

## Research Article

# Antioxidant Activity of the Bulb and Aerial Parts of *Ornithogalum sintenisii* L (*Liliaceae*) at Flowering Stage

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## Abstract

**Purpose:** *Ornithogalum sintenisii* is an Iranian species with little known about its pharmacological effects. The purpose of the present study was to investigate some antioxidant properties of the plant.

**Methods:** The antioxidant potency of the freeze-dried methanol extract of *O. sintenisii* bulbs and aerial parts were investigated by evaluating the following parameters: linoleic acid peroxidation, 1,1-diphenyl-2-picryl hydrazyl radical (DPPH), scavenging of nitric oxide and hydrogen peroxide as well as reducing power and Fe<sup>2+</sup> chelating activity, using standard methods. Phenol and flavonoid contents were determined as gallic acid and quercetin equivalents, respectively.

**Results:** The aerial parts contained higher phenol and flavonoid contents than the bulbs. IC<sub>50</sub> for DPPH radical-scavenging activity was 368 ± 15 and 669 ± 25 µg ml<sup>-1</sup> for aerial parts and bulbs, respectively. The reducing power of the extracts was less than that of vitamin C (p < 0.01) with the aerial parts showing stronger activity than the bulbs (p < 0.01). The extracts did not show any activity in the peroxidation test but displayed good H<sub>2</sub>O<sub>2</sub> radical scavenging activity compared with quercetin (IC<sub>50</sub>= 52.0 ± 3.1 µg ml<sup>-1</sup>) which was used as positive control.

**Conclusion:** The bulb and aerial parts of *O. sintenisii* aerial parts (at flowering stage) exhibited good but varying levels of antioxidant activities in nearly all the models studied.

**Keywords:** *Ornithogalum sintenisii* ; Bulbs; Aerial parts; Antioxidant activity; Flavonoids; Phenols.

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## INTRODUCTION

Living tissues that derive energy from aerobic metabolism are under constant threat of damage by reactive oxygen derivatives. Such free radicals are usually short-lived species but they possess a single unpaired electron, rendering them highly reactive against biologically important macromolecules including DNA, proteins and membrane lipids.[1] To counteract this threat to their integrity, cells have evolved a variety of defense systems based on both water-soluble and lipid-soluble antioxidant species, and on antioxidant enzymes.[1] A high proportion of the antioxidant systems of the human body are dependent on dietary constituents [1]. Consequently, the search for natural antioxidants, especially of plant origin, has notably increased in recent years [2]. *Ornithogalum sintenisii* is an Iranian species of the genus *Ornithogalum* L. (*Liliaceae*) that encompasses well over 150 perennial bulbous species, mostly distributed in the temperate regions of Europe, Asia, and Africa [3,4]. To the best of our knowledge, no biological and phytochemical investigation has been carried out on *O. sintenisii*. However, previous studies on some other *Ornithogalum* species revealed the presence of steroidal glycosides [4,5], monoterpene lactone [6] and homoisoflavanone [7], some of which were found to possess antimicrobial, cytotoxic, cytostatic, anticancer, antioxidant, mould-inhibiting and insect deterrent properties [4,8]. The objective of this study was to determine some antioxidant properties of *O. sintenisii* bulbs and aerial parts using a set of *in vitro* antioxidant assays including scavenging of DPPH, nitric oxide and hydrogen peroxide, as well as reducing power, linoleic acid peroxidation test and iron ion chelating power.

## EXPERIMENTAL

### Chemicals

Ferrozine, linoleic acid, trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl radical

(DPPH), and potassium ferricyanide were purchased from Sigma Chemicals Co., USA. Gallic acid, quercetin, butylated hydroxyanisole (BHA), ascorbic acid, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, ethylenediamine tetraacetic acid. EDTA and ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

### Plant Material

The bulb and aerial part (flowering stage) of *O. sintenisii* were collected from Khazar abad area (Panbeh chooleh, north of Sari, Iran) and identified by Dr Bahman Eslami (Department of Biology, Islamic Azad University of Qhaemshahr, Iran). A voucher (no NS76-NS79) has been deposited in the Sari School of Pharmacy Herbarium. The materials were dried at room temperature and coarsely ground before extraction. Each part (100 g) was extracted by percolation method using methanol (400 ml) for 24 h at room temperature. The extract was then separated from the sample residue by filtration through Whatman No 1 filter paper. This procedure was repeated thrice. The resultant extract was concentrated in a rotary evaporator until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal. Yields were 10.5 and 8.2% for bulb and aerial part, respectively.

### Determination of total phenolic and flavonoid contents

Total phenolic contents were determined by the Folin-Ciocalteu reagent method [9]. The extract samples (0.5 ml) were mixed with 2.5 ml of 0.2N Folin-Ciocalteu reagent for 5 min and 2.0 ml of 75 g/l sodium carbonate was then added. The absorbance of the reaction mixture was measured at 760 nm after 2 h of incubation at room temperature. The results were expressed as Gallic acid equivalents. Total flavonoids were estimated using the method of Ebrahimzadeh *et al* [10]. Briefly, 0.5 ml of each plant extract in methanol was

separately mixed with 1.5 ml of methanol, 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid contents were calculated as quercetin from a calibration curve.

### **DPPH radical-scavenging activity**

DPPH radical was used for determination of free radical-scavenging activity of the extracts as previously described [11]. Different concentrations of each extract were added, in equal volume, to the methanolic solution of DPPH (100 $\mu$ M). The mixture was left for 15 min at room temperature and the absorbance was read at 517 nm. The experiment was repeated three times, using vitamin C, BHA and quercetin were used as standard controls. The results were calculated as IC<sub>50</sub> values, which denotes the concentration of sample required to scavenge 50 % of DPPH free radicals.

### **Reducing power determination**

The reducing power of the extracts was determined according to the method of Nabavi et al [12, 13]. Different amounts of each extracts (25 - 800  $\mu$ g ml<sup>-1</sup>) in water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide, K<sub>3</sub>Fe(CN)<sub>6</sub>, (2.5 ml, 1 %). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of 10 % trichloroacetic acid (10%) was added to the mixture to stop the reaction followed by centrifugation at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl<sub>3</sub> (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

### **Assay of nitric oxide-scavenging activity**

The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent [1]. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM), in phosphate-buffered saline, was mixed with different concentrations of each extracts dissolved in water and incubated at room temperature for 150 min. Following the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as positive control [14].

### **Metal chelating activity**

The chelation of ferrous ions by the extracts was estimated colorimetrically as described previously [15,16]. Briefly, the extract (0.2 - 3.2 mg/ml) was added to a solution of 2mM FeCl<sub>2</sub> (0.05 ml). The reaction was initiated by the addition of 0.2 ml of 5mM ferrozine; the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then read at 562 nm. Percent inhibition of ferrozine-Fe<sup>2+</sup> complex formation was calculated as  $(A_0 - A_s)/A_s \times 100$ , where A<sub>0</sub> was the absorbance of the control, and A<sub>s</sub> the absorbance of the extract/standard. Na<sub>2</sub>EDTA was used as positive control.

### **Determination of antioxidant activity**

The inhibitory capacity of extracts against oxidation of linoleic acid measured colorimetrically as previously described [10,17]. Twenty mg/mL of samples, dissolved in 4 mL of 95 % ethanol, were mixed with 4.1 mL of 2.51 %v/v linoleic acid in 99.5 % ethanol, 8 mL of 0.05 M phosphate buffer pH 7.0 and 3.9 mL of distilled water, and then kept in screwcapped containers at 40 °C in the dark. To 0.1 mL of this solution were

added 9.7 mL of 75% ethanol and 0.1 mL of 30 %w/v ammonium thiocyanate. Precisely 3 min after the addition of 0.1 mL of 20mM ferrous chloride in 3.5 %v/v hydrochloric acid to the reaction mixture, the absorbance of the resulting red solution was measured at 500 nm, and repeated at 24 h intervals until the absorbance of the control reached maximum value. Percent inhibition of linoleic acid peroxidation was calculated as in Eq 1.

$$\text{Inhibition (\%)} = 100 - [A_s/A_c] \times 100 \dots (1)$$

where  $A_s$  = absorbance increase of the sample and  $A_c$  = absorbance increase of the control. All tests were run in duplicate, and the mean taken. Vit C and BHA were used as positive control.

### Hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was spectrophotometrically [11,14]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The extract (0.1-1 mg ml<sup>-1</sup>) in distilled water was added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the extracts and standard was calculated as Eq 2.

$$\% \text{ scavenged (H}_2\text{O}_2) = (A_0 - A_1)/A_0 \times 100 \dots (2)$$

where  $A_0$  is the absorbance of the control and  $A_1$  the absorbance in the presence of the extract or standard.

### Statistical analysis

Experimental results were expressed as mean  $\pm$  SD. The data were analysed by Analysis of Variance and the means separated by Duncan's multiple range test. Data were considered significant at  $p < 0.05$ . The  $IC_{50}$  values were calculated from linear regression analysis.

## RESULTS

### Total phenol and flavonoid contents

Total phenol compounds, reported as gallic acid equivalents, were derived from a standard curve ( $y = 0.0063x$ ,  $r^2 = 0.987$ ). Total phenolic contents of *O. sintenisii* bulbs and aerial parts were  $8.4 \pm 0.3$  and  $28.9 \pm 1.1$  mg gallic acid equivalent/g of extract powder, respectively. The total flavonoid contents of *O. sintenisii* bulbs and aerial parts were  $5.9 \pm 0.2$  and  $23.5 \pm 1.3$  mg quercetin equivalent/g of extract powder, respectively, as derived from a standard curve ( $y = 0.0067x + 0.0132$ ,  $r^2 = 0.999$ ).

### DPPH radical-scavenging activity

The radical-scavenging activities of the extracts increased with increasing concentration.  $IC_{50}$  for DPPH radical-scavenging activity was  $368 \pm 15$  and  $669 \pm 25$   $\mu\text{g ml}^{-1}$  for the aerial parts and bulbs, respectively. The  $IC_{50}$  values for ascorbic acid, quercetin and BHA were  $5.05 \pm 0.12$ ,  $5.28 \pm 0.43$  and  $53.96 \pm 2.13$   $\mu\text{g ml}^{-1}$ , respectively.

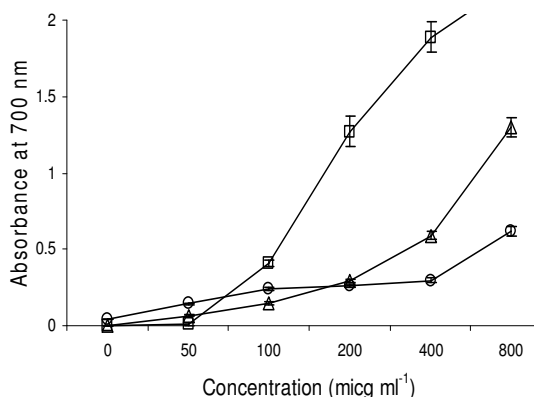
### Reducing power

Fig. 1 shows the dose-response curves for the reducing powers of the extracts. The reducing power of the extracts increased with increase in their concentrations. The extracts exhibited fairly good reducing power at 25 and 800  $\mu\text{g ml}^{-1}$  but were, however, less than that of vitamin C ( $p < 0.001$ ). Aerial parts showed stronger activity than the bulbs ( $p < 0.01$ ).

### Nitric oxide-scavenging activity

Table 1 shows the data for scavenging activities. The extract of the aerial parts showed moderately good nitric oxide-scavenging activity of between 0.2 and 3.2 mg ml<sup>-1</sup>. Inhibition increased with increasing concentration of the extracts. The aerial parts extract showed higher scavenging activity

with  $IC_{50}$  of  $339 \pm 13 \mu\text{g ml}^{-1}$ . The bulbs showed only 22.18 % scavenging activity at  $3.2 \text{ mg ml}^{-1}$ .  $IC_{50}$  of quercetin was  $20 \pm 0.3 \mu\text{g ml}^{-1}$ .



**Fig 1:** Reducing power of *O. sintenisii* bulbs (o) and aerial parts ( $\Delta$ ) at flowering stage, and of control (vitamin C) ( $\square$ ).

### $Fe^{2+}$ - chelating ability

The absorbance of  $Fe^{2+}$ -ferrozine complex was decreased in a dose-dependent manner, i.e., activity increased on increasing concentration from 0.2 to 0.8  $\text{mg ml}^{-1}$  (see Table 1). The aerial parts showed greater  $Fe^{2+}$  - chelating activity than the bulbs.  $IC_{50}$  was  $340 \pm 14$  for aerial parts and  $684 \pm 27 \mu\text{g ml}^{-1}$  for the bulbs. EDTA (standard) showed very strong activity ( $IC_{50} = 18 \mu\text{g ml}^{-1}$ ).

### Peroxidation inhibition

The plant extracts did not show any activity in peroxidation inhibition test (Table 1). Vitamin C and BHA, which were used as controls, showed 91 - 98 % inhibition at different incubation times (24 - 96 h).

### Hydrogen peroxide scavenging

The extracts scavenged hydrogen peroxide in a concentration-dependent manner (Table 1). Both of them showed very good scavenging activity.  $IC_{50}$  for  $H_2O_2$  scavenging was  $54.6 \pm 1.9$  and  $294 \pm 11 \mu\text{g ml}^{-1}$  for the bulbs and aerial parts, respectively while  $IC_{50}$  values for ascorbic acid and quercetin were  $21.4 \pm 0.1$  and  $52.0 \pm 3.1 \mu\text{g ml}^{-1}$ , respectively (Table 1).

## DISCUSSION

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [16]. Studies have shown that increasing levels of flavonoids in the diet could reduce the incidence of certain human diseases [10].

DPPH radical scavenging is a widely used method to evaluate the free radical scavenging ability of various materials [11]. DPPH is a stable nitrogen-centred free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation.

**Table 1:** Antioxidant activities of the bulb and aerial parts of *O. sintenisii* at flowering stage

Antioxidant material	DPPH free radical scavenging, $IC_{50}$ ( $\mu\text{g ml}^{-1}$ )	Nitric oxide scavenging, $IC_{50}$ ( $\mu\text{g ml}^{-1}$ )	$H_2O_2$ scavenging activity, $IC_{50}$ ( $\mu\text{g ml}^{-1}$ )	$Fe^{2+}$ chelating ability, $IC_{50}$ ( $\mu\text{g ml}^{-1}$ )
Aerial parts	$368 \pm 15$	$339 \pm 13$	$294 \pm 11$	$340 \pm 14$
Bulbs	$669 \pm 25$	22.2% <sup>a</sup>	$54.6 \pm 1.9$	$684 \pm 27$
BHA	$53.96 \pm 2.31$	-	-	-
Vitamin C	$5.05 \pm 0.12$	-	$21.4 \pm 1.1$	-
EDTA	-	-	-	$18 \pm 1.5$
Quercetin	$5.28 \pm 0.43$	$20 \pm 0.3$	$52 \pm 2.6$	-

Substances which are able to perform this reaction can be considered as antioxidants and, therefore, radical scavengers [10]. It was found that the radical-scavenging activities of both extracts increased with increasing concentration. High total phenol and flavonoid contents of aerial parts of plant may be a reason for its higher DPPH-scavenging activity than that of the bulb.

Fe (III) reduction is often used as an indicator of electron- donating activity, which is an important mechanism of phenolic antioxidant action [13]. Although, the reducing power of extracts was less than that of Vit C ( $p < 0.01$ ), the aerial parts showed stronger activity than the bulbs ( $p < 0.01$ ). It was evident that *O. sintonisii* showed reductive potential and could serve as an electron donor for terminating radical chain reaction. It would seem that the higher total phenol and flavonoid contents of the aerial parts of the plant led to the higher reductive potential of the extract.

The aerial parts extract showed moderately good nitric oxide-scavenging activity of between 0.2 and 3.2 mg ml<sup>-1</sup>. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions [11]. Consequently, this plant has potentials for counteract the effect of NO formation and, therefore, may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Furthermore, NO scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health.

Iron chelators mobilise tissue iron by forming soluble, stable complexes that are then excreted in faeces and/or urine. Chelation therapy reduces iron-related complications in humans and thereby improves quality of life and overall survival in some diseases such as thalassemia major [16]. In addition, brain iron dysregulation and its association with amyloid precursor protein plaque formation are implicated in Alzheimer's disease (AD)

pathology and so iron chelation could be considered a rational therapeutic strategy for AD [14]. Foods are often contaminated with transition metal ions which may be introduced by processing methods [10]. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry [14]. These processes can be delayed by iron chelation and deactivation. The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease [18]. Because Fe<sup>2+</sup> also has been shown to cause the production of oxyradicals and lipid peroxidation, minimising Fe<sup>2+</sup> concentration in Fenton reactions affords protection against oxidative damage. In this assay, both the extracts and EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that the extracts have chelating activity and capture ferrous ion before ferrozine. The absorbance of Fe<sup>2+</sup>- ferrozine complex was decreased dose-dependently, i.e., the activity increased on increasing extract concentration from 0.2 to 3.2 mg ml<sup>-1</sup>.

Metal chelating capacity was significant since the extract reduced the concentration of the catalyzing transition metal in lipid peroxidation [18]. It has been reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilising the oxidised form of the metal ion [17]. The aerial part extract showed higher Fe<sup>2+</sup> - chelating activity than the bulbs but it was not any where as strong as that of EDTA. The higher total phenol and flavonoid contents of the aerial parts might have led to the higher reductive potential of the extract.

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specifically, linoleic acid and arachidonic acid are targets of lipid peroxidation [10,17]. The inhibition of lipid peroxidation by antioxidants is due to their

free radical-scavenging activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical [2]. Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids, which results in lipid peroxidation. The plant extracts did not show any peroxidation inhibition activity probably due to other compounds that might be present in the plant that exert lipid peroxidation activity.

Scavenging of H<sub>2</sub>O<sub>2</sub> by the extracts may be attributed to their phenolics, which can donate electrons to H<sub>2</sub>O<sub>2</sub>, thus neutralising it to water. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner and they both showed very good scavenging activity. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H<sub>2</sub>O<sub>2</sub> is very important in food processes [11].

## CONCLUSION

*O. sintenisii* aerial parts (at flowering stage) and bulbs methanol extracts exhibited good but varying levels of antioxidant activity in nearly all the models studied. Overall, probably due to the higher total phenol and flavonoid contents of the aerial parts, this part showed higher antioxidant activity than the bulbs. Isolation of the individual compounds of the extracts, investigation of their *in vivo* antioxidant activities and the elucidation of their antioxidant mechanisms are required to confirm the findings of this study.

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