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Other Traditional Medicine

Biological activities of the *Ornithogalum orthophyllum* and its *in silico* ADMET profile

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Author contributions

Taskin D conceived the idea. Taskin D, Senturk S, Güler EM, Ermanoglu M, Taskin T, Doğan A and Omurtag GZ contributed to the acquisition, analysis, and interpretation of data. Taskin D and Taskin T wrote the manuscript. Taskin D and Omurtag GZ reviewed the paper and provided comments. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

HPLC, high-performance liquid chromatography; AD, Alzheimer's disease; AChE, acetylcholinesterase; ADMET, absorption, distribution, metabolism, excretion, and toxicity; FCR, Folin-Ciocalteu reagent; GAE, gallic acid equivalents; FRAP, ferric reducing antioxidant power; BHT, butylated hydroxytoluene; ABTS, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; CUPRAC, cupric ion reducing/antioxidant power; BHA, butylated hydroxyanisole; DMSO, dimethylsulfoxide; ATP, adenosine triphosphate; TEAC, Trolox equivalent antioxidant capacity; TE, Trolox equivalent.

Citation

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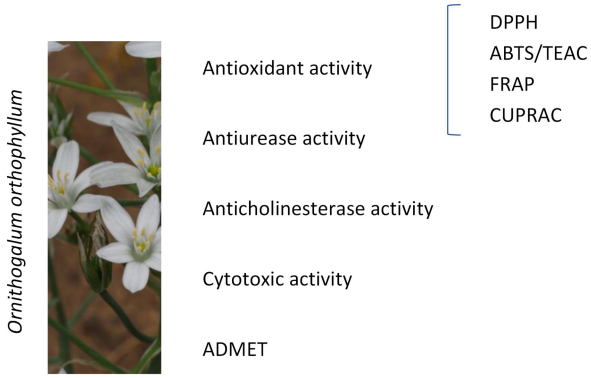
Abstract

Background: The bulbs and aerial parts of *Ornithogalum* are used in Turkey both as food and to treat various ailments, and some of its medical applications are well known. However, the biological activities of *Ornithogalum orthophyllum* have not yet been investigated. The objective of this study was to examine the antioxidant, urease and cholinesterase enzyme inhibition, and cytotoxic activities of different extracts obtained from the bulb and aerial parts of the *O. orthophyllum* plant. In addition, the absorption, distribution, metabolism, excretion, and toxicity properties of some phenolic compounds in plants were examined *in silico*. **Methods:** The antioxidant activity of the extracts obtained from the aerial parts of *O. orthophyllum* was investigated using the 2,2-diphenyl-1-picrylhydrazyl, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid), ferric reducing antioxidant power, and cupric reducing antioxidant capacity methods. Urease and cholinesterase enzyme inhibition were determined by using the indophenol and Ellman methods, respectively. The cytotoxic activity of the extracts was measured using a test based on the luminometric readings of the adenosine triphosphate levels of the cells. pkCSM, a free online web server (http://structure.bioc.cam.ac.uk/pkcsm) was used to predict the properties of the compounds analyzed. **Results:** The methanol extract of the aerial parts of *O. orthophyllum* was observed to have strong antioxidant and acetylcholinesterase enzyme inhibition potential. The petroleum ether extract of the aerial parts showed the highest anti-urease activity. The chloroform extract of the aerial parts exhibited the highest cytotoxic effect against A431 human epidermoid carcinoma cells. The absorption percentages of protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and *p*-coumaric acid compounds from the small intestine were between 71.17% and 93.49%, which were quite high. All the compounds were predicted to be unable to penetrate the central nervous system due to their inability to cross the blood-brain barrier. Not all compounds analyzed were predicted to have mutagenic, hepatotoxic, or minnow toxicity effects. **Conclusion:** The extracts obtained from the aerial parts of *O. orthophyllum* have strong biological activity and contain compounds that are well-absorbed and do not have mutagenic, hepatotoxic, or minnow toxicity effects, suggesting that they can be used as natural medicinal and nutritional sources in the future.

Keywords: *Ornithogalum orthophyllum*; antioxidant; cytotoxic; anticholinesterase; anti-urease; ADMET



Tradition

The traditional uses of *Ornithogalum orthophyllum* bulbs in stomach diseases were first described in an article titled "Investigations of ethnobotanical aspects of some geophytes growing in Alaşehir (Manisa) and the surrounding area." written by Salgın et al. of the *Journal of Graduate School of Natural and Applied Sciences* at Erciyes University in 2013. Hexanal and heneicosane compounds are major components of the essential oil obtained from the plant bulbs. In addition, it was determined that protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and *p*-coumaric acid compounds were the major phenolic compounds present in the aerial parts of these plants.

Background

The *Ornithogalum* genus, belonging to the Asparagaceae family, grows in many places worldwide [1]. It is widely distributed in Europe, around the Mediterranean and the Balkan Peninsula, Asia (reaching Afghanistan in the East), Africa (excluding the countries near the Tropic of Cancer), and Madagascar [2]. The name *Ornithogalum* dates back to ancient times; "ornithogalen" means "bird's milk" in Greek [3]. *Ornithogalum* is an annual bulbous plant that usually has scapes with shoots or small onions. *Ornithogalum* is represented by approximately 50 taxa in the flora of Turkey. Twenty of these species are endemic, and the endemism rate is approximately 40% [4]. The bulbs of these plants have been reported to be economically valuable. *Ornithogalum* species have been used as a treatment against abscesses and have been employed as emetic agents since the time of Dioscorides. In Turkey, some of the aerial parts and bulbs of *Ornithogalum* species are eaten. Phytochemical studies on *Ornithogalum* species revealed that they contain cholestane glycosides, cholestane bisdesmosides, cardenolide glycosides, and flavonoid glycosides. Furthermore, it was determined that they also contain homoisoflavones, alkaloids, glycosides, flavonoids, phytosterols, and have antioxidant, cytotoxic, antimicrobial, antimutagenic, and antitumor effects. Although cardenolid or cholestane glycosides are highly toxic compounds, some herbs from this genus have been used by traditional healers to treat a variety of medical conditions, including diabetes, heart disease, hepatitis, and even some types of cancer [1, 2].

Guner et al. (2012) used the *Turkish Plant List* (*Vascular Plants*) and Baytop (1999) in the book *Therapy with Medicinal Plants in Turkey* that utilized the vernacular names of *Ornithogalum orthophyllum* [5, 6]. The traditional use of *O. orthophyllum* bulbs in stomach diseases was first described in an article titled "Investigations of ethnobotanical aspects of some geophytes growing in Alaşehir (Manisa) and the surrounding area." (by Salgın and colleagues, written in the *Journal of Graduate School of Natural and Applied Sciences* at the Erciyes University in 2013) [7]. In addition, this species is preferred as an ornamental plant [7]. Analysis by gas chromatography-mass spectrometry showed the presence of hexanal and heneikosan as the major compounds present in the essential oil obtained from the plant bulbs. In addition, quantitative and qualitative analysis of phenolic compounds from the aerial parts of plants performed using high-performance liquid chromatography (HPLC)-diode array detection showed that they were comprised mainly of protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and *p*-coumaric acid compounds [1, 2].

Helicobacter pylori is the causative agent of duodenal, gastritis, and gastric ulcers and is one of the most important factors in the development of gastric adenocarcinoma [8]. *H. pylori* causes the breakdown of urea into ammonia and carbon dioxide to form virulents that create bacteria. Therefore, *H. pylori* is not affected by stomach acids [9]. Owing to the effects of the drugs used in the treatment against *H. pylori* and the eventual resistance of *H. pylori* to these antibiotics, the discovery of effective antimicrobial compounds against this bacterium is needed [10].

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by impaired cognitive abilities and reduced ability to perform daily vital activities accompanied by neuropsychiatric symptoms and behavioral changes. However, the etiological cause of AD is not yet known [11]. Acetylcholine is not sufficiently produced in patients with AD, so it is thought that AD can be slowed by inhibiting the action of acetylcholinesterase (AChE), the enzyme that breaks down acetylcholine [12].

Cancer, one of the most significant challenges in modern medicine, is the leading health concern in almost every country worldwide in terms of mortality and morbidity rates. Despite the development of new treatment approaches, cancer-related deaths still rank second in the developed societies [13]. Free radicals are formed as a product of normal metabolic pathways in aerobic organisms or under the influence of various external factors such as environmental agents (pesticides, aromatic hydrocarbons, toxins, solvents, etc.), stress, and radiation. Free radicals are thought to be responsible for aging, as well as many diseases such as cancer, cardiovascular diseases, immune system diseases, cataracts, diabetes, and kidney and liver diseases. Therefore, the body's endogenous defense system must be strengthened by antioxidant compounds via supplementation [14].

Currently, chemoinformatics software provide important information about whether a compound can be used as a medicine without conducting experimental studies. Because some laboratory-based bioactivity tests are expensive, important investigation regarding the path from plant to drug requires theoretical determination of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the plant compounds. Moreover, such *in silico* analyses are also required to investigate the potential of these compounds to be used as drugs or as raw materials for drugs [15].

A limited number of studies have been conducted on the biological activity of *O. orthophyllum*. Therefore, our aim was to examine the antioxidant, urease and cholinesterase enzyme inhibition, and cytotoxic activities of different extracts obtained from the bulb and aerial parts of *O. orthophyllum*. As polyphenolic compounds are known to be responsible for the biological activities (antioxidant, cytotoxic, anti-urease, Alzheimer, etc.) of medicinal plants [1, 2], the ADMET properties of protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and *p*-coumaric acid compounds, which were analyzed in this species in a previous study [1], were determined *in silico*.

Materials and Methods

Plant material

The *O. orthophyllum* Ten. species was identified by Ahmet Doğan, and the identified sample was stored in the Herbarium of Marmara University Faculty of Pharmacy with the code MARE: 19850.

Preparation of *O. orthophyllum* extracts

The bulb and aerial parts of *O. orthophyllum* were dried under ambient conditions. The dried samples were powdered using an herb grinder. Subsequently, petroleum ether, chloroform, and methanol were added to the bulb and aerial parts for 72 h at room temperature. At the end of the extraction process, the extracts were concentrated using a rotary evaporator at a low pressure and temperature. The raw extracts were stored at 4 °C in a refrigerator until further experiments on biological activity were performed.

Total phenolic content assay

4.5 mL of distilled water was added to the 0.1 mL extracts prepared at concentrations of 0.5, 1, 2, 3, and 5 mg/mL. The mixed was then added with 0.1 mL Folin-Ciocalteu reagent (FCR) (diluted with 1:3 distilled water) and 0.3 mL 2 percent sodium carbonate solution, and the absorbance of mixed was measured at 760 nm 2 hours later against reference. In a flask, gallic acid (25 mg) was dissolved in 25

mL water to obtain a stock solution of 1 mg/mL which was diluted with distilled water to prepare working solutions of different concentrations (0.05, 0.10, 0.20, 0.30, and 0.40 mg/mL). Gallic acid solutions prepared at concentrations of 0.05–0.40 mg/mL were evaluated using the FCR for the determination of phenolic content. For the further procedures, (1) absorbance versus concentration plots were constructed, (2) calibration curves were prepared, (3) the corresponding linear regression equations were obtained. The calibration equation for gallic acid was $A = 35.0612x + 0.1214$ ($R^2 = 0.9966$). The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per mg of extract [16].

Ferric reducing antioxidant power (FRAP) assay

The FRAP reagent (25 mL 300 mM acetate buffer (pH 3.6), 2.5 mL of 2,4,6-tri(2-pyridyl)-1,3,5-triazine solution, and 2.5 mL of 20 mM $FeCl_3{\cdot}6H_2O$) was kept at 37 °C for 30 min. The absorbance was measured at 593 nm in the 4th minute after dissolution of 190 µL of the FRAP reagent with 10 µL of the plant extracts prepared at concentrations of 0.5, 1, 2, 3, and 5 mg/mL, against the reference prepared by adding distilled water instead of the extract. A 1 mM stock solution of $FeSO_4{\cdot}7H_2O$ was prepared to obtain the $FeSO_4$ standard curve equation. Subsequently, working solutions of 0.5, 0.4, 0.2, 0.1, and 0.05 mM concentrations were prepared by diluting the stock solution with water. The $FeSO_4{\cdot}7H_2O$ solutions prepared at different concentrations were also evaluated using the FRAP method. The FRAP method was applied to butylated hydroxytoluene (BHT) solutions prepared at different concentrations (0.5, 1, 2, 3, and 5 mg/mL) and were used as a standard. For the further procedures, (1) absorbance versus concentrations plots were constructed, (2) calibration curves were prepared, and (3) the corresponding linear regression equations were obtained. The calibration equation for Fe^{2+} was $A = 12.8603x - 0.0066$ ($R^2 = 0.9986$). The FRAP values of the extracts are presented as mg Fe^{2+}/mg extract [17].

2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay

A 7 mM solution of ABTS ammonium salt (0.38 g of ammonium salt dissolved in 100 mL with water) was reacted with 2.45 mM potassium peroxydisulfate (0.066 g potassium peroxydisulfate was dissolved in 100 mL with water) for 12 h at room temperature to prepare $ABTS^{\cdot+}$ stock solution. Then the $ABTS^{\cdot+}$ stock solution was diluted with water at 734 nm to prepare a working solution with an absorbance of 0.70 (± 0.02). To 40 µL of the extracts prepared at different concentrations, 3,960 µL $ABTS^{\cdot+}$ working solution was added, and the resulting reduction in color intensity was measured spectrophotometrically at 734 nm against distilled water at 6 minutes. A control was prepared under the same conditions using 40 µL of distilled water instead of the extract or the standard. The control samples were measured daily. To obtain the Trolox standard curve, a 10 mM stock Trolox solution was prepared which was diluted to working solutions of 1, 0.8, 0.6, 0.5, 0.4, and 0.2 mM Trolox using 75 mM phosphate buffer (pH 7.4). The ABTS radical scavenging assay was applied to the Trolox solutions prepared at different concentrations. The ABTS radical scavenging experiments were also performed using the ascorbic acid solutions prepared at different concentrations (0.5, 1, 2, 3, and 5 mg/mL), which were also used as a standard. The calibration curve for Trolox is $A = 37.2214x + 1.66$ ($R^2 = 0.9899$). The results from this study are presented as mg Trolox/mg extract [18].

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

To the 0.1 mL extracts prepared at various concentrations (5, 3, 2, 1, and 0.5 mg/mL), 240 µL DPPH solution (0.1 mM) was added. The prepared mixtures were mixed for 1 minute before being incubated for 30 minutes at 25 °C. The absorbances of the mixtures were determined daily at 517 nm. Determining the absorbance of the control sample was carried out under the same conditions using 10 µL of methanol instead of the extract. DPPH radical scavenging experiments were also performed using ascorbic acid solutions prepared at different concentrations (0.5, 0.4, 0.2, 0.1, 0.05 mg/mL) and were used as a standard.

The % DPPH radical scavenging activity was calculated by the formula:

% DPPH radical inhibition = $((A_0 - A_1)/A_0) \times 100$

Where A_0 is the absorbance of the control solution and A_1 is the absorbance of plant extract or standard solutions.

The IC_{50} is defined as the extract/standard concentration that causes a 50 percent reduction in DPPH radical concentration. The IC_{50} value was calculated using the equation obtained by calculating the % radical scavenging activity against the concentrations studied. The data obtained from the investigation are given as IC_{50} = mg/mL. The assays were performed three times, and the averages and standard deviations of the results were calculated [19].

Cupric ion reducing/antioxidant power (CUPRAC) assay

In brief, 60 µL of $Cu(II){\cdot}2H_2O$, 60 µL of neocuproine, and 60 µL of 1 M NH_4Ac were mixed followed by the addition of 60 µL of the extracts at different concentrations (0.5, 1, 2, 3, and 5 mg/mL) and 10 µL of ethanol to the mixture. After 60 min, the absorbances of the mixtures were spectrophotometrically measured at 450 nm against the reference solution, which was prepared using adding ethanol instead of the plant extracts. To obtain the Trolox standard curve, a 1 mM stock Trolox solution was prepared which was diluted to working solutions of 1, 0.8, 0.6, 0.4, 0.2, and 0.1 mM using ethanol. Trolox solutions prepared at different concentrations were evaluated using the CUPRAC method.

The CUPRAC method was also applied to butylated hydroxyanisole (BHA) solutions prepared at different concentrations (0.5, 1, 2, 3, and 5 mg/mL) and used as a standard. For the further procedures, (1) absorbance versus concentrations plots were constructed, (2) calibration curves were prepared, (3) the corresponding linear regression equations were obtained. The calibration equation for Trolox was $A = 3.0550x + 0.2344$ ($R^2 = 0.9933$). The CUPRAC values of the extracts were given as mg Trolox/mg extract [20].

Determination of AChE inhibitory activities

Twenty microliters of the extracts prepared at different concentrations (0.5, 1, 2, 3, and 5 mg/mL) and 20 µL of AChE enzyme solution were added to 40 µL of 0.1 M phosphate buffer solution (pH = 8). The solution was incubated at 25 °C for 10 min. After incubation, 100 µL of 5,5'-dithiobis(2-nitrobenzoic acid) reagent and 20 µL of AcI (substrate) were added. The same procedure was applied to different concentrations galantamine (0.5, 0.4, 0.2, 0.1, and 0.05 mg/mL), which was also used as a standard. The yellow-colored 5-thio-2-nitrobenzoic acid formed as a result of the reaction of thiocholine with 5,5'-dithiobis(2-nitrobenzoic acid), which is released through the hydrolysis of the substrates (AcI), was spectrophotometrically monitored at a wavelength of 412 nm against a reference solution prepared by adding phosphate buffer solution instead of AChE enzyme solution. A control was prepared under the same conditions using 20 µL of phosphate buffer solution instead of the extract. AChE inhibitory activities of the extracts were calculated as percentage inhibition relative to the control [21] using the following equation: % inhibition = $(A_{control} - A_{sample}/A_{control}) \times 100$.

Urease assay

Plant extracts (100 µL) prepared at different concentrations (0.5, 1, 2, 3, and 5 mg/mL) and 500 µL of a urease enzyme solution (1 mg/mL) were mixed and kept in an incubator at 37 °C for 30 min. Later, 1,100 µL of urea (60 µg/mL) was added to this mixture, which was kept in an incubator at 37 °C for 30 min. Subsequently, after removing it from the incubator, the reagents R_1 (1% phenol, 0.005% sodium nitroprusside) and R_2 (0.5% NaOH, 0.1% sodium hypochlorite) were added to the mixture, and the resulting mixture was kept at 37 °C in an incubator for 2 h. The absorbance of the mixture was measured against a reference solution prepared by adding a buffer solution (1mM EDTA, 0.01 M K_2HPO_4, 0.01 M LiCl) instead of the urease enzyme solution at 635 nm. A control was also prepared under the same conditions using 100 µL of a buffer solution instead of the

extract. The same procedure was applied to a thiourea solution at different concentrations (0.5, 0.4, 0.2, 0.1, and 0.05 mg/mL), which was used as a standard [22].

The % inhibition of urease was calculated by the formula:

% enzyme inhibition = $((A_0 - A_1)/A_0) \times 100$

Where A_0 is the absorbance of the control solution and A_1 is the absorbance of plant extract or standard solutions.

Determination of cytotoxicity of the plant extracts

Chemicals and reagents. Fetal bovine serum, Dulbecco's minimum essential medium, Eagle's minimum essential medium, and penicillin/streptomycin were purchased from Sigma-Aldrich (Seelze, Germany). All other reagents and chemicals used in the study were of cell culture quality. All extracts were prepared by dissolving them in dimethylsulfoxide (DMSO) and diluting with diphosphate-buffered saline. The maximum amount of DMSO at all concentrations prepared was 0.1%. All the solutions were freshly prepared prior to use.

Cell culture. The human epidermoid carcinoma cell line A431 (ATCC® CRL1555™) and normal skin fibroblast cells CCD1079Sk (ATCC® CRL2097™) were obtained from the American Type Culture Collection. The complete medium contained 10% fetal bovine serum and 1% penicillin/streptomycin. The number of viable cells per passage was determined using trypan blue.

Cytotoxicity study. The cytotoxic activity of the extracts was measured by a test based on the luminometric reading of the adenosine triphosphate (ATP) level of the cells. 15,000 cells/well were seeded into 96 white-opaque cell culture wells. After 24 hours of incubation, culture media were refreshed, and extracts were added at different concentrations (18.75, 37.50, 75, 150 µg/mL). As a control, DMSO was added to a final concentration of 0.1% in the cells and incubated at 37 ˚C in 5% CO2 in the incubator for 24 hours. After the incubation, ATP solution was added to each well, and luminometric measurement was made on a luminometer (Thermo Scientific Varioskan Flash by Thermo Fisher Scientific, Waltham, MA USA). Results were calculated relative to the control [23].

ADMET prediction

Anticipating the pharmacokinetic properties of the potential pharmaceutical molecules increases the likelihood of identifying their target more quickly and accurately. The acronym ADMET is an abbreviation for absorption, distribution, metabolism, excretion, and toxicity, which define the pharmaceutical activities of drug candidates. pkCSM, a free online web server (http://structure.bioc.cam.ac.uk/pkcsm) was used to predict the properties of the compounds analyzed. The molecular polar surface area and molecular lipophilicity potentials of the analyzed molecules were also calculated using Molinspiration Cheminformatics (www.molinspiration.com/cgi-bin/properties) software [24, 25].

Statistical analysis

Results were expressed as the means ± standard deviation of three independent and parallel measurements. One-way analysis of variance was performed, and significant differences between means were determined using Tukey's multiple comparisons test. Statistical significance was set at $P < 0.05$.

Results

In vitro antioxidant activity results

Determination of DPPH radical scavenging activity. The free radical scavenging activities of the different plant extracts were determined using the DPPH method. The antioxidant activities of the extracts and ascorbic acid were evaluated by comparing their IC_{50} values. The results presented in Table 1 suggest that the chloroform extract (IC_{50}: 0.5530 mg/mL) from the bulb of the plant exhibited stronger radical scavenging activity compared to the petroleum ether and methanol extracts from bulb. In addition, the methanol extract (IC_{50}: 0.1800 mg/mL) obtained from the aerial parts of the plant had the highest radical scavenging potential compared to all extracts. Contrastingly, the petroleum ether extracts of the bulb and the aerial parts did not show DPPH radical-scavenging activity. When the IC_{50} values of all extracts were compared with ascorbic acid as a standard, it was found that the DPPH radical scavenging activity of all extracts was lower than that of ascorbic acid (IC_{50}: 0.0028 mg/mL).

ABTS/Trolox equivalent antioxidant capacity (TEAC) assay. This method is based on the spectrophotometric measurement of the decrease in color intensity of the solution as a result of the removal of the ABTS cation radical, which is a blue-green colored compound. Since this method is suitable for both hydrophilic and lipophilic compounds, it is frequently used to measure the antioxidant activity of plant extracts [18]. The results from this study are given as mg Trolox equivalent (TE)/mg extract (mg TE/mg extract) (Table 1). TEAC of the extracts from the bulb and aerial parts is as follows: ascorbic acid (0.1300 mg TE/mg) > aerial parts, methanol (0.1000 mg TE/mg) > aerial parts, chloroform (0.0650 mg TE/mg) > bulb, chloroform (0.0300 mg TE /mg) > bulb, methanol (0.0120 mg TE/mg) > bulb, petroleum ether (0.002 mg TE/mg). Based on these results, the methanol extract from the aerial parts showed the strongest ABTS radical scavenging potential compared to the other extracts. It was also observed that the petroleum ether extracts of the aerial parts did not show ABTS radical scavenging activity, and the petroleum ether extract of the bulb showed the lowest radical scavenging activity.

Table 1 The antioxidant activities and total phenolic contents of different extracts obtained from the plant

Extracts/ compounds	DPPH (IC_{50}: mg/mL)		FRAP (mg $FeSO_4$/mg extract)		CUPRAC (mg TE/mg extract)		ABTS/TEAC (mg TE/mg extract)		TPC (mg GAE/mg extract)	
	Bulb	Aerial parts	Bulb	Aerial parts	Bulb	Aerial parts	Bulb	Aerial parts	Bulb	Aerial parts
Petroleum ether	NA	NA	NA	NA	0.0420 ± 0.0110[a]	0.0300 ± 0.0090[a]	0.0020 ± 0.0008[a]	NA	NA	0.0080 ± 0.0030[a]
Chloroform	0.5530 ± 0.1470[a]	0.3310 ± 0.0090[a]	0.0070 ± 0.0180[a]	0.0110 ± 0.0150[a]	0.0720 ± 0.1070[b]	0.0950 ± 0.0900[b]	0.0300 ± 0.0022[b]	0.0650 ± 0.0006[a]	0.0120 ± 0.0030	0.0380 ± 0.0010[b]
Methanol	0.6530 ± 0.0150[b]	0.1800 ± 0.0030[b]	0.0003 ± 0.0040[b]	0.0015 ± 0.0040[b]	0.0130 ± 0.0430[c]	0.1480 ± 0.0430[c]	0.0120 ± 0.0009[c]	0.1000 ± 0.0010[b]	NA	0.0420 ± 0.0030[c]
Ascorbic acid	0.0028 ± 0.0004						0.1300 ± 0.0100			
BHA					0.1150 ± 0.0020					
BHT			0.0612 ± 0.0010							

NA, no activity; BHA, butylated hydroxyanisole, pozitive control for CUPRAC assay; DPPH, 2,2-diphenyl-1-picrylhydrazyl; CUPRAC, cupric ion reducing/antioxidant power; FRAP, ferric reducing antioxidant power; ABTS, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid); BHT, butylated hydroxytoluene, positive control for FRAP assay; TE, Trolox equivalent; TPC, total phenolic contents; GAE, gallic acid equivalent. Ascorbic acid, pozitive control for DPPH and ABTS assays; values are mean of triplicate determination (n = 3) ± standard deviation; [a] $P < 0.05$ compared with the positive control, [b] $P < 0.01$ compared with positive control, [c] $P < 0.001$ compared with positive control.

Determination of FRAP. The iron (III) ion reducing power of plant extracts is essential for evaluating their antioxidant potential. The iron reduction power is based on the ability of the extract to reduce Fe^{3+} to Fe^{2+} and is measured via spectrophotometry at 593 nm. In this method, a high absorbance indicates a high iron reduction potential [17]. The antioxidant power via the iron (III) ion reduction of different extracts obtained from the plants was examined. The petroleum ether extracts obtained from the bulb and aerial parts did not have iron (III) reduction potential. The chloroform extracts of the aerial parts (0.0110 mg $FeSO_4$/mg) had highest FRAP values. All the plant extracts showed lower FRAP values than the BHT compound (0.0612 mg $FeSO_4$/mg) (Table 1).

Copper (II) ion-reducing antioxidant capacity. The copper (II) ion-reducing antioxidant capacity of the different plant extracts was evaluated using the CUPRAC method. The copper (II) ion-reducing antioxidant activity (mg TE/mg extract) of the extracts from the bulb and aerial parts is as follows: aerial parts, methanol (0.1480 mg TE/mg) > BHA (0.1150 mg TE/mg) > aerial parts, chloroform (0.0950 mg TE/mg) > bulb, chloroform (0.0720 mg TE/mg) > bulb, petroleum ether (0.0420 mg TE/mg) > aerial parts, petroleum ether (0.0300 mg TE/mg) > bulb, methanol (0.0130 mg TE/mg). The results showed that the methanol extract of the aerial parts exhibited the highest copper (II) ion-reducing antioxidant activity compared with the other extracts, including that of BHA (Table 1).

Determination of the total phenolic content of the extracts. The total phenolic compounds found in the different extracts from the bulb and aerial parts of *O. orthophyllum* were determined using the FCR method. The results obtained are given in Table 1 and are presented as mg GAE/mg extract. It was determined that the chloroform extract contained a low amount of phenolic compounds from the extracts prepared from the bulb. The extracts obtained from the aerial parts contained phenolic contents, and the methanol extract (0.0420 mg GAE/mg extract) from these extracts had the highest phenolic content compared to the other extracts.

Enzyme inhibitory activity

Urease enzyme inhibition potential. The urease enzyme inhibition IC_{50} values of the different extracts were determined using the indophenol method, the results of which are shown in Table 2. The petroleum ether (IC_{50}: 0.104 mg/mL) and methanol (IC_{50}: 0.102 mg/mL) extracts from the bulb had similar urease enzyme inhibition potentials. The petroleum ether extract (IC_{50}: 0.038 mg/mL) obtained from the aerial parts of the plant showed stronger anti-urease activity than the chloroform (IC_{50}: 0.262 mg/mL) and methanol (IC_{50}: 0.213 mg/mL) extracts. When the IC_{50} values of all extracts obtained from the plant were taken into consideration, all extracts showed lower enzyme inhibition potential than the thiourea compound (IC_{50}: 0.002 mg/mL). In this study, the petroleum ether extract of the aerial parts showed the highest anti-urease activity.

AChE enzyme inhibition potential. The AChE enzyme inhibition potential of the different plant extracts was analyzed according to the Ellman method (Table 2). The AChE enzyme inhibition activity (IC_{50}, mg/mL) of the extracts from the bulb and aerial parts was as follows: galantamine (0.012 mg/mL) > aerial parts, methanol (0.019 mg/mL) > aerial parts, petroleum ether (0.023 mg/mL) > bulb, methanol (0.028 mg/mL) > aerial parts, chloroform (0.029 mg/mL) > bulb chloroform (0.035 mg/mL) > bulb, petroleum ether (0.042 mg/mL). These findings suggest that the methanol extract from the aerial parts had the highest enzyme inhibition potential when compared with the other extracts. In addition, the methanol extract of the aerial parts (0.019 mg/mL) showed enzyme inhibition activity close to that of galantamine (0.012 mg/mL).

Cytotoxic activity. The cytotoxic activities of the different plant extracts against human skin cancer cells and healthy cells were measured via a luminometric method, and the results obtained are shown in Table 3. The final concentrations of the extracts prepared in petroleum ether, chloroform, and methanol solutions were prepared ranging from 18.37 to 150 µg/mL and incubated with the cells for 24 h. Of the different extracts from the bulb, the methanol extracts showed the highest cytotoxicity, which were 10% more cytotoxic against cancer cells than healthy cells ($P < 0.001$). Among the extracts obtained from the aerial parts, chloroform extracts showed the highest cytotoxicity, which were 30% more cytotoxic against cancer cells than healthy cells ($P < 0.001$). In summary, all extracts were found to be more cytotoxic against cancer cells than healthy cells.

***In silico* ADMET prediction.** The results of the ADMET studies of the compounds are shown in Table 4. The absorption percentages of the compounds for the small intestine were between 71.17% and 93.49%, which are quite high. It was predicted that the Caco-2 permeability values of *p*-hydroxybenzoic acid and *p*-coumaric acid would be high among the compounds analyzed. It was estimated that all of the compounds can pass through the skin and have a low volume of distribution. All of the compounds were predicted to be poorly distributed in the blood-brain barrier and to be unable to penetrate the central nervous system. Not all compounds analyzed were predicted to have mutagenic, hepatotoxic, and minnow toxicity effects. It was shown that p450 enzymes, mostly found in the liver, do not metabolize the analyzed compounds. ADMET estimates of the phenolic compounds showed that these compounds are well absorbed and do not have toxic effects (Table 4).

According to Lipinski, in order to estimate the oral bioavailability of a drug, it must have favorable physico-chemical properties. These values are: MlogP ≤ 5, molecular weight ≤ 500 g/mol, number of hydrogen bond acceptors (total of N and O atoms) ≤ 10, number of hydrogen bond donors (total of OH and NH groups) ≤ 5, number of rotatable bonds ≤ 10, and polarized surface area Å² < 140 Å² in the form. As seen in Table 5, the theoretically calculated ADMET results of the analyzed compounds are in agreement with the five rules of Lipinski.

Table 2 The enzyme inhibition potential of different extracts from plant

Extracts/compounds	Urease enzyme inhibition (IC_{50}: mg/mL)		AChE inhibition (IC_{50}: mg/mL)	
	Bulb	Aerial parts	Bulb	Aerial parts
Petroleum ether	0.104 ± 0.052[a]	0.038 ± 0.019[a]	0.042 ± 0.004[a]	0.023 ± 0.002[a]
Chloroform	0.155 ± 0.144[b]	0.262 ± 0.088[b]	0.035 ± 0.004[b]	0.029 ± 0.002[b]
Methanol	0.102 ± 0.083[c]	0.213 ± 0.006[c]	0.028 ± 0.001[c]	0.019 ± 0.008[c]
Thiourea	0.002 ± 0.001	0.002 ± 0.001		
Galantamine			0.012 ± 0.001	0.012 ± 0.001

Values are mean of triplicate determination (n = 3) ± standard deviation; [a]$P < 0.05$ compared with the positive control, [b]$P < 0.01$ compared with positive control, [c]$P < 0.001$ compared with positive control. AChE, acetylcholinesterase.

Table 3 Cytotoxic activity of different extracts obtained from the plant

Extracts		Concentration (μg/mL)	% Viable cell ratio	
			CCD-1079Sk	A-431
Bulb	Petroleum ether	18.75	88.95	81.62[a]
		37.50	78.08	71.59[a]
		75.00	70.69	61.66[a]
		150.00	51.64	44.56[b]
	Chloroform	18.75	88.57	79.56[b]
		37.50	69.65	64.59[a]
		75.00	51.54	49.59
		150.00	47.41	41.58[a]
	Methanol	18.75	85.99	77.11[a]
		37.50	65.96	62.10
		75.00	65.09	57.14[a]
		150.00	44.33	40.20
Aerial parts	Petroleum ether	18.75	91.75	81.07[b]
		37.50	71.81	66.11
		75.00	56.90	51.11
		150.00	51.43	45.13[a]
	Chloroform	18.75	60.97	51.05[b]
		37.50	51.00	41.08[b]
		75.00	42.39	31.08[c]
		150.00	22.25	15.14[a]
	Methanol	18.75	85.94	82.54
		37.50	70.95	71.58
		75.00	62.97	58.58
		150.00	60.28	47.57[c]

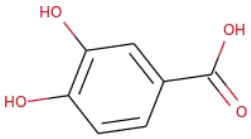
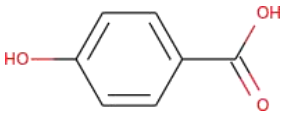
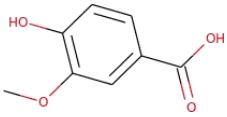
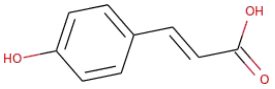
Cytotoxicity of different extracts from plant was evaluated using the luminometric ATP assay following 24 hours incubation of human epidermoid carcinoma (A-431) and normal skin fibroblast (CCD1079Sk) cells with doses of 18.75–150 μg/mL. Statistical differences between A-431 and CCD-1079Sk cells; [a] $P < 0.05$ compared with the control, [b] $P < 0.001$ compared with control, [c] $P < 0.001$ compared with control. ATP, adenosine triphosphate.

Table 4 ADMET profile screening of compounds in the plant

Compounds	Absorption		
	Caco2 permeability (log Papp in 10^{-6} cm/s)	Intestinal absorption (human) (% absorbed)	Skin permeability (log Kp)
Protocatechuic acid	0.490	71.174	−2.727
p-Hydroxybenzoic acid	1.151	83.961	−2.723
Vanillic acid	0.330	78.152	−2.726
p-Coumaric acid	1.210	93.494	−2.715
	Distribution		
	VDss (human) (log L/kg)	BBB permeability (log BB)	CNS permeability (log PS)
Protocatechuic acid	−1.298	−0.683	−3.305
p-Hydroxybenzoic acid	−1.557	−0.334	−3.210
Vanillic acid	−1.739	−0.380	−2.628
p-Coumaric acid	−1.151	−0.225	−2.418
	Metabolism		Excretion
	CYP 450 substrate	CYP 450 inhibitor	Total clearance (log mL/min/kg)	Renal OCT2 substrate
Protocatechuic acid	No	No	0.551	No
p-Hydroxybenzoic acid	No	No	0.593	No
Vanillic acid	No	No	0.628	No
p-Coumaric acid	No	No	0.662	No
	Toxicity			
	AMES toxicity	Hepatotoxicity	Oral rat acute toxicity (LD50) (mol/kg)	Skin sensitisation
Protocatechuic acid	No	No	2.423	No
p-Hydroxybenzoic acid	No	No	2.255	No
Vanillic acid	No	No	2.454	No
p-Coumaric acid	No	No	2.155	No

ADMET, absorption, distribution, metabolism, excretion, and toxicity.

Table 5 Molecular properties of active compounds of *O. orthophyllum*

Compounds		Molecular weight (g/mol)	Acceptor H-bonds	Donor H-bonds	Rotatable bonds	LogP	TPSA
Protocatechuic acid		154.12	3	3	1	0.80	77.75
p-Hydroxybenzoic acid		138.12	2	2	1	1.09	57.53
Vanillic acid		168.15	3	2	2	1.10	66.76
p-Coumaric acid		164.16	2	2	2	1.49	57.53

LogP, calculated logarithm of partition coefficient of a compound between octanol and water; TPSA, topological polar surface area; rotatable bonds, single non-ring bond, bounded to nonterminal heavy atom (excepted for C-N bonds).

Discussion

According to the available literature, only one study has been conducted on the antioxidant activity and another on essential oil content of *O. orthophyllum*. In the first study, the antioxidant activities of methanol extracts from the aerial parts and bulb of the plant were investigated using 1,1-diphenyl-2-picryl-hydrazyl, superoxide radical scavenging, ferrous ion-chelating, phosphomolybdenum-reducing, and ferric-reducing antioxidant power assays. In addition, the contents of the different extracts (ethyl acetate, diethyl ether, and water extracts) obtained from the leaves and flowers were analyzed using HPLC-UV. According to the results of this study, the aerial parts of the plant showed stronger DPPH radical scavenging (aerial parts, IC_{50}: 0.39 mg/mL; bulb, IC_{50}: 2.36 mg/mL) and ferric-reducing antioxidant (aerial parts: 27.39 BHAE mg/g extract; bulb: 4.80 BHAE mg/g extract) activity than those obtained from the bulb. Moreover, the aerial parts (11.00 GAE mg/g extract) contained higher amounts of phenolic compounds than the bulb (2.04 GAE mg/g extract). In addition, the protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and *p*-coumaric acid content in the different extracts from the leaves and flowers of the plant were analyzed via HPLC-UV [1]. Unlike the previous study mentioned above, in our study, petroleum ether, chloroform, and methanol extracts were obtained from the aerial parts and bulbs of the plant via maceration. The antioxidant activities of the different extracts were determined through the DPPH, FRAP, ABTS, and CUPRAC methods. In this study, consistent with the literature, different extracts from the aerial parts of the plant showed higher antioxidant activity than those obtained from the bulb. It was observed that the methanol extracts from the aerial parts had higher DPPH radical scavenging potential (IC_{50}: 0.1800 mg/mL) and total phenolic content (42 mg GAE/g extract) than that reported in the previous study mentioned above (IC_{50}: 0.39 mg/mL; 11 mg GAE/g extract). In addition, it was reported that methanol extract (1.5 mg/g extract) obtained from aerial parts in our study showed lower ferric-reducing antioxidant activity (27.39 mg/g extract) than the previous study. In another study, the essential oil content of the flowers and bulbs was analyzed using gas chromatography-mass spectrometry. A total of 22 and 36 compounds were identified from the extracts obtained from the flowers and bulbs of the plant, respectively. It was determined that furan, nonanal, and heptanal compounds were the major components of the essential oil obtained from the flower. On the other hand, hexanal and heneikosan compounds were found to be abundant in the essential oil obtained from the bulb parts [2]. No biological activity studies other than the one performed above were encountered in the relevant literature. The cytotoxic, anti-urease, and anticholinesterase activities of different extracts obtained from this plant and the ADMET properties of some phenolic compounds were examined by our team for the first time.

Conclusion

In this study, certain biological activities of *O. orthophyllum* were investigated for the first time for both food and medicinal purposes. Although all these results showed lower biological activity than the standards used in the experiment, it was thought that the methanol and petroleum ether extracts of the aerial parts of *O. orthophyllum* can be used as anticholinesterase, antioxidant, and anti-urease agents, respectively. The chloroform extract obtained from the aerial parts showed higher cytotoxic activity than the other extracts. All extracts were also found to be more cytotoxic against cancer cells than healthy cells. The ADMET results suggest that extracts from this plant species could be used as a natural medicinal and nutritional source in the future, after performing more detailed biochemical analyses.

References

1. Renda G, Özel A, Akyüz-Turumtay E, et al. Comparison of phenolic profiles and antioxidant activity of three *Ornithogalum* L. species. *Turk J Biochem*. 2019;44(3):299–306. https://doi.org/10.1515/tjb-2018-0011
2. Renda G, Tosun G, Yaylı N. SPME GC/MS analysis of three *Ornithogalum* L. species from Turkey. *Rec Nat Prod*. 2016;10(4):497–502. https://acgpubs.org/doc/2018080619403459-RNP-EO_1505-024.pdf
3. Obermeyer AA. *Ornithogalum*: a revision of the southern African species. *Bothalia*. 1978;12(3):323–376. https://doi.org/10.4102/abc.v12i3.1793
4. Öztürk D, Koyuncu O, Yaylacı ÖK, et al. Karyological studies on the four *Ornithogalum* L. (Asparagaceae) taxa from Eskişehir (Central Anatolia, Turkey). *Caryologia*. 2014;67(1):79–85. https://doi.org/10.1080/00087114.2014.892279
5. Guner A, Aslan S, Ekim T, Vural M, Babac MT. *Turkey Plant List* (*Vascular Plants*). Istanbul: Nezahat Gökyiğit Botanical Garden

and Flora Research Association Press;2012:98–100. https://isikguner.com/en/project-blog/33/5/turkey-plant-list-vascular-plants
6. Baytop T. *Therapy with Medicinal Plants in Turkey*. Istanbul: Nobel Medical Bookstores;1999. ISBN: 975-420-021-1
7. Sargın SA, Selvi S, Akçiçek E. Investigations of ethnobotanical aspect of some geophytes growing in Alaşehir (Manisa) and surrounding area. *J Grad School Nat Appl Sci Erciyes Univ*. 2013;29(2):170–177. https://dergipark.org.tr/tr/download/article-file/236121
8. Nakamura H, Yoshiyama H, Takeuchi H, et al. Urease plays an important role in the chemotactic motility of *Helicobacter pylori* in a viscous environment. *Infect Immun*. 1998;66(10):4832–4837. https://doi.org/10.1128/iai.66.10.4832-4837.1998
9. Argueta EA, Moss SF. Treatment of *Helicobacter pylori*. *Curr Opin Gastroenterol*. 2019;35(6):544–550. https://doi.org/ 10.1097/MOG.0000000000000578
10. Önder T, Anuk T, Heybeli C. The relationship between oral hygiene index and gastric *Helicobacter pylori* positivity. *Dicle Med J*. 2016;43(1):112–116. https://doi.org/10.5798/diclemedj.0921.2016.01.0648
11. Taşkın T, Taşkın D, Çam ME, Bulut G. Phenolic compounds, biological activities and trace elements of *Capparis ovata* var. *canescens*. *Rev Biol Trop*. 2020;68(2):590–600. https://doi.org/10.15517/rbt.v68i2.40215
12. Standridge JB. Pharmacotherapeutic approaches to the treatment of Alzheimer's disease. *Clin Ther*. 2004;26(5):615–630. https://doi.org/10.1016/S0149-2918(04)90064-1
13. Prutipinyo C, Kamontip MBA, Sirichotiratana N. Self-care behaviours of chemotherapy patients. *J Med Assoc Thai*. 2012;95(6):30–37. https://pubmed.ncbi.nlm.nih.gov/23130486/
14. Taşkın T, Çam ME, Taşkın D, Rayaman E. In vitro and in vivo biological activities and phenolic characterization of *Thymus praecox* subsp. *skorpilii* var. *skorpilii. J Food Meas Charact*. 2019;13:536–544. https://doi.org/10.1007/s11694-018-9967-1
15. Ferreira LLG, Andricopulo AD. ADMET modeling approaches in drug discovery. *Drug Discov Today*. 2019;24(5):1157–1165. https://doi.org/10.1016/j.drudis.2019.03.015
16. Taskın T, Balkan IA, Taskın D, Dogan A. Characterization of phenolic constituents and pharmacological activity of *Achillea vermicularis*. *Indian J Pharm Sci*. 2019;81(2):293–301. https://doi.org/10.36468/pharmaceutical-sciences.510
17. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996;239:70–76. https://doi.org/10.1006/abio.1996.0292
18. Taşkın T, Gezmiş T, Çam ME, et al. The in vitro and in vivo investigation of biological activities and phenolic analysis of *Helichrysum plicatum* subsp. *plicatum.* Braz. *J Pharm Sci*. 2020;56:e18345. https://doi.org/10.1590/s2175-97902019000418345
19. Fu W, Chen J, Cai Y, et al. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch. et Sav.) Ching. *J Ethnopharmacol*. 2010;130(3):521–528. https://doi.org/10.1016/j.jep.2010.05.039
20. Taşkın D, Dilek BA, Dölen E. Evaluation of antioxidant capacity and analysis of major phenolic compounds in *Achillea grandifolia* by HPLC-DAD with Q-TOF LC/MS confirmation. *Chiang Mai J Sci*. 2018;45(1):287–298. https://epg.science.cmu.ac.th/ejournal/journal-detail.php?id=8749
21. Ellman GL, Courtney KD, Andress V, et al. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*. 1961;7(2):88–95. https://doi.org/10.1016/0006-2952(61)90145-9
22. Ghous T, Akhtar K, Nasim FUH, et al. Screening of selected medicinal plants for urease inhibitory activity. *Biol Med*. 2010;2(4):64–69. https://www.biolmedonline.com/Articles/Vol2_4_64-69.pdf
23. Guler EM, Sisman BH, Kocyigit A, Hatiboglu MA. Investigation of cellular effects of thymoquinone on glioma cell. *Toxicol Rep*. 2021;8:162–170. https://doi.org/10.1016/j.toxrep.2020.12.026
24. Pires DE, Blundell TL, Ascher DB. pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J Med Chem*. 2015;58(9):4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104
25. Patlewicz G, Jeliazkova N, Safford RJ, Worth AP, Aleksiev B. An evaluation of the implementation of the Cramer classification scheme in the Toxtree software. *SAR QSAR Environ Res*. 2008;19(5–6):495–524. https://doi.org/10.1080/10629360802083871