Assessment of antioxidant potential and α-amylase and acetylcholinesterase inhibitory activities of Urginea maritima (L.) Baker bulbs

A. M. Maazoun1,2, F. Belhadj1, J. M. Ben Jemâa2, M. N. Marzouki1

1Laboratory of Protein Engineering and Bioactive Molecules, (LIP-MB), National Institute of Applied Sciences and Technology, INSAT, Tunis Cedex 1080, University of Carthage, Ministry of Higher Education and Scientific Research, Tunisia

2Laboratory of Biotechnology Applied to Agriculture (LBAA); National Agricultural Research Institute of Tunisia, INRAT, Tunis Cedex 2080, University of Carthage, Ministry of Higher Education and Scientific Research, Tunisia

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asmamaazoun@gmail.com
Phone: +21629883165

Abstract
Recently, there has been worldwide interest in finding new and safe antioxidants from natural sources because of their significance in treating various metabolic syndromes and chronic disorders. In this study, we aimed to evaluate the antioxidant potential of Urginea maritima bulb extract. Numerous in vitro models were used in order to highlight the antioxidant potential of the methanolic extract of U. maritima bulbs. In addition, the effects of U. maritima bulbs extract against α-amylase and acetylcholinesterase activities were explored. Results indicated that U. maritima bulbs extract exhibited a high antioxidant potential in the various in vitro systems. Moreover, the investigation of the protective effect of U. maritima bulb extract against induced lipid peroxidation in a biological matrix showed its effective protection. In addition, U. maritima bulb extract revealed an important inhibitory power against α-amylase (IC50 = 95.03 ± 1.29 µg/mL) and acetylcholinesterase (IC50 = 72.19 ± 1.48 µg/mL) activities.

1. Introduction
In recent years, there has been a growing interest regarding free radicals, due to the key role played in the oxidant reactions, which occur in human body [1]. Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in orbitals. The most important class of radical species generated in living systems is reactive oxygen species (ROS) [1]. It is a well-documented fact that exposure of organisms to exogenous factors generates an overproduction of the ROS, inducing oxidative stress [2]. The overproduction of the ROS plays pivotal roles in various pathogenesis such as cancer, cardiovascular diseases, atherosclerosis, hypertension and neurodegenerative [3]. Therefore, many synthetic antioxidants were used to strengthen the defense of the endogenous system but recently works reported that they have dangerous effects to human health [4]. Hence, antioxidants from natural sources such as plants are looked for their potential to replace synthetic ones [4]. In fact, plant derived antioxidants present a potentially safe solution to protect the human body and to reduce the risk of illnesses.

Urginea maritima isa medicinal plant in the Liliaceae family commonly found in Africa and Asia [5-6]. Urginea species are used in traditional medicine for various diseases due to their cardiotonic, antiepileptic, antiasthmatic, dermatological and diuretic properties [7-8-9]. The medicinal parts are the bulbs [10]. However, despite the widespread folklore uses of U. maritima bulbs in the management of numerous pathogenesis [11], there is no comprehensive data reported on the antioxidant activity of U. maritima bulbs. In addition, it is reported that plants belonging to the genus Urginea are a rich source of phenolic compounds. In this context, Banani et al., (2015) [12] reported polyphenols as major components of Urginea indica bulbs. Similarly, Maazoun et al., (2017) [13] showed that phenolic compounds formed U. maritima bulbs, majorly.

Indeed, *U. maritima* bulbs were found to be rich in polyphenols (130.88 ± 0.44 mg GAE/g FW), flavonoids (50.81 ± 0.25mg CE/g FW) and condensed tannins (6.76 ± 0.10 mg CE/g FW) [13]. These phytochemicals are well known for their antioxidant potential [14]. Their antioxidant potential, due to their redox properties, make them able to act as reducing agents, hydrogen donors, free radical scavengers and metal chelators [15].

Therefore, the aim of this research work was to assess the antioxidant activity and to bring out α-amylase and acetylcholinesterase inhibition power of *U. maritima* bulb extract.

2. Material and Methods

2.1. Chemicals

Methanol, sodium hydroxide (NaOH), hydrochloric acid (HCl), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferrous sulfate (FeSO₄ 7H₂O), potassium ferrocyanide [K₃Fe(CN)₆], trichloroacetic acid (TCA), ferric chloride (FeCl₃), ethylenediamine-tetraacetic acid (EDTA), ferrous chloride (FeCl₂), ferrous sulfate (FeSO₄), ferrozine, iron-EDTA (ferrous ammonium sulfate, EDTA), riboflavin, nitro blue tetrazolium (NBT), butylated hydroxytoluene (BHT), thiobarbituric acid (TBA), ascorbic acid, dimethyl sulfoxide (DMSO), Nash reagent, starch, 3,5-dinitrosalicylic acid (DNS), acarbose, acetyltihocholine iodide (AChI), 5,5’-dithiobis [2-nitrobenzoic acid] (DTNB), AChE from *Electrophorus electricus*, galanthamine hydrobromide from *Lycoris* sp.; All chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Plant material

Fresh bulbs of *U. maritima* (L.) Baker were collected from the region of RasJbel in the North East of Tunisia (37° 12’ 54” North 10° 07’ 26” East). The bulbs were washed with water to remove all debris and cut into small pieces. Plant materials were then ground in a mortar with a pestle.

2.3. Preparation of Urginea maritima bulb extract

The ground material (5 g) was macerated with organic solvent (50 mL of methanol) for 24 h at room temperature. Light exposure was avoided during the extraction process. The mixture was then filtered and centrifuged at 3500 rpm for 30 min. The solvent was removed by evaporation to dryness under reduced pressure by a Rotavapor.

2.4. Biological matrix

A freshly dissected sheep brain was purchased from a local market and was used as a biological matrix for lipid peroxidation assay.

2.5. Evaluation of antioxidant activities

2.5.1. DPPH radical scavenging assay

The DPPH radical scavenging activity was measured by the method of Braca et al., (2001) [16]. Different concentrations of the methanolic extract of *U. maritima* bulb were prepared (10-1000 µg/mL). Each sample (25 µL) was incubated in the dark at room temperature with 1 mL of DPPH (0.04 mM). After incubation for 30 min, the absorbance was measured at 517 nm. Three determinations were made for each sample. BHT was used as standard. The DPPH radical scavenging activity was calculated using the following equation:

\[
\text{DPPH radical scavenging activity} \hspace{1mm} (%) = \frac{\left( A_0 - A_1 \right)}{A_0} \times 100
\]

Where \( A_0 \) was the absorbance of the control and \( A_1 \) was the absorbance of the sample.

2.5.2. Reducing power

The reducing power was determined according to the method of Oyaizu, (1986) [17]. A volume of 100 µL of various concentrations (10-1000 µg/mL) of *U. maritima* bulbs extract was mixed with 100 µL of phosphate buffer (0.2 mM, pH 6.6) and 100 µL of \( K_3Fe(CN)_6 \) (1%). The mixture was heated at 50°C for 20 min. Then, 100 µL of TCA (10%) was added and the solution was centrifuged at 3000 rpm for 10 min. The supernatant (400 µL) was mixed with 400 µL of \( H_2O \) and 80 µL of \( FeCl_3 \) (0.1%). After 10 min at room temperature, absorbance was measured at 700 nm. Three tests were performed for each sample. Ascorbic acid was used as standard.

2.5.3. Chelating effect on ferrous ions

The chelating effect on ferrous ions was estimated using Decker and Welch, (1990) [18]. Different concentrations of *U. maritima* bulb extract were prepared (2-50 µg/mL). Each sample (300 µL) was mixed with 300 µL of FeSO₄, 6H₂O (0.125 mM). After 5 min at room temperature, 300 µL of ferrozine (0.31 mM) were
buffer (20 mM, pH 7.5) were incubated during 2 µL.
The acetylcholinesterase activity inhibition was measured using an adaptation of the Ellman, (1961) method. Where $A_0$ was the absorbance of the control and $A_1$ was the absorbance of the sample.

2.5.4. Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity was estimated using the method of Beauchamp and Fridovich, (1971) [19] as follows. The methanolic extract of *U. maritima* bulb was mixed with phosphate buffer (50 mM, pH 7.6) to obtain different concentrations (5-400 µg/mL). Each sample (945 µL) was mixed with 20 µL of riboflavin (1 mg/mL), 24 µL of EDTA (12 mM) and 11 µL of NBT (5 mg/mL). The samples were exposed to strong illumination for 90 sec then the absorbance was measured at 590 nm against a blank (the blank contains all the chemicals except the extract and was kept in the dark until reading the absorbance). BHT was used as a standard. The superoxide anion radical scavenging activity was calculated using the equation below:

$$\text{Superoxide anion radical scavenging activity} (\%) = \left( \frac{A_0 - A_1}{A_0} \right) \times 100$$

Where $A_0$ was the absorbance of the control and $A_1$ was the absorbance of the sample.

2.5.6. Lipid peroxidation assay

The lipid peroxidation assay was performed according to the method of Ohkawa et al., (1979) [21]. Freshly dissected sheep brain was used as a biological matrix to evaluate the protective effect of *U. maritima* bulb extract against lipid peroxidation. The brain was homogenized with polytron in ice cold phosphate-buffered saline (20 mM, pH 7.4) at a ratio of 1:10 (w:v). The produced homogenate was centrifuged at 3000 rpm for 10 min. A volume of 2 mL of the supernatant was incubated with 100 µL of *U. maritima* bulbs extract at different concentrations (100-800 µg/mL), 100 µL of ferrous sulfate (FeSO$_4$, 10 µM) and 100 µL of ascorbic acid (0.1 mM) at 37 °C for 60 min. After incubation, 500 µL of TCA (28%) and 2 mL of TBA (2%) were added and the mixture was heated at 80 ºC for 20 min then, centrifuged at 3000 rpm for 10 min to remove the precipitated proteins. The absorbance of the supernatant was measured at 532 nm. BHT was used as standard. The results were expressed as milligrams malondialdehyde equivalent per kilogram of sheep brain sample (mg MDAE/kg).

2.6. α-amylase activity inhibition

This test was performed according to the method of Shobana et al., (2009) [22]. Two hundred microliters of *U. maritima* bulb extract, dissolved in 1% DMSO, were incubated with 500 µL of phosphate buffer(0.02 M pH 6.9) containing 0.5 mg/mL of pancreatic porcine α-amylase for 10 min at 25°C. One percent starch solution (500 µL in0.02 M phosphate buffer pH 6.9) were added. After 10 min at 25 °C, 500 µL of DNS was added and the mixture was incubated for 5 min at 100°C.The reaction was stopped by adding 1 mL of H$_2$O and the absorbance was measured at 540 nm. Acarbose was used as reference. α-amylase activity inhibition was determined by the following formula:

$$\text{α-amylase activity inhibition} (\%) = \left( \frac{A_0 - A_1}{A_0} \right) \times 100$$

Where $A_0$ was the absorbance of the control and $A_1$ was the absorbance of the sample.

2.7. Acetylcholinesterase activity inhibition

The acetylcholinesterase activity inhibition was measured using an adaptation of the Ellman, (1961) method [23] described by Ferreira et al., (2006) [24]. Briefly, 100 µL of the enzyme solution (AChE, 0.28 U/mL), 200 µL of *U. maritima* bulb extract dissolved in 1% DMSO at different concentrations and 355 µL of Tris-HCl buffer (20 mM, pH 7.5) were incubated during 20 min at 30°C. Subsequently, 200 µL of AChI solution (0.075 M), and 100 µL of DTNB (0.01 M) were added. The final mixture was incubated for 20 min at 30°C.
Absorbance was measured at 412 nm. Galanthamine hydrobromide was used as reference. AChE activity inhibition was evaluated using the following equation:

$$\text{AChE activity inhibition (\%) = } \frac{[(A_0-A_1)/A_0] \times 100}{\text{Where } A_0 \text{ was the absorbance of the control and } A_1 \text{ was the absorbance of the sample.}}$$

2.8. Data analysis

The results were expressed as the mean ± standard deviation of three determinations. The median inhibitory concentrations IC$_{50}$ were calculated by Graph Pad Prism 6 for windows. Analysis of variance (ANOVA) was performed in order to determine the significant differences. Significance of difference was defined at the 5% level ($P<0.05$) using the SPSS 20.0 software (SPSS Inc.). Pearson’s correlation coefficient ($r$) was calculated using the same statistical analysis program.

3. Results and discussion

An antioxidant can act according to several mechanisms such as prevention of an oxidation chain by scavenging initiating radicals, donating its own hydrogen to prevent the free radicals continued abstraction of hydrogen in addition to the reducing capacity, binding of transition metal ion catalysts and free radical scavenging [25]. Thus, for assessing the antioxidant activity of $U. \text{maritima}$ bulbs extract, several assays have been used.

The DPPH method has been used extensively to screen for in vitro antioxidant activity [26]. DPPH is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of the plant extract [27]. When the DPPH radical accepts an electron from the antioxidant compound, its violet color was reduced to yellow colored diphenylpicrylhydrazine radical [28]. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [29]. The DPPH radical scavenging activity was expressed in terms of inhibition percent as shown in Figure 1. $U. \text{maritima}$ bulb extract was characterized by a high DPPH radical scavenging property. DPPH radical scavenging of a plant extract may indicate the ability of its antioxidants to donate hydrogen ions [30-31]. Therefore, it may be postulated that $U. \text{maritima}$ bulb extract has a DPPH scavenging activity by reducing the DPPH radical to corresponding hydrazine because of hydrogen ion-donating ability of its phenolic compounds. The concentration of $U. \text{maritima}$ bulb extract required to achieve 50% of DPPH radical reduction was evaluated to 57.86 ± 0.63 µg/mL for $U. \text{maritima}$ bulbs extract and to 18.48 ± 1.36 µg/mL for BHT (Table 1).

Ferrous ions can induce many radical reactions in biological systems. Hence, Fe$^{2+}$ chelation is considered as a useful therapeutic approach. In fact, Fe$^{2+}$ chelation may render important antioxidative effects by retarding metal-catalyzed oxidation [32]. As described in Figure 1, $U. \text{maritima}$ bulb extract showed a good chelating activity on ferrous ions with an IC$_{50}$ equivalent to 6.11 ± 0.26 µg/mL (Table 1). Thus, the results demonstrated that $U. \text{maritima}$ bulb extract seemed to be capable to prevent the initiation of free radicals generation by chelating the transition metal ions. Its chelating effect on ferrous ions may be attributed to the presence of phenolic compounds.

Hydroxyl radical is the most reactive free radical in biological systems. It causes severe oxidative damage to biomolecules, with subsequent tissue injury [33]. The hydroxyl radical scavenging activity of $U. \text{maritima}$ bulb extract was evaluated by generating the hydroxyl radicals using ascorbic acid–iron EDTA system. The hydroxyl radicals were formed by the oxidation reaction with the dimethyl sulfoxide (DMSO) to yield formaldehydes[34]. $U. \text{maritima}$ bulb extract was found to be effective in scavenging the hydroxyl radical (Figure 1) and showed an IC$_{50}$ value of 80.41 µg/mL (Table 1). The hydroxyl radical scavenging activity may be associated to the presence of phenolic compounds in the tested extract and their hydrogen donating ability.

It is well known that superoxide anion damages biomolecules directly or indirectly by forming H$_2$O$_2$ [35]. The results from superoxide anion scavenging activity assay demonstrated that $U. \text{maritima}$ extract inhibited Nitro Blue Tetrazolium (NBT) reduction efficiently (Figure 1). In addition, $U. \text{maritima}$ bulbs extract inhibited the production of superoxide anions by 50% when a concentration equivalent to 85.50 µg/mL of the bulbs extract was added to the reaction solution (Table 1). The results revealed that the extract under investigation had the ability to scavenge superoxide anion. Phenolic compounds of $U. \text{maritima}$ bulb extract may be involved in this potential.

The Fe$^{3+}$ ion is a reduced agent used to estimate a molecule's reducing power. Fe$^{3+}$ reduction is often considered as an indicator of electron donating activity, which is an important mechanism of antioxidant action [36]. The reducing ability of a plant extract generally depends on the presence of phenolic compounds, which exert the antioxidant activity by donating an electron [37]. As depicted in Figure 2, the absorbance at 700 nm in the presence of $U. \text{maritima}$ bulb extract clearly increased in a concentration dependent manner, due to the reduction of Fe$^{3+}$/ferricyanide complex to the ferrous form. At the maximum dosage of 1000 µg/mL, the
absorbance in the presence of *U. maritima* bulb extract was found to be 0.64 and that in the presence of the ascorbic acid was in the range of 0.78 (Figure 2). Therefore, it may be suggested that the phenolic compounds of *U. maritima* bulb extract caused the reduction of Fe$^{3+}$ ion and thus proved its reducing power ability thereby acting as efficient reducing agents.

**Table 1:** Antioxidant activities of the methanolic extract of *Urginea maritima* bulbs expressed in IC$_{50}$ (µg/mL) (mean ± standard deviation)

<table>
<thead>
<tr>
<th>samples</th>
<th>DPPH scavenging activity</th>
<th>Chelating effect on ferrous ions</th>
<th>Hydroxyl radical scavenging activity</th>
<th>Superoxide anion scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. maritima</em> bulb extract</td>
<td>57.86 ± 0.63</td>
<td>6.11 ± 0.26</td>
<td>80.41 ± 3.41</td>
<td>85.50 ± 6.26</td>
</tr>
<tr>
<td>Standard</td>
<td>18.48 ± 1.36</td>
<td>3.14 ± 0.30</td>
<td>29.20 ± 0.32</td>
<td>35.89 ± 0.64</td>
</tr>
</tbody>
</table>

Results were expressed as means of triplicate determinations ± standard deviation; *: BHT for DPPH, hydroxyl radical and superoxide anion scavenging activities; EDTA for chelating effect on ferrous ions.

**Figure 1:** Antioxidant activities of *Urginea maritima* bulb extract; results were expressed as means of triplicate determinations ± standard deviation; statistical differences have been done within concentrations and marked by different letters according to Tukey test at $P = 0.05$.

Malondialdehyde (MDA), a principal byproduct of polyunsaturated fatty acids degradation induced by ROS, is widely considered an indicator of lipid peroxidation. Interaction of this highly reactive and toxic aldehyde with biomolecules has often been referred to as potentially mutagenesis and atherogenic. On the other hand, the increase of lipid peroxidation level is involved in cell lysis and correlates with several diseases such as cancer, diabetes, and liver and cardiovascular diseases [38]. Thus, inhibition of lipid peroxidation can be a valuable strategy to protect biomolecules and reduce the risk of diseases associated to lipid peroxidation. In this context, protective effect of *U. maritima* bulb extract against induced lipid peroxidation was evaluated using sheep brain as biological matrix. The induced oxidation status in the absence of the studied extract was measured and was estimated to 0.39 ± 0.014 mg MDAE/kg. Compared with this value, a significant decrease in MDA levels was detected when the brain homogenate was treated with different concentrations of *U. maritima* bulbs extract ($P < 0.05$). Indeed, the amount of TBARS decreased to reach 0.14 ± 0.019 mg MDAE/kg in the presence of *U. maritima* bulb extract at a concentration of 800 µg/mL, which corresponds to an inhibition of lipid peroxidation of about 64.10% (Figure 3).
Based on these findings, *U. maritima* bulb extract seemed to be a good inhibitor of lipid peroxidation in biological matrix. Phenolic compounds could be responsible for antiliperoxidant activity of *U. maritima* bulb extract. In this regards, phenolic compounds have been reported to be able to act as antioxidants and inhibit lipid peroxidation and therefore protect the biological systems from oxidative damage [39-40].

**Figure 2:** Reducing power of *Urginea maritima* bulb extract and standard (ascorbic acid); results were expressed as means of triplicate determinations ± standard deviation; statistical differences have been done within concentrations and marked by different letters according to Tukey test at $P = 0.05$

$\alpha$-amylase is key enzyme in the digestion of carbohydrates. This enzyme is used as targets for $\alpha$-amylase inhibitors in attempts to treat several diseases such as diabetes mellitus [41]. Inhibitors of this enzyme delay the breakdown of starch and lower the postprandial blood glucose levels in diabetic patients [42]. Many synthetic $\alpha$-amylase inhibitors have been used for the treatment of this metabolic disorder. However, natural inhibitors have been recognized as a cost effective alternative to synthetic ones since those molecules are safe and present less side effects [43]. The $\alpha$-amylase activity inhibition of *U. maritima* bulb extract was investigated. Results indicated that the extract under study exhibited considerable inhibitory effect against the enzyme activity at all tested concentrations ($F = 510.74$, $df = 4$, $P < 0.001$) (Figure 4) with an $IC_{50}$ evaluated to 95.03 ± 1.29 $\mu$g/mL. In addition, the $\alpha$-amylase activity inhibition was significantly correlated to the concentrations of *U. maritima* bulb extract ($r = 0.96$, $P < 0.05$). The observed substantial anti-amylase activity of the *U. maritima* bulb extract could possibly be due to its richness in phenolic compounds. In fact, Da silva et al., (2014) [44] reported that phenolic...
compounds are able to bind to the α-amylase in saliva thereby causing their inhibition. In the same context, Hanhineva et al., (2010) [45] and Williamson, (2013) [46] explained that phenolic compounds inhibited the α-amylase activity due to their ability to bind with proteins, which confirmed our results, since our extract was found to be rich in phenolic compounds. Based on these results, it may be postulated that phenolic compounds of *U. maritima* bulb extract could be used to delay absorption of dietary carbohydrates in the meal, leading to suppression of an increase in postprandial blood glucose level without adverse effects.

![Figure 4: α-amylase activity inhibition by *Urginea maritima* bulb extract; results were expressed as means of triplicate determinations ± standard deviation; statistical differences have been done within concentrations and marked by different letters according to Tukey test at *P* = 0.05](image)

The inhibition of AChE, the key enzyme in the breakdown of acetylcholine, is considered as one of the treatment strategies that help in managing deficient acetylcholine level [47]. Therefore, it was considered that AChE inhibitors might be helpful in attenuating the symptoms against several neurological disorders such as Alzheimer’s disease and senile dementia [48]. In order to search for effective AChE inhibitors from natural sources, *U. maritima* bulb extract was screened for its AChE inhibition power. As summarized in Figure 5, AChE activity was significantly inhibited in the presence of *U. maritima* bulb extract in a concentration dependent manner (*r* = 0.92, *P* < 0.05).

![Figure 5: AChE activity inhibition by *Urginea maritima* bulb extract; results were expressed as means of triplicate determinations ± standard deviation; statistical differences have been done within concentrations and marked by different letters according to Tukey test at *P* = 0.05](image)
At a concentration of 500 µg/mL, the inhibitory effects were evaluated to 77.46 ± 0.43% and 90.05 ± 0.11% for *U. maritima* bulb extract and galanthamine, respectively. Our results showed that *U. maritima* bulb extract contained AChE inhibitors, which could probably bind to active site and form inactive complex. Phenolic compounds of *U. maritima* bulb extract could possibly act as AChE inhibitors. In this context, Bivar Roseiro et al., (2012) [49] reported that phenolic compounds induced AChE activity inhibition and explained that the presence of hydroxyl groups in phenolic compounds may be involved in their inhibitory potential. These phenolics from *U.maritima* bulb extract are potentially safer, and therefore, may be considered as preferred alternatives for inhibition of AChE activity.

**Conclusion**

The results of this study showed that the *U. maritima* bulb extract exhibited a substantial antioxidant activity, which is highly related to the presence of antioxidants, and an efficient capacity to inhibit α-amylase and acetylcholinesterase activities indicating its potential use as a source of natural antioxidants and suggesting its therapeutic value. The biological activities reported in this study must be more investigated to elucidate the bioactive compounds and the molecular mechanism responsible for the biological properties of *U. maritima* bulb extract. In addition, further in vivo studies are needed to confirm the effects highlighted in this work.

**References**
