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Research Article

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In vitro antioxidant properties and phenolic contents of *Zygophyllum album* L. from Algeria

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ABSTRACT

The ethanolic extract of Zygophyllum album was extracted successively with chloroform, ethyl acetate and butanol. Using colorimetric methods, the higher content of phenols was recorded in crude extract and water fraction (5.118 ± 0.105 and 2.088 ± 0.012 mg GAE/g DW respectively) , the higher content of flavonoids was found in crude extract and butanol fraction (2.393 ± 0.061 and 0.506 ± 0.0 13 mg QE/g DW respectively), and the higher content of tannins was recorded in crude extract and water fraction (197.875 ± 25.46 and 103.611 ± 49.235 mg RE/g DW respectively). All extracts showed very good activity of ferric reducing power, the higher power was in crude extract and butanol fraction (11.262 ± 0.38 and 30.177 ± 2. 397 mM respectively) more effective than BHA and BHT. IC_{50} of inhibition of radical DPPH in chloroform fraction was 22.127±0.837µg/ml more effective than BHT.

Keywords: DPPH; Ferric reducing activity; Flavonoid; Phenol; Tannin; *Zygophyllum album L.*

INTRODUCTION

Plants are the main source of phenolic compounds as other secondary products. Polyphenols are known as natural antioxidant [1]. Antioxidants have been used to protect food from oxidative degradation, also used in cosmetology and dermopharmacy [2, 3]. Synthetic antioxidants like BHA and BHT have been widely used in industry but suspected to have toxicological effects [4].

Plants growing in Sahara and salty areas (Sabkha) are exposed to extreme conditions as salinity, heat and luminosity which contribute in the quality of their antioxidants [5].

Zygophyllum album is a Saharian plant belonging to the genus *Zygophyllum*, family Zygophyllaceae. The local population in Algeria used it as remedy for rheumatism, gout, asthma, diuretic, diabetes, dermatitis, spasm, dysmenorrhea, hypertension, local anesthetic and antihistamic [6, 7].

There are studies on crude ethanolic and water extract of *Z. album* suggest that the antioxidant properties of this extracts could be responsible for their antidiabetic activity and antihypercholesterolemic [8, 9]. Another study provides the possibility of the use of *Z. album* in the development of antiobesity drugs [10].

The aim of our study was to evaluate the antioxidant properties of the crude extract of *Z. album* 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.

EXPERIMENTAL SECTION

2.1 Plant material

The aerial parts of *Z. album* were collected in the month of April 2013 from Ouargla Sahara, southeast of Algeria. The identification was done on the basis of Quezel and Santa [11] by Halis youcef researcher in Touggourt's Scientific and Technical Research Centre for Arid Areas.

2.2 Preparation of the extract and fractions

Air-dried aerial parts (100 g) of *Z. album* 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 and then re-dissolved with minimum of ethanol or water and kept at 4C°.

2.3 Determination of total phenolic content

The total phenolic content in the crude extract and the fractions of *Z. album* was estimated by using Folin-Ciocalteu reagent [12].Briefly; 0.1 ml of the extract sample 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).

2.4 Determination of total flavonoid content

The total flavonoid content in the crude extract and the fractions of *Z. album* was estimated by using aluminum chloride colorimetric method [13]. 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).

2.5 Determination of total tannin content

The total tannin content in the crude extract and the fractions of *Z. album* was estimated by colorimetric method [14]. 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).

2.6 Determination of ferric reducing power

Reducing power of the crude extract and the fractions of Z. *album* was determined by the method of Oyaizu [15]. Different concentrations of the sample (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.

2.7 Determination of antiradical activity

The free radical scavenging activity of *Z. album* was measured by using DPPH assay [16]. 1 ml of diluted plant extract was added to 1 ml of a 0.25 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.

RESULTS AND DISCUSSION

3.1 Total phenolic, flavonoid and tannin contents

The total phenolic content of the crude extract and the fractions, expressed as gallic acid equivalent per gram dry weight (mg GAE/g DW), varied between 5.118 ± 0.105 and 0.116 ± 0.002 mg GAE/g DW (Table 1). The highest total phenolic content was found in the crude extract and the water fraction while the lowest was in the chloroform fraction. The total flavonoid content of the extracts, expressed as quercetin equivalent per gram dry weight (mg QE/g DW), ranged from 2.393 ± 0.061 to 12 ± 0.582 µg QE/g DW (Table 1). Crude extract and butanol fraction contained high amount of flavonoids while chloroform fraction contained lower amount of flavonoids. For the

determination of the total tannin content of the crude extract and the fractions, expressed as catechin equivalent per gram dry weight (mg CE/g DW), the crude extract and the butanol fraction exhibited a highest content of tannins, on the other hand, the ethyl acetate fraction showed a lowest value of tannin content (Table 1).

Flavonoid and triterpene glycosides, tannins and saponins were the major compounds found and isolated from *Z. album*. These families of compounds are known as great antioxidant and widely used in industrial fields [17-21].

Table 1: Total phenolic, flavonoid and tannin contents

Extract	Total phenolics (mg GAE/g)*	Total flavonoids (µg QE/g)*	Total tannins (µg CE/g)*
Crude extract	5.118 ± 0.105	2393.25 ± 61.905	197.875 ± 25.46
Chloroform fraction	0.116 ± 0.002	12.152 ± 0.582	8.194 ± 1.554
Ethyl acetate fraction	0.122 ± 0.004	32.762 ± 0.771	3.162 ± 0.248
Butanol fraction	0.919 ± 0.021	506.305 ± 13.317	13.746
Water fraction	2.088 ± 0.012	340.926 ± 4.295	103.611 ± 49.235

**Results are expressed as mean of 3 values ± standard deviation*

3.2 Antioxidant activities

The ability of the extracts to reduce iron (III) to iron (II) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Yellow color of the test solution changes to green or blue color depending on the reducing power of antioxidant samples. A higher absorbance indicates a higher ferric reducing power. The reducing power of the crude extract and the fractions of *Z. album* are summarized in Table 2. The values of reducing activity varied between 30.177 ± 2.397 and 7.689 ± 0.562 mmol/ml. The butanol fraction gave the higher result of reducing activity while the lowest was recorded in the chloroform fraction (Figure 1). All the extracts showed a very good activity of reducing power and better than the synthetic antioxidants BHA (0.55±0.01 mmol/ml), BHT (0.75±0.005 mmol/ml) and gallic acid (1.122±0.49 mmol/ml).

DPPH is used to evaluate the free radical scavenging activity of natural antioxidants and extracts, the method is based on the reduction of the stable free radical DPPH by donating hydrogen from the phenolic hydroxyl groups. This reduction can be monitored at 517 nm by measuring the bleaching of DPPH (violet) to DPPH-H (yellow). Figure 2 shows DPPH inhibition activities of crude extract and fractions of *Z. album*. The values of IC_{50} ranged from 84.104 ± 3.989 to 22.127 ± 0.837 µg/ml (Table 2). The highest scavenging activity was observed in the chloroform fraction (22.127 ± 0.837 µg/ml) and ethyl acetate fraction (26.138 ± 01.542 µg/ml), the lowest scavenging activity was recorded in the crude extract and butanol fraction (84.104 ± 3.989 and 62.1506 ± 10.213 µg/ml respectively). The fractions showed better scavenging action than the crude extract. IC_{50} values of all these compounds were greater than that of BHT where IC_{50} was achieved at 62.652±3.016 µg/ml but lower than ascorbic acid (14.657±0.698 µg/ml).

Table 2: reducing power and DPPH scavenging

Extract	Reducing power (mM)*	DPPH (IC_{50} in µg/mL)*
Crude extract	11.262 ± 0.38	84.104 ± 3.989
Chloroform fraction	8.723 ± 0.732	22.127 ± 0.837
Ethyl acetate fraction	7.689 ± 0.562	26.138 ± 01.542
Butanol fraction	30.177 ± 2.397	62.15 ± 10.213
Water fraction	26.637 ± 2.928	33.254 ± 0.162
Ascorbic acid	-	14.657 ± 0.698
BHA	0.556 ± 0.012	13.145 ± 0.304
BHT	0.751 ± 0.005	62.652 ± 3.016
Gallic acid	1.122 ± 0.049	-

** Results are expressed as mean of 3 values ± standard deviation*

Polyphenol possess ideal structural chemistry for radical scavenging, this properties arise from its high reactivity as hydrogen or electron donator, and the stability and delocalization of the impaired electron of polyphenol-derived radical [22]. Many flavonoids have strong antioxidant capacities. Flavonoids can prevent injury caused by free radicals by direct scavenging of reactive oxygen species, activation of antioxidant enzymes and increasing in antioxidant properties of low molecular antioxidants [23].

Concerning the correlation between phenolic, flavonoid and tannin contents and DPPH scavenging activity, the values of correlation coefficient were 0.66, 0.81 and 0.48 respectively. According to this result, the contribution of phenols, flavonoids and tannins was 66%, 81% and 48% in radical scavenging activity.

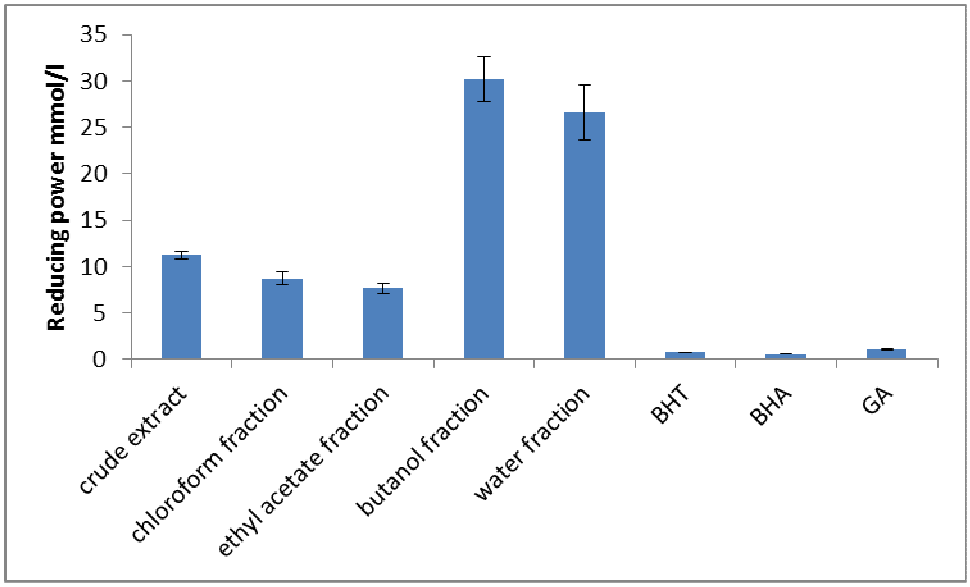

Figure 1: Reducing power activities of crude extract and fractions of *Z. album*

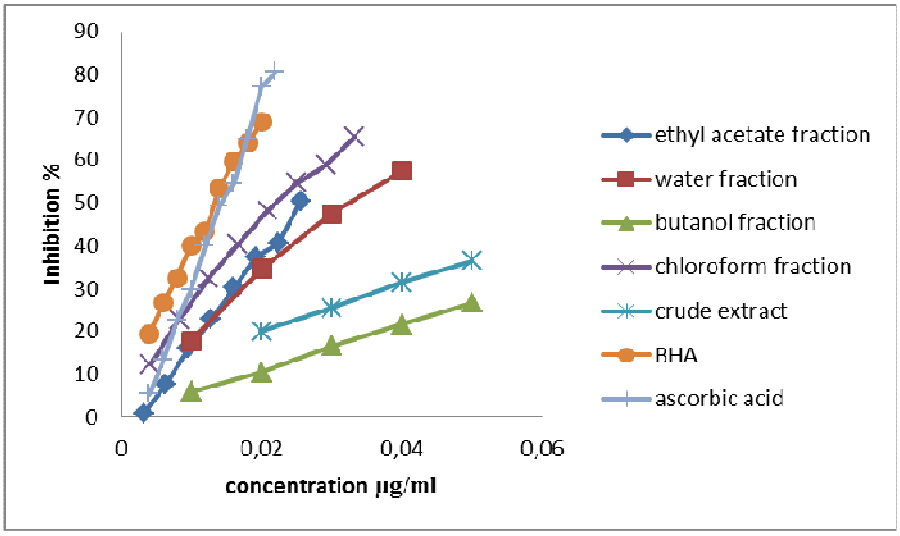

Figure 2: Percentage scavenging of DPPH in crude extract and fractions of *Z. album*

CONCLUSION

The crude ethanolic extract and four fractions of *Z. album* were investigated for their polyphenolic contents and antioxidant activities. The results obtained from this study indicate that the fractions of *Z. album* have a good antioxidant activity. All extracts have strong reducing activity, while chloroform and ethyl acetate have good antiradical activity. The antioxidant activity in the fractions of *Z. album* arising from the rich content on polyphenol in this plant.

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