KSÜ Tarım ve Doğa Derg 27 (4), 782-792, 2024
KSU J. Agric Nat 27 (4), 782-792, 2024
DOI:10.18016/ksutarimdoga.vi.1339345

Exploring the Potential of *Psephellus huber-Marathi* (Wagenitz) Wagenitz: A Comprehensive UHPLC-MS/MS Analysis of Phytochemical Composition and Evaluation of Antioxidant, Antimicrobial, and Antiproliferative Activities

Tugrul Cagri AKMAN[1]✍, Samed SİMSEK[2], Zeynep AKSİT[3], Ali AYDİN[4], Mustafa Abdullah YİLMAZ[5]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, Erzincan Binali Yildirim University, 25240, Erzincan, Türkiye, [2]Department of Medical Services and Techniques, Çayırlı Vocational School, Erzincan Binali Yıldırım University, 24500, Erzincan, Türkiye, [3]Department of Food Engineering, Erzincan Binali Yildirim University, 25240, Erzincan, Türkiye, [4]Department of Basic Medical Science, Faculty of Medicine Bozok University, 66900, Yozgat, Türkiye, [5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Dicle University, Diyarbakır, Türkiye

[1]https://orcid.org/0000-0002-5439-950X, [2]https://orcid.org/0000-0001-8451-3425, [3]https://orcid.org/0000-0002-0349-0223
[4]https://orcid.org/0000-0002-9550-9111, [5]https://orcid.org/0000-0002-4090-7227
✉: eczcagri87@gmail.com

ABSTRACT

Psephellus huber-morathii (PH) is an endemic species in the Eastern Anatolia Region. In this study, besides the biological activities of the 70% methanol extract of PH, its comprehensive phytochemical composition was investigated. The phenolic composition of the PH extract was analyzed using the UHPLC-MS/MS method. To evaluate its antimicrobial characteristics, the microdilution technique was employed. The antioxidant capabilities and total phenolic and flavonoid contents of the extract were determined using the spectrophotometer. Additionally, the effects of the extracts on cell proliferation and NCI-60 survival parameter values were assessed using the MTT assay. Quinic acid and chlorogenic acid were major compounds. The extract showed high antioxidant activity in DPPH (IC_{50}:13.9±0.4 µg mL^{-1}) and FRAP (61.3±2.3 mg TE g $extract^{-1}$) methods. The total phenolic and flavonoid contents of the extract were found as 52.2±1.9 mg GAE g $extract^{-1}$ and 28.6±0.9 mg QE g $extract^{-1}$, respectively. Discussion: The extract showed low antiproliferative activity against normal lung and retinal cell lines, promising anticancer effects on gynecological and colon cancer cells, and moderate antimicrobial activity against *Bacillus cereus* and *Enterococcus faecium*. The study demonstrated the medicinal potential and value of PH extract against infectious diseases and cancer.

Biochemistry

Research Article

Article History
Received : 08.08.2023
Accepted : 19.12.2023

Keywords
Psephellus huber-Marathi
Antimicrobial
Antioxidant
Antiproliferative
LC-MS/MS

Psephellus huber-Marathi (Wagenitz) Wagenitz'in Potansiyelini Keşfetme: Fitokimyasal Bileşiminin Kapsamlı Bir UHPLC-MS/MS Analizi ve Antioksidan, Antimikrobiyal ve Antiproliferatif Aktivitelerinin Değerlendirmesi

ÖZET

Psephellus huber-morathii (PH), Doğu Anadolu Bölgesi'nde endemik bir türdür. Bu çalışmada PH'nin %70 metanol ekstraktının biyolojik aktivitelerinin yanı sıra kapsamlı fitokimyasal bileşimi de incelenmiştir. PH ekstraktının fenolik bileşimi, UHPLC-MS/MS yöntemi kullanılarak analiz edildi. Antimikrobiyal özelliklerini değerlendirmek için mikrodilüsyon tekniği kullanıldı. Ekstrenin antioksidan özellikleri, toplam fenolik ve flavonoit içerikleri spektrofotometre kullanılarak belirlendi. Ek olarak, ekstraktların hücre proliferasyonu ve NCI-60 sağkalım parametre değerleri üzerindeki etkileri, MTT deneyi kullanılarak değerlendirildi. Kinik asit ve klorojenik asit majör bileşiklerdi. Ekstrakt, DPPH (IC_{50}:13.9±0.4 µg mL^{-1}) ve FRAP (61.3±2.3 mg TE g $ekstrakt^{-1}$) yöntemlerinde yüksek antioksidan aktivite göstermiştir. Ekstraktın toplam fenolik ve flavonoid içerikleri sırasıyla 52.2±1.9 mg GAE g $ekstrakt^{-1}$ ve 28.6±0.9 mg QE g $ekstrakt^{-1}$ olarak bulundu. Ekstrakt, normal akciğer ve retina hücre hatlarına karşı düşük antiproliferatif aktivite, jinokolojik ve kolon kanser hücreleri üzerinde umut verici bir antikanser etki ve

Biyokimya

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 08.08.2023
Kabul Tarihi : 19.12.2023

Anahtar Kelimeler
Psephellus huber-morathii
Antimikrobiyal
Antioksidan
Antiproliferatif
LC-MS/MS

Bacillus cereus ve *Enterococcus faecium*'a karşı orta derecede antimikrobiyal aktivite gösterdi. Çalışma, PH ekstraktının bulaşıcı hastalıklara ve kansere karşı tıbbi potansiyelini ve değerini göstermiştir.

Atıf İçin : Akman, T. C., Şimşek, S., Aksit, Z., Aydın, A., & Yılmaz, M. A. (2024). Exploring the potential of Psephellus huber-morathii (Wagenitz) Wagenitz: A comprehensive UHPLC-MS/MS analysis of phytochemical composition and evaluation of antioxidant, antimicrobial, and antiproliferative activities. *KSÜ Tarım ve Doğa Derg 27* (4), 782-792. https://doi.org/10.18016/ksutarimdoga.vi.1339345.

To Cite: Akman, T. C., Şimşek, S., Aksit, Z., Aydın, A., & Yılmaz, M. A. (2024). Exploring the potential of Psephellus huber-morathii (Wagenitz) Wagenitz: A comprehensive UHPLC-MS/MS analysis of phytochemical composition and evaluation of antioxidant, antimicrobial, and antiproliferative activities. *KSU J. Agric Nat 27* (4), 782-792. https://doi.org/10.18016/ksutarimdoga.vi.1339345

INTRODUCTION

Medicinal plants have been used for a long time by many communities as they are rich in natural phytochemicals (phenolic acids, flavonoids, tannins, phenolic diterpenes, terpenoids, vitamins, essential oils, and other biological metabolites). It is crucial to discover the phytochemicals of medicinal plants, to research and assess their therapeutic and bioeconomic potentials, the possible applications in the medical industries, and biological functions. Particularly phenolic derivatives of bioactive chemicals have biological effects that are anti-inflammatory, antibacterial, antimicrobial, and antioxidant. They are also used successfully as a natural medicine and various treatment methods due to their antiproliferative properties that suppress various tumors or prevent a poor prognosis (Ríos & Recio, 2005; Chanda & Nagani, 2013; Tarhan et al., 2022). Türkiye has a unique and diverse botanical heritage with over 11,700 plant species. Numerous species are both endemic and have medicinal uses. Plants show many medicinal properties and biological activities with many important compounds such as alkaloids, sesquiterpene lactones, and flavonoids in their compositions (Güçlü et al., 2022). For this reason, elucidating the phytochemical compositions and biological activities of plants will guide their medicinal uses. One of the significant plant families in Türkiye is Asteraceae, which comprises 190 species, 112 of which are endemic. *Psephellus huber-morsathii* (PH) (*synonym Centaurea huber-morathii*) is a member of the Asteraceae family and an endemic species in Erzincan, Türkiye (Aydin et al., 2013). There is no study in the literature about the traditional use of PH in Türkiye. However, many plants from the Psephellus genus are utilized in traditional medicine in Türkiye. It was reported that plants from the Psephellus genus have antipyretic, hemorrhage, and wound-healing effects (Altundağ et al., 2013; Özcan, 2013) and they are used as antidiabetic, antibacterial, and for stomach disorders (Orallo et al., 1998; Arif et al., 2004; Güven et al., 2005). Also worldwide, Psephellus species are traditionally used for treating diarrhea, dandruff, rheumatism, inflammation, digestive issues, hypertension, fever, infections, and menstrual problems alone or in combination with other herbs (Farrag et al., 1993; Barrero et al., 1997; Orallo et al., 1998; Khammar & Djeddi, 2012).

The pharmacological and medicinal properties of PH are not investigated in detail. There are limited studies in the literature that investigate the biological activities and phytochemical profile of PH. In previous studies, the phytochemical profile of the methanol extract of PH seeds by HPLC (Shoeb et al., 2007), its antibacterial, and antioxidant activity (Sarker, 2005), and its antiproliferative activity against colon cancer (Sarker et al., 2007) were reported. In addition, the essential oil profiles of the seeds were determined (Başer et al., 2006). However, there is only one study examining the total phenolic content (TPC), antioxidant, antimicrobial, antityrosinase, and anticholinesterase activities of the methanol extract of PH aerial parts (flowers, leaves, stem, and roots) (Korkmaz et al., 2019). Several studies reported the phytochemical compositions, and antioxidant, cytotoxic, and anti-inflammatory activities of other *Psephellus* species (Shoeb et al., 2007; Zengin et al., 2010; Polatoglu et al., 2015; Demiroz, Nalbantsoy, Kose, & Baykan, 2020). The phytochemical composition of PH was determined by the high-sensitivity liquid chromatography-mass spectrometry (UHPLC-MS/MS) technique in any study. Also, the total flavonoid content (TFC), the cytotoxic activity, and its efficacy against other types of cancer (brain and gynecological) of PH were not studied. In this study, the phytochemical composition of the hydroalcoholic extract of PH was determined by the UHPLC-MS/MS method. Apart from the antioxidant and antibacterial properties of PH extract, total phenolic, and flavonoid contents, antiproliferative and cytotoxic activities of the plant were evaluated for the first time.

MATERIALS and METHODS

Plant materials

PH was collected during the flowering period from subalpine grasslands on Pöske Mountain in Erzincan Province, Türkiye, on 26 June 2022 (2069 meters above sea level). The plant was dried in dark suitable drying areas at room temperature and the taxonomic descriptions of its materials were provided by

taxonomist Prof. Dr. Ali Kandemir, Erzincan Binali Yildirim University, Faculty of Arts and Sciences, Department of Biology. Authentic samples (ID number: 10862) were stored in the Biology Department Herbarium, Erzincan Binali Yıldırım University.

Preparation of extracts

Air-dried aerial parts of the plant were finely ground using a laboratory mill (5-20 mm). 10 g plant materials were macerated overnight with 500 mL of 70% methanol (the ratio of methanol: water, 70:30, v/v) and filtered. The extraction process was repeated three times. The methanol was evaporated, and the remaining water was lyophilized to give an amorphous solid. The yield of the extraction procedure was 19%. The obtained powder (1.9 g) was stored at +4 °C for LC-MS/MS characterization and antioxidant, antimicrobial, and antiproliferative activity studies.

Mass and chromatographic conditions

A Shimadzu-8040 model ultra-high performance liquid chromatography device (UHPLC) equipped with an autosampler (SIL-30AC), a column oven (CTO-10ASvp), dual pumps (LC-30AD), a degasser (DGU-20A3R) and MS/MS detector (8040) was used for the phytochemical characterization of PH. The phenolic compounds were quantified using a validated UHPLC method. Table 1 displays the chromatographic conditions of the method (Yilmaz, 2020).

Table 1 Chromatographic conditions.
Çizelge 1 Kromatografik şartlar

Parameters	*Conditions*
Column	Agilent Poroshell 120 EC-C18 model (150 mm×2.1 mm, 2.7 µm)
Temperature	40°C
Mobil phase	Mobile phase A: (Water: 5 mM ammonium formate: 0.1% formic acid) Mobile phase B: (Methanol: 5 mM ammonium formate: 0.1% formic acid)
Flow rate	0.5 mL min^{-1}
Injection volume	5 µL
Gradient Elution	20-100% B (0-25 min), 100% B (25-35 min), 20% B (35-45 min)

A Shimadzu LCMS-8040 model mass spectrometer with negative electrospray ionization mode (ESI) in the UHPLC system was used for detection. Mass conditions and collision energies (CE) were optimized for the qualitative and quantitative determination of phytochemical compounds. Mass conditions are given in Table 2. Data from the mass detector was recorded and processed by LabSolutions software (Shimadzu). The 56 compounds were identified and quantified using the multiple reaction monitoring (MRM) mode.

Table 2 Mass conditions
Çizelge 2 Kütle şartları

Parameters	*Conditions*
Mass spectrometer	Shimadzu LCMS-8040
Ionization Mode	Electrospray ionization (ESI)
Drying gas (N2) flow	15 L min^{-1}
Nebulizing gas (N2) flow	3 L min^{-1}
DL temperature	250°C
Heat block temperature	400°C
Interface temperature	350°C

Antioxidant activity assays

In-vitro free radical scavenging (DPPH·), the ferric ion reducing antioxidant power (FRAP), TPC, and TFC of extract of PH were assessed as antioxidant parameters by using spectrophotometric techniques.

DPPH· free radical scavenging activity

Shimada's method was used for the free radical scavenging activity of the extract with a small modification (Sipahi et al., 2022). Stock solutions of extract and standard (1 mg mL^{-1}) were prepared. A 0.026 M DPPH· (2,2-diphenyl-1-picryl hydrazine) solution was prepared using methanol. 5, 10, 25, 50, 75, 100, 200, and 250 µg mL^{-1} solutions were prepared by diluting the stock solution to 3 mL with methanol. 1 mL of DPPH· solution was added to each solution. The mixture was vortexed and incubated in the dark at room temperature for 30 minutes. The absorbances of all solutions at 517 nm against a blank were recorded using a spectrophotometer. The absorbance values obtained were converted to % activity, and the IC_{50} (µg mL^{-1}) for PH extract was calculated. Trolox was used as a positive control. All tests were repeated three times, and the test data were given as mean ± standard deviation.

$$\%\ activity_{DPPH} = 100 \times [Acontrol-(Asample-Ablank)] / Acontrol$$

Ferric-ion reducing antioxidant power (FRAP) assay

Oyaizu's method was applied with minor modifications for the ferric-ion-reducing power of the extract (Aksit et al., 2022). Trolox was used as the reference standard. Stock solutions of the extract and Trolox were prepared at a concentration of 1 mg mL^{-1}, separately. 0.25 mL of the extract was made up to 1.25 mL with 0.2 M phosphate buffer (pH 6.6) and then 1.25 mL $K_3Fe(CN)_6$ (1%) was added. The mixture was incubated at 50 °C for 20 minutes and cooled at room conditions. Following incubation, 1.25 mL trichloroacetic acid (10%) and 0.25 mL iron (III) chloride (0.1%) were added and the final mixture was vortexed. The absorbance of the mixture at 700 nm was measured. A calibration curve was created from different concentrations (5-400 µg mL^{-1}) of Trolox and, the result was converted to mg Trolox equivalent (TE)

activity g extract^{-1} using that calibration curve. The tests were done six times, and the results were given as mean values with standard deviations.

Total phenolic content

The TPC of the extract was determined by spectrophotometer using the Folin-Ciocalteu's reagent (Aksit et al., 2022). Gallic acid was used as a standard in the study. The stock solutions of both gallic acid and extract were prepared (1 mg mL^{-1}). 0.1 mL of the stock solution of the extract was diluted with 4.5 mL of distilled water. It was made up of 5 mL with 0.3 mL of Na_2CO_3 (2%) and 0.1 mL of Folin-Ciocalteu reagent. After 10 minutes at room temperature, it was vortexed and kept in the dark for 120 minutes for incubation. The absorbance at 760 nm of the solution was recorded. The calibration curve (y=0.117x-0.011) of gallic acid consisting of different concentrations (1, 5, 10, 25, 50, 100, 250, 400, and 800 µg mL^{-1}) was used for calculations. TPC was expressed as mg gallic acid equivalent phenolic substance per g of extract.

Total flavonoid content

Aluminum chloride was used to determine the TFC in the extract with the spectrophotometric method (Aksit et al., 2022). Quercetin was used as a standard. 1 mg mL^{-1} stock solution of the extract and quercetin was prepared in methanol. 4.7 mL of methanol, 0.1 mL of $AlCl_3$ (10%), and 0.1 mL of 1 M ammonium acetate solution were added to 0.1 mL of the stock solution of the extract, and vortexed. The mixture was incubated for 45 minutes. After the incubation, the absorbance at 415 nm of the mixture was recorded. The calibration curve of quercetin was generated using different concentrations (1, 5, 10, 25, 50, 100, 250, 400, and 800 µg mL^{-1}). Finally, after the results were converted to mg quercetin equivalent (QE) g extract^{-1} using the calibration curve, the TFC of the extract was reported as the mean and standard deviation.

Determination of the minimum inhibitory concentration (MICs)

The microdilution technique was utilized in the antimicrobial test to determine the antimicrobial characteristics of PH extract against four Gram-positive: (Elshikh et al., 2016) *S. aureus* (ATCC 6538), *L. monocytogenes* (ATCC 51774), *B. cereus* (ATCC 10876), *E. faecium* (ATCC 8459), and as well as four Gram-negative; *P. fluorescens* (ATCC 13525), *P. aeruginosa* (ATCC 15442), *E. coli* (ATCC 25922), *S. enteritidis* (ATCC 13076). To obtain serial dilution of test material (from 0.1 to 1.95 µg mL^{-1}), 100 µL of test solution prepared in dimethyl sulfoxide (DMSO) (10%, w/v) was added to the first row of the 96-well plate. The other wells were filled with 50 µL of sterile Muller Hinton Broth (MHB), then 50 µL volume of the first well was transferred to the latter wells. 10 µL of bacterial suspension (1x10^8 CFU mL^{-1}) was added to the corresponding well and incubated at 37 ℃ for 18 hours. After, the addition of 30 µL of resazurin solution (0.02%) to each well the plates were incubated for 6 hours above-mentioned condition. For the setting of the sterile control well, 50 µL of DMSO solution (10% w/v), 10 µL of MHB, 30 µl of indicator solution, and 10 µL of bacterial solutions were added for each bacterium. Negative control was prepared by the addition of 30 µL of indicator solution and 60 µL of MHB. The color change from purple to pink indicated a non-inhibited bacterial action. The concentration level at which color change was observed was noted as MIC values in µg mL^{-1} for each bacterial strain assayed. Tetracycline was used as the positive control.

Antiproliferative activity

Cancer cell lines and cell culture

In this work, the following cell types were used: HeLa (ATCC, CCL-2) and A2780 (RRID, CVCL-0134) gynecological cancer cell lines, SW620 (ATCC, CCL227) and HT29 (ATCC, HTB-38) colon cancer cell lines, A172 (ATCC, CRL-1620) and C6 (ATCC, CCL-107) brain cancer cell lines, and Beas2B (ATCC, CRL-9609) normal lung cell lines, RPE (ATCC, CRL-4000) normal retinal cell lines, and HSF (ATCC, CRL-7449) normal skin cell lines. In a laminar cabinet, all cell preparation processes were completed in a sterile setting. The cell lines were employed once they had reached confluence in a supplemented DMEM medium with 10% FBS and 2% PenStrep solution at 37°C, 5% CO_2 conditions. 10,000 cells per well were sown on measuring plates. Test extracts were introduced after 16 hours of pre-incubation, and after 24 hours of incubation, measurements were made.

Antiproliferative activity assay

The effects of the extracts on cell proliferation and NCI-60 survival parameter values were assessed using the MTT assay. Following a 24-hour incubation period with test extracts and cancer cell lines, this test technique was used. The optical density of the cells treated with the DMSO was taken to be 100% and the findings were expressed as % cell inhibition. So, using the formula [1-(A test substance/A solvent control) x 100, the % inhibition was determined. The IC_{50} concentrations of test extracts were determined using the MTT technique on cells with increasing concentrations of each test extract (1.96, 3.91, 7.81, 15.63, 31.25, 62.5, and 125.0 µg mL^{-1}) across a predetermined range. It was examined by applying a logarithmic function to the absorbance-derived logarithmic curve after the NCI-60 survival parameters were measured using the following formulas (GI_{50}, TGI, and LC_{50}):

Cell proliferation:

[(Ti-Tz)/(C-Tz)]x100 if Ti>/=Tz (cytolytic effect) or

[(Ti-Tz)/Tz]x100 if Ti<Tz (cytocidal or cytotoxic effect)

(Tz; zero point, C; control growth, Ti; inhibition by test substance).

GI_{50}: Concentration value that reduces growth by 50%

([(Ti-Tz)/ (C-Tz)]x100=50),
TGI: Concentration value that reduces growth by 100% (Ti=Tz),
LC_{50}: concentration value that by 50% kills cells in the medium ([(Ti-Tz)/Tz]x100=-50).

Cytotoxicity test

The LDH technique was used to assess if the test extracts were cytotoxic or cytostatic to cells. Depending on the extracts examined, a rise in LDH in the culture supernatant will occur if more cells perish throughout the incubation time. The cytoplasmic enzyme lactate dehydrogenase (LDH), which is stable, is present in the majority of cells. The manufacturer's instructions for using the LDH cell cytotoxicity kit were followed for this purpose. Briefly, NAD^+ is reduced to $NADH/H^+$ by the LDH resulting in lactate-to-pyruvate conversion. Then, a catalyst (diaphorase) transfers H/H^+ from $NADH/H^+$ to the tetrazolium salt which is reduced to formazan. All measurements were made in triplicate. IC_{50} concentrations of test substances were used. In a nutshell, the formula below was used to calculate and assess the change in the quantity of formazan produced as a result of LDH enzyme activity:
% Cytotoxicity = [(Substance Absorbance - Low Control / High Control - Low Control) x 100].

Statistical analysis

The results of LC-MS/MS analyses, and antioxidant and antiproliferative activity assays were statistically analyzed using the SPSS Statistics (IBM v.20, Chicago, IL, USA). The results were expressed as mean and standard deviation.

RESULTS and DISCUSSION

In the study, the dried aerial parts of endemic *Psephellus huber-morathii* were used and these parts of the plant were extracted with 70% methanol. The phytochemical profile of the extract was determined and the total phenolic content, total flavonoid content, and antioxidant, antimicrobial, and antiproliferative activities of the hydroalcoholic extract were investigated.

LC-MS/MS characterization

There is no tandem mass spectrometry and electrospray ionization to liquid chromatography application in the literature for the evaluation of the phenolic composition of PH. In the study, the plant extract was screened by the UHPLC-MS/MS method, which can recognize 56 phytochemicals. In the method, negative ionization, which shows higher sensitivity for phenolic compounds and flavonoids, was preferred. These phytochemicals were determined by examining the molecular ions and MS/MS fragments and the associated collision energies for these fragments, and their amounts were expressed as mg analyte g extract^{-1}. (Figure 1)
The phenolic composition of the extract is listed in Table 3. In the extract of PH, the flavonoid phytochemicals such as protocatechuic aldehyde, vanillin, coumarin, luteolin-*7-O*-glucoside, quercetin-*3*-glucuronide, rutin, isoquercitrin, hesperidin, apigenin-*7*-glucoside, quercitrin, kaempferol-*3-O*-glucoside, nicotiflorin, quercetin, naringenin, luteolin, kaempferol, apigenin, chrysin, acacetin were detected and quantified.

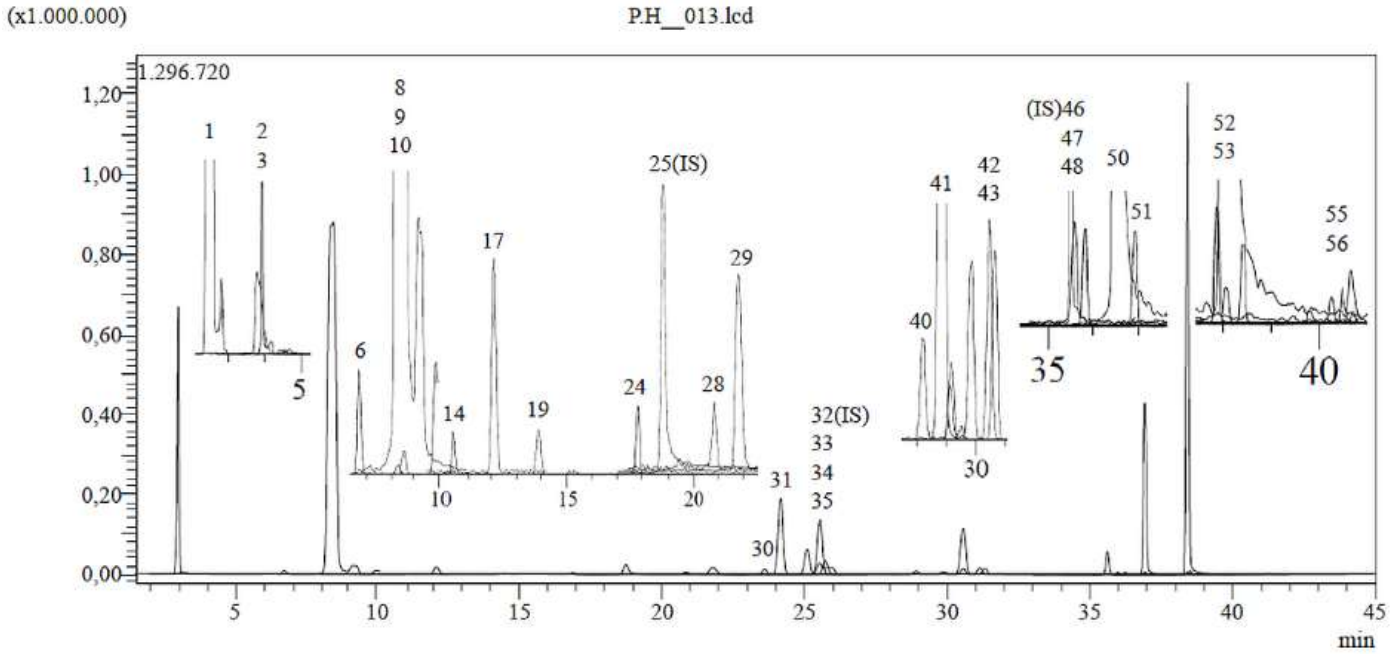

Figure 1. MRM chromatogram of the hydroalcoholic extract of PH obtained by UHPLC-MS/MS.
Şekil 1. UHPLC-MS/MS ile elde edilen PH hidroalkolik ekstraktının MRM kromatogramı

Table 3 Phytochemical compounds of PH extract analyzed by UHPLC-MS/MS (mg g extract^{-1})
Çizelge 3 UHPLC-MS/MS ile analiz edilen PH ekstraktının fitokimyasal bileşikleri (mg g extract^{-1})

No	*Analyte*	*mg g extract^{-1}±SD*	*No*	*Analyte*	*mg g extract^{-1}±SD*
1	Quinic acid	44.95±3.17	29	Salicylic acid	0.10±0.00
2	Fumaric acid	0.22±0.00	30	Luteolin-*7-O*-glucoside	0.40±0.01
3	Aconitic acid	0.24±0.01	31	Quercetin-*3*-glucuronide	9.04±1.34
4	Gallic acid	<LOD	32	Rutin-D3-IS	n.a.
5	Epigallocatechin	n.d.	33	Rutin	3.22±0.89
6	Protocatechuic acid	0.30±0.04	34	Isoquercitrin	6.62±1.47
7	Catechin	<LOD	35	Hesperidin	1.26±0.07
8	Gentisic acid	0.02±0.00	36	*O*-Coumaric acid	<LOD
9	Chlorogenic acid	29.00±4.63	37	Genistin	<LOD
10	Protocatechuic aldehyde	0.04±0.00	38	Rosmarinic acid	n.d.
11	Tannic acid	n.d.	39	Ellagic acid	n.d.
12	Epigallocatechin gallate	<LOD	40	Apigenin-*7-O*-glucoside	0.24±0.01
13	1,5-dicaffeoylquinic acid	n.d.	41	Quercitrin	6.82±1.09
14	4-*OH* Benzoic acid	0.15±0.04	42	Kaempferol-*3-O*-glucoside	0.78±0.09
15	Epicatechin	<LOD	43	Nicotiflorin	0.74±0.08
16	Vanillic acid	n.d.	44	Fisetin	<LOD
17	Caffeic acid	0.13±0.03	45	Daidzein	<LOD
18	Syringic acid	<LOD	46	Quercetin-D3-IS	n.a.
19	Vanillin	0.05±0.01	47	Quercetin	0.15±0.03
20	Syringic aldehyde	<LOD	48	Naringenin	0.02±0.00
21	Daidzin	<LOD	49	Hesperetin	<LOD
22	Epicatechin gallate	<LOD	50	Luteolin	1.30±0.06
23	Piceid	<LOD	51	Genistein	0.01±0.00
24	*p*-Coumaric acid	0.03±0.00	52	Kaempferol	0.04±0.01
25	Ferulic acid-D3-IS	n.a.	53	Apigenin	2.43±0.53
26	Ferulic acid	<LOD	54	Amentoflavone	n.d.
27	Sinapic acid	<LOD	55	Chrysin	0.01±0.00
28	Coumarin	0.02±0.00	56	Acacetin	0.01±0.00

*n.d.: Not detected, n.a.: Not Applicable, SD: Standard deviation, LOD: Limit of detection

Many organic acids and phenolic acids in the plant extract were identified and quantified: quinic acid, fumaric acid, aconitic acid, protocatechuic acid, gentisic acid, chlorogenic acid, 4-*OH* benzoic acid, caffeic acid, *p*-coumaric acid, salicylic acid. According to these results, quinic acid (44.95 mg analyte g extract^{-1}) and chlorogenic acid (30.00 mg analyte g extract^{-1}) are major compounds of PH. Besides the major compounds, PH contains high amounts of quercetin-*3*-glucuronide (9.04 mg analyte g extract^{-1}), isoquercitrin (6.62 mg analyte g extract^{-1}), quercitrin (6.82 mg analyte g extract^{-1}) and apigenin (2.43 mg analyte g extract^{-1}).

Two HPLC studies were reported for the phenolic compounds of PH (Korkmaz et al., 2019; Shoeb et al., 2007). Korkmaz et al. identified 7 compounds in the methanol extract of the plant and quantified two of them. In parallel with this study, they reported that the plant contains *p*-coumaric acid (2.21 mg g extract^{-1}). They also suggested that the plant contains benzoic acid (11.55 mg g extract^{-1}) as the main compound. Shoeb et al. reported that they isolated kaempferol and afzalin from plant seeds by HPLC. Only the qualitative analysis of phytochemical compounds was performed in this study, and their amounts were able to not determined. Octanol, hexadecanoic acid, *p*-cymene, and caryophyllene oxide were found in the essential oil analysis of plant seeds (Başer et al., 2006). This study is the first comprehensive report on the organic acid and flavonoid compounds of PH hydroalcoholic extract.

Antioxidant activities, TPC and TFC

The results of the TPC, TFC, FRAP, and DPPH· activities of the extract are shown in Table 4.

Both TPC and TFC as well as DPPH· and FRAP activities of the plant extract were determined quickly by UV-visible spectrophotometer. In this investigation, the TPC and TFC of PH extract were calculated as 52.19±1.97 mg GAE (gallic acid equivalent) and 28.57±0.94 mg QE per gram extract. Additionally, the extract concentration required to achieve 50% inhibition of the DPPH· radical (IC_{50}) was calculated as 13.18±0.91 µg mL^{-1}, compared to 8.25±1.16 µg mL^{-1} for the positive control. Similar results were observed for the FRAP test, which measures the ability to reduce Fe^{3+} to Fe^{2+}, where one gram of extract had a reducing power equal to 61.27±2.33 mg Trolox.

Table 4 The results of the TPC, TFC, FRAP, and DPPH · activities of the extract
Çizelge 4 Ekstraktın TPC, TFC, FRAP ve DPPH · aktivitelerinin sonuçları

Example	*DPPH· scavenging IC_{50} (µg mL^{-1}) ±SD*	*Total phenolics mg GAE g extract^{-1} ±SD*	*Total flavonoids mg QE g extract^{-1} ±SD*	*Ferric Ion Reducing power mg TE g extract^{-1}±SD*
The extract	13.90±0.5	52.19±2.0	28.57±1.0	61.27±2.3
Trolox	8.25±1.2	-	-	-

SD: Standard deviation, *IC_{50}:* Half maximal inhibitory concentration, GAE: gallic acid equivalent, QE: quercetin equivalent, TE: Trolox equivalent

In the literature, TPC values of water and methanol extracts of PH were reported as 13.9±0.460 mg GAE g extract^{-1} and 10±0.268 mg GAE g extract^{-1}, respectively. The DPPH· activity of these extracts was expressed as IC_{50} value of 0.3379±0.00 mg mL^{-1} and 0.2073±0.00 mg mL^{-1}. It was discovered that FRAP activities were 841±4.70 µM TE and 666±3.21 µM TE. (Korkmaz et al., 2019b) In another study, the methanol extract of plant seeds showed 10 times higher antioxidant activity than Trolox in the DPPH test (Sarker et al., 2005).

In this study, the antioxidant activity, TPC, and TFC of PH hydroalcoholic extract were investigated for the first time. The extract showed high antioxidant activity in DPPH and FRAP tests. It also has rich TPC and TFC. These findings can be explained by the high yield (19%) of the 70% methanol extraction procedure. The rich phytochemical profile of the hydroalcoholic extract better reflects the antioxidant ability, TPC, and TFC of the plant. The antioxidant activity and TPC findings in this study support previous findings about PH (Sarker et al., 2005; Korkmaz et al., 2019).

Estimation of antimicrobial activities of PH

The minimum inhibitory concentration was defined if an extract has strong activity when the MIC is less than 100 µg mL^{-1}, moderate activity when it is between 100 and 500 µg mL^{-1}, and weak activity when it is between 500 and 1000 µg mL^{-1}. The extract is inactive if the MIC is more than 1000 µg mL^{-1} (Holetz et al., 2002; Khan et al., 2009). Antimicrobial activities of the extract are given with MIC values in Table 5.

Table 5 MIC values of PH extract (µg mL^{-1})
Çizelge 5 PH ekstraktının MIC değerleri (µg mL^{-1})

Microorganisms	*PH extract*	*Tetracycline*
Pseudomonas fluorescence	250	3.91
Pseudomonas aeruginosa	500	7.81
Escherichia coli	250	7.81
Salmonella enteritidis	250	3.91
Listeria monocytogenes	500	3.91
Staphylococcus aureus	250	1.95
Bacillus cereus	125	7.81
Enterococcus faecium	125	1.95

Accordingly, the extract showed moderate antimicrobial activity in almost all of *S. aureus, L. monocytogenes, B. cereus, E. faecium*, *P. fluorescens*, *P. aeruginosa*, *E. coli*, and *S. enteritidis.* The extract had a lower MIC value (125 µg mL^{-1}) against gram-positive bacteria *B. cereus* and *E. faecium*. On the contrary, the extract showed a low antimicrobial activity against *L. monocytogenes* and *P. aeruginosa* with a MIC value of 500 µg mL^{-1} (Table 5). This assay demonstrated that the hydroalcoholic extract of PH was more effective against *B. cereus* and *E. faecium* than other bacteria tested.

In the literature, the antimicrobial activity of PH was investigated by Korkmaz et al. (2019), the methanol extract of PH showed moderate antimicrobial activity against *S. aureus, P. aeruginosa,* and *E. coli*. (MIC values 100-500 µg mL^{-1}) However, it was reported that the methanol extract has weaker activity against *L. monocytogenes* (Korkmaz et al., 2019). In the study, it was determined that the hydroalcoholic extract showed moderate activity against *L. monocytogenes*. On the other hand, Sarker et al. (2005) reported that the seed extract did not show any antimicrobial activity against *S. aureus, P. aeruginosa,* and *E. coli.* It was reported that plant seeds show low and moderate antimicrobial activity only against *Citrobacter freundii* and *Enterococcus faecalis* (MIC values 100-1000 µg mL^{-1}) (Sarker et al., 2005).

Antiproliferative activity

Evaluation of PH extract according to NCI-60 screening methodology

Numerous antiproliferative medicines used in contemporary chemotherapy still do not exhibit the intended therapeutic qualities because of the tumors' dynamic mutational loads. Additionally, their therapeutic efficacy decreases over time due to chemotherapeutic agent resistance mechanisms and side effects. Because of this, a lot of research is being done to obtain new antiproliferative drugs. In this aim, the MTT test was used to assess the effects of PH extract on cell proliferation (NCI-60 screening approach) and IC_{50} values. It can be seen that the tested extract has a higher lethal concentration (LC_{50}) value than the control antiproliferative drug, 5-Fluorouracil (5FU) when LC_{50} values of the extract on Beas2B, RPE, and HSF control cells are examined. This suggests that the extract does not have unfavorable toxicity. High LC_{50} values suggest that the cytotoxic effects of the extract are lower, which is

desired, as indicated in the NCI-60 screening protocol. PH extract showed high LC_{50} (>1000 µg mL^{-1}) values on Beas2B, RPE, and HSF control cells. This result was interpreted as a desirable good situation when compared with 5FU (417.72 µg mL^{-1}) (Table 6).

Table 6 GI_{50}, TGI, LC_{50}, and IC_{50} values for PH extract (µg mL^{-1})
Çizelge 6 PH ekstraktının GI_{50}, TGI, LC_{50} ve IC_{50} değerleri(µg mL^{-1})

Cell Lines	*PH extract*				*5FU*			
	GI_{50}	*TGI*	*LC_{50}*	*IC_{50}*	*GI_{50}*	*TGI*	*LC_{50}*	*IC_{50}*
Beas2B**	1.04	309.23±9.9	>1000	97.66±4.1	1.43	33.08±1.8	417.72±10.8	34.62±1.7
RPE**	1.71	>1000	>1000	94.58±2.9	1.54	55.37±3.6	423.82±8.3	65.30±2.1
HSF**	1.97	42.91±2.2	>1000	61.68±2.4	1.41	30.64±2.2	357.86±7.8	32.43±2.0
A172**	1.54	>1000	>1000	106.40±3.2	1.38	45.67±2.0	336.86±9.4	45.88±2.1
C6**	1.52	>1000	>1000	126.78±3.8	1.39	39.87±1.5	348.65±9.1	46.11±2.4
HeLa**	2.35	209.83±3.5	>1000	82.28±2.9	1.27	37.18±1.4	393.06±9.5	32.74±1.7
A2780**	2.55	>1000	>1000	131.03±3.2	1.29	48.77±2.0	386.42±8.5	49.94±1.9
SW620**	2.54	>1000	>1000	142.72±3.2	1.59	47.12±1.9	391.24±10.1	53.41±2.7
HT29**	1.28	>1000	>1000	96.25±2.7	1.29	35.36±1.9	411.54±9.2	43.18±2.1

GI_{50}: Growth Inhibition, TGI: Total Growth Inhibition, LC_{50}: Lethal concentration, IC_{50}: Half maximal inhibitory concentration, 5FU: 5-Fluorouracil
*Percent inhibition noted is mean values ± SDs of three independent measures. (SD: Standard deviation)
** If percent inhibition is smaller than 10, the SD value is <0.5.

Considering the Total Growth Inhibition (TGI) and Inhibitory Concentration (IC_{50}) values of the extracts for control cells, the extract was not toxic against normal cells. It showed higher TGI and IC_{50} values on Beas2B, RPE, and HSF normal cells than 5FU (control drug), respectively. As can be observed, the extract's toxic values fell within acceptable ranges because its TGI and IC_{50} values for HSF normal cells were greater than those of 5FU. Examination of the Growth Inhibition (GI_{50}) values of the PH extract showed that the GI_{50} value of the extract on Beas2B control cells (1.04 µg mL^{-1}) was lower than the GI_{50} value of 5FU (1.43 µg mL^{-1}). In RPE and HSF control cells, the extract's GI_{50} values were higher than 5FU. A low GI_{50} value indicates greater cytostatic effects of the extract on Beas2B cells, which is desirable. This feature allows the extract to be used in a wide and safe dose range for chemotherapeutic treatment (Table 6).

The antiproliferative effect of the extract on glioblastoma (A172 and C6), gynecological (HeLa and A2780), and colon (SW620 and HT29) cancer cell lines were also evaluated in the study (Table 6).

The extract showed similar efficacy to 5FU on colon and glioblastoma cell lines in terms of GI_{50} growth inhibition. However, on HeLa and A2780, the extract had a higher GI_{50} value (2.35 and 2.55 µg mL^{-1}). TGI and IC_{50} values of the extract were also found to be higher than 5FU for all these cancer cell lines (Table 6). The high inhibition values measured indicate that the extract is more effective at killing cancer cells than the control antiproliferative drug. The high LC_{50} (389.16->1000 µg mL^{-1}) values caused by the extract in glioma, gynecological, and colon cancer cells compared to 5FU indicate that the safe dose range of the extract in cancer treatment is wide. As a result, concentration modifications will be carried out more easily in a wide range (Table 6).

The different inhibition effects of the extract in each cell line are due to the differences in the cellular biological mechanisms that take an active role against the extract. In conclusion, when we look at all NCI-60 survival parameters, the fact that the extract has low GI_{50} and high LC_{50} values against cancer cells shows that it is a potential candidate for antiproliferative drug development. On the other hand, the GI_{50}, TGI, LC_{50}, and IC_{50} values of the extract on normal Beas2B, RPE, and HSF cell lines are within the desired limits is proof of its reliability (Table 6). All these findings prove that the extract of PH is specifically effective against cancer.

Tumor Specificity Index (TSI) was obtained by dividing the sum of the IC_{50} values from normal cells (Beas2B, RPE, and HSF) by the sum of the IC_{50} values of each cancer cell (A172, C6, HeLa, A2780, SW620, and HT29). According to this data, the tumor-specificities of PH extract to cancer cells are ideal because of the same selectivity as the positive control drug. In addition, the TSI value of the extract was the best for HeLa cells (TSI, 1.04) (Table 7).

Cytotoxicity test

For this, a kit is used to assess the activity of cytoplasmic lactate dehydrogenase (LDH), which leaks from the damaged plasma membrane into the environment. The level of leakage that might result in indirect membrane damage is indicated by LDH activity. Utilizing IC_{50} concentrations, the percent cytotoxicity caused by the extract was evaluated in this investigation. Accordingly, PH extract showed 10.0±0.8, 12.0±1.0, and 9.5±0.6 percent cytotoxicity values against Beas2B, RPE, and HSF at IC_{50}

concentration, respectively. On the other hand, the percent cytotoxic effect of the extract on cancer cell lines was determined as 11.4±1.0% for A172, 10.5±0.9% for C6, 9.9±0.6% for HeLa, 9.7±0.8% for A2780, 10.0±0.9% for SW620 and 11.9±1.0% for HT29 (Table 8). In the literature, the cytotoxicity activity of PH was investigated in a single study. In this study, it was reported that methanol extracts of plant seeds have remarkable cytotoxic effects against colon cancer using the MTT test. (IC_{50}: 33.0 g mL^{-1}) As can be seen, the plant extract showed higher cytotoxic activity against colon cancer cells compared to the seed extract.

Table 7 Tumor Specificity Index (TSI) for PH extract*
*Çizelge 7 PH ekstraktı için Tümör Özgüllük İndeksi (TSI)**

Cell Lines	*PH extract (µg mL^{-1})*		*5FU (µg mL^{-1})*	
	IC_{50}	*TSI*	*IC_{50}*	*TSI*
Beas2B, RPE, and HSF	84.64	1	44.11	1
A172	106.40	0.81	45.88	0.96
C6	126.78	0.68	46.11	0.96
HeLa	82.28	1.04	32.74	1.35
A2780	131.03	0.66	49.94	0.88
SW620	142.72	0.60	53.41	0.82
HT29	96.25	0.90	43.18	1.02

IC_{50}: Half maximal inhibitory concentration, TSI: Tumor Specificity Index, 5FU: 5-Fluorouracil
*TSI is calculated by dividing the average normal cell line IC_{50} by the IC_{50} of each cancer cell line.

Table 8 % Cytotoxicity values for PH extract at IC_{50} concentrations against the cells*
*Çizelge 8 Hücrelere karşı IC_{50} konsantrasyonlarında PH ekstraktı için % sitotoksisite değerleri**

	Beas2B	*RPE*	*HSF*	*A172*	*C6*	*HeLa*	*A2780*	*SW620*	*HT29*
The extract	10.0±0.8	12.0±1.0	9.5±0.6	11.4±1.0	10.5±0.9	9.9±0.6	9.7±0.8	10.0±0.9	11.9±1.0
5FU	10.1±1.0	10.7±1.0	10.9±1.1	10.8±1.0	11.5±1.1	11.9±1.1	10.1±1.0	11.0±1.3	10.9±1.2

*Percent cytotoxicity was noted as mean values ± SDs of three independent measures. (SD: Standard deviation)

CONCLUSION

The study is the first to report comprehensive phytochemical compounds and antiproliferative activity of the *Psephellus huber-morathii* which is endemic in Türkiye. When the MTT proliferation test and LDH cytotoxicity test findings were interpreted together, the extract showed an acceptable antiproliferative effect against cancer cells. It has minimal cytotoxicity against normal cells and a wide range of concentrations for dose adjustment. For this reason, we suggest further investigation of *Psephellus huber-Marathi* extract through preclinical and clinical studies. In this study, the extract showed moderate antimicrobial activity (100-500 µg mL^{-1}) against various Gram-positive (*S. aureus, L. monocytogenes, B. cereus,* and *E. faecium*) and Gram-negative bacteria (*P. fluorescens, P. aeruginosa, E. coli,* and *S. enteritidis*) that cause many infectious diseases. Moreover, the hydroalcoholic extract both exhibited high antioxidant activity and contained significant TPC and TFC. The phenolic compounds determined in the extract play an important role in these biological activities. Additionally, the extract exhibited promising antiproliferative effects against gynecological (IC_{50}=82.28±2.9) and colon (IC_{50}=96.25±2.7) cancer lines. These findings contribute to the understanding of *Psephellus huber-Marathi* as a valuable source of bioactive compounds with potential therapeutic applications. Further investigations are warranted to explore the underlying mechanisms and validate their efficacy in preclinical and clinical studies. The medical potential of *Psephellus huber-marathon* against cancer and infectious diseases should be supported by *in vivo* studies and used in alternative treatments.

ACKNOWLEDGMENT

The authors thank Dr. Hüseyin AKŞİT and Prof. Dr. Ali Kandemir for the collection and taxonomic description of the plant samples.

Author's contribution

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Aksit, H., Aksit, Z., Simsek, S., Aydin, A., Yilmaz, M. A., Kandemir, A., & Koksal, E. (2022). LC-MS/MS profiling phytochemical content of *Echinophora chrysantha* (Apiaceae) and antiproliferative, antioxidant activity. *Pharmacy & Pharmacology International Journal, 10*(5), 190–194. https://doi.org/10.15406/PPIJ.2022.10.00384

Altundağ, E., Altundağ, B. G. E., & Gürdal, B. (2013). Anatomical characteristics of *Centaurea glastifolia* L. (*Asteraceae*) used as folk medicine in East Anatolia. *Journal of Faculty of Pharmacy of Istanbul University, 40*, 57-64. Retrieved from https://dergipark.org.tr/en/pub/iujfp/issue/578/5676

Arif, R., Küpeli, E., & Ergun, F. (2004). The biological activity of *Centaurea* l. species (Review). *Fen Bilimleri Dergisi, 17*(4), 149–164.

Aydin, Ö., Çoşkunçelebi, K., Gültepe, M., & Güzel, M. E. (2013). A contribution to the taxonomy of *Centaurea* including *Psephellus* (*Asteraceae*) based on anatomical and molecular data. *Turkish Journal of Botany, 37*(3), 419–427. https://doi.org/10.3906/bot-1204-25

Barrero, A. F., Herrador, M. M., Arteaga, P., Cabrera, E., Rodriguez-Garcia, I., Garcia-Moreno, M., & Gravalos, D. G. (1997). Cytotoxic activity of flavonoids from *Carthamus arborescens*, *Ononis natrix* ssp. *ramosissima* and *Centaurea malacitana*. *Fitoterapia (Milano), 68*(3), 281–283.

Başer, K. H. C., Özek, G., Özek, T., & Duran, A. (2006). Composition of the essential oil of *Centaurea huber-Marathi Wagenitz* isolated from seeds by micro distillation. *Flavor and Fragrance Journal, 21*(3), 568–570. https://doi.org/10.1002/FFJ.1620

Chanda, S., & Nagani, K. (2013). In vitro and in vivo methods for anticancer activity evaluation and some Indian medicinal plants possessing anticancer properties: An Overview. *Journal of Pharmacognosy and Phytochemistry, 2*(2). Retrieved from www.phytojournal.com www.phytojournal.com

Demiroz, T., Nalbantsoy, A., Kose, F. A., & Baykan, S. (2020). Phytochemical composition and antioxidant, cytotoxic, and anti-inflammatory properties of *Psephellus goeksunensis* (Aytaç & H. Duman) Greuter & Raab-Straube. *South African Journal of Botany, 130*, 1–7. https://doi.org/10.1016/ J.SAJB.2019.11.021

Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M., Marchant, R., & Banat, I. M. (2016). Resazurin-based 96-well plate microdilution method for the determination of the minimum inhibitory concentration of biosurfactants. *Biotechnology Letters, 38*(6), 1015–1019. https://doi.org/10.1007/S10529-016-2079-2/ TABLES/3

Farrag, N., Abdel-Aziz, E., El-Domiaty, M., & El Shafea, A. (1993). Phytochemical investigation of *Centaurea araneosa* growing in Egypt. *Zagazig Journal of Pharmaceutical Sciences, 2*(1), 29–45. https://doi.org/10.21608/ZJPS.1993.188986

Güçlü, G., Ergül, M., Uçar, E., Eruygur, N., Ataş, M., & Aşkın AKPULAT, H. (2022). Anticancer, Antioxidant, Antimicrobial and Enzyme Inhibitory Activities of *Inula aucheriana*. *2022 KSU J. Agric Nat, 25*(5), 946–954. https://doi.org/10.18016/ksutarimdoga.vi.985837

Güven, K., Çelik, S., & Uysal, I. (2005). Antimicrobial activity of *Centaurea* species. *Pharmaceutical Biology, 43*(1), 67–71. https://doi.org/10.1080/13880200590903390

Holetz, F. B., Pessini, G. L., Sanches, N. R., Cortez, D. A. G., Nakamura, C. V., & Dias Filho, B. P. (2002). Screening of some plants used in Brazilian folk medicine for the treatment of infectious diseases. *Memórias Do Instituto Oswaldo Cruz, 97*(7), 1027–1031. https://doi.org/10.1590/S0074-02762002000700017

Khammar, A., & Djeddi, S. (2012). Pharmacological and Biological Properties of some *Centaurea* Species. *European Journal of Scientific Research, 84*(3), 398–416. Retrieved from http://www.EuropeanJournalofscientificresearch.com

Khan, N. A., Quereshi, S., Pandey, A., & Srivastava, A. (2009). Antibacterial Activity of Seed Extracts of Commercial and Wild *Lathyrus* Species. *Turkish Journal of Biology, 33*(2), 165–169. https://doi.org/10.3906/biy-0806-25

Korkmaz, N., Sener, S. O., Balturk, N., Kanbolat, S., Badem, M., Aliyazicioglu, R., Ozgen, U., Kandemir, A., Karaoglu, S. A. (2019). Determination of Phenolic Contents by HPLC, Antioxidant, Antimicrobial, Antityrosinase, and Anticholinesterase Activities of *Psephellus huber-marathon*. *Journal Of Pharmaceutical Research International, 26*(1), 1–10. https://doi.org/10.9734/JPRI/2019/V26I130125

Orallo, F., Lamela, M., Camiña, M., Uriarte, E., & Calleja, J. M. (1998). Preliminary study of the potential vasodilator effects on rat aorta of centaurein and centaureidin, two flavonoids from *Centaurea corcubionensis*. *Planta Medica, 64*(2), 116–119. https://doi.org/10.1055/S-2006-957386/BIB

Özcan, M. (2013). Morphological and anatomical aspects of *Psephellus pulcherrimus* (syn: *Centaurea pulcherrima* var. *freynii*) (*Cardueae, Asteraceae*) growing in Turkey. *Journal of Forestry Faculty, 14*(1), 104-112.

Polatoglu, K., Sen, A., Bulut, G., Bitis, L., & Gören, N. (2015). The essential oil composition of *Centaurea stenolepis* Kerner. from Turkey. *Http://Dx.Doi.Org/10.1080/0972060X.2014.935090, 17*(6), 1268–1278. https://doi.org/10.1080/ 0972060X.2014.935090

Ríos, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology, 100*(1–2), 80–84. https://doi.org/10.1016/J.JEP.2005.04.025

Sarker, S. D., Shoeb, M., Celik, S., Jaspars, M., Nahar, L., Kong-Thoo-Lin, P., & MacManus, S. M. (2007). Extracts of *Centaurea bornmuelleri* and *Centaurea huber-morathii* inhibit the growth of colon cancer cells in vitro. *Advances in Traditional Medicine, 7*(4), 336–340. https://doi.org/10.3742/OPEM.2007.7.4.336

Sarker, S. D., Kumarasamy, Y., Shoeb, M., Celik, S., Eucel, E., Middleton, M., & Nahar, L. (2005). Antibacterial and antioxidant activities of three

Turkish species of the genus *Centaurea*. *Advances in Traditional Medicine, 5*(3), 246–250. https://doi.org/10.3742/OPEM.2005.5.3.246

Shoeb, A., MacManus, S., Jaspars, M., Nahar, L., Kong-Thoo-Lin, P., Celik, S., & Sarker, S. (2007). Lignans and Flavonoids from the Seeds of *Centaurea bornmuelleri* Hausskn. Ex. Bornm. and *Centaurea huber-morathii* Wagenitz. *Polish Journal of Chemistry, 81*(1), 39–44. https://doi.org/10.2/JQUERY.MIN.JS

Sipahi, H., Orak, D., Reis, R., Yalman, K., Şenol, O., Palabiyik-Yücelik, S. S., … Yesilada, E. (2022). A comprehensive study to evaluate the wound healing potential of okra (*Abelmoschus esculentus*) fruit. *Journal of Ethnopharmacology, 287*, 114843. https://doi.org/10.1016/J.JEP.2021.114843

Tarhan, L., Ozturk Urek, R., Oner, A., & Nakiboglu, M. (2022). Evaluation of phenolic profiles, antioxidant activities, and cytotoxic and apoptotic potentials of *Phlomis angustissima* and *Phlomis fruticosa*, medicinal plants from Turkey. *European Journal of Integrative Medicine, 55*, 102188. https://doi.org/10.1016/J.EUJIM.2022.102188

Yilmaz, M. A. (2020). Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: A detailed, robust and comprehensive LC-MS/MS method validation. *Industrial Crops and Products, 149*, 112347. https://doi.org/10.1016/J.INDCROP.2020.112347

Zengin, G., Cakmak, Y. S., Guler, G. O., & Aktumsek, A. (2010). In vitro antioxidant capacities and fatty acid compositions of three *Centaurea* species collected from the Central Anatolia region of Turkey. *Food and Chemical Toxicology, 48*(10), 2638–2641. https://doi.org/10.1016/J.FCT.2010.06.033