Investigation of Phenolic Composition, Antioxidant Capacity, and Antidiabetic Effect of *Ornithogalum lanceolatum* L.: An *in vitro* Study

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Abstract: Diabetes Mellitus is a global health problem that leads to macro- and microvascular diseases associated with hyperglycemia. Phytotherapy is one of the alternative ways to cope with this type of disease. The genus *Ornithogalum* is consumed as a wild edible plant and traditionally used for ailments. This study aims to investigate the phenolic composition using High-Performance Liquid Chromatography as well as antioxidant and antidiabetic effects using spectrophotometric method of *Ornithogalum lanceolatum* L. aerial parts and bulb. In order to determine the antioxidant capacity total phenolic content, total flavonoid content and DPPH and ABTS free radical scavenging activities were analyzed in *O. lanceolatum*. Moreover, in vitro inhibitory effects of the *O. lanceolatum* aerial parts and bulb on digestive enzymes were determined by evaluating the α-amylase and α-glucosidase activities. Protocatechuic acid was found to be the main compound in both plant parts. However, the amounts of the total phenolic acids and flavonoids were found higher in the aerial parts than those in bulb as well. Furthermore, *O. lanceolatum* aerial parts exhibited more radical scavenging activity than bulb. The α-amylase and α-glucosidase IC₅₀ inhibition activities of aerial parts were found more efficient than those for bulb. It can be concluded that *O. lanceolatum* can enhance the antioxidant status and also can prevent nutraceutically postprandial hyperglycemia by inhibiting α-amylase and α-glucosidase enzymes. These findings reveal the importance of traditional remedies in the ethnopharmacological use of herbs.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic and metabolic disease characterized by elevated blood glucose level that occurs when insulin cannot be produced enough or used effectively. An estimated of 422 million people suffer from DM worldwide and 1.6 million people solely died in 2016. The prevalence and incidence of DM in the world is increasing rapidly. Diabetes is a major cause of cardiovascular disease, high blood pressure, neuropathy, nephropathy, retinopathy, foot damage, and skin complications (WHO, 2016). The metabolic abnormalities of diabetes are caused by hyperglycemia such as non-enzymatic glycosylation and glucose
auto-oxidation leading continuous production of free radicals. Increased free radical production causes the activation of some major pathways such as polyol pathway, protein kinase C, increase in formation of advanced glycation end-products, and overactivity of the hexosamine pathway. At the same time, these pathways lead to the production of free radicals again and play a role in the pathogenesis of complications (Zhang et al., 2020), therefore; lowering the high blood glucose is crucial in reducing the risk of diabetic complications. Various medications are already used to reduce hyperglycemia. Therapeutic herbal approaches are also traditionally used in the treatment and management of diabetes. Herbs have some bioactive compounds such as phenolic acids and flavonoids that exert a protective effect against various diseases. Besides, augmenting the antioxidant status helps both reducing free radicals and preventing the activation of the above-mentioned pathways (Ayepola et al., 2014).

Recently, medicinal and wild edible plants have gained attention as they offer both an important source of food and natural remedies for various ailments (Milella et al., 2014). They also have nutritive and dietetic value, largely owing to the presence of complex carbohydrates, mineral salts, vitamins, and polyphenolic compounds (Sekeroglu et al., 2006; Temiz, 2021). Turkey is one of the countries that have rich flora in terms of medicinal, aromatic, and wild edible plants. Ornithogalum lanceolatum known as burlumbuş, bulumbuç, burlumbuç, bulumbüşık, is a wild edible plant, a member of Asparagaceae family, which shows propagation from Turkey to north Israel. It is collected in spring season and used in salads or consumed after cooking. O. lanceolatum is also traditionally used against arthralgia as topical painkiller in Turkey. Some Ornithogalum species are used especially in Turkish traditional and folk medicine against liver diseases, digestive system disorders, cough, asthma, edema, renal insufficiency, rheumatism, and diabetes (Koyuncu et al., 2018; Plančić et al., 2014; Renda et al., 2018). Based on ethnobotanical reports, this study was designed for scientific evaluation of O. lanceolatum. To the best of our knowledge, no antidiabetic investigation has been carried out until today, and very limited experimental studies have been reported in the literature on O. lanceolatum. This study aimed to evaluate folkloric information on the antidiabetic effect and antioxidant capacity as well as to determine phenolic composition of the extract from the aerial parts and bulb of O. lanceolatum.

2. MATERIAL and METHODS

2.1. Chemicals

All chemicals and reagents used in the study were HPLC and analytical grade and were procured from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Acarbose (Glucobay®, Turkey) was procured from pharmacy.

2.2. Plant Material and Extraction

Ornithogalum lanceolatum was picked up from Sertavul, Mersin, Turkey, in April 2020. The plants were immediately transported to the laboratory and foreign materials were removed. The plant was identified by a specialist. The plant specimens are being kept at the plant laboratory of Vocational School of Technical Sciences Karamanoğlu Mehmetbey University. The plants were dried outdoors and under shade. The all dried parts of the plant (aerial parts and bulb) were separately crumbled and extracted twice with 80% aqueous ethanol at 50°C for 3 h by continuous stirring (Wisd WiseStir MSH-20D). After being filtered with nylon cloths, the extract was filtered with 22 µm ptfe syringe filter. The Ornithogalum lanceolatum extracts (OLE) were stored in amber bottles at -20 °C for 4 weeks until further analyses.

2.3. HPLC Analysis of Plant Samples

The phenolic profiles of samples were determined as previously described (Özcan et al., 2018), with some modifications using the HPLC system (Agilent Technologies 1260 Infinity, USA).
Separation of phenolic compounds was performed using an Inertsil ODS-3 C18 (250 × 4.6 mm, 5 μm) column through gradient solvent system at 25°C. Elution was carried out at the flow rate of 1.0 mL/min using a binary mobile phase mixture of water/acetic acid (98:2 v/v) (A) and acetonitrile/water/acetic acid (50:49.5:0.5 v/v) (B). The gradient program was used as follows: 0 min 85% A, 30 min 80% A, 60 min 55% A. Diode array detector was used for monitoring at 254, 280, and 320 nm. The identification of each compounds was based on their retention times and spectral matching by comparison with external standards.

2.4. Determination of Total Phenolic and Total Flavonoid Content
Total phenolic content (TPC) in the OLE was determined by the modified Folin-Ciocalteu reagent method (Singleton and Rossi, 1965) using gallic acid as a standard. TPC were calculated as mg gallic acid equivalent 100 g⁻¹ dry weight (mg GAE/100 gr dw). Total flavonoid content (TFC) was determined by the AlCl₃ method (Zhishen et al., 1999) using quercetin as a standard. TFC were calculated as mg quercetin equivalent 100 g⁻¹ dry weight (mg QE/100 gr dw).

2.5. DPPH Radical Scavenging Activity
DPPH (2,2-Diphenyl-1-picrylhydrazyl) method was performed to evaluate the free radical scavenging activity with minor modifications as described by Pyo et al. (2004). Briefly, 100 μL diluted OLE or Trolox standard series and 3.90 mL methanolic solution of DPPH• (6x10⁻⁵ M) were mixed in a tube and vortexed. The tubes were incubated for 60 min at room temperature in the dark; thereafter, absorbance was measured against methanol at 517 nm (Shimadzu UV-3600, Kyoto, Japan). DPPH activity was expressed as IC₅₀, which was calculated graphically.

2.6. ABTS Assay
ABTS (2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) (7 mM) was reacted in potassium persulfate (2.45 mM) to produce the ABTS radical cation (ABTS⁺⁺) in the dark at room temperature for 12-16 h. The ABTS⁺⁺ working solution was diluted with distilled water to give an absorbance of 0.70±0.02 at 734 nm (Re et al., 1999). Briefly, 20 μL diluted OLE or Trolox standard series was added to 1980 μL adjusted ABTS⁺⁺ solution in tubes and vortexed. The tubes were left exactly for 6 min at room temperature in the dark, and then the absorbance was immediately measured at 734 nm. The percent inhibition was calculated graphically and expressed as IC₅₀.

2.7. Determination of α-amylase and α-glucosidase Inhibition Activity
α-amylase inhibition activity of the OLE was determined as previously described (Kim et al., 2005) with some modification. A total of 250 μL α-amylase (0.05 U/mL) in phosphate buffer (0.02 M, pH 6.9) was mixed with 200 μL various concentrations of the extract or acarbose and incubated at 37°C for 10 min. Thereafter, 250 μL 1% starch solution as the substrate was added and incubated at 37°C for 15 min. After the reaction was quenched with 500 μL 1% dinitrosalicylic acid, the tubes was boiled in water for 10 min. After cooling the tubes, the mixture was diluted with 5 mL distilled water. The absorbance of mixture was recorded at 540 nm. Acarbose was used as a standard. The α-amylase inhibition activity of the extract was expressed as IC₅₀, which was calculated graphically.

α-glucosidase inhibition activity was carried out with the method described by Kim et al. (2005) with some modification. The 60 μL of 1 U/mL α-glucosidase in phosphate buffer (0.1 M, pH 6.8) was mixed with 120 μL various concentrations of the extract or acarbose and incubated at 37°C for 10 min. Thereafter, 120 μL 4-nitrophenyl α-D-glucopyranoside (5 mM) was added as the substrate and tubes were kept at 37°C for 15 min. The reaction was quenched by adding 300 μL Na₂CO₃ (0.1 M) and the absorbance was recorded at 405 nm. α-glucosidase inhibition activity of the extract was expressed as IC₅₀, which was calculated graphically.
2.8. Statistical Analyses

All measurements were performed in triplicate. The data were expressed as mean and standard deviation (X±SD). One-way ANOVA and t-test was performed and the results were correlated.

3. RESULTS and DISCUSSION

In the present study the antioxidant and antidiabetic activities as well as the phenolic composition of *Ornithogalum lanceolatum* ethanolic extract were evaluated. The amount of phenolic compounds of *O. lanceolatum* aerial parts and bulb is shown in Table 1 and HPLC chromatograms are depicted in Figure 1. Protocatechuic acid was the main compound in both plant parts, as well as rutin, quercetin, and p-coumaric acid were the most predominant phenolic compounds in the aerial parts. Syringic acid was not detected in both plant parts. Chlorogenic acid, o-coumaric acid, and p-coumaric acid were not also detected in bulb. On the other hand, the unidentified compound at 15.826th sec in HPLC might tentatively be a flavonoid which accumulated in bulb. Although the amount of phenolic compound in the current analysis was not fully compatible with the previous investigation on *O. lanceolatum* bulb, it has a similar phenolic composition (Özcan et al., 2018). The most abundant phenolic compounds in the bulb were reported to be catechin, gallic acid, catechol, and protocatechuic acid (Özcan et al., 2018). Biosynthesis of various phenolic compounds in plants depends on some factors, such as; species specificity, vegetation period, and growing conditions (climatic factors, water, light, altitude, and soil properties, etc.) (Chepel et al., 2020). Moreover, plants can produce various phenolic compounds at different growth stages and accumulate them in their bulb and/or other parts (Feduraev et al., 2019). In the current study, chlorogenic acid, o-coumaric acid, and p-coumaric acid that could not be detected in the bulb may be due to the plant which is in the flowering stage and which has not yet been accumulated in the bulb. Feduraev et al. (2019) also stated that at the fruiting stage, leaves and generative parts accumulated 3-7-fold much more of phenolic compounds in comparison to those in roots and stems.
Table 1. Amount of phenolic compounds of *Ornithogalum lanceolatum* (mg/kg dw).

|        | GA     | PCA    | Cath   | ChA    | CA     | SyA    | Que    | pCou   | FA     | Ru     | oCou   |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Aerial | 88.3±0.2 | 1856.2±6.4 | 48.8±0.7 | 176.0±1.3 | 15.7±0.1 | n.d | 329.2±2.0 | 244.9±1.6 | 37.7±0.1 | 646.8±4.6 | 192.7±1.5 |
| Bulb   | 32.8±0.1 | 120.8±1.0  | 36.4±0.7 | n.d     | 21.5±0.1 | n.d | 42.0±0.1 | n.d     | 18.2±0.1 | 70.3±0.8 | n.d     |

Phenolic profile of ethanolic extracts obtained from *O. lanceolatum* aerial and bulb parts. Data shows mean ± standard deviation, dw: dry weight, GA: gallic acid, PCA: protocatechuic acid, Cath: catechin, ChA: chlorogenic acid, CA: Caffeic acid, SyA: syringic acid, Que: quercetin, pCou: p-coumaric acid, FA: Ferulic acid, Ru: rutin, oCou: o-coumaric acid. n.d: not determined.
Figure 1. HPLC chromatograms of: a) the standards mixture overlay of all wavelengths (254, 280, and 320 nm), b) the obtained ethanolic extract from *O. lanceolatum* aerial parts at 280 nm wavelength, and c) the obtained ethanolic extract from *O. lanceolatum* bulb parts at 280 nm wavelength. Column: Inertsil ODS-3 C18 (250 × 4.6 mm, 5 μm); flow rate, 1.0 mL/min, temperature 25°C, gradient system. GA: gallic acid, PCA: protocatechuic acid, Cat: catechin, ChA: chlorogenic acid, CA: caffeic acid, SyA: syringic acid, Qe: quercetin, pCou: *p*-coumaric acid, FA: ferulic acid, Ru: rutin, oCou: *o*-coumaric acid.

Moreover, the phenolic composition findings were supported by TPC and TFC results (Figure 2A and B). The amounts of the total phenolic acids and flavonoids were higher in the aerial parts than those in bulb. Aerial parts and bulb had the total phenolic content with 835±79 and 249±23 mg GAE/100 g dw, respectively. The total flavonoid content of aerial parts and bulb were found to be 886±91 and 487±49 mg QE/100 g dw, respectively. It was reported that the TPC of methanolic *O. lanceolatum* and *Ornithogalum armeniacum* bulb extracts were calculated as 13.61 and 15.14 mg GAE/100 g fw, respectively (Özcan et al., 2018). In a previous study conducted on *O. sintenisii* aerial part and bulb were measured as 28.9±1.1 and 8.4±0.3 mg GAE/g dw for TPC, respectively. Also, in the same study, the TFC in aerial part and bulb
was determined to be 23.5±1.3 and 5.9±0.2 mg QE/g dw, respectively (Ebrahimzadeh et al., 2010). In another species of Ornithogalum, Apaydın and Yolcu (2017) reported TPC and TFC of methanol extracts of fresh Ornithogalum umbellatum possessed 5.82±0.008 mg GAE/g dw and 3.258±0.028 mg QE/g dw, respectively. Ornithogalum orthophyllum aerial parts and bulbs were recently determined to be 11.00±0.18 and 2.04±0.22 mg GAE/g extract for TPC (Renda et al., 2018). TPC and TFC results in current and previous studies showed that Ornithogalum species could be a promising source of antioxidants.

Figure 2. Amount of total phenolic content by Folin-Ciocalteu spectrophotometric method (A) and total flavonoid content by AlCl₃ spectrophotometric method (B) of ethanolic (80%) extracts from O. lanceolatum aerial and bulb parts. GAE: Gallic acid equivalent, QE: Quercetin equivalent, dw: dry weight. Data shows mean ± standard deviation, n=3, t-test was performed, p<0.05, †: Significantly different from bulb

The IC₅₀ values of DPPH and ABTS of aerial part of O. lanceolatum were determined approximately 2-fold more efficient compared to its bulb (Table 2). Basically, a lower IC₅₀ value indicates a higher inhibition activity. Herein, O. lanceolatum extracts exhibited DPPH and ABTS scavenging activity in proportion to their total phenolic and flavonoid content. It was reported that DPPH IC₅₀ values of aerial parts and bulb of Ornithogalum sintenisii at flowering stage was found to be 368±15 and 669±25 µg/mL, respectively (Ebrahimzadeh et al., 2010). However, DPPH % inhibition of O. umbellatum stem and flower parts for concentration of 200 µg/mL were determined 24.92±0.012 % and 15.31±0.002 %, respectively (Aydın, 2020). In a recent study, methanol and water extracts of bulb of Ornithogalum narbonense were calculated as 12.60±0.30 and 4.02±0.55 mg TE/g extract, respectively for DPPH (Zengin et al., 2015). Besides, Zengin et al. (2015) reported O. narbonense bulb methanol and water extracts had 18.16±1.21 and 7.52±0.64 mg TE/g extract, respectively for ABTS scavenging activity. However, the ABTS IC₅₀ value of polysaccharides extracted from O. billardieri bulb (Syn. O. lanceolatum) was interestingly determined as 1.51±0.1 mg/mL (Medlej et al., 2021). The extracts of aerial parts and bulb of O. lanceolatum have shown remarkable free radical scavenging ability comparing to other Ornithogalum species extracts.

Table 2. DPPH and ABTS IC₅₀ values of Ornithogalum lanceolatum (dw).

|                | DPPH (mg TE/g) | ABTS (mg TE/g) | DPPH (mg/mL) | ABTS (mg/mL) |
|----------------|----------------|----------------|--------------|--------------|
| Aerial parts   | 5.09±0.02†     | 4.92±0.00†     | 12.53±0.03†  | 11.08±0.01†  |
| Bulb           | 10.00±0.00     | 11.46±0.18     | 22.53±0.03   | 23.42±0.46   |

Antioxidant capacities of ethanolic (80%) extracts obtained from O. lanceolatum aerial and bulb parts. TE: Trolox equivalent, dw: dry weight. Data shows mean ± standard deviation, n=3, t-test was performed, p<0.05, †: Significantly different from bulb
α-amylase and α-glucosidase inhibition activities of *O. lanceolatum* are shown in Table 3. α-amylase inhibition IC$_{50}$ value of aerial parts was more efficient than bulb (3.06±0.02 and 6.02±0.04 mg/mL, respectively). Similarly, α-glucosidase inhibition activity of aerial parts was higher than that for the bulb. The α-glucosidase inhibition IC$_{50}$ values were 4.98±0.08 and 8.86±0.16 mg/mL in aerial part and bulb, respectively. Besides, α-amylase and α-glucosidase IC$_{50}$ values for acarbose were found to be 0.38±0.02 and 0.57±0.02 mg/mL, respectively. The plant parts had higher IC$_{50}$ inhibition values compared to acarbose, meaning that, it was less effective in digestive enzymes inhibition. Huyssteen et al., (2011) stated that the aqueous extract of *O. longibracteatum* bulb showed 131.9% significant increase in vitro glucose utilization activity in Chang liver cells. Moreover, in a previous study, it was reported that administration of *Ornithogalum caudatum* alcohol extract to diabetic mice for 14 days declined blood glucose concentration (Cao et al., 2015). Phytochemicals in herbs can exert antidiabetic effects through various mechanisms. These mechanisms may be through inhibition of digestive enzymes and/or by acting like insulin-mimetic to reduce postprandial blood glucose (Temiz & Temur, 2019). Besides they might have antidiabetic effects such as reducing glucose absorption in the small intestine, stimulation of insulin secretion from islets, improving insulin sensibility, and increasing the uptake and bioavailability of glucose to the cells (Temiz and Temur, 2019; Temiz, 2021; Yang et al., 2015; Zhang et al., 2020). Backgrounds of these mechanisms have complex pathways in the cells. The antidiabetic action mechanisms of medicinal plants have not been fully clarified. Therefore, these studies may lead to the elucidation of the antidiabetic effect mechanisms with future studies.

Table 3. α-amylase and α-glucosidase inhibition IC$_{50}$ values of *Ornithogalum lanceolatum* (dw).

|                | α-amylase (mg/mL) | α-glucosidase (mg/mL) |
|----------------|-------------------|-----------------------|
| Aerial parts   | 3.06±0.02†‡       | 4.98±0.08†‡           |
| Bulb           | 6.02±0.04†        | 8.86±0.16†            |
| Acarbose       | 0.38±0.02         | 0.57±0.02             |

Carbohydrate digestive enzyme inhibition of ethanolic (80%) extracts obtained from *O. lanceolatum* aerial and bulb parts, Acarbose: positive control, Data shows mean ± standard deviation, n=3, One-way ANOVA was performed, p<0.05, †‡: Significantly different from bulb, †: Significantly different from acarbose

4. CONCLUSION

The aerial parts and bulb of *O. lanceolatum* have remarkable antioxidant capacity. *O. lanceolatum* aerial parts can exert an antidiabetic effect through α-amylase and α-glucosidase inhibitory activities. The favorable antioxidant and antidiabetic effects of *O. lanceolatum* have revealed the potential efficacy of its use as a traditional medication in the maintenance of oxidant/antioxidant balance and in the management of diabetes mellitus. Investigation of traditional phytotherapeutic interventions may provide important new insights into the treatment of diabetes.

Declaration of Conflicting Interests and Ethics

The author declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

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