

EXPERIMENTAL PAPER

The antimicrobial and antioxidant activity of *Muscari neglectum* flower ethanol extract

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Summary

Introduction: *Muscari neglectum* has been used as food in some countries. **Objective:** The antimicrobial and antioxidant activities of *M. neglectum* were the subject of the study. **Methods:** In this study, the total phenolic, flavonoid, monomeric anthocyanin contents, antioxidant and antimicrobial activity of *M. neglectum* flowers ethanol extract were determined by different methods against some food poisoning microorganisms. **Results:** Total phenolic, flavonoids and anthocyanin contents of the extract were 18.2, 0.94 and 0.11%, respectively. The IC₅₀ for *M. neglectum* was higher than that of BHT. *M. neglectum* extract showed no inhibition zone (IZ) against *Staphylococcus aureus* and had small IZ against *Candida albicans* and *Aspergillus niger*. *Shigella flexneri* and *Escherichia coli* had the higher IZ than the others. The lower MIC and MLC values were for *C. albicans*, followed by *Sh. flexneri* and *E. coli*. *S. aureus* had the higher MIC and MLC values than the others. **Conclusion:** Therefore, the *M. neglectum* flower extract can be used as a natural preservative and coloring agent in foods as replacement of synthetic ones.

Key words: *Muscari neglectum*, flower, preservatives, food, phenolics, anthocyanins

INTRODUCTION

Food poisoning is a process, in which food decomposes due to attack by microorganisms (bacteria, mold and yeast) or oxidation processes and enzyme activities. Therefore, the food deteriorates to the level in which the nutrient is not suited for human purposes. For preventing from such conditions, some preservatives were used for prolonging the shelf life of foods. The preservatives are natural or man-made substances that prevent the products from decomposition by microbial growth or can be antioxidants which inhibit the oxidation of food components [1]. Furthermore, the safety of these preservatives is the topic of debate among many scientists. They have shown that some modern synthetic preservatives cause respiratory or other health problems such as asthma or hyperactivity in children [2]. Hence, the popularity of medicinal plants as natural preservatives has been increased by scientists [3]. One of the least studied medicinal plants is *Muscari neglectum* (Liliaceae family). *M. neglectum* is a perennial bulbous plant with the common name "Grape Hyacinth". It naturally prefers well drained sandy, poor ground with acid to neutral conditionse. The violet blue flowers were emerged in springtime. The flower buds, bulbs, leaves [4], fruits [5, 6] and roots [7] are used as food in different cultures. The leaves, flowers and flower buds are edible as raw, boiled, grilled or pickled. The decoction of *M. neglectum* fruits are used internally for the treatment of rheumatism in Turkey [5, 6]. *M. neglectum* roots have pectoral stimulatory effects, anti-inflammatory, anti-allergic and aphrodisiac effects [7]. *M. neglectum* flowers are used in raw form as snack [8]. Furthermore, *M. neglectum* has been the most important plant in dyeing industries from the ancient times. Likewise, the flowery aerial parts of *M. neglectum* were boiled with eggs during the "Nowroz" in Iran for making the eggs purplish [9]. There are some studies that evaluate the chemical composition and biological activities of *M. neglectum* [4, 10, 11]. Homo-isoflavanones [4], flavonoid, alkaloid, terpenoid and steroid [11] compounds were identified in *M. neglectum* extracts. The *M. neglectum* prevents the woods from fungal spoilage like "*Trametes versicolor*" [10].

Thus, due to little information in regard to *M. neglectum*, the subject of this research was to evaluate the preservative potency (antimicrobial and antioxidant) of *M. neglectum* ethanol extract with estimation of total phenols, flavonoids, anthocyanins of *M. neglectum* flower ethanol extracts.

MATERIAL AND METHODS

Plant material and extraction

Muscari neglectum Guss. flowering aerial parts were gathered from Mahalat Road, Arak, Iran at a full flowering stage in March-April 2013 and were authenticated under number 1631-1. Fresh *M. neglectum* flowering aerial parts were mixed with

ethanol-water (70:30 v/v) for 24 h at the ambient temperature in the percolator. The extract was separated, and the ethanol was removed from the solution under vacuum.

Antioxidant activity of *M. neglectum* ethanol extract by DPPH

Radical scavenging effects of extract were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution. Different concentrations of ethanol-water extract were mixed with 2 ml of DPPH methanol solution. Butylated hydroxytoluene (BHT) ethanol solution was used as synthetic antioxidant. The absorbance of the solutions was read against a blank at 517 nm after 70 min incubation period at 37°C. Suppression of free radicals by DPPH was calculated in percent (I%) in the following way:

$$I\% = [A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}] \times 100$$

where A is the absorbance of reading at 517 nm after 70 min incubation period at 37°C. The IC₅₀ was determined as the concentration of compound that inhibits 50% of DPPH solution. Tests were carried out in triplicate [12].

Total phenolic content

Total phenolic content of extract was determined by a spectrophotometer using the Folin-Ciocalteu reagent [13]. 10 mg of dried extract was dissolved in 10 ml of ethanol (1 mg/ml). 0.2 ml of extract with 3 ml of water and 0.25 ml of Folin-Ciocalteu's reagent were mixed and swirled. After 3 min, 0.75 ml of 20% (w/v) sodium carbonate solution was added and mixed. Deionized water was added until the volume reached exactly 5 ml. The solution was mixed thoroughly and allowed to stand at ambient temperature for 2 h. The absorbance of reaction mixture was evaluated at 760 nm. All trials were conducted in triplicate and averaged. Quantification of total phenolic content was based on a standard curve generated by Gallic acid and the following equation was created:

$$A = -0.007 + 0.1142w,$$

where A is absorbance and w is the weight. The results were expressed as percent (w/w) of dry extract as gallic acid equivalent.

Total flavonoid content

The aluminum chloride colorimetric method was used [14]. 1 mg/ml solution of extract was used for calculating the total flavonoid content. 0.5 ml of extract was mixed with 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and diluted to 5 ml with distilled water. The absorbance was measured at 415 nm after

30 min. The total flavonoid content of extract was determined using a standard curve established with quercetin and the outcomes were expressed as percent (w/w) of dry extract in the following equation:

$$A = -0.0099 + 0.0129w,$$

where A is absorbance and w is the weight.

Total monomeric anthocyanins content

Monomeric anthocyanin pigments were determined by changing the color at pH 1.0 and pH 4.5 at 520 nm. Degraded anthocyanins in the polymeric forms were not included in the measurements. The appropriate dilution factor was prepared by diluting the test portion with pH 1.0 buffer, until absorbance was in the linear range of 520 nm. Using this dilution factor, two dilutions of the test sample were prepared, one with pH 1.0 buffer and the other with pH 4.5 buffer.

Absorbance of diluted samples at both 520 and 700 nm was determined 20–50 min after preparation. Anthocyanin pigments concentration was expressed as cyanidin-3-glucoside equivalents (mg/g), as follows:

$$\frac{A \times MW \times DF \times 10^3}{\epsilon \times l}$$

where A = ($A_{520\text{nm}}$ - $A_{700\text{nm}}$) pH 1.0 - ($A_{520\text{nm}}$ - $A_{700\text{nm}}$) pH 4.5; MW (molecular weight) = 449.2 g/Mol for cyanidin-3-glucoside (Cyd-3-Glu); DF – dilution factor established; l – path length in cm; ϵ – 26 900 molar extinction coefficients, in $\text{g} \times \text{Mol}^{-1} \times \text{cm}^{-1}$, for cyd-3-Glu; and 10^3 = conversion factor [15].

Microbial strains and antimicrobial evaluations

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028, *Shigella flexneri* PTCC 1234 and fungi *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404 were used. Antimicrobial evaluations were evaluated using disc diffusion [16] and micro broth dilution assays [17].

Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to a concentration of approximately 10^8 CFU/ml using standard routine spectrophotometric methods. Suspensions of fungi (10^6 CFU/ml) were made with Sabouraud dextrose broth and using a sterile cotton swab, the microbial suspensions were cultured on appropriate media. Subsequently, sterile blank discs (6 mm in diameter) were saturated with different concentrations of extract and were put on the cultured media. The plates were incubated at 37°C for 24 and 48 h for bacteria and fungi, respectively. The inhibition zones (IZ) diameters were measured in millimeters (mm) and average of IZ was recorded as means \pm SE.

The minimum inhibitory concentration (MIC) and minimal lethal concentration (MLC) values of the extract were determined by micro broth dilution assay. The extract was twofold serially diluted (896–18.65 $\mu\text{g/ml}$). MOPS-buffered RPMI 1640 with L-glutamine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and cation adjusted Muller Hinton broth were used as broth media for fungi and bacteria, respectively. After shaking, 100 μl of the extract was added to each well. The above microbial suspensions were diluted to 1×10^6 and 1×10^4 CFU/ml and then 100 μl of this suspension were added to each well and incubated at $35 \pm 2^\circ\text{C}$. MIC was defined as the lowest concentration of extract that inhibits bacteria after 24 and fungi after 48 h. MLC value was the first well that showed no growth on suitable media.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

Chemical attributes of *M. neglectum* ethanol extract revealed that the total phenolic and flavonoid contents of the extract were 18.2% and 0.94%, respectively. The total anthocyanin content of *M. neglectum* extract was 0.11%. The antioxidant activity of *M. neglectum* was lower than BHT. Furthermore, the IC_{50} for *M. neglectum* was 450 $\mu\text{g/ml}$ and was higher than that of BHT (18 $\mu\text{g/ml}$) (tab. 1, fig. 1).

Table 1

Chemical attributes of *M. neglectum* ethanol extract

	TPC	TFC	TMAC	IC_{50} [$\mu\text{g/ml}$]
Extract	18.2%	0.94%	0.11%	450
BHT	-	-	-	18

TPC – total phenolic content; TFC – total flavonoids content; TMAC – total monomeric anthocyanin content, IC_{50} – inhibitory concentration

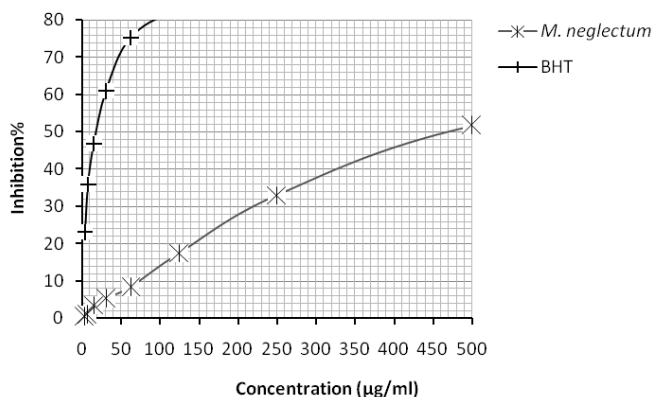


Figure 1.

The antioxidant activity evaluation of *M. neglectum* ethanol extract by DPPH assay

The analysis of *M. neglectum* demonstrated the presence of phenolic compounds in the flowering aerial parts of its ethanol extract. It is shown that the diet containing phenolic compounds is associated with lower incidence of non-communicable diseases, including atherosclerosis, cancer, neurodegenerative and cardiovascular diseases [18, 19]. Indeed a diet rich in phenolic compounds decreases or prevents LDL oxidation [20], oxidative DNA damage [21] and reactive oxygen species, inflammation markers [22] and has many other beneficial effects [23, 24]. Although the quantity of flavonoids in *M. neglectum* was low, flavonoids are hydroxylated phenolic substances with benzo- γ -pyrone structure of protective effects against infectious and degenerative diseases [25]. Therefore, flavonoids like phenolic compounds have an essential role in human nutrition.

Anthocyanins are naturally soluble color compounds that have received less attention than flavonoids. In addition to their biological activities, such as anti-inflammatory effect [26, 27], anti-carcinogenic, pro-apoptotic [28] and many other biological activities [29, 30], anthocyanins are used for coloring applications as food colorant.

The antioxidant activity of *M. neglectum* is related to components such as phenolics, flavonoids and anthocyanins or many other unknown compounds. Anthocyanins are potent antioxidants [31], flavonoids and phenolic compounds suppress or scavenge radical oxygen species either by inhibition of enzymes or by chelating trace elements in free radical generation; or upregulating and protecting the antioxidant enzymes involved in the defenses [32]. Therefore, in addition to nutritional value of *M. neglectum*, it can be used as antioxidant and color agents instead of synthetic ones.

The antimicrobial activity of *M. neglectum* ethanol extract against some food pathogens, including *S. aureus*, *E. coli*, *S. typhimurium*, *Sh. flexneri*, *C. albicans* and *A. niger* was evaluated by disc diffusion method and micro broth dilution assays (tab. 2). In disc diffusion method, the IZ diameter of *M. neglectum* extract was enhanced by increasing the concentration of extract and this increasing process was dose-dependent. In disc diffusion method, different concentrations of *M. neglectum* extract showed no antibacterial activity against *S. aureus* (6.8 ± 0.0). *M. neglectum* extract showed a smaller IZ diameter against *C. albicans* and *A. niger*. *C. albicans* showed more sensitivity to *M. neglectum* extract than *A. niger* (12.8 ± 0.2 vs. 7.9 ± 0.32 mm). *Sh. flexneri* and *E. coli* had the higher IZ (mm) than the others, followed by *S. typhimurium* with more sensitivity to *M. neglectum* extract with IZ of 11.7 ± 0.35 mm. The MIC and MLC evaluations of extract against these microorganisms showed the lower MIC, MLC values were for *C. albicans* (37.3 and 84 $\mu\text{g/ml}$) and followed by *Sh. flexneri* (56 and 224 $\mu\text{g/ml}$) and *E. coli* (87.11 and 211.5 $\mu\text{g/ml}$). The MLC value of *M. neglectum* extract against *A. niger* (448 $\mu\text{g/ml}$) was manifold of its MIC value (56 $\mu\text{g/ml}$). Thus, *M. neglectum* extract showed inhibitory activity against *A. niger*. The Gram-negative bacteria showed more sensitivity to *M. neglectum* ethanol extract than Gram-positive ones. *S. aureus* had a higher MIC and MLC values than others (224 and 448 $\mu\text{g/ml}$).

Table 2.

The antimicrobial evaluation of *M. neglectum* ethanol extract

	Inhibition zone diameter [mm]			Minimal concentrations [$\mu\text{g/ml}$]	
	37.4 μg	56.1 μg	74.8 μg	MIC	MLC
<i>S. aureus</i>	6.8 \pm 0.0	6.8 \pm 0.0	6.8 \pm 0.0	224	448
<i>E. coli</i>	8.03 \pm 0.49	10.5 \pm 0.1	12.5 \pm 0.19	87.11	211.5
<i>S. typhimurium</i>	8.2 \pm 0.18	10.2 \pm 0.21	11.7 \pm 0.35	112	448
<i>Sh. flexneri</i>	7.3 \pm 0.22	11.7 \pm 0.07	13.5 \pm 0.09	56	224
<i>C. albicans</i>	6.8 \pm 0.0	8.1 \pm 0.2	12.8 \pm 0.2	37.3	84
<i>A. niger</i>	6.8 \pm 0.0	6.9 \pm 0.13	7.9 \pm 0.32	56	448

MIC – minimal inhibitory concentration, MLC – minimal lethal concentration

The results showed that some of the most important food pathogens like *E. coli*, *Sh. flexneri* and *S. typhimurium* were sensitive to *M. neglectum* extract and this extract can be used as a preservative in food industries for preventing the food spoilage. The antimicrobial activities of *M. neglectum* extract relates to components, especially phenolic and flavonoid compounds. Phenolic compounds are well known antimicrobial agents [33], and flavonoids are able to complex with proteins and bacterial cell walls and show their antimicrobial properties [34]. There are many studies that report Gram-positive bacteria as sensitive ones to essential oils and extracts than Gram-negative bacteria [35-37] and this resistance is related to an outer membrane and periplasmic space of Gram-negative bacteria, while *M. neglectum* extract showed more action against Gram-negative ones and Gram-positive bacteria were resistant to this extract. Therefore, the extract contains components that are able to permeate to Gram-negative bacteria *via* outer membrane and unique periplasmic space while it cannot be transmitted from the thick cell wall of Gram-positive bacteria.

CONCLUSION

M. neglectum along with its application in many cultures as food has many beneficial and functional compounds such as flavonoids, phenolics and anthocyanins that may help human health. Too, the extract has antioxidant and antimicrobial activity against some food pathogens, especially against Gram-negative bacteria that introduces *M. neglectum* ethanol extract as suitable natural preservative in foods for increasing the shelf life of products in food industries. The soluble pigment of *M. neglectum* is valuable as natural coloring agent in foods and products.

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Conflict of interest: Authors declare no conflict of interest.

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PRZECIWDROBNOUSTROJOWE I ANTYOKSYDACYJNE WŁAŚCIWOŚCI ETANOŁOWEGO WYCIĄGU Z KWIATÓW *MUSCARI NEGLECTUM*

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Streszczenie

Wstęp. *Muscari neglectum* (szafirek groniasty) był stosowany w niektórych krajach jako środek spożywczy. **Cel.** Celem prowadzonych analiz było zbadanie właściwości przeciwdrobnoustrojowych oraz antyoksydacyjnych *M. neglectum*. **Metodyka.** Przy pomocy szeregu metod określono zawartość sumy związków fenolowych, flawonoidów, monomerycznych antocyjanów oraz aktywność przeciwdrobnoustrojową i antyoksydacyjną w stosunku do niektórych mikroorganizmów powodujących zatrucia pokarmowe. **Wyniki.** Zawartość sumy związków fenolowych, flawonoidów i antocyjanów wynosiła odpowiednio 18,2, 0,94 oraz 0,11%. Wartość IC₅₀ dla *M. neglectum* była wyższa niż dla BHT (butylowany hydroksytoluen). Wyciąg z *M. neglectum* nie powodował wystąpienia IZ (inhibition zone) w stosunku do *Staphylococcus aureus*; w przypadku *Candida albicans* oraz *Aspergillus niger* wielkość IZ była niewielka. W badaniach z zastosowaniem *Shigella flexneri* i *Escherichia coli* wartości IZ były większe, niż w przypadku innych drobnoustrojów. **Wnioski.** Powyższe wyniki wskazują, że wyciąg z kwiatów *M. neglectum* może być stosowany jako naturalny środek konserwujący oraz barwiący żywność w miejsce środków pochodzenia syntetycznego.

Słowa kluczowe: *Muscari neglectum*, kwiaty, środki konserwujące, żywność, związki fenolowe, antocyjany