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Antioxidant and Quantitative Estimation of Phenolic and Flavonoids of Three Halophytic Plants Growing in Libya

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ABSTRACT

Halophytic plants are more susceptible for oxidative stress and damage due to high contents of salt and minerals inside these plants. Therefore, self defence against this oxidative stress appeared in the high phenolics particularly, flavonoids content are abundant in these plants. *Mesembryanthemum crystallinum, Limoniastrum guyonianum* and *Anabasis articulate* are three of halophytic plants growing in Mediterranean coast of Libya and most of North African countries, were taken as example for estimating the phenolic and flavonoids contents as well as antioxidant evaluation in order to understanding the effect of habitat of these plant imitation on the by-products production. Our present work suggested that, there are high relations between the qualitative and quantitative constituent of these halophytic plants which growing near to each other in the same environment.

Keywords: Antioxidant activity; Phytochemical screening; *Mesembryanthemum crystalline; Limoniastrum guyonianum; Anabasis articulate*.

1. Introduction

Oxidation is the transfer of electrons from one atom to another [1]. It represents an essential part of our metabolism and aerobic life in general, since oxygen is the ultimate electron acceptor in the electron flow systems that transport energy in the form of ATP [2]. Problems may arise however when the electron flow generates free radicals, such as O_2-centred free radicals, known as reactive oxygen species (ROS), and including superoxide (O_2^{-}), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$), hydroxyl ($HO^{\cdot}$) and nitric oxide ($NO^{\cdot}$) radicals [3].

ROS may be very damaging, since they can attack lipids in cell membranes, proteins in tissues or enzymes, carbohydrates and DNA to induce oxidative modifications, which cause membrane damage, loss of protein function and DNA damage [4]. This oxidative damage is considered to play a causative role in ageing and several degenerative diseases associated with it, such as heart disease, congestive dysfunction and cancer[5]. Humans have evolved antioxidant systems to protect against free radicals. These systems include some antioxidants produced in the body (endogenous antioxidants) and others obtained from the diet (exogenous antioxidants).

Flavonoids are a group of naturally occurring polyphenolic compounds ubiquitously found in plants [6]. Flavonoids first appeared in green algae 500 million years ago, resulting from the fusion of two biogenetic pathways, namely the cinnamate and the ancient polyketide route and they have then become more and more complex with plant evolution [7].

flavonoids have long been recognized to possess anti-inflammatory, antioxidant, ant-allergic and hepato-protective properties. They are also believed to be antithrombotic, antibacterial, antifungal, antiviral, and cancer protective, and also to protect against cardiovascular disease [8-10].

Numerous publications have investigated the antioxidant activities of flavonoids and how they can contribute to the treatment of several diseases. Considering these publications, they indicate that biological and pharmacological effects of flavonoids may depend upon their behaviour as either antioxidants or as prooxidants [11]. Some flavonoids can behave as both antioxidants and prooxidants, depending on concentration and the redox environment present. For instance, certain flavonoids act as antioxidants against free radicals, yet demonstrate prooxidants activity when a transition metal such as Cu_2+is present [12].

The chemistry of the flavonoids are predictive of their free radical scavenging activity as the reduction potentials of flavonoids and the consequently radical form, are lower than those of alkyl peroxyl radicals and the superoxide radical, which therefore means the flavonoids may inactivate these radical species and prevent the deleterious consequences of their reactions [13-16].

Halophytes grow in a wide variety of saline habitats, from coastal regions, salt marshes and mudflats to inland deserts. In these saline areas, unfavourable environmental conditions increased production of reactive oxygen species (ROS) in plants, leading to cellular damage, metabolic disorders, and senescence processes [17, 18].

Halophytes are known for their ability to overcome and quench these toxic ROS, since they are equipped with a powerful antioxidant system [19, 20].

In our present work, three of halophytic rich polyphenolic plants with a great history usage in the folk medicine as shown in table (1), under quantitative estimation for the general phenolic and specific flavonoids contents. Exerting the antioxidant activity of these plants extracts may help understanding why physicians and traditional medicine practitioners give these plants a great interest in folk medicine.

Table 1: Systemic names, common names and traditional uses of investigated plants.

Botanical name	Common name	Family	Traditional use	Ref
Mesembryanthemum crystallinum	Crystalline ice plant Slender-leaf ice plant	Aizoaceae	Physicians used leaf juice to soothe inflammation of the mucous membranes of the respiratory or urinary system. In Europe, the fresh juice has been used to treat water retention, painful urination and soothe lung inflammation	(Bouftira Ibtissem, 2012)[21]
Limoniastrum guyonianum	Alzaia	Plumbaginaceae	treat gastric infections and bronchitis	Mounira Krifa et al. 2013 [22]
Anabasis articulata	Ajrem	Chenopodiaceae	treat diabetes, fever, kidney infections, headache and skin diseases such as eczema	(Hammiche and Maiza, 2006; Hmamouchi, 1999)[23,24]

2. Materials and Methods

2.1 Plant collection and preparation of extracts:

Fresh herbs of plants under investigation were collected from the coast of the Mediterranean Sea near Benghazi, Libya. The plants were identifies by comparison with standard sample in the herbarium of faculty of science, Benghazi University. The plants were washed with tap water and left for drying in the open air. 100 gram of the plants dried powders was gradually extracted by continuous soxhlation with petroleum ether, chloroform, ethyl acetate and ethanol (500ml), respectively. All fractions were evaporated to dryness using rota vapour (IKA-WERKE, GMBH& Co. Kg, D-79219 Staufen, Germany) and the extractive value for each fraction was calculated and mentioned in table (2). The different fractions were reconstituted in their extraction solvent to give the required concentration needed in this study.

Table 2: The extractive values obtained for the plants with the different solvents by gram percent.

Plants Solvent	petroleum ether	chloroform	ethyl acetate	Ethanol
Mesembryanthemum crystallinum	2.5 g	1.1 g	0.33 g	3.0 g
Limoniastrum guyonianum	3.5 g	2.1g	1.2	2.8
Anabasis articulate	0.9 g	1.2 g	0.75 g	2.8 g

2.2 Preliminary Phytochemical Screening of the Different Plants Extracts:

Preliminary screening of the different extracts were performed to investigate the presence or absence of the different phytochemical constituents such as phenolics, flavonoids, tannins, saponins and alkaloids using standard procedures described by Alex *et al.* [25]. The result showed in table (3).

2.3 Quantitative Estimation of Total Phenolic Constituents:

Total phenol contents of different extracts were determined by the modified Folin-ciocalteu method according to Omoruyi *et al* [26]. An aliquot of 0.5 ml of each extract (1 mg/ml) was mixed with 2.5ml Folin- Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 2ml (75% w/v) of sodium carbonate (Na_2CO_3). The tubes were vortexed for 15 s and allowed to stand for 30min at 40 °C for colour development. Absorbance was then measured at

765nm using spectrophotometer (Spectro UV-VIS double, 110V, 60Hz, Serial No. Double 001158, Labomed, Inc. U.S.A.). Total phenolics content of different extracts were expressed as mg/g tannic acid equivalent using the following equation from the calibration curve: Y = 0.4879×, R2= 0.9064, where × is the absorbance and Y is the tannic acid equivalent in mg/g. The experiment was conducted in triplicate and the results were expressed as mean ± SD values.

2.4 Quantitative estimation of total flavonoids:

Total flavonoid contents of different extracts were determined by method described by Ordonez *et al*. based on the formation of a flavonoid-aluminium complex [27]. 0.5ml of various solvent extracts (1mg/ml) was mixed with 0.5ml of aluminium chloride prepared in (2% in ethanol). The resultant mixture was incubated for 60min at room temperature for yellow colour development which indicated the presence of flavonoid. Absorbance was measured at 420nm using UV–VIS spectrophotometer. Total flavonoid content was calculated as quercetin equivalent (mg/g) using the following equation based on the calibration curve: Y = 0.217x, R^2 = 0.9582, where × is the absorbance and Y is the quercetin equivalent.

2.5 Quantitative estimation of total flavonols:

Total flavonoid contents of different extracts were determined by method described by Omoruyi *et al.* [26]. The reaction mixture consisting of 2 ml of the sample, 2 ml of aluminium chloride prepared in (2% in ethanol) and 3 ml of sodium acetate solution (50 gm/l) was allowed to incubate for 2.5 h at 20 °C. Absorbance at 440 nm was measured. Total flavonol content was calculated as mg/g of quercetin equivalent from the calibration curve using the equation: Y Y = 0.217×, R^2 = 0.9582 where × is the absorbance and Y is the quercetin equivalent.

2.6 Scavenging activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical:

The effect of extracts on DPPH radical was estimated using the method of Hosny M. *et al.* [28], with some modification. 1.9ml of DPPH-ethanol solution (300µM) was mixed with 0.1ml of different concentrations (6.5–500µg/ml) of various extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30min. The absorbance of the mixture was measured spectrophotometrically at 517nm. Quercetin, vitamin C and Butylated hydroxyl anisole (BHA) were used as standard drugs. The percentage of free radical scavenging was calculated according to the following equation: % scavenging = (1-Sample absorbance517/blank absorbance517) X 100.

2.7 Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates.

3. Result

3.1 Result of phytochemical screening and quantitative analysis:

The result of the preliminary phytochemical screening give a clear evidence for the presence of phenolics, flavonoids, tannins and alkaloids in addition to carbohydrates and sterols. The tests also revealed that the absence of anthraquinones. and saponins. Table 3 showed the results in a qualitative manner. Quantitative analysis results for the total phenolic and flavonoids of different plants extracts showed in table 4**.**

Table 3: Results of phytochemical screening for the different plants extracts

Constituents / Plants extracts	*Mesembryanthemum crystallinum*				*Limoniastrum guyonianum*				*Anabasis articulate*			
	pe	ch	ac	Et	pt	Ch	ac	Et	Pe	ch	ac	Et
Phenolics	-	+	++	+++	-	+	+	++	-	+	++	+++
Flavonoids	-	+	++	+++	-	+	+	++	-	+	++	+++
Tannins	-	-	++	+++	-	-	+	+++	-	-	++	+++
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	++	+++
Saponins	-	-	-	+	-	-	-	-	-	-	+	+
Carbohydrates	-	-	-	++	-	-	+	++	-	-	-	++
Sterols	+++	+	-	-	+++	++	+	-	++	+	-	-

Key: −ve (Absent), + (Low in abundance), ++ (Moderate in abundance), +++ (High in abundance), pe: petroleum ether extract, ch: chloroform extract, ac: ethyl acetate extract, et: ethanol extract.

Table 4: Results of quantitative estimation of total phenolics, flavonoids and flavonols

Plants	Extracts	Total phenolics	Total flavonoids	Total flavonols
Mesembryanthemum crystallinum	ethanol extract	467.89±0.28	156.12±3.52	5.36±4.14
	e. acetate extract	139.53±0.84	70.74±1.56	0.97±1.29
	chloroform extract	48.30±3.97	29.76±2.12	0.48±0.56
Limoniastrum guyonianum	ethanol extract	361.04±3.42	101.32±3.94	5.60±7.04
	e. acetate extract	40.00±2.12	18.54±5.17	1.95±0.56
	chloroform extract	5.85±1.97	6.34±6.83	0.73±0.74
Anabasis articulate	ethanol extract	491.80±3.97	121.08±0.12	25.90±2.09
	e. acetate extract	195.16±0.01	63.52±0.24	15.73±0.14
	chloroform extract	177.10±1.84	41.44±0.12	14.43±0.33

Result were expressed as mean ± slander deviation

3.2 Result of antioxidant activity:

The result of antioxidant activity of the plants extracts was exerted as the inhibitory effects of these extracts against DPPH stable free radical. The IC_{50} inhibitory concentration of ethanolic, ethyl acetate and chloroformic extract of the plants as well as quercetin, ascorbic acid and butaylated hydroxy anisole (positive control), are expressed in the table **5**. Inhibitory effects of plants extracts at 250µg/ml also showed in the figure **1**.

Table 5: Result of DPPH inhibition by the plants extracts as well as positive controls (the results expressed IC_{50} in µg of extracts /ml)

Plants and controls	IC_{50} of DPPH inhibition in µg/ml		
	Ethanolic extracts	Ethyl acetate extracts	Chloroform extracts
Mesembryanthemum crystallinum	354.16±8.80	391.82±5.05	Nd
Limoniastrum guyonianum	325.66±4.12	397.51±6.41	Nd
Anabasis articulata	149.26±3.23	246.38±4.01	416.61±6.19
Quercetin	9.54± 1.33		
Ascorbic acid	11.42±3.17		
Butaylated hydroxy anisole	13.10±5.99		

Key: nd: IC_{50} not detected up to 500 µg/ml

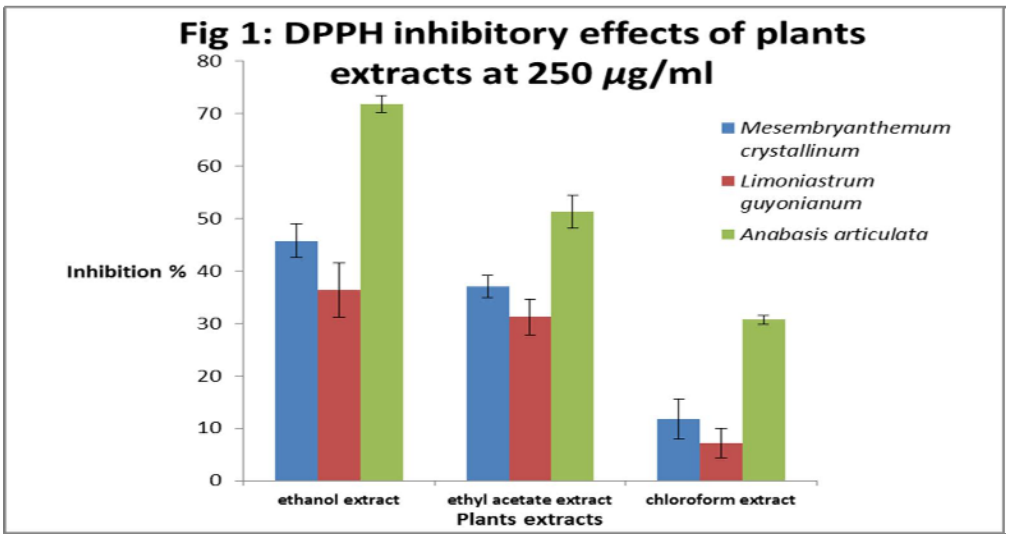

Fig 1: Dpph inhibitory effects of plants extracts at 250 ug/ml

4. Discussion

The result of phytochemical screening revealed the strong phytochemical relation within plants under investigation. Generally, phenolic, flavonoids and tannins represented in the chloroform, ethyl acetate, and ethaolic extracts with a special concern to the later one. Carbohydrates and sterols also detected in ethanolic and petroleum ether extracts of plants, respectively. Furthermore, Phytochemical screening tests revealed the absence of anthraqinoun and alkaloids except for the presence of the later one in the ethyl acetate and ethanol extract of *Anabasis articulate* (table **3**).

On the other hand, quantitative estimation of the phenolics and flavonoids in the extracts of our present plants give a clear evidence for the high quantities of these by- products percentage. With some variation within investigated plants, there is some kind of relation between these plants phenolic and flavonoids contents (table 4).

The point of difference in the quantities estimation is the presence of high contents of phenolic and flavonoids in the anabasis *articulate* and *Mesembryanthemum crystallinum* compared to *Limoniastrum guyonianum*. The estimation revealed also a remarkable high quantity of flavonols in *anabasis articulate* extracts compared to other plants under investigation (table 4). These results may be give a reason for the activity of these plants as antioxidant and how these plants extracts enable to scavenge the free radicals.

The result of scavenging activity of plants extracts as well as controls indicate that, all controls were more active than those of plants extracts with IC_{50} 9.54, 11.42 and 13.10 µg/ml for quercetin, ascorbic acid and butaylated hydroxy anisole, respectively (table **5**). Among all plants extracts, *Anabasis articulate* ethanol and ethyl acetate extracts showed a highest activity compared with all other plants extracts. Furthermore chloroformic extract of *Anabasis articulate* showed IC_{50} 416.61 µg/ml that cannot be detected in the same extract of other two plants (table **5**). Figure **1** on the other hand gave comparative pars for the antioxidant activity of ethanolic, ethyl acetate and chloroformic extracts of plants at 250µg/ml. Result showed in figure **1** revealed that, *Anabasis articulata* extracts were more active compared to other plant extracts with inhibition activity against DPPH colour 71.83, 51.27 and 30.67 for the extracts, respectively. The figure **1** also indicated that *Mesembryanthemum crystallinum* extracts showed intermediate activity and lower activity was measured or *Limoniastrum guyonianum* extracts.

Comparing the previous antioxidant results with those of quantitative estimation of total phenolic, flavonoids and flavonols for all plants extracts give clear evidence for the effects of these constituents as antioxidant and increasing in the quantities of these constituent conflicts increasing in scavenging power. The study also may give interesting point for the flavonols contents of these plants and effects of these flavonols in the scavenging power of the plants extract and strong antioxidant activity of *Anabasis articulate* compared to other plant mostly due to its high contents of flavonols.

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