



E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2016; 4(6): 158-161
Received: 13-09-2016
Accepted: 14-10-2016

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Free radicals scavenging activity and reducing power of two Algerian Sahara medicinal plants extracts

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Abstract

The aim of this study is to evaluate antioxidant activity of aqueous (Aq.E) and methanolic (Met.E) extracts of *Zygophyllum album* L. (Zygophyllaceae) and *Arthrophytum scoparium* (Chenopodiaceae), two medicinal plant from Algerian Sahara used in traditional pharmacopoeia. The results showed that Aq.E have the heights yields 24% and 22.3% for *Z. album* and *A. scoparium* respectively. Qualitative analysis of showed that the Met.E of *Z. album* of had the highest amount of polyphenols (202.26±4.73 µg GAE/mg E) and flavonoids (15.77±0.94 µg QE/mg E). The antioxidant activity of extracts was evaluated by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assay. Results showed that the extracts of *A. scoparium*. Have the highest free radical scavenger activity in DPPH test (EC₅₀=0.096±0.001 and 0.108±0.002 mg/ml for Met.E and Aq.E respectively). In addition, the results of reducing power assay demonstrated that Met.E of *Z. album* had the highest effect with 2399.65±12.31 µg ascorbic acid equivalent/mg E. Our results showed that the methanolic extract reported a considerable free radicals scavenging activity and reducing effect, may be due to their richness on polyphenols and flavonoids.

Keywords: *A. scoparium*, *Z. album*, antioxidant activity, medicinal plants polyphenols, flavonoids

1. Introduction

Medicinal plants have been used since ancient times to relieve and cure diseases human. In fact, their therapeutic properties are due to the presence of thousands of bioactive natural compounds. There is increasing trend in correlating phytochemical constituents of plants with its pharmacological activities. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines [1]. Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products; they have several biological and pharmacological effects such as anti-inflammatory, antispasmodic, anti-allergic, anti-diabetic, antimicrobial and antiviral [2, 3]. The oxidative stress is the majority cause of pathological disorders and disease such as aging, diabetes, cardiovascular diseases and cancer. Whatever the case, the risk is increased with the accumulation of reactive oxygen species (ROS) such as superoxide anion (O₂⁻), hydroxyl radicals (OH[·]) and hydrogen peroxide (H₂O₂). ROS leading to a radical reaction chain which degrades organic molecules vital to know the DNA, lipids and proteins in the body, which have been implicated in the etiology and pathophysiology [4, 5]. In recent years increased interest is observed on the biological effects of natural antioxidant included in the fight against oxidative stress involved in the initiation and progression of several diseases. In addition, many of the phenolic compounds have been shown to contain high levels of antioxidant and antimicrobial activities [6]. The aim of this study consisted to valorization of medicinal and aromatic plants of the Algeria flora, in order to evaluate the total polyphenols contents and the antioxidant activity of extracts of *Z. album* and *A. scoparium*, tow Algerian Sahara plants used in various folk medicines.

2. Materials and Methods

2.1 Materials

Zygophyllum album L. (Zygophyllaceae) and *Arthrophytum scoparium* (Chenopodiaceae) are widely distributed in the south of Algeria. Locally named "Bougriba" and "Elremth" respectively, which have been used traditionally in Algerian folk medicine. The plant materials were collected in December 2014, from Biskra (south of Algeria), authenticated by Dr. Salemkour N, the center of scientific and technical research on arid regions (C.R.S.T.R.A), Biskra, Algeria. The aerial part of plants is dried at temperature room then powdered using a blender and is stored in the dark crushed in stock until use.

2,2-diphenyl-2-picryl-hydrazil (DPPH), aluminum chloride (AlCl₃), potassium ferricyanide [K₃Fe(CN)₆], trichloroacetic acid (TCA), iron chloride (FeCl₃) sodium carbonate (Na₂CO₃), ascorbic acid, Folin-Ciocalteu reagent, gallic acid, quercetin, rutin and 2, 6 di-tert-butyl-4-methyl phenol (BHT). These products were purchased from Sigma Chemicals, Sigma-Aldrich.

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2.2 Methods

2.2.1 Preparation of extract

The extractions were carried from the aerial part plants by methanol and distilled water according to the method of [7-8]. The extraction of powdered plant material was carried out by maceration process for 24 h. The powdered plant material (10 g) was soaked in 100 ml of methanol or distilled water. The extract was filtered using a Buchner funnel and Whatman No. 1 filter paper to obtain aqueous extract. The same procedure to obtain the methanolic extract. The extracts were stored at room temperature until use.

2.2.2 Determination of total phenolic and flavonoids contents

The total phenolic contents of the extracts were measured using the Folin-Ciocalteu colorimetric method described by [9], with gallic acid as standard. Basically, 0.2 ml of extract was mixed with 1 ml of Folin-Ciocalteu phenol reagent (10-fold diluted). After 4 min, 0.8 ml of saturated sodium carbonate Na_2CO_3 (7.5%) solution was added and the mixture was allowed to stand for 2 h. Absorbance was measured at 765 nm. The same procedure was repeated with all standard gallic acid solutions (10-200 $\mu\text{g}/\text{ml}$). The amount of total polyphenols in different extracts were expressed as μg of gallic acid equivalent (GAE)/ mg extract.

Flavonoids were quantified using aluminum chloride reagent (AlCl_3) method described by [10]. The flavonoids content was expressed as quercetin equivalents (QE). Briefly, 1 ml of extract, dissolved in corresponding solvent was added to 1 ml of AlCl_3 (2% in methanol). The absorbance was measured at 430 nm, after incubation at room temperature for 10 min.

2.2.3 DPPH radical scavenging assay

The free radical scavenging activity of extracts was evaluated by 2,2-diphenyl-2-picryl-hydrazil (DPPH) scavenging assay according to the method reported by assay [11]. Briefly, 50 μl of solution at different doses containing the compound to be tested were added to 1250 μl of solution of DPPH (2.4 mg in 100 ml methanol). The studied extracts were tested with methanol as control, BHT, gallic acid, rutin and quercetin as antioxidant-standards. Absorbance was measured at 517 nm that was determined after allowed to stand at room temperature for 30 min. The absorbance of the control and samples was measured, and the DPPH scavenging activity in percentage was determined was calculated according to the following formula: Scavenging effect% = $[(AC - AS) / AC] \times 100$

Where Ac: control absorbance and As: absorbance in

presence of sample (extract). The data are presented as mean of triplicate and the concentration required for a 50% (EC_{50}) reduction of DPPH radical was determined graphically.

2.2.4 Reducing power assay

The ability of extracts to reduce ferric ions (Fe^{+3}) was assessed by the method of [12]. 800 μl of extract was mixed with 400 μl phosphate buffer (0.2 M, $\text{pH}=6.6$) and 800 μl of a 1% potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$], then the mixture was incubated at 50 $^\circ\text{C}$ for 20 min. About 800 μl (10%) of trichloroacetic acid (TCA) was added to the mixture and centrifuged for 10 min (3000 r/t). Finally, 400 μl of the supernatant solution was mixed with 400 μl of distilled water and 80 μl FeCl_3 (0.1%) and the absorbance was recorded at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The results were expressed as μg ascorbic acid equivalent/mg extract [13].

2.3 Statistical analysis

Experimental results were expressed as mean \pm SD of triplicate. The data were analysed by Student's *t*-test to determine statistical significance. *p*-values and $p < 0.05$ was considered as indicative of significance. The EC_{50} values were calculated from linear regression analysis. All statistical analysis and graphing of data were performed using Graph pad prism 5 software.

3. Results and discussion

3.1 Extraction and Total phenolic and flavonoid contents

Extract was dried with rapport of 1/10 (w/v) under reduced pressure giving different yields. The total phenolic and flavonoid contents in the extracts was determined spectrophotometrically, using Folin-Ciocalteu and aluminum chloride methods, respectively (Table 1). Results showed that the methanolic extract of the two plants contain the highest amounts of phenolic compounds and flavonoids (Table 1). Moreover, the Methanolic extract of *Z. album* L. contained the highest amount of polyphenols and flavonoids compared to the Met.E of *A. scoparium*. In addition, The Aq.E of *A. scoparium* showed the highest amount of polyphenols compared to the Aq. E of *Z. album* L. extract of total phenolic contents in plant extracts depend on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation [14, 15].

Table 1: Total polyphenol and flavonoids contents of extracts.

	Yield (%)		Polyphenols contents (μg GAE/mg E)		Flavonoids (contents μg QE/mg E)	
	Met.E	Aq.E	Met.E	Aq.E	Met.E	Aq.E
<i>Z. album</i> L.	11.6	24	202.26 \pm 4.73	46.37 \pm 2.20	15.77 \pm 0.94	1.95 \pm 0.13
<i>A. scoparium</i>	10.8	22.3	142.98 \pm 3.76	133.52 \pm 2.47	6.94 \pm 0.89	4.72 \pm 0.79

The results of [16] have shown that the polyphenol contents of the methanol extract that is greater than the aqueous extract although the study is carried out on the *Capparis spinosa* L. Therefore, many studies demonstrated that polar solvents give higher yields than apolar solvents, since the polar solvents have the ability to spread within the plant powder, reaching the vegetable matrix and therefore recover the possible metabolites. While non-polar solvents which are immiscible with water, does not have the ability to extract the maximum amount of bioactive molecules because of the presence of the

water contained in the plant tissue [17].

3.2 The antiradical activity using DPPH assay

DPPH radicals are widely used in the model system to investigate the scavenging activities of several natural compounds. When DPPH radical is scavenged, the color of the reaction mixture changed from purple to yellow with decreasing of absorbance at wavelength 517 nm. The antioxidant activity of different extracts was determined using DPPH radical scavenging test then antiradical activity was

expressed by effective concentration (EC_{50}). DPPH radical scavenging activity of the extracts is shown in Fig. 1. The results showed that both extracts of *A. scoparium* possess a height antiradical activity with ($EC_{50}=0.096\pm 0.001$ and 0.108 ± 0.002 mg/ml for Met. E and Aq. E respectively), this EC_{50} values are not significantly different compared with BHT). Moreover, The Z. Met. E and Z. Aq. E present less DPPH free radical scavenging activity with ($EC_{50}=0.46\pm 0.01$ and 1.23 ± 0.02 mg/ml, respectively). It has been reported that the antiradical activity of tea extracts is probably due to their

hydrogen donating ability attributed to polyphenols components [18]. In addition, the antioxidant properties of phenolic compounds are attributed in part to the ability of these compounds to scavenge free radicals such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals (OH^{\cdot}) and hydrogen peroxide (H_2O_2) [19]. The antiradical activity of natural antioxidants such as phenolic compounds and flavonoids may be due to their richness by hydroxyl-ring. The spatial configuration and the number of OH group of flavonoid structures can influence the different antioxidant mechanisms [20].

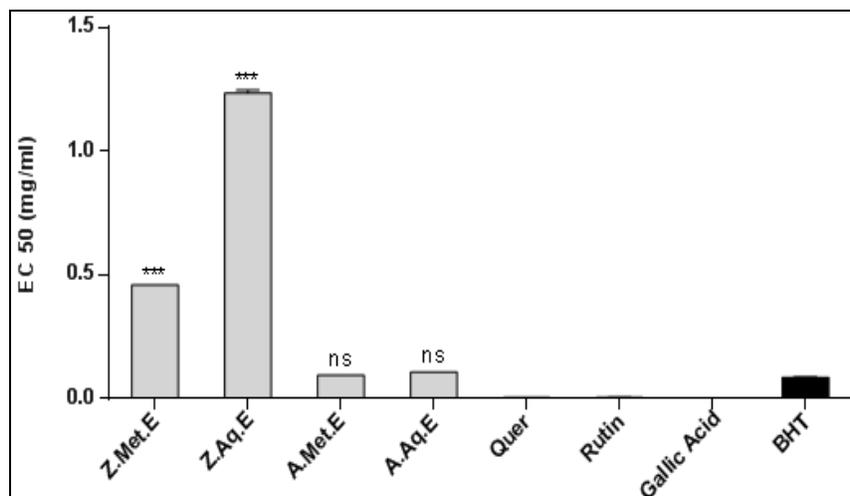


Fig 1: EC_{50} values in Free radical scavenging activity of the extracts against DPPH free radical. Values were means \pm SD of triplicate (ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$).

3.3 Reducing power

The ability of extract to reduce Fe^{+3} to Fe^{+2} (reducing effect) was determined according to the method described by [12]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [21]. Dietary antioxidant such as ascorbic acid was used for comparison. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [22]. The results were expressed as μ g ascorbic acid equ/mg extract. The Met.E of *Z. album* have strong reducing capacity (2399.65 ± 12.31 μ g AAequ/mg E) this capacity is more best than capacity of quercetin (2109.72 ± 15.49 μ g AAequ/mg Q). However, the reducing capacity of *A. scoparium* is less than the reducing capacity of *Z. album* extracts.

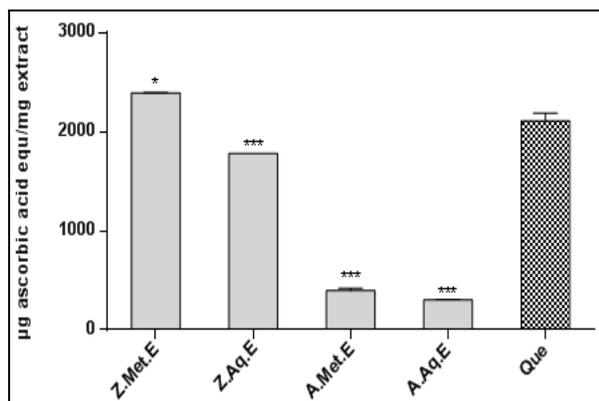


Fig 2: The reducing power of extracts and quercetin. Values were expressed as means \pm SD of triplicate. (ns: $p > 0.05$; * $p \leq 0.05$; ***: $p \leq 0.001$).

The reducing power of a compound set referred to its electron transfer capacity in a redox reaction, leading to the conversion of free radicals in less reactive or inert products. However, in addition to stabilizing the radical cation, the buffer systems by controlling the ratio of the protonated or deprotonated states of the antioxidants could result in an "induced antioxidant" activity [23]. The reducing capacity of antioxidant be due to their electron transfer property such as polyphenols flavonoids. Many studies demonstrated that the plants extract possess a strong reducing capacity. In other hand, many researchers has been widely reported the relationship between polyphenol structure and their and ferric reducing capacity [24, 26]. Phenolic compounds are called antioxidants because of their ability to scavenge free radicals, lipid peroxidation inhibition, and reducing effect [27, 20].

4. Conclusion

On the basis of the results of this study, the methanolic have a height antioxidant activity may be due to their phenolic contents in extracts, it possessed excellent antioxidant properties, including scavenging abilities against DPPH and reducing power. Moreover, The *A. scoparium* extracts exhibited a strong antiradical activity against DPPH free radical. When, the *Z. album* extracts exhibited a highest reducing capacity. In conclusion, the antioxidants activity of extract may be due to the nature of compounds in each extract. In view of the potential use of *A. scoparium* and *Z. lotus* extracts in therapeutic benefits and bioactive compounds warrant for further *in vitro* and *in vivo* investigations.

5. Acknowledgments

We would like to thank by Dr. Salemkour N, the center of scientific and technical research on arid regions (C.R.S.T.R.A), Biskra, Algeria, for the identification of the plant material.

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