Antioxidative And Free Radical Of Limonium Axillare From Qatarian Coasts

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Abstract

In the present study, we carried out the antioxidant and antiradical activity of a methanol extract from the leaves of the halophyte plant *Limonium axillare* (family: Limoniaceae, order: Plumbaginales). Antiradical activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and antioxidant activity was characterized by NBT/Riboflavin system. The antiradical effect of *L. axillare* (95 ± 0.5% DPPH scavenging at 1mg/ml) was greater than the synthetic antioxidant BHA. The antioxidant activity (92.96 ± 0.5% of superoxide inhibition) was higher than the standard quercitin. The phytochemical screening of the leaves samples indicates the presence of flavonoids, steroids and alkaloids.

Introduction

The Arabian Peninsula is today one of the most important regions in the world in terms of halophyte development and research. In the same time, the Arabian Peninsula is one of the most severely affected regions in the world in terms of water scarcity, and soil salinisation as well as groundwater salinisation [1]. Qatar is a peninsula located half way along the western coast of the Arabian Gulf. It covers an area of 11,437 sq. km including a number of coastal islands. The total area of Qatar’s sea-water is approximately 35,000 sq. km. The high evaporation of the water especially in summer, the very low rainfall, and the low inflow of freshwater are the main factors which produce high sea water salinity. Halophytes are highly evolved and specialized organism with well-adapted morphological and physiological characteristics, allowing them to proliferate in the soils possessing high salt concentrations [2]. There is increasing evidence that salinity is one factor leading to oxidative stress in plants cells [3,4,5]. To mitigate the oxidative damage initiated by ROS, plant have developed a complex antioxidative system [6]. Non-enzymatic antioxidants such as ascorbic acid, α-tocopherol, flavonoids and phenolics acids are present in the halophytes plants. For example, the halophyte plant *Crithmum maritimum* or sea fennel (family: Apiaceae) contains vitamin C, carotenoids [7]. *Limonium axillare* (family: Limoniacea, order: Plumbaginales.) contain flavonoids [8]. In the halophyte plant *Mesembryanthemum crystallinum* (family: Aizoaceae, order: Caryophyllales) high photosynthetic active radiation and ultraviolet irradiance induce the accumulation of flavonoids [9]. Flavonoids are a group of polyphenolic compounds with known properities which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [10]. Many phenolics, such as flavonoids, have antioxidants capacities that are much stronger than those of vitamin C and E [11]. Saponins are glycosidic compounds composed of a steroid (c-27) or triterpenoid (C30) saponin nucleus with one or more carbohydrate branches. Saponins produced by plants cells used to solubilize membrane proteins, are capable of decreasing tumor cell proliferation [12]. The proposed mechanism of anticarcinogenic properties of saponins includes antioxidant effect, direct and select cytotoxicity of cancer cells. *Limonium axillare* is a low-branched, salt-secreting, woody shrub belongs to the superorder Malviflorae, the order Plumbaginales and Limoniacea family. Plants from this family are characterized by medicinal properties and biological activity such as antiviral activity of *Limonium sinens* (family: Limoniacea, order: Plumbaginales), antimicrobial activity of *Limonium californicum* (family: Limoniacea, order: Plumbaginales) and antitumoral activity of *Limonium axillare* [13].

*Limonium axillare* is distributed in high coastal marshes as well as rocky grounds. It is a highly-salt tolerant plant that grows in coastal areas under high salinity. The economic potential of this species as an ornamental plant for coastal saline areas is great. It is also an important component of littoral ecosystem of Qatar.

The aim of the present study consisted in screening the potential antioxidant properties of *Limonium axillare* extract.
Methods

Preparation of the extract

Plant materials were collected since their native biotope in May 2006, by El Khour city (Qatar) at flowering stage. Limonium axillare is the plant of the year 2006 for the program flower each spring (Freinds of the Environment Center, Qatar).

Plants samples were dried on wire gauze at 40 °C. For extraction, 10.5 g of powdered dry plant material was mixed with 100 ml methanol and stirred at 500rpm for 24h at 20°C. After stirring and filtering under vacuum, the filtrate was evaporated to dryness in Rotavapor [yield 0.49 g (4.8%)] [14].

Phytochemical screening

Chemical screening was carried out on the powdered samples using standard procedures to identify the constituents [15, 16, 17, 18].

Tannins: About 0.5g of powdered samples was boiled in 6ml water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for blue-black coloration.

Saponins: About 2g of the powdered plant sample was boiled in 10ml of distilled water. 6ml of the filtrate was shook vigorously and observed for the formation of emulsion.

Flavonoids: A portion of the powdered plant sample was boiled with methanol and then filtered. 1ml of hydrochloric acid and Magnesium turner was added to the filtrate. A red coloration was observed indicating a positive test for flavonoids.

Steroids: A portion of powdered plant sample was heated with chloroform and filtered. To 1/5 of the filtrate was added acetic anhydride and H₂SO₄. The color changed from violet to blue or green indicating the presence of steroid.

Alkaloids: 0.5g of dried powdered samples was boiled in 6ml water and then filtered. Reactive of Drangendorf was added to the filtrate, the presence of alkaloid was indicated by the apparition of a precipitate.

Coumarines: A portion of powdered plant sample was mixed with alcohol 95%, heated over a steam bath and filtered. NaOH was added and the changes in color observed under UV lamp indicate the presence of coumarines.

Iridoides: About 2g of the powdered plant sample was boiled with distilled water for 10 min. After filtration, 1ml hydrochloric acid was added to the filtrate and heated over a steam bath. The apparition of black precipitate indicates the presence of iridoïdes.

Antioxidant activity

1) DPPH free radical scavenging activity

Free radical scavenging activity of plant extract was determined by using a stable free radical, (1, 1-diphenyl-2-picrylhydrazyl) DPPH [19]. DPPH solution was prepared at the concentration of (0.024mg/ml DPPH in ethanol). During assay 1 ml of the crude extract was mixed with 1 ml DPPH solution. The mixture was incubated in the room temperature for 30 min; absorbance was recorded at 517 nm (Cam spec M230/330 UV visible spectrophotometer, United Kingdom). Butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) were used as a standard for the investigation of the antiradical activity.

The percentage of remaining DPPH⁺ (%DPPHREM) at the steady state was determined as follows:

%DPPHREM = 100 C_{DPPH(t=0)} / C_{DPPH(t=0)}

Where C_{DPPH(t=0)} is the initial DPPH concentration and C_{DPPH(t=0)} is the DPPH concentration at the steady state.

2) Scavenger effect on superoxide anion

Antioxidant activity can be measured by NBT/Riboflavin method [20]. 100µl of the analysed solution was kept with (516.12 mM) phosphate buffer, (6.45 mM) EDTA, (0.096 mM) NBT and (3.87 10⁻³ mM) Riboflavin. The reaction was started by exposing the mixture to cool white fluorescent light at a photosynthetic photon flux of 50 μmol m⁻² s⁻¹ for 5 min. The absorbance of the blank (contain all composites with exception of NBT) and the 100% solution (contain all composites with exception of plant extract) was measured at 560nm (Cam spec M230/330 UV visible spectrophotometer, United Kingdom). Quercitin was used as a standard for the investigation of the antioxidant activity.

The percentage of antioxidant activity was determined as follows:

PI= (1-(DO_{Extract}/DO_{100%})) x 100

DO_{Extract}: absorbance of the extract solution
DO_{100%}: absorbance of 100% solution

Results

Antiradical activity was evaluated using DPPH scavenging activity, the synthetic antioxidant BHT and BHA were used as a standard (figure 1). L. axillare extract showed a higher antiradical activity (95 ± 0.5% at 1mg/ml) in comparison with BHA (95 ± 0.1% at 5mg/ml).
The antiradical activity of *L. axillare* extract was similar to the antioxidant BHT when the concentration increased to 2 and 5 mg/ml.

Antioxidant activity was determined in our study using NBT/Riboflavin method. The capacity of extract to inhibit the formation of superoxide anions was compared with a standard quercitin (figure 2). Antioxidant activity was higher in *Limonium axillare* (92.96 ± 0.5% at 0.3 mg/ml) extract in comparison with quercitin (75 ± 0.2% at 0.3 mg/ml).

The antioxidant activity in the medicinal plant *L. axillare* was confirmed in our study by the presence of chemical compounds (table 1) such as flavonoids, steroids, saponins and alkaloids.

### Discussion

A large number of epidemiological studies have indicated that reactive oxygen species (ROS) are implicated in the development of numerous pathological processes such as atherosclerosis and cancerogenosis [21]. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds [22].

The result of phytochemical screening showed that the leaves of the halophyte plant *Limonium axillare* were rich in flavonoids, alkaloids and steroids. The absence of tannins in *L. axillare* in the present study is in contrast with the opinion of Ahmed et al. [23] who noted that the tannins in *L. axillare* exhibited a high antitumoral activity. The presence of flavonoids in *L. axillare* has been reported by other researchers. A flavonol glycosides was isolated from *L. axillare* [24]. This isolated compound was investigated for the assay of cytotoxic activity in comparison with leaves alcoholic extract [13]. Our results showed that *L. axillare* contained steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones [25].

Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical methods is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [26]. This method was confirmed in our study by the use of NBT/Riboflavin system for the investigation of scavenger effect on superoxide anion.

The methanol leaf extract from *L. axillare* exhibited more than 70% scavenging activity in comparison with standard (figure 1 and 2). The antioxidant activity was higher than quercitin and the antiradical activity was higher than BHA. The extract of *L. axillare* tested in the present study was in crude form, at this stage, it is not possible to say which compounds are responsible for the observed effects. However, our data suggest that the high antioxidant activity exhibited by this plant, under these experimental conditions could be related to the presence of flavonoids in plant leaves. Flavonoids are phenolic substances isolated from a wide range of vascular plants [27]. They act in plants as antioxidants and an important structure-activity relationship of the antioxidant activity have been established. Many medicinal plants are investigated for their antioxidant activity in correlation with the metabolites present in plants materials. For example, Pourmorad et al. [28] showed that the antioxidant activity in *M. officinalis* was greater than that of the synthetic antioxidant BHT in scavenging of DPPH free radical. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract. In the medicinal plant *Prunella vulgaris* the organic fraction posses a marked antioxidant activity confirmed by the presence of phenolic acids and other phenolic compounds [29]. The sweet potatoes (*Ipomoea batatas*) ethanol extract (100 mg dry material/ ml) had a DPPH scavenging activity directly related to the total amount of phenolic and flavonoid found in the sweet potato’s extract [30].

### Conclusion(s)

In conclusion, our experimental evidence shows that *L. axillare* extract exhibits interesting antioxidant properties correlated with its chemical composition and expressed by its capacity to scavenge DPPH and $O_2^-$. These finding suggest that medicinal plants like *L. axillare* hold promise as source of chemical leads for the development of new drugs.

### Acknowledgement(s)

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Reference(s)

Illustrations

Illustration 1

Table 1. Chemical screening of plant samples

<table>
<thead>
<tr>
<th>Plant</th>
<th>Limonium axillare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
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<tr>
<td>Alkaloids</td>
<td>+</td>
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<tr>
<td>Coumarins</td>
<td>-</td>
</tr>
<tr>
<td>Irridoides</td>
<td>-</td>
</tr>
</tbody>
</table>
Illustration 2

Fig 1. Antiradical activity (BHT and BHA were used as a standard)
Illustration 3

Fig 2. Antioxidant activity (Quercitin was used as a standard)
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