractions of *Z. cornutum* was estimated by using aluminum chloride colorimetric method [16]. Briefly, 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of extract. After 30 min incubation at room temperature, the absorbance was measured at 430 nm and the results were expressed as mg quercetin equivalent per gram of plant dry weight (mg QE/g).

2. 6. Total tannin content TTC

The total tannin content in the crude extract and the fractions of *Z. cornutum* was estimated by colorimetric method [17]. 3 ml of 4% ethanol vanillin solution and 1.5 ml of concentrated hydrochloric acid were added to 0.4 ml of extract. The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm. The results were expressed as mg catechin equivalent per gram of plant dry weight (mg CE/g).

2. 7. Determination of ferric reducing power

Reducing power of the different extracts of Z. *cornutum* was determined by the method of Oyaizu [18]. Different concentrations of the extract (1 ml) were mixed with 2.5 ml phosphate buffer solution (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The resulting solutions were incubated at 50ºC for 20 minutes. After incubation, the reaction mixture mixed with 2.5 ml of 10% TCA and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was taken and 2.5 ml distilled water and 0.5 ml of ferric chloride (0.1%) were added to it. The absorbance was measured at 700 nm, using ascorbic acid as a positive control, and the results were expressed as mM equivalent ascorbic acid.

2. 8. Determination of antiradical activity

The free radical scavenging activity of *Z. cornutum* was measured by using DPPH assay [19]. 1 ml of diluted plant extract was added to 1 ml of a 0.250 mmol/l DPPH• ethanol solution. The solutions were placed in the dark at room temperature for 30 min. The absorbance of the resulting solution was then read at 517 nm and ascorbic acid was used as a positive control. Inhibition of DPPH radical was calculated as follows:

DPPH scavenging effect (%) = $[A_0\text{-}A_1/A_0] \times 100$

Where A_0 and A_1 are the absorbance at 30 min of the control and the sample, respectively.

2. 9. Determination of total antioxidant activity

The total antioxidant activity of the different extracts of Z. *cornutum* was determined by the phosphomolybdenum assay [20]**.** Different concentrations of the extract (0.1 ml) were mixed with 1 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The resulting solutions were incubated in a water bath at 95°C for 90 min. The mixture was left to cool at room temperature, the absorbance of the mixture was measured at 695 nm, using ascorbic acid as a positive control, and the results were expressed as mM equivalent ascorbic acid.

Statistical analysis

All tests were taken in triplicate. The results were expressed as means ± SD. Curve was drawn by Microsoft Excel 2010. IC_{50} was calculated from linear regression.

RESULTS AND DISCUSSION

3. 1. Extraction yield, total phenolic, flavonoid and tannin contents

The extraction yield for the crude hydro-alcoholic extract of *Z. cornutum* was 28.089 %. For the fractions, the highest yield was in WF (18.242 %) followed by BF (4.776 %). In the other hand, the lowest yield of extraction was in EF (0.221 %) (Table 1).

TPC of *Z. cornutum* extracts, expressed as gallic acid equivalent per gram dry weight (mg GAE/g DW), ranged from 0.133±0.003 to 3.755±0.050 mg GAE/g DW (Table 1). The highest content was found in CE and WF, the lowest content was registered in CF. TPC of the crude extract of *Z. cornutum* and its fractions were in the following order: CF < EF < BF < WF < CE.

TFC of the crude extract and the fractions of *Z. cornutum*, expressed as quercetin equivalent per gram dry weight (µg QE/g DW), was between 18.473±0.602 and 1320.500±54.848 µg QE/g DW. The highest amounts of flavonoids were present in the CE and WF, while the lowest were recorded in the CF. TFC increased in the following order: CF < EF < BF < WF < CE. The amount of phenol and flavonoid present in the crude extract was very low when comparing with other studies [21, 22]

TTC in the fractions and the crude extract of *Z. cornutum*, expressed as catechin equivalent per gram dry weight (µg CE/g DW), varied between 3.737±0.248 and 143.350±22.960 CE µg/g DW. The highest content was found in CE and WF, while the lowest was found in EF and CF. The values of tannin content in the crude extract was lower compared with Borago officinalis L. [21].

Table 1 Extraction yield, total phenolic, flavonoid and tannin contents

	Yield %	TPC mg GAE/g DW	TFC µg QE/g DW	TTC µg CE/g DW
Crude extract	28.089	3.755 ± 0.050	1320.500 ± 54.848	143.350 ± 22.962
Chloroform fraction	0.229	0.133 ± 0.003	18.473 ± 0.602	10.925 ± 1.085
Ethyl acetate fraction	0.221	0.166 ± 0.011	50.657 ± 0.203	3.737 ± 0.248
Butanol fraction	4.776	0.820 ± 0.005	306.881 ± 0.462	20.161 ± 25.397
Water fraction	18.242	2.184 ± 0.067	543.859 ± 8.590	142.605 ± 45.501

We have remarked that phenolic and flavonoid contents are bigger in the polar fraction (water and butanol fractions), that may indicate that these polyphenol compounds are more hydroxylated and/or glycosydated .The content of phenolic or flavonoid compounds in fractions was affected by their solubility in solvent used for extraction. Polar fractions had more polyphenols than non-polar fractions, similar results was found by Khedher *et al* [23].

Seven known saponins were isolated from the methanolic extract of the whole plant of *Zygophyllum cornutum* Coss [24],β-sitosterol, isorhamnetin-3-rutinoside [25] and one flavonoid [26].

When comparing this results with those of *Z. album* [27], we found that crude extract also the butanol and water fractions of *Z. album* contain more TPC than the same fractions of *Z. cornutum*. Whereas, chloroform and ethyl acetate fractions of *Z. cornutum* are richer than those of *Z. album*.

The amount of flavonoids found in crude extract and butanol fraction of *Z. cornutum* are less than those of *Z. album*, while the other fractions of *Z. cornutum* are richer than the same fractions of *Z. album*. The amount of tannins found in *Z. cornutum* was a little lower than *Z. album* [27], but generally the results are similar.

3. 2. Antioxidant activities

The antiradical activity of the crude extract and fractions of *Z. cornutum* was measured by the DPPH assay. The method is based on the reduction of the stable radical DPPH with a violet color to non-radical DPPH-H with a yellow color. The disappearance of the violet color can be monitored spectrophotometrically at 517 nm. Fig 1 shows the percentage of scavenging DPPH in crude extract and fractions of *Z. cornutum*. The values of IC_{50} varied between 24.955±1.983 and 67.059±4.727 µg/ml (Table 2). The best activity was found in the WF with an IC_{50} value of 24.935±1.983 µg/ml followed by CF with an IC_{50} value of 38.478±2.085 µg/ml. All fractions had better activity than crude extract. All extracts showed a good antiradical activity and better than BHT (62.652±3.016 µg/ml).

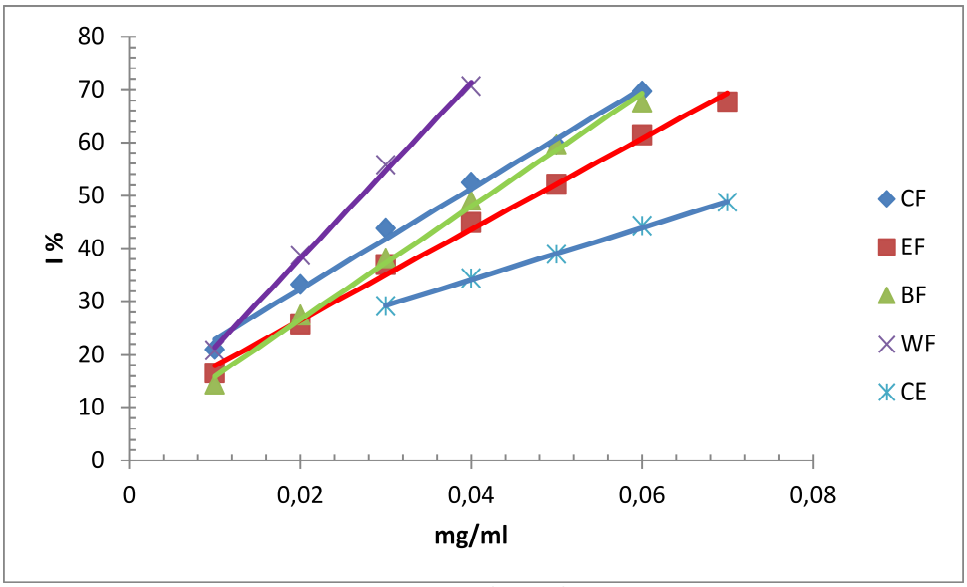

Fig 1 Free radical scavenging activity of chloroform (CF), ethyl acetate (EF), butanol (BF), water (WF), crude (CE) extracts of *Z. cornutum*

The ability of the different extracts of *Z. cornutum* to reduce the ferricyanide complex (Fe3+) to the ferrous form (Fe2+) was recorded by measuring the formation of Perl's Prussian blue at 700 nm.

Fig 2 shows the reducing power activities of crude extract and fractions of *Z. cornutum* expressed as absorbance in terms of the inverse of dilution factor. Ferric reducing activity of the different extracts of *Z. cornutum* ranged from 3.827 ± 0.131 to 15.461 ± 0.282 mM. WF had the best reducing activity with a value of 15.461 ± 0.282 mM, the lowest reducing activity was recorded in CF and EF (3.964 ± 0.071 and 3.827 ± 0.131 mM, respectively). All fractions are better than crude extract. All extracts showed a very good ferric reducing activity, better than BHA, BHT and gallic acid.

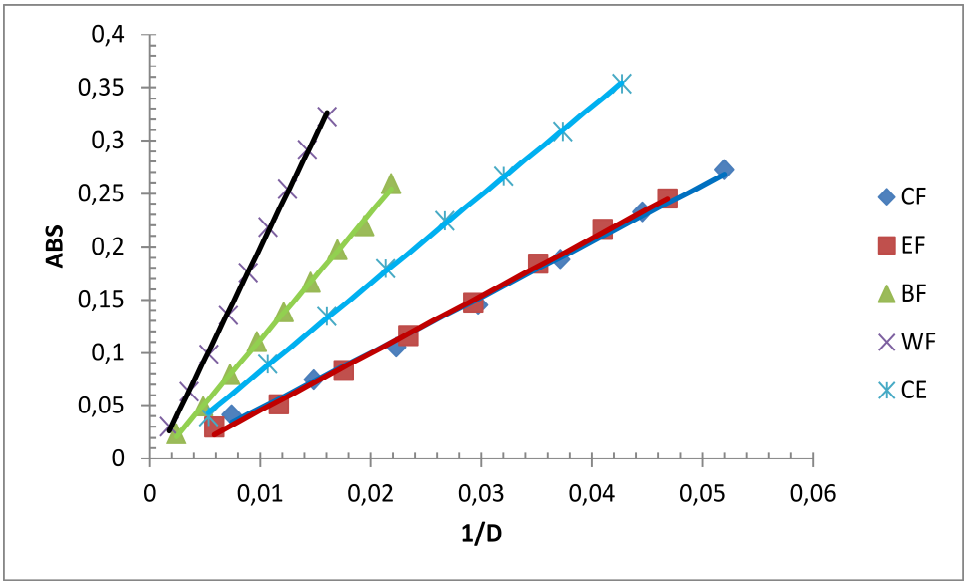

Fig 2 Reducing power activities of chloroform (CF), ethyl acetate (EF), butanol (BF), water (WF), crude (CE) extracts of *Z. cornutum*

The total antioxidant activity of the different extracts of *Z. cornutum* was measured by the phosphomolybdenum method, which is based on the reduction of Mo (VI) to Mo (V). The formation of green phosphate/Mo (V) compounds measured at 695. Fig 3 shows the total antioxidant activity of crude extract and fractions of *Z. cornutum* expressed as absorbance in terms of the inverse of dilution factor. Total antioxidant activity of all extracts varied between 14.434 ± 0.263 and 98.707 ± 0.382 mM. WF had a strong antioxidant activity with a value of 98.707 ± 0.382 mM followed by BF with a value of 45.576 ± 3.341 mM. The lowest antioxidant activity was recorded in EF with a value of 14.434 ± 0.263 mM. The crude extract and the fractions of *Z. cornutum* showed a very good antioxidant activity better than BHA and BHT (0.978 ± 0.041 and 0.841 ± 0.031 mM, respectively).

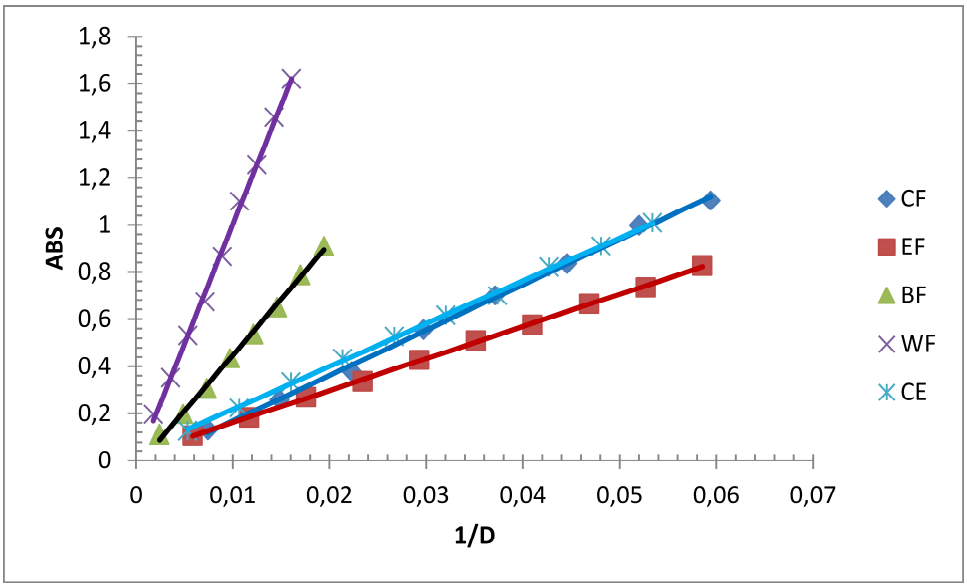

Fig 3 Total antioxidant activity of chloroform (CF), ethyl acetate (EF), butanol (BF), water (WF), crude (CE) extracts of *Z. cornutum*

There are a weak correlation between the phenolic, flavonoid and tannin contents and the antioxidant activities. , similar results were found in three Veronica species [28]. The contribution of phenols and flavonoids in the DPPH scavenging activity was 20.15% and 35.34% respectively. We have found also 43.92% of the ferric reducing activity and 22% of the activity to reduce molybdate were due to tannins. The chloroform fraction had a very good scavenging activity in spite of the low content of polyphenols, that is explained by the interference of non-phenolic compounds [29] like terpenoids already isolated from *Z. cornutum* [24].

The mechanism for the reaction of DPPH$^{\bullet}$ with phenols depend on the reactivity as hydrogen or electron donator. the high activity is due to the number of hydroxyl groups available [30]. Flavonoids and tannins are among the main groups of polyphenols. Flavonoids have a high redox potential, permitting them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelating potential [31]. Flavonoids are known to scavenger various oxidizing species and to have an ability to stabilize membranes by decreasing membrane fluidity [32]. Tannins are metal ion chelators, protein precipitating agents and biological antioxidants [31].

Table 2 DPPH scavenging, reducing power and total antioxidant activity

	DPPH IC_{50} µg/ml	FRAP mM	Molybdate mM
Crude extract	67.059 ± 4.727	6.487 ± 0.454	19.152 ± 0.077
Chloroform fraction	38.478 ± 2.085	3.964 ± 0.071	18.103 ± 1.104
Ethyl acetate fraction	47.767 ± 1.571	3.827 ± 0.131	14.434 ± 0.263
Butanol fraction	42.159 ± 3.029	8.440 ± 0.578	45.576 ± 3.341
Water fraction	24.935 ± 1.983	15.461 ± 0.282	98.707 ± 0.382
Ascorbic acid	14.657 ± 0.698	-	-
BHA	13.145 ± 0.304	0.556 ± 0.012	0.978 ± 0.041
BHT	62.652 ± 3.016	0.751 ± 0.005	0.841 ± 0.031
Gallic acid	-	1.122 ± 0.049	-

CONCLUSION

The crude extract of *Z. cornutum* and its fractions were found to have very good antioxidant activities and to contain a considerable content in polyphenolic compounds. These results may confirm the traditional use of the plant. Further work must be done like biological, antihyperglycemic and antihypercholesterolemic activities.

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