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Antioxidant Activity of Leaf Extracts from *Zygophyllum coccineum* L. Collected from Desert and Coastal Habitats of Egypt

El-Shora, H M*, El-Amier, Y. A. and Awad, M.H

Botany Department, Faculty of Science, Mansoura University, Egypt
**Corresponding author*

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A B S T R A C T

Zygophyllum coccineum L. (Family: Zygophyllaceae) was collected from coastal and desert habitats from Egypt. The plant leaves from each habitat were collected and used in preparation of the various extracts in hexane, methylene chloride, ethyl acetate and butanol.ZCD leaf extracts exhibited higher scavenging activity than ZCC in the four extracts. The antioxidant activity was measured using DPPH(2, 2'–diphenyl–1–picrylhydrazyl) test. Butanol fraction exhibited the highest scavenging activity. The antioxidant activity measured in every extract was dependent on the concentration of the extract. Various compounds were obtained by GC/MS and they were identified. 1-nonadecene, 9-octadecenoic acid, 2-methyl propanoic acid, *β*-sitosteol, tricosane and tetracosane were found in leaves of coastal plant (ZCC) and absent from the desert plant (ZCD). Stigmast-5-en-3-ol was found in leaves of the desert plant and not detected from the coastal plant. Docosene, 1-eicosanol, hexacosane, heptacosane, nonacosane, 6-Ethyl-5-hydroxy-2,3,7-trimethoxynaphtho quinone and pentacosane were found in the leaves of *Z. coccineum* from both habitats under investigation.

Introduction

Species of genus *Zygophyllum* represent a group of succulent plants that are salt tolerant and/or drought resistant. *Z. coccineum* is a small perennial herb with succulent leaves and somewhat whitish flowers of saline and sandy habitats near the sea. The growth and distribution of *Zygophyllum* are dependent on the soil chemical structure of its habitats (Hammoda *et al.,* 2013, Yaser *et al.,* 2015).

Plant primary metabolism produces products that enhance the growth and development of plants, howeverthey are not required for the plant to survive. This primary metabolism composed of chemical reactions which allow the plant to live. Secondary metabolism plays a pinnacle role in keeping all the of plants' systems working properly. Secondary metabolism facilitates the primary metabolism in plants (El-Shora *et al.,* 2015a).

It has been reported that secondary metabolites play important role in protection of plants from salt stress, UV light and other physical stress. Secondary metabolites utilized for engage pollinators as well as

seed dispersers, as signals (Wink, 2008).

Z. coccineum has been reported in previous studies as medicinal plant (Batanouny *et al.*, 1999, El-Shora *et al.*, 2016a). The leaves, fruits and stems are used in folk medicine as a drug active against gout, rheumatism, asthma, and hypertension. The plant is also used as a diuretic, antihistaminic, local anesthetic and antidiabetic agent(Osman and Badawy (2014).

Higher plants are the main source of medicine throughout the human history. Many plant species are still used for the traditional as well as modern symptoms of medicine since they contain several active compounds. These active compounds are normally extracted from the various plant parts (Shon *et al.*, 2003).

Some of the secondary metabolites are known by their antioxidant activity that can retard, or prevent oxidation processes. They react with free radicals, acting as oxygen scavengers, react with chelating metals and donate hydrogen atoms to the free radical (Kitazuru *et al.*, 2004, El-Shora *et al.*, 2015b).

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources (Abdallaand Roozen, 1999).

Antioxidant compounds have been used in prevention of oxidative degradation, in cosmetology and dermopharmacy (Belguidoum *et al.*, 2015). The synthetic antioxidants including BHT (butylated hydroxytoluene) and BHA and have been used widely in industry butit has been reported to have toxicological effect s(butylated hydroxyanisole).

The aim of the present investigation was to determine the antioxidant activity of various extracts and the chemical components of Zygophyllum coccineum from desert and coastal habitats.

Materials and Methods

Plant Materials

Zygophyllum coccineum L. was collected from naturally growing population in coastal (Deltaic Mediterranean coast) and inland desert (WadiHagul) of Egypt. The plant was identified by Prof. NaserBarakat, Professor of Plant Ecology, Botany Department, Minia University, Egypt.

Preparation of Leaf Powder of *Z. coccineum*

Z.coccineum leaves from the coastal plant (ZCC) and from the desert plant (ZCD)were handily cleaned, washed several times with distilled water to remove dust and other residues. The leaves were then dried at room temperature for several days till complete dryness. After drying the leaves were ground into powder and then preserved in well stopped bottles.

Processing of the Plant Material

The dried leaves of *Z.coccineum* were extracted by separating funnel with n-hexane followed by methylene chloride, ethylacetate and then butanol to give the corresponding extracts which were used for determination of antioxidant activity.

Determination of Antioxidant Activity

DPPH (2,´2– diphenyl–1–picrylhydrazyl) free radical scavenging activity of the

various prepared extracts from *Z. coccineum* leaves was determined following the method of Bozin*et al*.(2006).

Freshly prepared (0.004 % w/v) methanol solution of DPPH radical was prepared and stored at 10 C in the dark. A methanol solution of the test compound was prepared. A 10 µl aliquot of the methanol solution was added to 3 ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer at 515 nm.

% Radical scavenging activity = [1-(A_{sample}/$A_{control}$)] x 100, where A is the absorbance at 515 nm.

GC/MS Analysis of Hexane Extract

A sample of hexane extract of*Z. Coccineum* leaves from both locations were analyzed by GC/MS. The resulting compounds were identified by comparing their mass spectra with those of their analogous by NIST library (Adams, 1995). The identification of phytochemical compounds is based on the peak area (which representsthe percentage of that compound), molecular weight and molecular formula.

Results and Discussion

DPPH is a test used widely as free radical to evaluate the reducing substances (Cotelle *et al.*, 1996). It is a reagent used for estimation of the free radical scavenging activity of various plant extracts (Duan *et al.*, 2006).

Antioxidant Activity of the Various Extracts

The antioxidant activity of the various extracts including hexane, methylene chloride, ethyl acetate and butanol was determined by DDPH. It is apparent from the results that ZCD leaves exhibited higher scavenging activity than ZCC in all extracts as well as in the control as shown in Table 1. Also, butanol fraction exhibited the highest scavenging activity.

Antioxidant Activity of Hexane Extract

The antioxidant activity of hexane fraction was measured at various concentrations 100, 200, 400, 600, 800 and 1000 ppm. The results are shown in (Table 2). It is apparent that the scavenging activity of hexane extract was higher in ZCD leaves compared with ZCC.

Antioxidant Activity of Methylene Chloride Extract

The antioxidant activity of methylene chloride fraction was measured at different concentrations 100, 200, 400, 600, 800 and 1000 ppm. The results are shown in Table 3. It is evident that the scavenging activity was higher in ZCD leaf extract of methylene chloride compared with ZCC and in both cases it was dependent on the extract concentration.

Antioxidant Activity of Ethyl Acetate Extract

The antioxidant activity of ethyl acetate fraction was measured at different concentrations 100, 200, 400, 600, 800 and 1000ppm. The results are shown in Table 4. It is clear that the scavenging activity was higher in ZCD leaves compared with that of ZCC and it was dependent on the concentration.

Antioxidant Activity of Butanol Extract

The antioxidant activity of butanol extract was measured at different concentrations 10, 20, 40, 60, 80 and 100 ppm for the ZCD and 200,400,600,800 and 1000ppm. The results are shown in Table 5 andshow that for ZCD expressed higher activity than ZCC.

Table.1 DPPH Antioxidant Scavenging Activity of the Selected Fractions

Solvent	% Scavenging of . ZCC extract	% Scavenging ZCD extract
Control	1.67±0.05	1.73±0.04
Hexane	8.88±0.2	10.80±0.3
Methylene chloride	13.26±0.4	21.91±0.6
Ethyl acetate	23.13±0.3	27.60±0.5
Butanol	28.45±0.5	76.24±0.9

Table.2 DPPH Antioxidant Scavenging Activity of Hexane Fraction

Concentration (ppm)	%Scavenging of ZCC extract	%Scavenging of ZCD extract
100	4.5±0.1	4.6±0.3
200	5.5±0.2	6.5±0.2
400	6.5±0.4	7.7±0.4
600	9.5±0.3	10.3±0.5
800	13.7±0.5	14.2±0.6
1000	17.1±0.3	19.4±0.3

Table.3 DPPH Antioxidant Scavenging Activity of Methylene Chloride Fraction

Concentration (ppm)	% Scavenging of ZCC extract	% Scavenging of ZCD extract
100	5.8±0.2	12.1±0.4
200	7.1±0.2	20.4±0.5
400	10.4±0.3	21.6±0.3
600	13.2±0.6	22.6±0.5
800	17.1±0.4	24.7±0.6
1000	19.0±0.5	26.1±0.3

Table.4 DPPH Antioxidant Scavenging Activity of Ethyl Acetate Fraction

Concentration (ppm)	% Scavenging of ZCC extract	% Scavenging of ZCD extract
100	1.4±0.06	5.6±0.2
200	31.5±0.5	32.2±0.4
400	33.0±0.4	34.8±0.3
600	33.9±0.7	40.7±0.7
800	37.5±0.5	43.3±0.9
1000	37.5±0.8	49.3±0.7

Table.5 DPPH Antioxidant Scavenging Activity of Butanol Fraction

	Concentration (ppm)	% Scavenging activity
ZCD extract	10	07.6±0.2
	20	21.8±0.4
	40	23.2±0.6
	60	24.7±0.6
	80	27.6±0.5
	100	29.2±0.4
ZCC extract	100	05.7±0.2
	200	22.7±0.4
	400	26.3±0.5
	600	39.8±0.7
	800	47.9±0.5
	1000	46.7±0.8

Table.6 MS Data of Compounds Identified by GC/MS Analysis

The phytochemical compounds	
Compounds in ZCC only	**MW**
1-nonadecene	268
9-Octadecenoic acid	282
2-methyl propanoic acid	350
Stigmasterol	412
β-sitosteol	414
Tricosane	324
Tetracosane	338
Compounds in ZCC and ZCD	**MW**
Docosene	308
1-Eicosanol	298
Hexacosane	366
Heptacosane	380
Nonacosane	408
6-Ethyl-5-hydroxy-2,3,7-trimethoxynaphtho quinone	292
Pentacosane	352
Compounds in ZCDonly	**MW**
Stigmast-5-en-3-ol	414

Thus, the present results showed antioxidant activity in all various extracts. In support, previous studies on *Z. coccineum* reported antioxidant and antimicrobial activities as showed in literature survey (Gibbons and Oriowo, 2001).

Chemical Analysis

Sample from n-hexane extract of *Z. coccinum* leaves from costal and desert habitats were analyzed by GC/MS technique, which resulted in identification of several compounds (Table 5).These compounds were identified by comparing their mass spectra with those of their analogous reported by NIST library (Admas, 1995).

It was observed that stigmast-5-en-3-ol is present in leaves of desert *Z. coccineum* and absent from the coastal one (Table 6). However, there are 7 compounds present in leaves of the coastal plant but absent from the desert plant. They were tricosane, tetracosane, 1-nonadecene, 9-octadecenoic acid, *β*-sitosteol and stigmasterol.

As general, phytochemicals include two main groups according to their functions in the plant : primary and secondary metabolites. The primary metabolites are amino acids, proteins, sugars and chlorophyll whereas the secondary metabolites consist of alkaloids, tannins, saponins, flavonoids, terpenoids, and phenolic compounds (Krishnaiah *et al.,* 2007). *Z. coccineum* exhibited appreciable amounts of saponins, flavonoids, alkaloids and total phenol (El-Shora *et al.,* 2016b).

Higher content of flavonoids as secondary metabolites was found in the crude extractand butanol fraction of *Z. album*(El-Ghoul*et al*., 2011; Belguidoum *et al.*, 2015). Flavonoids are well known as antioxidants and are compounds of low molecular weight, involving fifteen carbon atoms and organized in a C6–C3–C6 configuration. The structure consists of two aromatic rings, A and B, connected by a 3-carbon bridge, usually in the form of a heterocyclic ring, C (Merken and Beecher, 2000).

Thus, it seems likely from the results that the antioxidant activity of *Z. coccineum* leaf extracts may be due to the various bioactive compounds detected in the plant leaves.

In conclusion, the present results showed that the various extracts of Z. *coccinum* obtained from the leaves exhibited an antioxidant activity particularly those of the desert plant. This proves that *Z. coccinum* can be used as natural source for antioxidant instead of the synthetic antioxidants which are carcinogenic and harmful to mankind.

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