Phenolic Contents, Antioxidant Properties Phenolic Contents and Antioxidant Properties of *Muscari parviflorum* Desf.

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Summary: Oxidative stress can be the result of normal metabolic activity or environmental factors. Geophytes, especially the *Muscari* Mill. species are very important in the plant groups. Many kinds of flavonoids have been identified in *Muscari* Mill plants. Flavonoids have many functions in the biochemistry, physiology and ecology of plants. In this study we determined that phenolic contents of the ethanolic extracts higher than the methanolic extracts. The phenolic contents of the extracts as follows: Bulb ethanolic extracts (MBE) (52.23 ± 1.32%) > Bulb methanolic extracts (MBM) (19.47 ± 2.56%) and leaf ethanolic extracts (MLE) (31.68 ± 0.92%) > leaf methanolic extracts (MLM) (26.73 ± 1.08%). The absorbance of the *Muscari parviflorum* Desf. plant extracts at 470 nm were evaluated using β -carotene–linoleic acid assay to determine the antioxidant activity. MBE showed the highest antioxidant activity with 76.8 ± 1.51% and MLM showed the lowest antioxidant activity with 60 ± 1.32% respectively. These values are higher than the controls (methanol - 48 ± 1.31%; ethanol - 31 ± 2.12%). The free radical scavenging activities of the extracts were determined as follows: MLE (61.16 ± 1. 42%), MLM (29.08 ± 0.42%), MBE (44.80 ± 0.81%), MBM (31.50 ± 0.74%) respectively. Results show that, *Muscari parviflorum* has the antioxidant activity.

Introduction

Oxidative stress can be the result of normal metabolic activity or environmental factors. The balance between antioxidants-prooxidants is dynamic and it is essential for energy creation [1]. Antioxidants contribute to the protection of organism against oxidative stress and insufficient entry of antioxidants in organism can lead to the damages on DNA, lipids and proteins [2-3].

Geophytes, especially the *Muscari* Mill. species are very crucial in plant groups. The genus *Muscari* Mill. (Hyacinthaceae) is native in the Mediterranean area and includes about 50 species and they are garden and ornamental plants [4]. There are a lot of floristic studies about genus *Muscari* [5-11]. They have the economic and pharmaceutical importance [12-13] and *Leopoldia comosa* (bulbs, syn.: *M. comosum*) demonstrated strong antioxidant activity in 1.1-diphenyl-2-picrylhydrazil (DPPH) radical assay [14]. Also, there are some genetical studies and studies about content of *M. comosum* [15-19].

Phenolic compounds are secondary metabolites which synthesized in plants and they affect plant foods appearance, taste, odor and oxidative stability [20-21]. Flavonoids are polyphenolic compounds and they have many functions in the biochemistry, physiology and ecology of plants [22]. Isoflavonoids belong to plant secondary metabolites and they are structurally similar to estrogens, exerting both estrogenic and antiestrogenic properties in various tissues [23-24]. Homoisoflavonoids have one more carbon atom than normal isoflavone skeleton [25]. It was demonstrated that chemical constituents of the genus *Muscari* are homoisoflavonoids, [26-28] water-soluble polysaccharides, [29] and glycosides [30-31]. It has been shown that *M. racemosum* (L.) homoisoflavonoids have the antimutagenic properties and may be important for the prevention from cancer [32].

Muscari parviflorum Desf. including the pseudomuscari group and there are limited surveys about *M. parviflorum*. Galil, [33] studied the vegetative reproduction and reproductive strategy of *M. parviflorum*. Also, some floristic records found about *M. parviflorum*, [34-35] and there are a few studies about the antioxidant properties of the *M. parviflorum* [36].

The aim of the study was to examine the *in vitro* antioxidant activities of the methanolic and ethanolic extracts of *M. parviflorum*. In literature survey, there were not many studies about the antioxidant activity of this species for analyzing them. Therefore, we think that the results presented here will provide new information on the species examined.

Results and Discussion

Phenolic compounds including flavonoids and phenolic acids are known to be responsible for

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antioxidant capacities in fruits, and the fruits with higher phenolic contents generally show stronger antioxidant capacities [37]. Bayberry fruits (*Myrica rubra*) contain flavonols and its radical scavenging activities were reported by Bao et al [38]. We found that, the phenolic contents of the ethanolic extracts are higher than the methanolic extracts. The phenolic contents of the extracts as follows: MBE (52.23 ± 1.32%) > MBM (19.47 ± 2.56%) and MLE (31.68 ± 0.92%) > MLM (26.73 ± 1.08%) (Table-1).

Table-1: Total phenolic content (µg of PEs[a] / mg of extract) of extracts from different parts of *M. parviflorum* using various solvents

Solvent	Bulb	Leaves
Methanol	**19.47 ± 2.56%**	**26.73 ± 1.08%**
Ethanol	**52.23 ± 1.32%**	**31.68 ± 0.92%**

[a] PEs, pyrocatechol equivalents.
[b] Values are means and standart deviations of three replicate analyses

Total Antioxidant Activity

In recent years a number of studies have been published about the antioxidant capacities of plant materials, which antioxidant characteristics of phenol compounds have been tested through different methods [39-42]. Because of that it is difficult to compare final results, even though there are the same plant species.

The absorbance of the *M. parviflorum* plant extracts at 470 nm was evaluated using β -carotene–linoleic acid assay. Absorbance of the sample that was measured every 30 min using a UV spectrophotometer (Shimadzu UV–1601, Japanese). Measurements were carried out in triplicate and the mean values of the measurements were calculated. Methanolic and ethanolic extracts of *M. parviflorum* presented different absorbance values. The highest initial absorbance value is detected as 213 for MBE, and the lowest initial absorbance value is detected as 168 for MBM. At the end of the 120 minutes, the highest absorbance value is detected as 174 for MBE, the lowest absorbance value is detected as 118 for MLM (Fig. 1).

The antioxidant activities of the plant extracts were detected as follows: MBE (76.8 ± 1.51%) > MLE (71.2 ± 1.14%) > MBM (65.2 ± 0.87%) > MLM (60 ± 1.32%). Differences of the absorbance values showed that, MBE (76.8±1.51%) has the highest and MLM (60± 1.32%) has the lowest antioxidant activities. These values are higher than the controls (methanol - 48 ± 1.31%; ethanol - 31 ± 2.12%) (Fig. 2). But there were no significant differences were observed among the antioxidant activities of the extracts. Results showed that *M. parviflorum* has high phenolic contents. Because of that, it exhibited the high antioxidant and free radical scavenging activities.

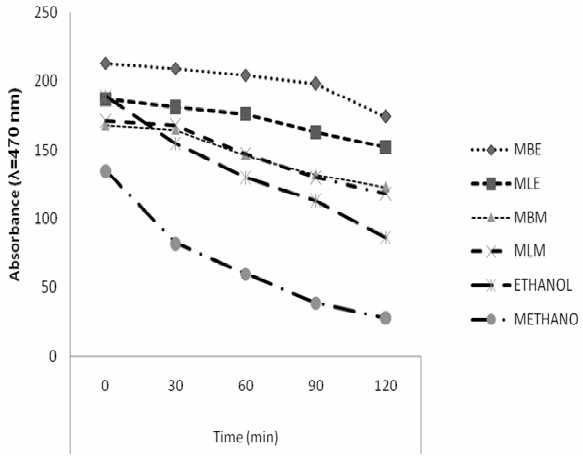

Fig. 1: Absorbance values of methanol (Control A) and ethanol (Control B) extracts determined with β-carotene-linoleic acid model system . MBE, *Muscari* Bulb-ethanol; MLE, *Muscari* Leaf-ethanol; MLM, *Muscari* Leaf-methanol; MBM, *Muscari* Bulb-methanol.

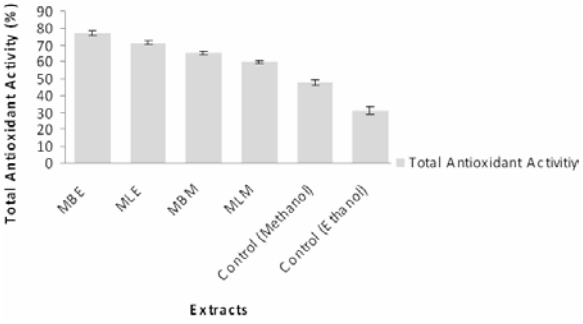

Fig. 2: The antioxidant activities in the methanol and ethanol extracts. MLM: *Muscari* Leaf-methanol; MBM: *Muscari* Bulb-methanol; MLE: *Muscari* Leaf-ethanol; MBE: *Muscari* Bulb-ethanol.

Free Radical Scavenging Activity

The free radical scavenging activity of the extracts depends on the hydrogen donating ability and the structural conformation of the compounds [21-43]. Free radical scavenging capacities of *M. parviflorum* leaves and methanolic and ethanolic extracts of bulbs were evaluated and determined as follows: MLE (61.16 ± 1.42%), MBE (44.80 ± 0.81%), MBM (31.50 ± 0.74%) and MLM (29.08 ± 0.42%) (Fig. 3).

Experimental

Chemicals, Plant Materials and Extracts

Chemicals

Ascorbic acid, Na_2CO_3, β-carotene, methanol (70%), ethanol (70%), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), chloroform, tween-20, linoleic acid, Folin–Ciocalteu (FC) was obtained from Merck (Darmstadt, Germany). Butylated hydroxytoluene (BHT) was obtained from Sigma Chemical Co. (St. Lois, MO). Other used chemicals were of analytical grade.

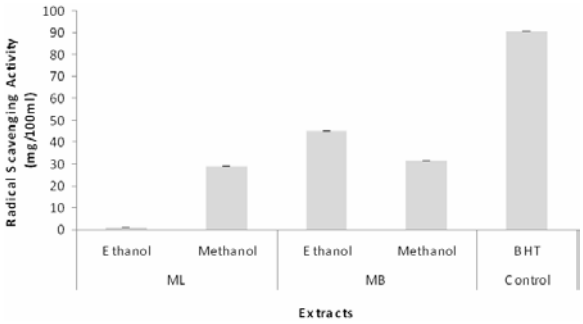

Fig. 3: The free radical scavenging capacity of the extracts with methanol and ethanol through DPPH method. BHT: Butylated Hydroxytoluene; ML: *Muscari*-Leaf; MB: *Muscari*-Bulb.

Plant Materials

M. parviflorum was collected in the spring from Marmaris and Datca (Muğla province, in Turkey) locality. The fresh underground and above ground parts of the plant materials were cleaned and dried in the shadow for extraction. Extraction processes and antioxidant studies have been done in Pamukkale University Department of Biology.

Plant Extracts Preparations

Plants were collected at flowering stage; their bulbs and leaves were dried, chopped up with a blender, and prepared for the experiment. Ethanol and methanol were added in the ratio of 1:10 and they were put in water bath (ST 402) at 55ºC for 6 h. Then, mixture was filtered by a filter paper (Whatman No: 1), and the solvents were evaporated in a rotary evaporator (IKA RV 10D) at 42-49°C. The water extract was lyophilized using a freeze dryer (Thermo Savant Modulyo D**).** Anhydrous extracts were stored at -20°C until analysis. All extraction procedures were performed in duplicate.

Plant Extracts

Methanolic and ethanolic extracts were obtained from leaves and bulbs of *M. parviflorum*. These extracts are: *Muscari* Bulb-Methanol (MBM), *Muscari* Bulb-Ethanol (MBE), *Muscari* Leaf-Methanol (MLM), *Muscari* Leaf-Ethanol (MLE).

Total Phenolic Contents (TPC)

The total phenolic content (μg of PEs /mg of extract) of extracts were determined using Folin-Ciocalteu (FC) method [41-45] and was estimated colourimetrically [43-44]. One milliliter of *M. parviflorum* leaf and bulb extracts which appropriately diluted using 70% ethanol and 70% methanol, were added to 9 mL distilled water, 5 mL of 5% Na_2CO_3 solution and FC phenol reagent (0.5 mL) and the mixtures were vigorously shaken. Then solution was diluted to 25 mL with distilled water immediately and mixed thoroughly. The absorbance of the solution was measured at 760 nm using a UV–spectrophotometer (Shimadzu UV–1601, Japanese). The total phenolic contents calculated using standard curve of gallic acid and the concentration of total phenolic compounds were expressed in mg of Gallic Acid Equivalents (GAE) per g of dried extracts (DE). All measurements were carried out in five replicates.

Antioxidant Activity

β-Carotene–linoleic Acid Assay

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [45-46]. A stock solution of β -carotene–linoleic acid mixture was prepared as follows: 0.5 mg β -carotene was dissolved in 1 mL of chloroform (HPLC grade), and 25 μl linoleic acid and 200 mg tween 20 were added. Chloroform was completely evaporated (42°C) using a vacuum evaporator. Then, 100 mL distilled water was added by vigorous shaking. 2500 μl of this reaction mixture were dispensed to test tubes and 350 μl portions of the extracts, which was prepared at 2 g L^{-1} concentration was added and the emulsion system was incubated up to 48 h at room temperature. The same procedure was repeated with synthetic antioxidant, BHT, as positive control and a blank. After this incubation period, absorbances of the mixtures were measured at 470 nm. Standards at the same concentration with samples were used as comparison. 0.2 mL of 70% methanol and 70% ethanol, in 5 mL of the above emulsion was used as the control.

Antioxidant activity (AA) was measured in terms of successful bleaching of β -carotene by using

a slightly modified version of the formula from Jayaprakasha et al [47] and the absorbance was measured during 120 minutes.

$AA= (1- (A_0-A_t / A_0^o - A_t^o)) \times 100*$

Where A_0, OD of the sample at time t=0 minutes; A_t, OD of the sample at time t=120 minutes; A_0^o, OD of the control at time t=0 minutes; A_t^o, OD of the control at time t=120 minutes.

DPPH Assay

The DPPH is a stable free radical which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants [21, 48-50]. Hydrogen donating activity was quantified in presence of stable DPPH radical on the basis of Blois method [51]. The hydrogen atoms or electron-donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of a purple-colored ethanol and methanol solutions of DPPH. This spectrophotometric assay uses the stable DPPH radical as a reagent [52-57]. *M. parviflorum* extract (0.62 - 4.96 mg/mL) and BHT (0.04 - 1.28 mg/mL) were mixed with 800 µl of 100 mM Tris - HCl buffer (pH 7.4). The mixture was then added to 1 mL of 500 µM DPPH. This was made up to a DPPH final concentration of 250 µM. The mixture was shaken vigorously and left to stand at room temperature for 30 min in a dark room.

Absorbance at 517 nm was measured using a UV spectrophotometer (Shimadzu UV–1601, Japanese) until the reading reached a plateau. Inhibition of free radical DPPH in percent (*I* %) was calculated in following way:

$$\text{Scavenging activity (\%)} = \left[\frac{A_{(-ve)} - A_S}{A_{(-ve)} - A_{(+ve)}}\right] \times 100$$

where, A_s is the absorbance of the sample, A(-ve) and A(+ve) are the absorbance values of negative and positive controls. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. Tests were carried out in triplicate.

Conclusion

In summary, our results demonstrated that all extracts of *M. parviflorum* have efficient phenolic compounds. The MBE extract has the highest phenolic contents (52.23 ± 1.32%) and it exhibited the highest antioxidant activity (76.8 ± 1.51%). But, MLE exhibited the highest free radical scavenging activity (61.16 ± 1.42%).

It was concluded that all extracts of the *M. parviflorum* have antioxidant activity because of high content of phenolic compounds. Results suggested that strongest free radical scavenging activity may be related with the higher levels of phenolic content. As mentioned above, MLE exhibited the strongest free radical scavenging activity but it has least phenolic contents as compared with MBE. In this case, the polarity of the active compounds of the extract is important. If the polarities of the active compounds lower than the scavenged radicals, the radical scavenging activities are reduced. With reference to these findings, more researches are needed to identify the active compounds of the extracts and their health effects of these substances.

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