RESEARCH IN PHARMACY AND MEDICINE
PHARMEDICINE JOURNAL

2024; 1(1):20-26. doi: 10.62482/pmj.2

Original Article

The Antioxidative, Antimicrobial Activity and HPLC Analysis of *Ornithogalum pyrenaicum*

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Received: 2024.12.28; Revised: 2024.02.09; Accepted: 2024.02.13

Abstract

Introduction: The aim of this study was to specify the antioxidant, antimicrobial activity and phenolic contents of *Ornithogalum pyrenaicum.*

Methods: The antioxidant capacity of *O. pyrenaicum* was determined by 2,2-diphenylpicrylhydrazyl (DPPH) method and ferric reduction antioxidant power by FRAP method. The total phenolic content (TPC) was of the samples was determined using spectrophotometric method. The phenolic contents the samples were analyzed by reverse phase-high performance liquid chromatography (RP-HPLC). Antimicrobial activity was researched on 9 microorganism by agar diffusion method.

Results: As a result of the study, high phenolic contents and strong antioxidant capacitiy were observed. Phenolic compounds were detected as *p-coumaric* acid and benzoic acid. Additionally, it was determined that *O. pyrenacium* had considerable antimicrobial activity on *Yersinia pseudotuberculosis, Staphylococcus aureus* and moderate activity on *Pseudomonas aeruginosa* and *Mycobacterium smegmatis.*

Conclusion: In conclusion, *O. pyrenaicum* extract could be evaluated in the pharmaceutical and cosmetic fields due to their antioxidant and antimicrobial potential and phenolic compounds.

Keywords: Antimicrobial activity, antioxidant activity, *Ornithogalum pyrenaicum*, RP-HPLC, total phenolic content

1. Introduction

The genus *Ornithogalum* L. comprises approximately 200 species. *Ornithogalum* L. which belong to the Liliaceae family are deployed along soft climates in Africa, Asia, and Europe (1). The genus is defined among this genus 36 species are found in Turkey (2). *Ornithogalum* species are used for various medical purposes. These species are well-known to have antimicrobial, anticarsonogenic, antioxidant and cytotoxic properties due to their various phytochemical components (3-5).

Natural antioxidants plants are of great interest in order to protect the human body against the attack of free radicals today (6). Phenolic compounds are the essential sources of antioxidant impacts in plant products. These products can prevent many radical diseases such as cancer, diabetes, Alzheimer's and heart disease by preventing free radical reactions (7).

Plants have unlimited components which are aromatic substances, most phenol or oxygen-substituted derivatives (8), most of these components have antimicrobial activity. For example caffeic acid is effective against viruses (9), bacteria (9, 10, 11) and fungi (9, 12). Catechol and pyrogallol are both compounds that have been shown to be toxic against microorganisms. The number of hydroxyl groups in the phenol group is thought to be associated with their toxicity to microorganisms. There is also proof that increased hydroxylation increases toxicity (13).

Nevertheless, there is insufficient information examining the phenolic contents and biological activity of *O. pyrenaicum* in the literature. That is why antioxidant, and antimicrobial activitiy of the plant were examined in present study. Additionally, phenolic profiles of the plant extracts was determined using HPLC.

2. Methods

2.1. Chemicals and instrumentation

The chemicals used in biological activity and HPLC studies are as follows: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), and sodium carbonate (Na_2CO_3) were bought from Sigma. Butylated hydroxytoluene (BHT), and Folin-Ciocalteau reagent were provided from Fluka and Supelco, respectively. Methanol and acetic acid were purchased from Merck. Ampicillin and Fluconazole used in antimicrobial activity were purchased from Mustafa Nevzat and Phizer, respectively. BMG LABTECH SPECTROstar® Nano spectrophotometer was used for antioxidant activity studies. Evaporation processes were realised by Heidolph (Schwabach, Germany) rotary evaporator system. HPLC studies were performed HPLC system (Shimadzu Corporation, Kyoto, Japan).

2.2. Plant material and preparation of samples

Ornithogalum pyrenaicum were collected from Dikmen, Akçaalan Region / Sinop in Turkey in May 2014 and identified by Prof. Zeki AYTAC and Murat EKICI (herbarium number: 26706). The aerial parts of *O. pyrenaicum* were dried in the ambient conditions and grinded. Then, 50 g of grinded plant mixed with 500 mL methanol and extracted shaking incubator overnight at room temperature (25 °C). This process was repeated three times and solvent was removed by a rotary evaporator. The methanolic extract was stored at +4 °C till all analyses. This extract obtained was used for biological studies. For HPLC study, this extract was prepared HPLC purity methanol to 10 mg/mL and filtered by membranes filter and stored at +4°C.

2.3. Antioxidant activity

The methanolic extract was prepared at a concentration of 10 mg/mL for TPC and FRAP studies. TPC in extract was specified using the Folin method (14). Gallic acid was prepared as standard in a range of concentrations (1000, 500, 250, 125, 62.5, 31.25 ve 15.63 µg/mL). In this study, the extract, Folin reagent, and Na_2CO_3 were mixed in test tubes and incubated at 20 °C and in dark conditions for about 2 hours. The absorbances were read at 760 nm using spectrophotometer. Whole processes was repeated in triplicate.

In order to determine the ferric reduction antioxidant power, FRAP method was performed as stated in the literature (15). Trolox was prepared as standard in a range of concentrations (62.5, 125, 250, 500, 1000 µM). The methanolic extract and FRAP reagent was added in test tubes. All samples were incubated in dark conditions at 37 °C during 20 minutes. And then, the absorbance was read 595 nm againist a blank. Whole processes was repeated in triplicate.

DPPH method was applied to investigate the radical scavenging effect of the plant (16). BHT was prepared as standard and methonolic extract

were prepared in a range of concentrations (0.005, 0.025, 0.0125, 0.00625 mg/mL). DPPH and extract were added in test tubes at the same proportions and mixed. After the test tubes were incubated in the darkness during 50 minutes, the absorbances of the samples were read at 517 nm. Whole processes was repeated in triplicate.

2.4. Antimicrobial actvity

2.4.1. Test microorganisms

The studied microorganisms were provided from Refik Saydam Hifzissihha Institute (Ankara, Turkey). *Bacillus cereus* 709 ROMA, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Mycobacterium smegmatis* ATCC607, *Pseudomonas auroginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, and *Yersinia pseudotuberculosis* ATCC 911 were chosen as test bacteria. *Candida albicans* ATCC 60193 and *Saccharomyces cerevisiae* RSKK 251 were chosen as yeast.

2.4.2. Antimicrobial assay

In order to detect the antimicrobial activity, some modifications were made in agar disk diffusion method (17). All bacteria were suspended in MH broth except M. smegmatis, which was augment in BHA. For yeast-like fungi, Sabouraud Dextrose Agar (SDA) was used. Each microorganism was diluted. A "flood inoculum" was applied to the surface of Mueller Hinton Agar (MHA) and SDA and then dried. Wells with a diameter of 5 millimeters were opened from the agar using a sterile cork borer and 50 µL of extract was added to the wells. The plates were then incubated at 35°C for 18 hours. Antimicrobial activity was studied by comparing the zone of inhibition with the test organism. In this study, ampicillin, fluconazole and streptomycin as the standard drug and dimethylsulfoxide as the control were preferred.

2.5. Determination of phenolic content by HPLC

HPLC analysis to detect the phenolic compounds were implemented as regardes the method employed by Aliyazıcıoglu et al (18). The standards used in this HPLC study are given in Table 1. In this study, a reverse phase column (250 × 4.6 mm i.d, 5 µm) and a gradient program with two solvents system were preferred. The solvent system was prepared as 2% acetic acid in water and 5% acetic acid in acetonitrile:water (1:1). The flow rate was adjusted to 1.2 ml per minute and all signals were determined by diode array detector (DAD) (18).

Table 1. Phenolic standards used in HPLC analysis

Benzoic acid
Caffeic acid
Chlorogenic acid
Gallic acid
Ferulic acid
Protocatechuic acid
Protocatechuic aldehyde
Sinapic acid
Syring aldehyde
Vanillic acid
Vanillin
p-coumaric acid
p-OH benzoic acid

3. Results

3.1. Antioxidant activities of *O. pyrenaicum* methanolic extract

Antioxidant activity of *O. pyrenaicum* methanolic extract was identified by using three different methods i.e. total phenolic contents (TPC), 2,2-diphenylpicrylhydrazyl (DPPH), and reducing antioxidant power (FRAP). TPC and FRAP results of the methanolic extract were found as 5.7 ± 0.2645 mg of GAE/g sample and 129 ± 3.7859 µM Trolox/g sample, respectively. According to DPPH assay result, SC_{50} value of the plant was 2.2186 ± 0.0418 mg/mL. The radical scavenging capacity of extract was lower than that of BHT (0.0099 ± 0.0002 mg/mL). The methanolic extract of *O. pyrenaicum* was exhibited potent antioxidant activity. All the results of antioxidant activities of

the plant are presented in Table 2.

3.2. Antimicrobial activities of *O. pyrenaicum* methanolic extract

The extract of the *O. pyrenaicum* (10 mg/mL) showed antimicrobial effects against *M. smegmatis*, *P. aeruginosa*, *S. aureus*, and *Y. pseudotuberculosis* (Table 3).

3.3. HPLC chromatogram of *Ornithogalum pyrenaicum* methanolic extract

Chromatograms of the all standards and the plant extract of the *O. pyrenaicum* have been given in Figure 1 and 2. In the extract of the *O. pyrenacium*, *p*-coumaric acid (0.6039 mg/g) and benzoic acid (8.1926 mg/g) were detected as phenolic compounds.

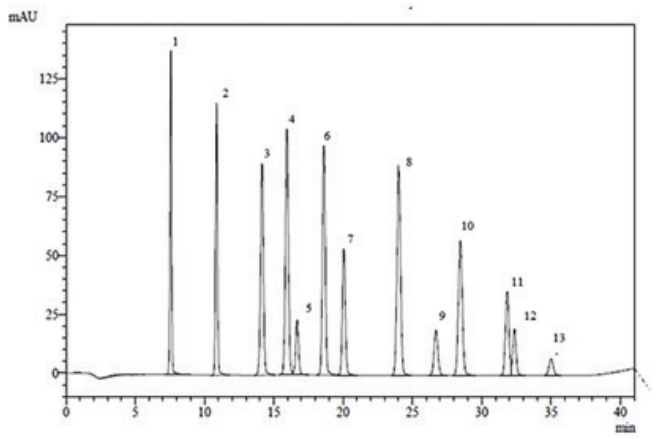

Figure 1. RP-HPLC chromatogram of phenolic standards investigated in O. pyrenacium. Peak identification: (1) gallic acid, (2) protocatechuic acid, (3) protocatechuic aldehyde, (4) p-OH benzoic acid, (5) chlorogenic acid, (6) vanillic acid, (7) caffeic acid, (8) vanillin, (9) syringaldehyde, (10) p-coumaric acid, (11) ferulic acid, (12) sinapic acid, (13) benzoic acid [18].

Table 2. The antioxidant activities of *Ornithogalum pyrenaicum* extract

Test Compounds	TPC[1]	FRAP[2]	DPPH[3]
Methanolic extract	5,7 ± 0,2645	129 ± 3,7859	2,2186 ± 0,0418
BHT			0,0099 ± 0,0002

[1]TPC stated in mg of gallic acid equivalent (GAE) per gram of dry plant weight.

[2] FRAP value stated as µM trolox equivalents (TE) per gram of dry plant weight.

[3]Concentration of test specimen (mg/mL) required to produce 50% scavenging (SC_{50}) of the DPPH radical.

Table 3. Inhibition zone values of extract of *Ornithogalum pyrenaicum*

Tested Compounds	Microorganisms and Inhibition Zone (mm)								
	Gram negative			Gram positive			No gram	Yeast Like Fungi	
	Ec	Pa	Yp	Ef	Bc	Sa	Ms	Ca	Sc
O. pyrenaicum	-	6	10	-	-	10	6	-	-
Ampicillin	10	18	10	35	15	10	-	-	-
Fluconazole							-	25	25
Streptomycin	-	-	-	-	-	-	35	-	-

Ec: *E. coli* ATCC 25922, Pa: *P. aeruginosa* ATCC 43288, Yp: *Y. pseudotuberculosis* ATCC 911, Ef: *E. faecalis* ATCC 29212, Bc: *B. cereus* 709 Roma, Sa: *S. aureus* ATCC 25923, Ms: *M. smegmatis* ATCC607, Ca: *C. albicans* ATCC 60193, Sc: *S. cerevisiae* RSKK 251, (-): no activity

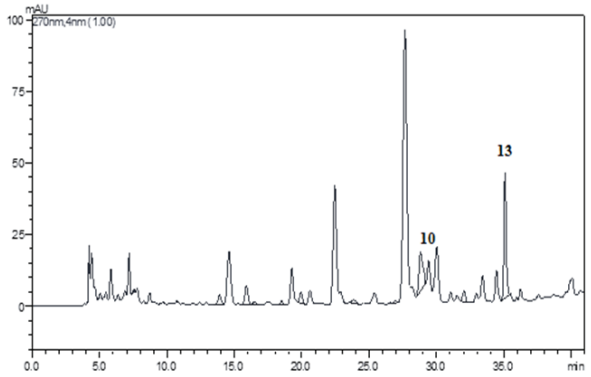

Figure 2. RP-HPLC chromatogram of *O. pyrenacium*.

4. Discussion

Antioxidant substances can reduce damages caused by free radicals which are known to have harmful effects on health. The antioxidant effects of herbal products are mainly due to their phenolic content. The protective effects of phenolic compounds against many diseases (i.e. cardiovascular diseases, cancer, diabetes mellitus, neurodegenerative diseases and osteoporosis) have shown in different studies (19-21).

The antioxidant activity of *O. pyrenacium* was examined and also HPLC studies were performed to determine phenolic content. Herewith, it was determined that due to its rich phenolic content, the plant showed strong antioxidant activity. In the study, benzoic acid and *p*-coumaric acid were found as major phenolic constituents of *O. pyrenacium*. Benzoic acid derivatives have antioxidant, antipyretic, antirheumatic, bacteriocidal and fungicidal and analgesic properties due to their phenolic groups. Also benzoic acid derivatives are a group of chemicals widely used as preservatives in the cosmetic and pharmaceutical industries (22).

Coumaric acid is a compound with antioxidant properties that suppress nitrosamine formation and thus reduce the risk of developing stomach cancer (23).

In a study by Chen et al. was found that *O. caudatum* Ait has possessed considerable antioxidant activity (24). In a biological activity study performed with *O. narbonse*, antioxidant and tyrosinase inhibition activity and phenolic profiles were researched by using different anatomical parts of the plant. In this study, the highest activity was observed in the bulb samples of the plant. *O. narbonse* was also found to be rich in phenolic content (2).

In a study using *O. sintenisii* L. was found that the bulb and aerial parts of this plant were demonstrated good antioxidant properties (25). When compared with these studies in the literature, it can be said that the plant shows potent antioxidant characteristics. However, in this study has found that *O. pyrenacium* has not effective on tyrosinase enzyme.

Interest in extracts of the plants are gradually increasing in the protection of foods and in the production of natural origin medicines. Because the side and toxic effects of sentetic medicines have giving damages to human healthy (26). These days, long duration treatment with certain antibiotics can lead to detrimental side effects. These side effects can be mitigated with phenolic compounds that are antioxidant sources. In addition, these compounds can be used as antimicrobial agents. In this study, antimicrobial properties of the plant were examined and the extract of the *O. pyrenacium* showed antimicrobial effects against *M. smegmatis*, *P. aeruginosa*, *S. aureus*, and *Y. pseudotuberculosis*. *Y. pseudotuberculosis* is a pathogen that causes symptoms such as swelling of lymph nodes, septicemia, typhoidal clinical effects in humans (27). *P. aeruginosa* is a gram-negative bacterium. These bacteria cause urinary tract, eye and ear infections. It also causes diseases such as burn and wound infections, meningitis and bronchitis (28). Spread infections lead to by *M. smegmatis* are frequently associated with immunosuppression (29). When the antimicrobial effect of the plant is taken into consideration, it is thought that it can be used from the plant in the production of drugs in diseases caused by these bacteria.

5. Conclusion

Consequently, it was shown that *O. pyrenaicum* posses significiant antimicrobial and antioxidant properties in the present study. The phenolic components which have antioxidant properties were analysed from the plant. So that *O. pyrenaicum* may represent an artificial source of antimicrobial

and antioxidant agents. There are not enough studies about *O. pyrenaicum*. In our opinion, the study is important in terms of pioneering further studies.

Funding: The authors declared that this study has received no financial support.

Conflicts of interest: The authors have no conflicts of interest to declare.

Acknowledgements: We thank for identify the plant material to Professor Dr Zeki AYTAC (Gazi University, Faculty of Arts and Science, Department of Biology, Ankara, TURKEY) and Professor Dr Murat EKICI (Gazi University, Faculty of Arts and Science, Department of Biology, Ankara, TURKEY).

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Cite this article: Aliyazicioglu R, Kanbolat S, Badem M, Sener OS, Korkmaz N, Ozgen U, Karaoglu Alpay S. The Antioxidative, Antimicrobial Activity and HPLC Analysis of *Ornithogalum pyrenaicum*. Pharmedicine J. 2024;1:20-26. DOI: https://doi.org/10.62482/pmj.2