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Biochemical Evaluation of Ornamental and Native *Muscari* and *Bellvalia* Genotypes with the Attitude of Using them as Valuable Edible Species

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Abstract

Muscari and *Bellvalia* are related ornamental bulbous plants that grow natively in several areas of Iran. These species are highly used as potted and outdoor ornamental plants. Also, these plants have been used as edible species in some regions, but they are not common in Iran. These ornamental plants have the potential to be introduced as valuable edible genotypes and enter the process of domestication and commercial production. Besides ornamental uses, these plants are used primarily as mucilage and filler in cooking. Moreover, various phenolic and saponin compounds affect flavor, too. In the present study, the physicochemical properties of three species of *Muscari*, including *comosum*, *botryoides*, and *neglectum*, and one species of *Bellevalia* from native places were evaluated. These antioxidant capacity, total phenol, and total content of saponin, mucilage, alkaloids, and steroids in leaf and bulb extracts were examined. By evaluating the various metabolites of proposed plants, it can be concluded that *M. comosum* is a suitable genotype for entering the domestication process and as a parent for hybridization. Also, the lesser-known genotype, *Bellevalia paradox*, in addition to being an ornamental plant, has valuable nutritional properties, and further studies on this genotype will have an appropriate approach.

Keywords: Antioxidant capacity, Grape hyacinth, Mucilage, Phenolic compound, Saponin.

INTRODUCTION

Some plants have been used for medicinal purposes for thousands of years. This group of plants is known for being the source of many natural ingredients that heal human health problems. *Muscari* and *Bellevalia* are two native genera that grow naturally across some regions of Iran, and they are usually known and consumed as edible and medicinal plants. Despite the different karyological aspects in these two genera, they have similar morphological and molecular features, making them be considered originating from the same clade (Azizi *et al*., 2016; Johnson, 2003; Pfosser and Speta, 1999).

Muscari, *Pseudomuscari*, and *Leopoldia* are three subgenera in the genus *Muscari,* which is classified in the Asparagaceae family (Speta, 1998). *Muscari* is also known as grape hyacinth due to the shape of the inflorescence. This genus has also been interested in its several ornamental usages, such as its usage as a garden plant, pot plant, and cut flower because of producing colored beautiful and sweet-scented inflorescence in spring (de Hertogh and le Nard, 1993; Qi *et al*., 2013). Some plants of this genus are consumed as edible plants in several countries, such as Iran, Italy, and Turkey. In Turkish traditional medicine, *M. neglectum* species are known as expectorants, appetizers, and diuretics and are used to treat warts, which seems that some compounds in these plants have antiviral properties (Özkan *et al*., 2017).

In Iranian traditional medicine, this species is known as Kalaghak and is used for disorders of the digestive system and uterus. Bulbs of this species are traditionally used in southern Italy as medicine to treat facial blemishes and toothache (Motti *et al*., 2009). The nutritional value and antioxidant capacity of bulbs of this species, which are widely used in Italian food, have been reported in several studies (Casacchia *et al*., 2017; Pieroni *et al*., 2002). The flowers of this species are sweet and are used as flavoring (Lim, 2014; Wright, 2001). There are also edible types among the species of the *Bellvalia* genus. For example, in Turkey, the leaves of *Bellevalia paradoxa* are used in foods (Altundağ, 2009). Phenolic compounds are a leading group of plant antioxidants (Kochan *et al*., 2019). A comparison of some ethnic vegetables in southern Italy showed that the antioxidant activity of *Leopoldia comosa* (L.) Parl. (syn. *Muscari comosum* (L.) Miller) bulbs were higher than that occurred in the other 26 studied species, including *Asparagus acutifolius* L. shoots (Pieroni *et al*., 2002). Another 2018 report indicated that the total phenolic compound in *Muscari armeniacum* Leichtlinex Baker was 88.19 mg GAE/100 g, higher than that found in the other eight tested species. Antioxidant activity in this research was about 11% for *M. armeniacum* (Özcan *et al*., 2018). Saponins are an important group of plant secondary metabolites, which include glycosylated triterpene or steroids. Some properties of saponins are hemolytic activity, cholesterol-binding properties, and bitterness. Most of the properties of saponins are beneficial. Therefore, plants with a specific saponin content are popular as folk medicine (Price *et al*., 1987). Extensive research about *Muscari*'s saponin content, especially *Bellevalia* species, is not satisfying. A few research studies have demonstrated the presence of saponins in the bulb and leaf of some *Muscari* species, such as *Muscari longipes* (Masum and Osw, 2016).

The use of various polysaccharides in the food industry (especially gums and mucilage) has increased due to their edible properties, including thickening and stabilizing the taste and color of food, as well as having medicinal properties in the control and prevention of cardiovascular diseases (Gao *et al*., 2017; Kaur *et al*., 2018) studies on some plants of Asparagaceae, such as *Asparagus racemosus* Wild. Indicate the appropriate ability of this family's plants to produce mucilage (Gheybi *et al*., 2021; Saju and Sivaraman, 2021). Studies have shown the presence of alkaloids and steroids in *Muscari* and *Bellevalia* genera, but their amount has yet to be measured. Some plant alkaloids are medicinal, and some are classified as toxic, so their identification in

edible plants is essential. Steroids are important in maintaining salt balance, preventing thyroid problems, and enhancing sexual power (Nasrudin, 2017).

Given that these two genera include many plant varieties valuable in their health and medicinal properties on one hand and offering many culinary values besides their remarkable worthy ornamental characteristics in the garden and interior spaces on the other hand, it would be highly reasonable to do more investigations for identifying those varieties rich in valuable and healthy compounds which can be later introduced as commercial cultivars in mass field production.

In this study, we aimed to identify and compare several selected genotypes regarding medicinal and edible value by measuring some of their biochemical traits. Hence, three *Muscari* and a *Bellvalia* were analyzed to determine the antioxidant, total phenols, mucilage, alkaloids, and steroids. These parameters are essential to perform the edible and medicinal properties of the selected plants.

MATERIALS AND METHODS

Materials

Some different species of *Muscari* and *Bellevalia* were collected from northwest Iran. These plants were identified by field research and, in some cases, the cooperation of villagers and foresters to find original habitats. Local people consumed all the genotypes collected in the primary habitat. The Center of Genetic Resources of Iran studied and identified the collected samples according to morphological characteristics and keys (Rechinger, 1990). The identified genotypes were classified into three species of *Muscari* and one species of *Bellevalia*, including *M. neglectum* (M1), *L. comosa* (M2), *Muscari botryoides* (M3), and *B. paradoxa* (B5) (Fig. 1). Based on this, the two studied genotypes were *Muscari comosum* species collected from Node village in Ardabil province and Sahand mountain hillsides. Also, *M. botryoides* species were collected from Khalkhal summer areas, *M. neglectum* species were collected from the original areas of Khalatposhan research station in the east of Tabriz, and *Bellevalia paradoxa* species were collected from relatively high areas of Sahand mountain. The identified samples were also matched with the herbarium samples of the Tabriz University Agriculture Faculty, and their codes were recorded in table 1. In the case of sample M3, there was no similar case in the herbarium of the Agriculture Faculty.

Fig. 1. Digital photographs of the collected initial plants used in this study. The width of leaves, shape, and color of flowers and bulbs are the significant characteristics that helped to identify the species. M1: *Muscari neglectum* Guss. ex Ten; M2: *Leopoldia comosa* (L.) Parl. From Ardabil province; M3: *Muscari botryoides*; M4: *Leopoldia comosa* (L.) Parl. From East Azarbaijan province; B5: *Bellevalia paradoxa* (Fisch. & C.A.Mey.) Boiss.

Thirty bulbs were randomly collected from each identified species (Table 1). In all identified regions, almost the same areas were considered first, and then, by throwing a piece of wood, the plants in the place where the wood landed were collected. All bulbs were planted in the same climate and substrate and harvested at the stage of the whole opening of the florets. The harvested plants were washed with distilled water, and after quick freezing in liquid nitrogen, they were kept in a freezer at -80 °C until the experiments.

Table 1. The location of plants of the Asparagaceae family was used to analyze this study.

Species	Another name	Location	Ltitude (M)	Longitude	Latitude	Herbarium code
Muscari neglectum Guss. ex ten (M1)		East Azarbaijan, Tabriz-Basmenj Rd, Khalatposhan	1564	38˚1ʹ54.214''	46˚23ʹ38.927''	13357
Leopoldia comosa (L.) Parl. (M2)	*Leopoldia comosa* (L.) Parl.	Ardabil, nowdeh village, Aq dagh mountain range.	1576	37˚21ʹ23.206''	48˚27ʹ38.892''	6915
Muscari botryoides (M3)		Ardabil, Asalem-Khalkhal Rd, Finaroud.	2024	37˚37'39.049''	48˚37'58.864''	---
Leopoldia comosa (L.) Parl. (M4)	*Leopoldia comosa* (L.) Parl.	East Azarbaijan, Isparaxan, Sahand mountain range.	2582	37˚48'49.716''	46˚24' 24.393''	6915
Bellevalia paradoxa (Fisch. & C.A.Mey.) Boiss. (B5)		East Azarbaijan, Isparaxan hotspring Rd, Sahand mountain range.	3094	37˚45'46.868''	46˚22' 56.392''	1669

Extraction of samples

The bulbs and leaves of samples were lyophilized (DENA®) at -20 °C and ground into a fine powder using mortar and pestle; the 200 mg of fine powder of each sample was added in 1800 µL of methanol and water (70:30). Samples were then being moved slightly for 30 minutes by shaker and centrifuged for 10 minutes at 10000 g. After separating the supernatant, 1800 µL of methanol and water (70:30) mixture was added to the vial, and the previous step was repeated. The supernatant obtained from two steps was transferred to new microtubes and mixed for further studies.

Total phenolic compound content

The total phenolic compound content of the bulbs and leaves extract was determined by spectrophotometric technique with the Folin-Ciocalteu reagent (Singleton and Rossi, 1965) with some modifications. As the standard for the calibration curve, pyrocatechol (Sigma C9510) (1-10 µg mL-) was used, following the mixing of 100 µL of extraction or standard and 2 mL of 2% Na_2CO_3 (Merck 106392). The samples were kept at room temperature for 2 minutes. After this time, 100 µL of Folin-Ciocalteu reagent (1/2 diluted) was added to the mixture, and samples were incubated in the dark for 30 minutes. The absorbance was recorded at 720 nm at the end with a UV-visible spectrophotometer (Specord, Analytik Jena, Germany). All the procedures were followed for the blank sample, though water was added instead of extracted. The results were interpreted as µg pyrocatechol equivalent (PEs) per mg dry weight of plant material.

Evaluation of antioxidant capacity by the DPPH assay

Radical scavenging capacity was determined with 2,2-Diphenyl-1-picrythydrazyl (DPPH) according to the method of Brand-Williams *et al*. (1995). A methanolic solution of DPPH (60 µM) was prepared for use as fresh. After adding of 50 µL of each extract to 1950 µL of DPPH solution, the reaction mixture was shaken vigorously and incubated in darkness at room temperature for 15 minutes. The absorbance of samples and control containing DPPH solution without extract was recorded at 517 nm using a UV-Visible. Methanol is used as a blank. The amount of DPPH radical scavenging activity by the methanolic extract of *Muscari* was calculated with the following formula:

Free radical scavenging activity = [1-(sample absorption- control absorption) / control absorption × 100]

After obtaining the percentage of free radical scavenging capacity, the IC_{50} value of the extract and ascorbic acid (Sigma A5960) were determined. The amount of sample concentration required for inhibition of 50% of the initial concentration of DPPH radicals was defined as the IC_{50} (µg of dry sample per mL), and its value is obtained by plotting different RSA values according to different sample concentrations and calculating the equation of the regression line.

Evaluation of saponin content by spectrophotometric analysis

The saponin content was determined using the method of Nickel *et al*. (2016). At first, 25 mL of 50% ethanol was added to 110 mg of fine powder of dry plant material and allowed to macerate for 72 h at room temperature. The extract was filtrated and marked up to 25 mL with 50% ethanol. To evaluate saponin content, 2 mL of extract or standard was added to 7 mL of Lieberman-Burchard reagent (16.7% of acetic anhydride in sulfuric acid concentrated). The mixture was incubated at room temperature for 30 minutes, and after that time, the absorbance of the mixture was recorded at 528 nm. A standard saponin curve (50-350 µg/mL) was used to measure saponin concentration in the samples, and the results were expressed as % dry weight of plant material.

Evaluation of total mucilage content

Dried samples powdered and defatted using petroleum ether solvent. After removing the solvent, the remaining materials were dried at room temperature. The 200 mg of dried plant material was kept in 1 mL of distilled water for 12 hours and then placed in a bain-marie at boiling temperature for one hour. The mixture was filtered and then mixed with the same volume of 96% ethanol to precipitate mucilage. Finally, the water from the samples was removed in a freeze-dryer, and mucilage powder was obtained. The amount of mucilage was expressed by its weight and its percentage over the weight of plant tissue (Deore and Khadabadi, 2008).

Evaluation of total alkaloid content

The total alkaloid of bulbs and leaf extracts was measured using the UV-visible technique (Sreevidya and Mehrotra, 2003). This method used a Dragendorff reagent (Sigma 44578), and the samples' absorbance was recorded at 435 nm. Different concentrations of bismuth nitrate (Sigma 254150) were used to plot the standard calibration curve, and the alkaloid concentration of all samples was measured using the calibration curve.

Evaluation of steroid content

The content of total steroids of sample extracts was measured by the Ray and Gupta

method (Ray and Gupta, 1994), with some adaption and the use of potassium hexacyanoferrate (Merck 702587). Sulfuric acid 4 N and 0.5% iron chloride (Merck 157740) were added to the methanolic extracts of samples and combined with 0.5% potassium hexacyanoferrate solution. The solution was incubated for 30 minutes at 70 °C; their absorbance was recorded at 780 nm. Cycloartenol (Merck 08172) was used as a standard, and the total steroid content of the samples was calculated in terms of mg of cycloartenol equivalent per gram of the sample's dry weight.

Statistical analysis

The Kolmogorov-Smirnov test analyzed data's normality and homoscedasticity using the Hartley test. The results were statistically evaluated by one-way analysis of variance (ANOVA), the Tukey test determined mean difference at a 5% level of probability, and correlations between variables were determined by the Pearson correlation coefficient ($P < 0.001$). All analyses were performed using IBM SPSS 21.0 software.

RESULTS AND DISCUSSION

Total phenols content

The total phenol content of leaves and bulbs of *Muscari* species and *Bellevalia* are given in table 2. Phenolic compounds are one of the primary metabolites of plants that correspond to the human diet. These compounds have various physiological properties such as anti-allergic, anti-arthrosis, anti-inflammatory, antimicrobial, antioxidant, anti-thrombosis, heart protection, and vasodilation. The beneficial effects of phenolic compounds have been attributed to their antioxidant activity (Balasundram *et al*., 2006). Therefore, total phenol and antioxidant capacity are the primary parameters to consider in plant samples. The content of total phenols in bulb extracts varied from 16.27 to 20.03 µg of PEs/mg of dry weight, and in leaf extracts ranged from 17.51 to 21.27 µg of PEs/mg of dry weight. In bulb extraction, *Bellevalia paradoxa* (B5) had the highest amount of total phenols content, and the lowest amount was determined in M1 and M3 ($P<0.05$). The B5 and M4 had the highest total phenol content in the leaf extracts ($P<0.05$). The leaves of M2 were provided lower phenols compared to the other species. The genotypes perfectly affected total phenol content, and the *Leopoldia* subgenus had more phenol in bulbs than the *Muscari* subgenus. The total phenols content of M2 and M4, which are the same species but coming from different locations, were the same in bulbs and leaves and had no significant difference ($P<0.05$). In the survey literature, the total phenol in *Allium oschaninii* was determined to be 17.18 mg gallic acid/g, which follows the results obtained from our examined cultivars (Lu *et al*., 2011). In the proposed research, the total phenol content of three varieties of edible onion, *Allium cepa* L., was much lower than *Allium toscanini*'s. Hence, these results confirm the high nutritional value of *Muscari*.

Table 2. Total phenolic compound (µg of PEs/mg) of bulb and leaf extracts.

Genotypes	Bulbs	Leaf
M1	16.27^{c*}	17.51^{c}
M2	17.68^{b}	18.56^{b}
M3	16.60^{c}	17.92^{c}
M4	17.37^{b}	19.37^{a}
B5	20.03^{a}	21.27^{a}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

The changes of secondary metabolites in the studied genotypes are affected by the genetic differences of the studied species, although, the climate will also affect the occurrence of these phenotypes. All species had higher total phenol content in their leaves than in their bulbs. Experiments conducted on *Muscari parviflorum* Desf. and *Allium jesdianum* (Ghasemi Pirbalouti, 2019; Mammadov *et al*., 2012), showed that the amount of total phenol in leaf extract was higher than in bulb extract, which is like our findings. It should be noticed that native people generally prefer leaves of M4 and M2 to those of other species.

Antioxidant capacity

Determination of antioxidant capacity by DPPH is an indirect method based on scavenging stable free radicals (DPPH) by hydrogen donors in plant extracts. This method is also the most common and oldest for antioxidant capacity determination (Roginsky and Lissi, 2005). Likewise, evaluating IC_{50} is an efficient way to compare the antioxidant activity of samples. In the current study, ascorbic acid was used as a standard index for measuring and comparing the antioxidant capacity of samples. The IC_{50} value has an inverse relationship with the antioxidant capacity; therefore, the higher the antioxidant capacity of a sample, the lower the IC_{50} value. Consumers are interested in edible flowers as food due to their benefits, such as their anti-allergy and anti-inflammatory properties. Scientific findings indicate that medicinal plants have other potential benefits, such as preventing oxidative damage, reducing blood sugar, and fighting cancer (Chensom *et al*., 2019; Fernandes *et al*., 2017). The antioxidant capacity of the bulb and leaf extracts of samples are listed in table 3. The bulb extract of B5 had significantly the lowest amount of IC_{50} at 195.5 µg/ml ($P<0.05$) comparing the extracts taken from the other bulbs. Therefore, the highest antioxidant capacity was related to the B5. The high antioxidant capacity in the *Bellevalia* bulb can be due to the presence of phenolic compounds in this plant; as seen in table 2, this plant has the highest concentration of phenolic compounds among the other samples. It has been proven in many reports that different levels of total phenol have a direct effect on the antioxidant activity of the plant (Santas *et al*., 2008; Sellappan and Akoh, 2002).

In Mexico, four types of common medicinal plants were examined, and *Myrtillocactus geometrizans* provided the highest antioxidant activity of 675.06 µmol TE/g DW. Also, this plant's phenol was obtained higher than other species (Pinedo-Espinoza *et al*., 2020). Both phenolic acids and flavonoid compounds, which are a subset of phenolic compounds, act as strong and well-known antioxidants by chelating various ions and combining with free radicals, especially superoxide, peroxyl, and hydroxyl radicals, therefore protecting against DNA damage and preventing the peroxidation of phospholipids, which can lead to the damage of biological membranes (Urquiza-Martínez and Navarro, 2016). In recent years, there has been interest in phenolic compounds and their antioxidant activity among consumers and the scientific community. Meanwhile, epidemiological studies have linked diets rich in natural antioxidants with a reduced risk of oxidative stress-related diseases such as cancer and cardiovascular disease (Chen *et al*., 2016). The lowest antioxidant capacity is obtained for M1, with 27.54% and 34.98% in bulb and leaf extracts, respectively. The antioxidant capacity of leaves was significantly higher than that in all species' bulbs ($P<0.05$), as it has been shown in a study that the antioxidant activity of the extract of the herbal parts of *M. neglectum* is higher than its bulb (Özkan *et al*., 2017). Comparing the samples taken from M2 and M4 plants revealed a significant difference in the antioxidant capacity of their extracts, and M2 had the highest antioxidant activity in both leaf and bulb extracts ($P<0.05$). It is probably caused by the climatic effect that ultimately led to changes in the production of metabolites and antioxidant

capacity. However, for the more correct and explicit conclusion, it is necessary to grow both plants in a similar climate at a greenhouse so that the plant's phenotype can be compared. Despite the low antioxidant capacity of genotype M1 compared to other studied genotypes, this species has a higher antioxidant capacity than many plants. For example, in the study about the antioxidant capacity of *Allium sativum* and *Allium ascalonicum* bulbs as two common edible species, the IC_{50} results were 5300 and 1330 µg/ml, respectively (Povichit *et al*., 2010), which are much higher than the genotypes of this study. Accordingly, it can be said that consuming a lower amount of *Muscari* bulbs compared to onions or shallots provides similar antioxidant compounds for the human body.

Table 3. Antioxidant activity of bulb and leaf extracts of different species.

Genotypes	Antioxidant capacity by DPPH			
	DPPH radical scavenging capacity (%)		IC_{50} (µg/ml)	
	Bulb	Leaf	Bulb	Leaf
M1	27.54^{e*}	34.98^{d}	277.3^{a}	322^{a}
M2	40.86^{b}	42.49^{b}	245.2^{c}	258^{c}
M3	30.19^{d}	39.14^{c}	246.4^{b}	273.3^{b}
M4	35.58^{c}	39.25^{c}	240.5^{b}	270.7^{b}
B5	44.55^{a}	66.83^{a}	179.0^{d}	195.5^{d}
Ascorbic acid	94.2	68.3		

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

T he study demonstrated a notable range in phenol content among the different genotypes. *Bellevalia paradoxa* (B5) exhibited the highest phenolic content in bulb and leaf extracts, suggesting a strong potential for antioxidant activity. This test was confirmed by the DPPH assay, where B5, with the highest antioxidant capacity, showed the lowest IC50 value, indicating its efficacy in scavenging free radicals. The higher phenol content and antioxidant capacity in leaves compared to bulbs across all species align with previous findings in similar studies (Ghasemi Pirbalouti, 2019; Mammadov *et al*., 2012; Pinedo-Espinoza *et al*., 2020). This could be due to the greater exposure of leaves to environmental stressors. The significant antioxidant capacity of *Muscari* species, even in the genotype with the lowest performance (M1), underscores their potential as dietary sources of antioxidants. The comparison with *Allium* species, commonly consumed for their health benefits, highlights *Muscari*'s competitive edge in providing similar or superior antioxidant benefits with potentially lower consumption quantities.

Saponins content

Saponin contents and their variation across the species and plant parts are illustrated in Fig. 2. Consuming saponins can reduce cholesterol concentration in the plasma, reducing the risk of heart disease and inducing cancer cell death through different pathways (Lorent *et al*., 2014). This is probably due to the stereo structure of the saponins. However, the health benefits of saponins make it attractive to find natural sources of saponins in the human diet. Among the species, this substance concentration varied from 1.85% to 4.03% in bulbs and from 1.30% to 3.21% in leaf extracts. In fiber-rich powders from asparagus, saponin content ranged from 2.14 to 3.64 mg/g (Fuentes-Alventosa *et al*., 2009). Saponins of some allium species, as common edible plants, were detected between 2 and 3 mg/g dry matter (Smoczkiewicz *et al*., 1982), which are close to the results of genotypes In this study. *Allium nigrum*, also known as black garlic, has a high saponin content in bulbs (19.38 mg/g DW), which is higher than that of leaves

(10.48 mg/g DW) (Mostafa *et al*., 2013). Plants of M2 and M4 showed the highest saponin content in bulb and leaf, respectively. This result illustrates the role of environmental factors in saponin fluctuations over different climatic regions.

Given that the saponin content of the bulbs was higher than in the leaves of all samples, this may be the reason for the higher degree of bitterness of the bulbs when compared to the leaves. The lowest saponin content belonged to the leaf extracts of M3, which were 1.30%. Although, saponin terpenoids increase the medicinal value of plants, due to the bitter and astringent taste, these parts may be less accepted by consumers, especially when eaten fresh.

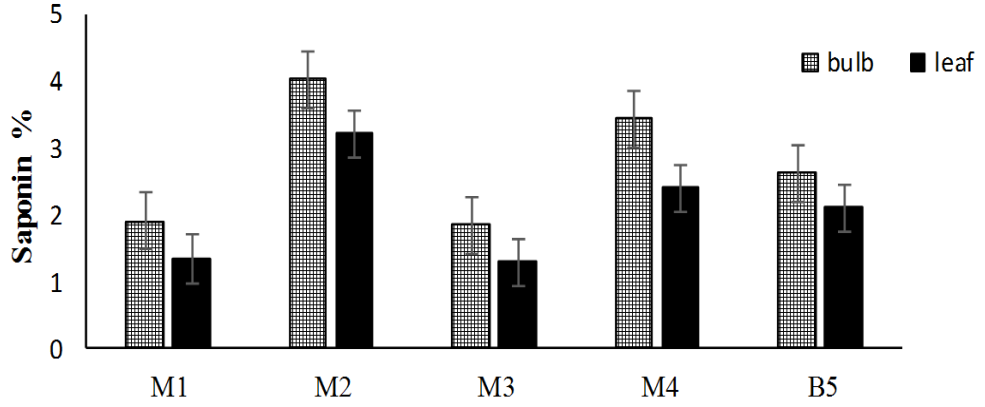

Fig. 2. Saponin content (%) of plant species' bulb and leaf extractions.*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

Saponins, known for their bitter taste and health benefits, varied significantly across the species. M2 and M4 showed the highest saponin content in bulbs and leaves, respectively. These findings suggest that environmental factors significantly influence saponin levels, which are higher in bulbs than in leaves (Ariyanti and Latifa, 2021; Mostafa *et al*., 2013). The bitter taste of high saponin content might affect consumer acceptance, especially for fresh consumption. However, their medicinal properties, such as cholesterol-lowering effects and immune system enhancement, make them valuable.

Total mucilage content

The mean comparison of mucilage percentage of bulb and leaf samples is given in table 4. Mucilage is a sticky, mucus-like substance produced by plants. Its main components are polysaccharides, proteins, minerals, lipids, and uric acid units. Researchers have proven that adding mucilage to food formulations improves nutritional quality (Goksen *et al*., 2023). This substance has also been reported to be beneficial for health; oral Mucilage consumption helps reduce blood cholesterol levels (Dhar, 2005). In general, the mucilage in bulb tissues was higher than in leaves in all plants, but the bulb of M3 had much lower mucilage than other species. Bulb of M2 consumed more than other bulbs and has the highest amount of mucilage, an essential factor in justifying its high use. The mucilage content of the bulb of M2 is higher than the yield of the *O. cuspidatum* bulb's mucilage reported in 2021, a plant of the same family. In this research, the mucilage percentage of the plant's root was obtained as 16.4%, and it was introduced as a suitable plant for use in the food industry with the property of thickening food and increasing its taste (Gheybi *et al*., 2021). Most of the Malvaceae family, such as *Adansonia digitata* L., *Gossypium* spp., *Hibiscus cannabinus*, *Plantago psyllium*, and *Abelmoschus* spp L., are known due to their significant mucilage content (Ahmad *et al*., 2009). The 21 species

of *Okra abelmoschus* spp L., one of the most famous mucilages edible plants, provide 6.52 and 37.67 mg/kg of mucilage in the fruits (Ahiakpa *et al*., 2014). The amount of mucilage in *Plantago ovata* and *P. psyllium* is obtained in the range from 13.52 to 18.60%, and these results showed that two different environments can differentiate genotypes in terms of the amount of mucilage (Shahriari *et al*., 2018).

In addition, mucilage has different properties depending on the type of monosaccharides that constitute it. Various commercial medicines have been formulated from mucilage. In this study, only the total amount of these substances has been evaluated, and accurate studies are needed to fully identify the mucilage components of *Muscari* genotypes.

Table 4. Percentage of mucilage extracted from plant samples.

Genotype	Bulb	Leaf
M1	12.73^{d*}	10.40^{c}
M2	19.29^{a}	15.32^{a}
M3	8.72^{e}	6.48^{e}
M4	16.60^{c}	7.06^{d}
B5	17.88^{b}	12.45^{b}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

The study confirmed that the mucilage content was higher in bulbs than in leaves, with M2 bulbs showing the highest content. This polysaccharide has various applications in the food industry due to its thickening properties and potential health benefits, including improved digestion and bowel health. The high mucilage content in *Muscari* species is advantageous for culinary and health applications, adding to their value as edible plants (Gao *et al*., 2017).

Total alkaloid content

The number of alkaloids in all samples was generally insignificant (Table 5). However, the highest amount was seen in both leaf and bulb extracts of B5 and M4. Alkaloids give the plant a bitter taste; in this sense, the public does not accept their presence and high amount in the edible plant. Some alkaloids, such as caffeine or quinine, cause bitterness in food products (Briand and Salles, 2016). The presence of alkaloids has been detected in *M. neglectum*, but its amount has not been measured (Nasrabadi *et al*., 2013). However, some alkaloids also have medicinal properties. A study reported that the main extracted alkaloids of *M. armeniacum* bulb included hyacinthine A1, A2, A3, and B3. Hyacinthacine alkaloids have glycosidase inhibitory activities. These alkaloids are anti-cancer, anti-viral, anti-diabetic, and anti-obesity compounds (Savaspun *et al*., 2014). In all samples, the amount of alkaloids in bulbs was higher than in leaves, which can justify the bitter taste in bulbs compared to leaves.

Table 5. Total alkaloids content (µg g^{-1} DW).

Genotype	Bulb	Leaf
M1	1.22^{e*}	1.10^{d}
M2	1.47^{d}	1.22^{c}
M3	1.59^{c}	1.45^{b}
M4	2.03^{b}	1.48^{b}
B5	2.21^{a}	2.06^{a}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

Toxicological studies on *Muscari* alkaloids are limited but can be toxic, particularly in high concentrations. However, *Muscari* species, such as hyacinthine A1, A2, A3, and B3, have been shown to exhibit significant bioactivity. These compounds can offer medicinal benefits, such as glycosidase inhibitory activities that may have anti-cancer, anti-viral, anti-diabetic, and anti-obesity effects (Savaspun *et al*., 2014). Still, they can also pose risks if consumed in large amounts.

Total steroids content

In previous studies, the presence of steroids in the leaves and bulbs of some *Muscari* species has been determined, but no study has been done on the total amount of steroids in this genus (Nasrabadi *et al*., 2013). Steroids are known for their medicinal properties, such as antitumor, liver protection, antibacterial, plant growth regulators, and antiparasitic, and they are attractive to herbalists (Petersen and Simmonds, 2003). The number of total steroids in the plant samples of this study is remarkable compared to the amount of the total steroid in the root of *Asparagus racemosus*, which is a well-known medicinal plant of the same family, and it was measured in previous studies as 27.5 µg/mg (Saraswathi *et al*., 2020). In a study on ten medicinal herbals in India, they set the amount of total steroid as the main parameter to determine the medicinal activity of the plant. The *Foeniculum vulgare* plant has shown the highest steroid amount of 68.39 µg/mg (Madhu *et al*., 2016). Comparing these results with our experiment highlights our studied genotypes. In general, the amount of steroid in leaves was higher than in bulbs, and the highest amount in both leaves and bulbs was related to B5, followed by M4 (Table 6).

The difference in the amount of steroids in genotypes was significant and considerable. Steroids do not have a noticeable effect on the taste and color of the product. In many cases, these substances do not have a known role in consumers' bodies, although some can act as compounds like animal steroid hormones. In this study, the total concentration of steroid compounds in the plant has been evaluated, and it is necessary to identify and accurately evaluate the steroids of *Muscari* in the following studies.

Table 6. Total steroids of plant samples (mg g^{-1} DW).

Genotype	Bulb	Leaf
M1	15.25^{d*}	67.45^{e}
M2	54.05^{c}	88.55^{c}
M3	50.80^{c}	77.42^{d}
M4	62.50^{b}	101.65^{b}
B5	70.62^{a}	110.12^{a}

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Tukey test.

The study also assessed alkaloid and steroid contents, which, although generally lower than other metabolites, were significant in B5 and M4. Steroids were more concentrated in leaves than bulbs, with B5 again showing the highest content. Alkaloids contribute to the bitter taste but also possess medicinal properties such as anti-cancer and anti-viral activities (Karolkowski *et al*., 2023; Rajput *et al*., 2022). This highlights the dual ornamental and medicinal potential of *Bellevalia paradoxa*, suggesting further exploration and utilization of these compounds.

The present study aimed to evaluate the biochemical properties of different *Muscari* and *Bellevalia* genotypes to assess their potential edible species. The investigation focused on various genotypes' total phenol content, antioxidant capacity, saponin content, mucilage

content, alkaloid content, and steroid content. The results reveal significant variations among the genotypes, highlighting the potential of specific species for both nutritional and medicinal uses.

CONCLUSION

This study demonstrated the considerable potential of the *Muscari* and *Bellvalia* species for cultivation and utilization as a valuable source of nutrition and medicinal plants. Specifically, when comparing different genotypes, the *M. comosum* species, widely recognized as an essential edible genotype in countries like Turkey and Italy, emerged as the most suitable candidate for inclusion in domestication programs among the genotypes examined.

Moreover, the experiment revealed promising results for the less familiar *Bellevalia paradox*, which in certain instances exhibited outcomes comparable to or even superior to those of *Muscari* species. This suggests that *Bellevalia paradox* could be regarded as a valuable genotype with antioxidant-rich compounds, capable of competing with *Muscari* for significant presence within agricultural production settings.

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