**An Acad Bras Cienc (2024) 96(2): e20230657 DOI 10.1590/0001-3765202420230657**
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

**CHEMICAL SCIENCES**

# Determination of phytochemical contents by LC/QTOF/MS and evaluation of *in-vitro* biological activities of 2 *Peltigera* lichens from Bursa

BURCU S. TÜZÜN, BAŞAK T. KARADAĞ, SEYHAN ORAN, ŞULE ÖZTÜRK, FETHİYE F. YILMAZ, TUĞÇE FAFAL & BİJEN KIVÇAK

**Abstract:** Lichens are symbiotic associations of algae and fungi. They are edible as food and have been used in traditional medicine for years. It is aimed to screen *Peltigera praetextata* (Flörke ex Sommerf.) Zopfand and *Peltigera elisabethae* Gyeln. phytochemically by LC/QTOF/MS and according to the constituents to evaluate the antioxidant, tyrosinase inhibitory, and antibacterial activities. In total 54 of metabolites detected by LC/QTOF/MS were common in both species. According to LC/QTOF/MS scanning results, alkaloids, iridoid glycosides, phenolics, cyanogenetic glycosides, and terpenic structures were detected. DPPH, ABTS, superoxide radical scavenging activities, and metal chelating capacity $IC_{50}$ values were 84.55, 9.349; 51.27, 9.127; 95.01, 58.65 and 20.57, 70.08 µg/mL., respectively. The CUPRAC reducing power was determined as 4.69 and 9.57 $TEAC_{CUPRAC}$, respectively. Tyrosinase inhibitor activity were found to be 86.95 and 196.7 µg/mL. Both lichens did not show antimicrobial effects. As a result of the antioxidant and tyrosinase inhibitor activities it was seen that their activities were significant and further *in vivo* studies could be carried out on this lichens.

**Key words:** Antioxidant, LC/QTOF/MS, Lichen phenolics, *Peltigera elisabethae* Gyeln., *Peltigera praetextata* (Flörke ex Sommerf.), Tyrosinase inhibitor.

## INTRODUCTION

Lichens are symbiotic associations that spread almost worldwide with twenty-five thousand taxa. Lichens are formed due to the physiological and morphological associations of algae and fungi. They can occur as long as there is sufficient moisture. With the lichen acids they secrete and they can even decompose the stones and form humus soil. Therefore, they are of great importance as pionir organisms. According to the morphological forms of the thallus; they are divided into crustose, foliose, and fruticose lichens. According to their anatomical structures, they are divided into the homeomeric type and heteromeric type (Ivanova & Ivanov 2009, Emsen 2019). Lichens also take place in ethnobotanical uses. Because it is rich in calcium and iron, it is consumed as food after boiling. *Peltigera*, like other lichens, is a genus often used in traditional medicine. It is known that they are used especially for skin disorders, wound healing, gynecological diseases, respiratory tract and digestive regulators (Rankovic & Kosanic 2015). They are very rich in secondary metabolites. They consist of aliphatic and aromatic compounds. Depsids, tridepsids and depsidones, dibenzofuran, cromon, and quinone derivatives are lichen secondary metabolites (Huneck & Yoshimura 1996). Lichen secondary metabolites have antioxidant, anticancer, antibiotic, antimicrobial, enzyme inhibitor, antiviral, and anti-genotoxic

effects (Huneck & Yoshimura 1996, Rankovic & Kosanic 2015, Sivas 2019).

In nature, most lichens are edible and provide nutrition for microflora and mammals. It is known that it is often consumed as a human food item. *P. rufescens* and *P. apthosa* lichens are used as flour. In addition to being consumed as food (flour etc.), their use in traditional medicine is also known. For example, it is known that *P. canina* is used in treating rabies among people, and it is known that it got its name from there. In addition, traditionally, *Peltigera* type lichens have been shown to have anti-inflammatory and antibacterial uses in jaundice (Rankovic & Kosanic 2015, Sivas 2019).

Today, lichens are one of the new focuses of attention, and the lack of studies about them has attracted our attention to contribute to the literature.

In this study, phytochemical screening of *P. praetextata* (PP) and *P. elisabethae* (PE) was performed with LC/QTOF/MS, and they were investigated for their antioxidant, antimicrobial, and tyrosinase inhibitor activities.

### List of abbreviations

ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt CUPRAC: Copper(II) Ion Reducing Capacity

DPPH: 2,2'-diphenyl-1-picrylhydrazil

HPLC: High Performance Liquid Chromatography NBT; Nitroblue tetrazolium

PE: *P. Elisabethea*

PP: *P. praetextata*

TFC: Total flavonoid content TPC: Total phenolic content

### Chemicals and reagents

Methanol (Sigma), Ethanol (Sigma), LC grade Formic acid (Sigma), LC grade acetonitrile (Sigma), Folin Ciocalteu reagent (Merck), $Na_2CO_3$ (Merck), Gallic acid (Sigma), $AlCl_3$ (Merck), Quercetin (Sigma), DPPH (Sigma), DMSO (Sigma), ABTS(Sigma), Neocuproine(Sigma), Copper (II) chloride(Sigma), EDTA(Sigma), NBT (Nitroblue tetrazolium) (Sigma), Riboflavin(Sigma), Phosphate buffer(Sigma), Ascorbic acid(Sigma), Ferrozine(Sigma), L-Dopa(Sigma), Kojic acid (Sigma), tyrosinase enzyme(Sigma).

## MATERIALS AND METHODS

### Lichen collection, determination, drying, and extraction

PP and PE species distributed in Bursa province were investigated. The collected lichen samples were identified with the help of identification keys in various flora books (Wirth 1995, Brodo et al. 2001, Smith et al. 2009).

Prof. Dr. Şule Öztürk and Assoc. Prof. Dr. Seyhan Oran identified them. The collected samples, especially the very moist ones of the species that spread on the soil, were kept in laboratory conditions for four days and dried as a pre-treatment proocedure. The thalli of the collected lichen samples were carefully cleaned from foreign materials such as moss, soil, and tree bark in a Leica MZ 6 stereomicroscope with dissection forceps, placed in paper bags, and stored in the refrigerator until the laboratory experiments began.

### Lichen extraction

For the extraction process, 1 gram of powdered lichen thallus was extracted three times with methanol in an ultrasonic bath (Bandolin Sonorex) (25 °C) for 30 minutes. After each filtering, the solvent was removed in the rotavapor (Buchi) at 35-40 °C, and the collected extracts were combined and then stored at + 4 °C (Adams et al. 1993, Zagoskina et al. 2013).

### Qualification of compounds by LC/QTOF/MS

Chromatographic separation was carried out using an HPLC Agilent 1260 Infinity series (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump, and an online degasser, an autosampler, and a Poroshell 120 EC-C18 (3.0X100 mm, particle size) 2.7 μm) (Agilent Technologies). A mobile phase system composed of water with 0.1% formic acid (A) and acetonitrile (B) was used in a gradient elution mode as follows: 0–0.5 min, 5% B; 0.5–7 min, 25% B; 7–16 min, 50% B; 16–23 min, 75% B; 23–30 min, 95% B; 30–40 min, 5% B for equilibration of the column. The column temperature was maintained at 35 °C. The injection volume was 10 μL, and the flow rate was 0.4 mL/min.

Ionization of chromatographic eluates was performed using an Agilent 6550 iFunnel high-resolution Accurate-Mass QTOF-MS, equipped with an Agilent Dual Jet Stream, and electrospray ionization (Dual AJS ESI) interface operating in negative ion was used at the following conditions: drying gas flow, 14.0 L/min; nebulizer pressure, 35 psi; gas drying temperature, 290 °C; sheath gas temperature, 400 °C; sheath gas flow, nitrogen at 14 L/min and nozzle voltage 1000 V. All spectra were collected in targeted MS/MS mode from *m/z* 50 to 1800 scan range for inducing MS/MS data collection. The collision energy was 20 eV during analysis.

Integration and data evaluation were performed using MassHunter Workstation software, and the MassHunter METLIN and the Accurate Mass Personal Compound Database and Library (METLIN_AM_PCDL) were used to detect phenolic compounds. (Agilent Technologies, Santa Clara, CA, USA). Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface operating in negative and positive ions.

### Determination of total phenol and flavonoid content

#### *Determination of Total Phenolic Content (TPC)*

The analysis was carried out according to the Folin-Ciocalteu method. 2.8 mL of deionized water was added to 100 μL of extract (concentration range of 5μg/mL-1mg/mL). Then, 2 mL of 2% $Na_2CO_3$ and 0.1 mL of 50% Folin reagent were added, mixed, and incubated at 25 ˚C for 30 minutes in the dark. Gallic acid calibration curve and total phenolic content of lichens were determined by measuring absorbance against water at 750 nm by Optima SP-3000 Nano Spectrophotometer (Cheung et al. 2003, Kanipandian et al. 2014). Results are expressed as mg gallic acid/g extract.

#### Determination of Total Flavonoid Content (TFC)

1.5 mL of 95% ethanol was added to 500 μL of extract solution (concentration range of 5μg/ml- 1mg/ml). After, 100 μL of $AlCl_3$ and 2.8 mL of deionized water were added to it and left to incubate for 40 minutes. The absorbance was measured against ethanol at 415 nm by Optima SP-3000 Nano Spectrophotometer. Quercetin was used as a standard (Cheung et al. 2003, Kanipandian et al. 2014). The results are reported as mg quercetin/g extract.

### *In vitro* Antioxidant Activity Studies

#### *DPPH (2,2'-diphenyl-1-picrylhydrazil) Radical Scavenging Activity*

The method described by Esmaeli et al. was used with little modification (Esmaeili & Khadadadi 2002). DPPH solution prepared in DMSO was added to the tested samples in a certain concentration range (10 μg/mL – 1 mg/mL) and then incubated in the dark for 30 minutes. The absorbance of the resulting mixture was then measured at 517 nm by Optima SP-3000 Nano

Spectrophotometer and methanol used as a blank. α-tocopherol was used as a standard. Results are given as $IC_{50}$ (μg/mL) (GraphPad Prism 5).

### $ABTS^+$ Radical Cation Scavenging Activity

It was evaluated by modifying the $ABTS^+$ decolorization method of Rer et al. (1999). In this method, ABTS radical was prepared by reacting stock ABTS solution with potassium persulfate (Rer et al. 1999). It was diluted with ethanol until an absorbance of 0.750 at 734 nm was obtained. Then, 0.1 mL of extract (concentration range of 20 μg/mL-1 mg/mL) and 4 mL of ethanol were added to 1 mL of diluted ABTS solution, and its absorbance at 734 nm at 6 minutes was read by Optima SP-3000 Nano Spectrophotometer. α-tocopherol was used as a standard. Results are given as $IC_{50}$ (μg/mL) (GraphPad Prism 5) Ethanol was used as a blank.

### CUPRAC (Copper(II) Ion Reducing Capacity) Method

The copper(II) reducing power was determined according to the method of Apak et al. (2004). After the mixture of neocuprine and Copper (II) was prepared at pH 7, sample solution (5 μg/mL-1 mg/mL) was added and incubated for 30 minutes. The absorbance against the blank methanol at 450 nm was measured by Optima SP-3000 Nano Spectrophotometer. Trolox solution was used as standard. Results are given as $TEAC_{CUPRAC}$.

### Superoxide Radical Scavenging Activity

It was carried out according to the research conducted by Patel et al. (2010). 10 μL of extracts and standards at different concentrations, 15 μL 12 mM EDTA, 10 μl 0,1 mg/mL NBT (nitro blue tetrazolium), 5 μL 0.2 mg/mL riboflavin, and 160 μL 0.067mM Potassium Phosphate Buffer (pH 7.4) are placed in a Thermo Scientific- Varioscan Flash microplate reader and incubated at fluorescence light for 5 minutes. Ascorbic Acid was used as a standard. The absorbance at 560 nm was then measured by Optima SP-3000 Nano Spectrophotometer. Results are given as $IC_{50}$ (μg/mL) (GraphPad Prism 5) (Patel et al. 2010).

### Metal Chelating Activity

The metal chelating activity was modified using the Fe (II)-Ferrozine method. In this method, 5 mL of test solutions at concentrations of 31.25-500 μg/mL were added to 100 μL of 2 mM $FeCl_2$ and incubated for 30 minutes at room temperature in the dark. At the end of the period, 200 μL of 5 mM ferrozine solution was added and incubated again for 10 minutes in the dark and at room temperature. EDTA was used as a standard. Its absorbance at 562 nm was measured by Optima SP-3000 Nano Spectrophotometer. Results are given as $IC_{50}$ (μg/mL) (GraphPad Prism 5) (Decker, 1997).

### *In vitro* Tyrosinase Inhibitor Activity

The method of Masuda et al. (2005) was modified. 110 μL phosphate buffer (0.01 M, pH: 6,8), 10 μL plant extract (different concentrations), and 20 μL tyrosinase solution (200 unit/mL) were mixed. After 10 minutes of incubation at 37 °C, the reaction was initiated by adding 20 μL of L-Dopa and allowed to incubate at 37 °C for 10 minutes. The absorbance was measured at 475 nm by Thermo Scientific Varioskan Flash Microplate Reader. Kojic Acid was used as a standard. Results are given as $IC_{50}$ (μg/mL) (GraphPad Prism 5) (Masuda et al. 2005).

### Antimicrobial Activity

In antimicrobial tests, Gram-positive bacteria *Bacillus cereus* (ATCC 7064), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 6538-P), Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883),

*Pseudomonas aeruginosa* (ATCC 27853), and two yeast strains *Candida albicans* (ATCC 90028), *Candida parapsilosis* (ATCC 22019) were used.

### *Agar Well Diffusion Method*

Fresh cultures of bacteria and standard yeast strains were adjusted to a 0.5 McFarland turbidity by suspending in physiological saline water and inoculated under aseptic conditions on cation-added Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates respectively. 50 µl of the stock solutions (4mg/ml) of the extracts prepared in deionized and sterilized water were filled into the opened wells on the agar plates. The final extract loads were 200 µg in each well. Ciprofloxacin 5 µg and fluconazole 25 µg were used as positive controls. An evaluation was made by measuring the zone diameters seen at the end of 24-48 hours of incubation at 37 ºC (Korkak et al., 2022).

### *Microdilution Method*

50 µL cation-added Mueller Hinton Broth (MHB) medium for yeasts, and Sabouraud Dextrose Broth (SDB) medium for yeasts were added to the wells in a sterile U-bottom microplate. Stock solutions of the extracts in water (4mg/mL) were distributed on the microplate with a concentration range of 2-2048 µg/mL. Microorganisms, whose fresh cultures prepared from the stock were suspended in physiological saline and adjusted to 0.5 McFarland turbidity, were diluted 1:100 with MHB and SDB media, then distributed in equal amounts to the wells. The first well without visible turbidity at the end of 24-48 hours of incubation was considered as minimum inhibitory concentration (MIC). Growth control and medium controls were also included. Ciprofloxacin and fluconazole were used as positive controls (Korkak et al., 2022).

### Statistical analysis

All results are expressed as mean ± *SD*. One Way ANOVA test at a level of $p < 0.05$ in IBM SPSS (ver. 25). Were performed (Table V).

## RESULTS AND DISCUSSION

As a result of the extractions, PE and PP extracts were obtained wtih a yield of 18.11% and 16.31% respectively. According to LC-QTOF-MS library scanning, a total of 441 items were identified (Supplemantary files-Tables), including both secondary and primary metabolites. All chromatograms are listed in Figure 1, 2, 3 and 4. Alkaloids, iridoid glycosides, phenolics,

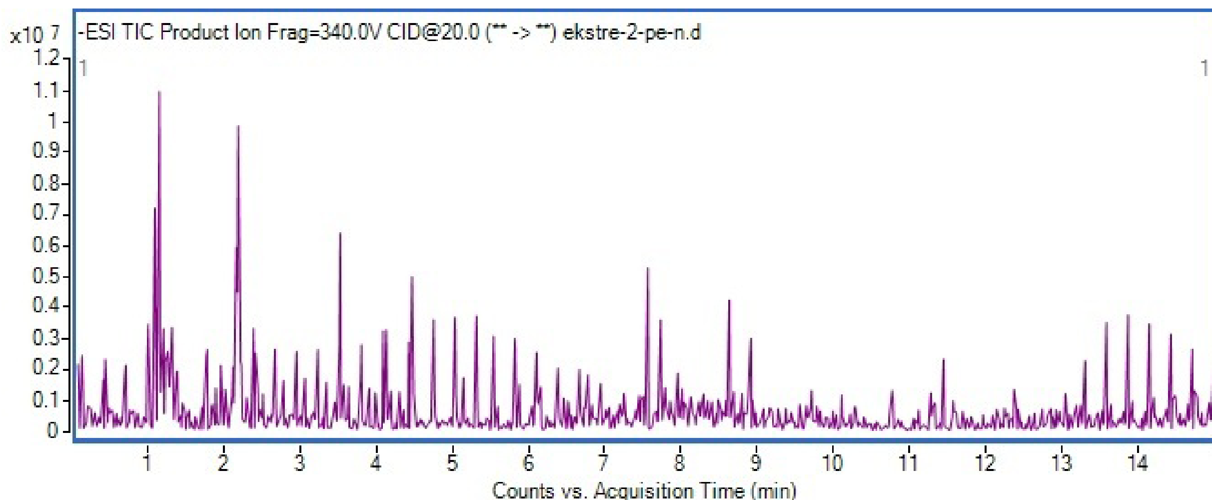


**Figure 1. Negative ion mode LC/QTOF/MS Chromatogram of PE.**

cyanogenetic glycosides, and terpenic compounds were determined as secondary metabolites. Xanthosine, C16 Sphinganine, Tortuosamine, Erythronolide B, Prosopinine, 14,19- Dihyroaspidospermatine, Tetradecylamine, Fenpiclonil, Eudesmin, n-Pentadecylamine, Triclofos, Gemfibrozil M1, 9-nonadecene, 1-Eicosene, Spermine derivates, Diisobutyl/diisononyl phthalate, Luteolin derivate, Nonoxynol-9 and Harderoporphyrin were biologically active compounds and detected in both PP and PE (Figure 1,2,3,4). According to the literature research (Table I), it was seen that these substances have antioxidant, tyrosinase inhibitor, and antimicrobial effects, and it was decided to screen the activities of the extracts based on this. TPC (Total phenolic content), TFC (Total flavonoid content) and *in vitro* antioxidant activities were evaluated and summarized (Tables II and III). It has been observed that the radical scavenging activities of PE, which has more TPC and TFC and contains more secondary metabolites known to be antioxidants, are much higher. Tyrosinase inhibitory activity results are summarized in Table IV. It was observed that activity of PP is higher than PE. This suggests

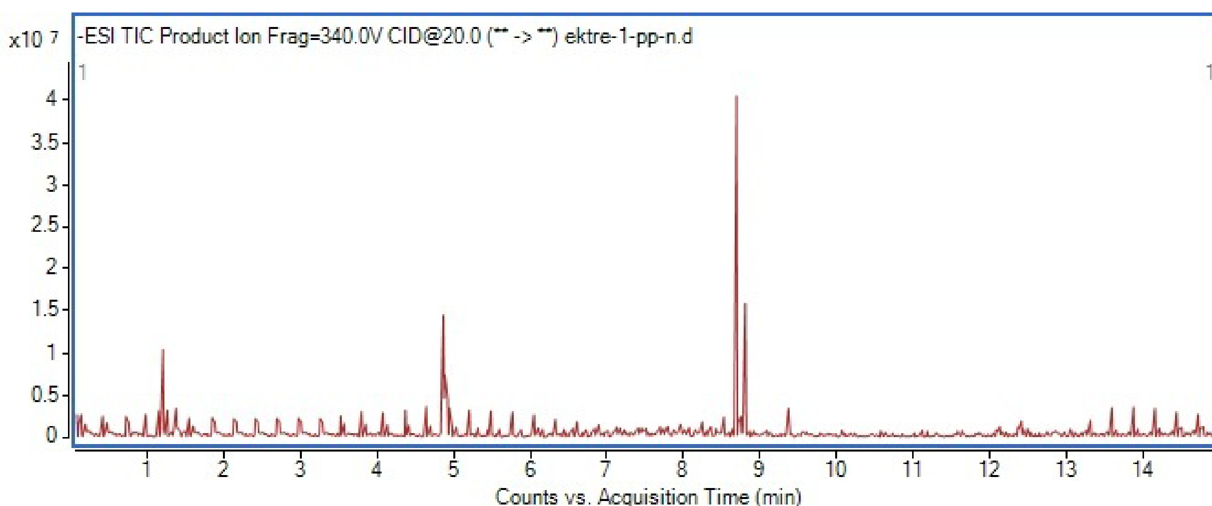


**Figure 2.** Negative ion mode LC/QTOF/MS Chromatogram of PP.

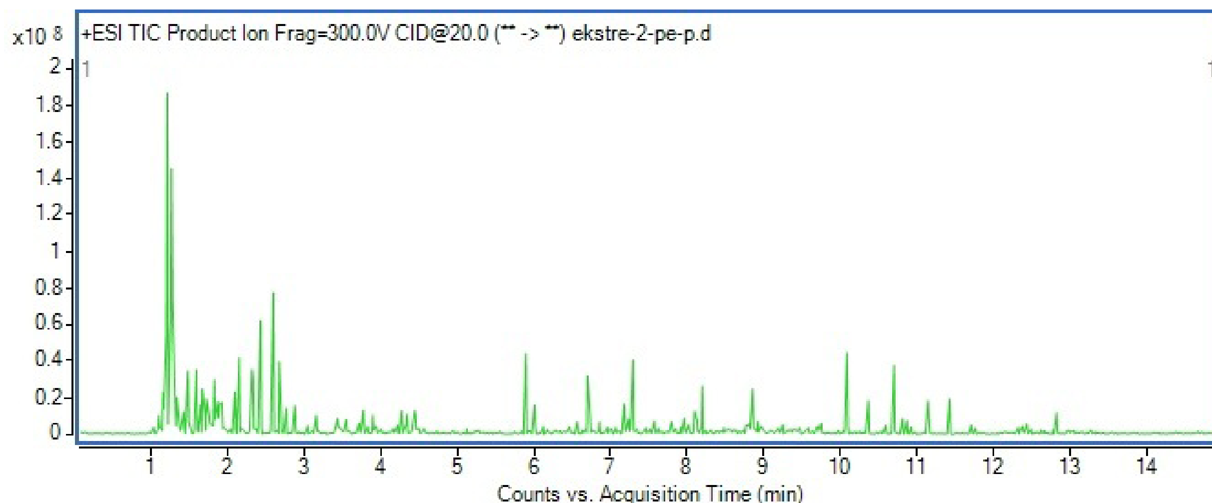


**Figure 3.** Positive ion mode LC/QTOF/MS Chromatogram of PE.

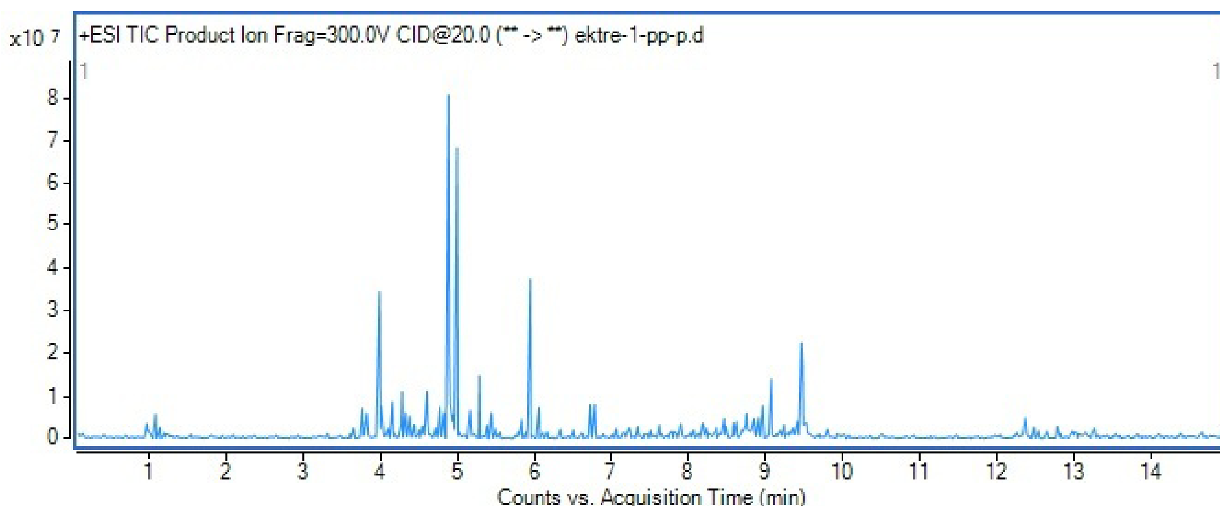


**Figure 4. Positive ion mode LC/QTOF/MS Chromatogram of PP.**

that this may be due to the amount of tyrosinase inhibitor substances and synergistic effects in their content. There are a few studies on PE and PP. When they were evaluated, it was determined that PP had an antimutagenic effect (Nardemir et al. 2015). Its antimutagenicity is important as it supports the consumability of edible lichens. In another study involving both PP and PE, their antiproliferative effects were determined. In the same study it was also stated that PP does not contain secondary metabolites in contrast to our study (Munzi et al. 2014).

When the studies on the other species in *Peltigera* genus were examined, it was seen that acetone extract of *P. horizontalis* were effective as an antioxidant. Still, it was not found to have an antibacterial effect in 5 different strains. In this study, while antioxidant activity was observed in our species, antibacterial activity was not observed in similar strains. The DPPH scavenging activity of *P. laciniata* ethanol extract was given as percent inhibition (80%). It can be seen that it shows a very strong scavenging activity. Since our results are given as $IC_{50}$ value in this study it cannot be compared exactly (Plaza et al. 2014). However we can indicate that the results are compatible. In a different study, DPPH radical scavenging and metal chelating activities of *P. canina* methanol extract were determined and expressed as $IC_{50}$ value. These values were 42,37, and 50,33 mg/L, respectively (Emsen, 2019). Compared to our current research results, *P. canina*'s activity is between PP (84,55-20,57 µg/mL) and PE (9,349-70,08 µg/mL) in our research. A research with *P. rufescens* stated that it is effective as an antioxidant (Aydin & Turkez 2011). Compared to our results, it is true that both of them have antioxidant effects. As a result of a study with Nepal mountain lichens, *Peltigera* sp. was ineffective against *Bacillus subtilis* and *Staphillococcus aurens* as our study. Again in the same study, the radical scavenging effects of DPPH and ABTS were examined, and their $IC_{50}$ values were reported as 5.6 and 6.9 µg/mL, respectively, closer to PE in our study (Paudel et al. 2012).

**Table I.** **Compounds detected in PP and PE and biological activities in literature**

| | Compound name | Chemical structure | Detected Lichens | Retention time | Mass | Ion mode | Biological activity | Ref.for activity |
|---|---|---|---|---|---|---|---|---|
| 1 | Feruloylagmatine | Cinnamamides | PE | 1.149 | 306.1702 | Positive | Antioxidant and Antibacterial | Wang et al. 2020, Zeiss et al. 2021 |
| 2 | Neolinustatin | cyanogenic glycoside | PE | 1.372 | 423.1744 | Positive | Antioxidant | Li et al. 2012a |
| 3 | Chavicol glycoside | Phenyl propanoid | PE | 1.383 | 296.1267 | Positive | Antioxidant and tyrosinase inhibitor | Oliveira et al 2021, Avetisyan et al 2017 |
| 4 | Dehydrofalcarinone | ynone | PE | 1.697 | 240.1515 | Positive | Antioxidant | Khalil et al. 2022 |
| 5 | Tetramethylquercetin 3-rutinoside | Flavonoid glycoside | PE | 2.223 | 666.2150 | Negative | Antioxidant | Olgun et al. 2018, Si et al. 2012 |
| 6 | Atraric Acid | Phenolic | PE | 2.725 | 196.0743 | Positive | Antioxidant and tyrosinase inhibitor | Mitrovic et al. 2014, Lie et al. 2022 |
| 7 | Levosimendan | Pyridazinone | PE | 3.173 | 280.1068 | Positive | Antioxidant | Abeer et al. 2017 |
| 8 | Pyridyl nicotine and derivates | Alkoloid | PE | 3.218 | 239.1419 | Positive | Antioxidant and tyrosinase inhibitor | Lin et al. 2012 |
| 9 | Donepezil | Piperidine derivate | PE | 3.274 | 379.2130 | Positive | Antioxidant | Umukoro et al. 2014 |
| 10 | Nitrendipine | Pyridine derivate | PE | 3.441 | 360.1328 | Positive | Antioxidant | Unuvar et al. 2021 |
| 11 | Austin | Terpene | PE | 3.508 | 500.2031 | Positive | Antioxidant and tyrosinase inhibitor | Burgos & Serranilos 2012, Kootheat et al. 2023 |
| 12 | Anabsin | Sesquiterpenoid | PE | 3.777 | 512.2750 | Positive | Antioxidant | Brunet et al. 2005 |
| 13 | Avenanthramide | Phenolic alkoloid | PE | 3.788 | 361.1167 | Positive | Antioxidant and tyrosinase inhibitor | Dimberg et al. 1993, Park et al 2021 |
| 14 | Tocopheronic Acid | Sesquiterpenoid | PE | 3.889 | 294.1471 | Positive | Antioxidant | Al-Fatlawi & Al-Fatlawi 2020 |
| 15 | 3- methoxy chalcones | Chalcone | PE | 3.934 | 448.1729 | Positive | Antioxidant | Bandgar et al. 2010, Akhtar et al. 2015 |
| 16 | Jasmonic acid glucoside | Jasmonate | PP | 4.034 | 388.1729 | Negative | Antioxidant | Sirhindi et al. 2016 |
| 17 | Hordatine B | heterodimer of feruloylagmatine | PE | 4.213 | 580.3141 | Positive | Antioxidant | Wang et al. 2020 |
| 18 | 5,6,7-Trimethoxycoumarin | Coumarin | PE | 4.437 | 236.0692 | Positive | Antioxidant and tyrosinase inhibitor | Matos et al. 2017, Menezes & Diederich 2019 |
| 19 | Mycophenolate mofetil | Carboxylic ester | PE | 4.549 | 433.2096 | Positive | Antioxidant | Dalmarco et al. 2009 |
| 20 | Marmin | Coumarin | PP | 4.779 | 360.1561 | Positive | Antioxidant | Chian et al. 2018 |
| 21 | Xanthosine | purine nucleoside | PP/PE | 5.048 | 284.0753 | Negative | Antibrowning agent | Tao et al. 2021 |
| 22 | Cyproterone acetate | Steroidal | PE | 5.242 | 416.1751 | Positive | Tyrosinase inhibitor | Tadokoro et al. 2003 |
| 23 | Hexyl glucoside | alkyl polyglycosides | PP | 5.622 | 264.1584 | Positive | Antioxidant | Adem et al. 2014 |
| 24 | Apigenin | Flavonoid | PE | 5.857 | 270.0539 | Positive | Antioxidant and tyrosinase inhibitor | Sadasivam & Kumeresan 2011, Karaoglan &Koca 2020 |
| 25 | Dihydroartemisinin | Sesquiterpenoid | PE | 5.969 | 284.1629 | Positive | Antioxidant | Ittarat et al. 2003 |
| 26 | C16 Sphinganine | Amino alcohol | PP/PE | 6.115 | 273.2672 | Positive | Antioxidant and antibacterial | Tamsir et al. 2020, Barreto-Bergter et al. 2006 |
| 27 | Cappariloside A | Alkoloid | PE | 6.193 | 334.1170 | Positive | Antioxidant and tyrosinase inhibitor | Aliyazicioglu et al 2013, Kirkan et al. 2021 |
| 28 | Tortuosamine | Alkoloid | PP/PE | 6.233 | 326.1978 | Negative | Antioxidant and Antimalarial | Olatunji et al. 2021 |
| 29 | Erythronolide B | Macrolide | PP/PE | 6.278 | 402.2605 | Negative | Antibacterial effect inducer | Saito & Mitsuhashi 1971 |
| 30 | Prosopinine | Alkoloid | PP/PE | 6.345 | 287.2465 | Positive | Tyrosinase inhibitor | Saleem et al. 2021 |

**Table I. Continuation.**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 31 | 14,19-Dihyroaspidospermatine | Alkoloid | PP/PE | 6.402 | 340.2136 | Negative | Antibacterial | Tanaka et al. 2006 |
| 32 | Tetradecylamine | Alkyl amine | PP/PE | 6.450 | 213.2464 | Positive | Antibacterial | Borick et al. 1959 |
| 33 | Perilloside C | Terpene glycoside | PP | 6.457 | 316.1893 | Positive | Antioxidant | Hou et al. 2022 |
| 34 | Fenpiclonil | Pyrrole | PP/PE | 6.718 | 235.9907 | Negative | Antibacterial | Leroux et al. 1992 |
| 35 | Eudesmin | Lignan | PP/PE | 6.726 | 386.1725 | Positive | Tyrosinase inhibitor, Antibacterial | Li et al. 2013, Patel & Patel 2022 |
| 36 | Oleamide | Fatty acid amide | PE | 6.808 | 281.2719 | Positive | Antioxidant | Reyes-Soto et al. 2022 |
| 37 | Vialinin A | Terpene | PE | 6.909 | 562.1624 | Positive | Antioxidant | Sonowal et al. 2018 |
| 38 | n-Pentadecylamine | Alkyl amine | PP/PE | 7.020 | 227.2619 | Positive | Antibacterial | Rammali et al. 2012 |
| 39 | Sulfaphenazole | Sulfonamide | PP | 7.064 | 314.0848 | Negative | Antioxidant | Khan et al. 2007 |
| 40 | Triclofos | Monoalkyl phosphate | PP/PE | 7.134 | 227.8907 | Negative | Antibacterial | Leroux et al. 1992 |
| 41 | Gemfibrozil M1 | Aromatic ether | PP/PE | 7.743 | 266.1522 | Positive | Antioxidant | Ashidate et al. 2002 |
| 42 | Dodecyl Phosphocholine | Ethanol amine | PE | 7.747 | 352.2614 | Positive | Antioxidant | Balakrishna et al. 2017 |
| 43 | 9-nonadecene | Fatty acid | PP/PE | 8.027 | 266.2979 | Positive | Antioxidant and Antibacterial | Premathilaka & Silva 2016, Mahamuni 2015 |
| 44 | Delphinidin glucoside | Anthocyanin | PE | 8.128 | 803.2019 | Positive | Antioxidant | Watsona & Schönlau 2015 |
| 45 | 1-Eicosene | Hydrocarbon | PP/PE | 8.240 | 280.3130 | Positive | Antibacterial | Lay-Jing et al. 2012 |
| 46 | Spermine derivates | Polyamine | PP/PE | 8.302 | 370.2721 | Positive | Tyrosinase inhibitor | Kim et al. 2018 |
| 47 | Scytonemin | Alkoloid | PE | 8.474 | 544.1423 | Positive | Antioxidant and tyrosinase inhibitor | He et al. 2022 |
| 48 | Diisobutyl/diisononyl phthalate | Benzene carboxylic acid | PP/PE | 8.537 | 278.1521 | Positive | Antibacterial | Huang et al. 2021 |
| 49 | 10-Gingerol | Phenol | PE | 8.586 | 350.2457 | Positive | Antioxidant | Lee & Ahn 1985 |
| 50 | Tianeptine | | PE | 8.633 | 436.1233 | Negative | Antioxidant | Dello et al. 2012 |
| 51 | Luteolin derivate | Flavonoid | PP/PE | 8.686 | 710.1720 | Negative | Tyrosinase inhibitor and Antibacterial | Zhang et al. 2017 |
| 52 | Nonoxynol-9 | Alkane | PP/PE | 8.817 | 616.4182 | Positive | Antibacterial | Hillier et al. 2005 |
| 53 | Annosquamosin B | Diterpenoid | PE | 9.268 | 308.2351 | Positive | Antioxidant | Pandey & Barve 2011 |
| 54 | Aeglin | Fatty acyl glycoside | PE | 9.324 | 510.2095 | Positive | Antioxidant | Patel et al. 2020 |
| 55 | Rubroskyrin | Bis-anthroquinone | PP | 9.373 | 574.1103 | Negative | Antioxidant and tyrosinase inhibitor | Bräse et al. 2012 |
| 56 | Quercetin-3- glucoside | Flavonoid | PP | 9.466 | 714.1554 | Positive | Antioxidant and tyrosinase inhibitor | Chen & Kubo, 2002, Heo et al. 2007, Baghel et al. 2012 |
| 57 | Harderoporphyrin | Heterocyclic m crocycle | PP/PE | 9.761 | 608.2627 | Positive | Tyrosinase inhibitor | Baldea et al. 2016 |
| 58 | Pheophorbide A | Heterocyclic compound | PE | 10.085 | 592.2687 | Positive | Antioxidant and tyrosinase inhibitor | Lanfer et al. 2005, Li et al. 2012b |
| 59 | Ginsenoside Rh4 | Saponin | PE | 10.152 | 620.4282 | Positive | Tyrosinase inhibitor | Kim 2015 |
| 60 | Melilotoside A | Hydroxycinnamic acid derivate | PE | 10.722 | 590.4177 | Positive | Antioxidant and tyrosinase inhibitor | Yang et al. 2007; Güven et al. 2023 |
| 61 | Paradol | Phenol | PE | 10.890 | 334.2504 | Positive | Antioxidant and tyrosinase inhibitor | Nile & Park 2015, Viggiano et al. 2023 |
| 62 | Biotin | Heterocyclic compound | PE | 11.259 | 614.4155 | Positive | Antioxidant | Al-Quadah & Ismail 2012 |
| 63 | Methenamine | Heterocyclic compound | PE | 12.791 | 140.1063 | Positive | Antioxidant | Wetchakul et al. 2022 |

Enzyme inhibitory activities in lichens have been studied before, and interesting results were obtained. Of these, lichens showing tyrosinase inhibitory activity were *Flavoparmelia caperata*, *Letharia vulpina, Cetraria juniperina, Parmotrema perlatum* (Malaspina et al. 2020), *Hypogymnia physodes, Bulbothrix setschwanensis, Usnea* species (Zhao et al., 2021), *Cetraria islandica* (Akbulut & Yildiz 2010) and *Himantormia* (Areche et al. 2022) species. However, studies of tyrosinase inhibitory activity in *Peltigera* lichens have not been done before. Since many substances showing tyrosinase inhibitory activity were detected in our study, the activities of the extracts were examined and determined as 86.95 and 196.7 μg/mL ($IC_{50}$) in PP and PE, respectively. Standart compound kojic acid's inhibitory activity was 37,40 μg/mL ($IC_{50}$) (Table IV). Compared to kojic acid, PP and PE's $IC_{50}$ values were found to be close and that indicated the strong tyrosinase inhibitor activity.

According to antibacterial and antifungal compounds C16 sphinganine, tetradecyl amine, diisobutyl phthalate, nonoxynol-9, erythronolide B, 14, 19- dihydroaspidospermatine, fenpiclonil, eudesmin, 1- eiocosene, 9- nonadecene, triclofoteolin and 8-hydroxyluciferol extracts, which are known to exist in both lichens, extracts antibacterial and antifungal effects were tested. They did not show activity against any of the strains. It was observed that similar results were obtained in antibacterial tests performed on *Peltigera* species before. It is thought that this may be due to the synergistic effects of different substances in the extract.

**Table II.** **TPC, TFC and *in vitro* antioxidant activity results.**

| | (mg GAE/ g extract)[a] | (mg QE/ g extract)[b] | ($IC_{50}$) (μg/mL)[c] | ($IC_{50}$) (μg/mL)[d] | ($IC_{50}$) (μg/mL)[e] | ($IC_{50}$) (μg/mL)[f] | ($TEAC_{CUPRAC}$)[g] |
|---|---|---|---|---|---|---|---|
| *PP* | 29,81±4,3 | 32,87±1,8 | 84,55±0,34 | 51,27±0,10 | 95,01±0,23 | 20,57±0,19 | 4,69±0,11 |
| *PE* | 168,47±4,1 | 34,88±1,5 | 9,349±0,14 | 9,127±0,08 | 58,65±0,26 | 70,08±0,11 | 9,57±1,1 |
| α-tocopherol | | | | | | | |
| Trolox | | | | 18.87±0.01 | | | |
| Ascorbic Acid | | | 4.474 ± 0.31 | | 5.992± 0.01 | | |
| EDTA | | | | | | 6,619±0,21 | |

**[a]Results of TPC. [b]Results of TFC. [c]Results of DPPH assay. [d]Results of ABTS assay. [e]Results of Superoxide scavenging activity. [f]Results of Metal chelating assay. [g]Results of CUPRAC assay.**

**Table III.** **CUPRAC slope values.**

| | slope |
|---|---|
| Trolox | 0,052 |
| PP | 0,2439 |
| PE | 0,4975 |

**Table IV.** **Tyrosinase inhibitor activity results.**

| | Tyrosinase inhibitory activity($IC_{50}$) (μg/mL) |
|---|---|
| *PP* | 86,95±0,11 |
| *PE* | 196,7±0,22 |
| Kojic acid | 37.40 ± 0.42 |

**Table V.** **Statistical Analysis (One way ANOVA).**

| Activity | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 10344.257 | 3 | 3448.086 | 11966.634 | .000 |
| Within Groups | 2.305 | 8 | .288 | | |
| Total | 10346.562 | 11 | | | |

## CONCLUSIONS

It has been determined that PP and PE lichens contain lichen phenolics, alkaloids, terpenic metabolites and flavonoids. They showed antioxidant and tyrosinase inhibitor activities, which are among the expected effects in line with the substances they contain. Although many substances with antibacterial and antifungal properties were detected, they were found to have no effect, suggesting that this result was due to the synergistic effect of the substances contained in it. It was determined that PP and PE have strong antioxidant and tyrosinase inhibitor activities and it was envisaged that further studies could be conducted. Peltigera lichens showed antioxidant and tyrosinase inhibitory activity in line with the secondary metabolites but showed no antimicrobial effect.

### Acknowledgments

The research was carried out with the support of Ege University BAP project no. 30782.

## REFERENCES

ABEER MR, ALZOUBİ KH & ATMEH A. 2017. Levosimendan enhances memory through antioxidant effect in rat model: behavioral and molecular study. Behav Pharmacol 29(4): 344-350. http://dx.doi.org/10.1097/FBP.0000000000000362.

ADAMS WW, DEMMİG-ADAMS W & LANGE OL. 1993. Carotenoid composition and metabolism in green and blue-green algal lichens in the field. Oecologica 94: 576- 584. https://doi.org/10.1007/bf00566975.

ADEM YT, MOLİNA P, LİU H, PATAPOF TW, SREEDHARA A & ESUE O. 2014. Hexyl Glucoside and Hexyl Maltoside Inhibit Light-Induced Oxidation of Tryptophan. J Pharm Sci 103: 409-416. http://dx.doi.org/10.1002/jps.23809.

AKBULUT G & YILDIZ A. 2010. An Overview to Lichens: The Nutrient Composition of Some Species. Kafkas Üniv Fen Bil Enst Derg 3(2): 79-86.

AKHTAR MN, SAKEH NM, ZAREEN S, GUL S, LO KM, UL-HAQ Z, SHAH SAA & AHMAD S. 2015. Design and synthesis of chalcone derivatives as potent tyrosinase inhibitors and their structural activity relationship. J Mol Struct 1085: 97-103. https://doi.org/10.1016/j.molstruc.2014.12.073.

AL-FATLAWI AAY & AL-FATLAWI AAY. 2020. Tocopheronic acid attenuates chlorpromazine induced liver injury in rats, targeting oxidative stress and same biochemical markers. Int J Pharm Res 12(4): 2001-2011. http://dx.doi.org/10.31838/ijpr/2020.12.04.285.

ALIYAZICIOGLU R, EYUPOGLU OE, SAHİN H, YILDIZ O & BALTOS N. 2013. Phenolic components, antioxidatn activity and mineral analysis of *Capparis spinosa* L. Afr J Biotechnol 12(47): 6643-6649. https://doi.org/10.5897/AJB2013.13241.

AL-QUADAH KM & ISMAIL ZB. 2012. The relationship between serum biotin and oxidant/antioxidant activities in bovine lameness. Res Vet Sci 92: 138-141. https://doi.org/10.1016/j.rvsc.2010.10.017.

APAK R, GÜÇLÜ K, ÖZYÜREK M & KARADEMİR SE. 2004. A novel total antioxidant capacity index for dietarypolyphenols, vitamin C and E, using their cupric ion reducing capability in the presence of neocuproine: The CUPRAC method. J Agric Food Chem 52: 7970-7981https://doi.org/10.1021/jf048741x.

ARECHE C, PARRA JV, SEPULVEDA B, GALCIA-BELTRAN O & SIMIRGIOTIS MJ 2022. UHPLC-MS Metabolomic Fingerprinting, Antioxidant, and Enzyme Inhibition Activities of *Himantormia lugubris* from Antarctica. Metabolites 12: 560-581. https://doi.org/10.3390/metabo12060560.

ASHİDATE K, KAWAMURA M, TOHDA H, MİYAZAKİ S, HAYASHİ H, TERAMOTO T & HİRATA Y. 2002. Ascorbic acid augments cytotoxicity induced by oxidized low-density lipoprotein. J Atheroscler Thromb 10(1): 7-12. https://doi.org/10.5551/jat.10.7.

AVETİSYAN A, MARKOSIAN A, PETROSYAN M, SAHAKYAN N, BABAYAN A, ALOYAN S & TRCHOUNIYAN A. 2017. Chemical composition and some biological activities of the essential oils from basil *Ocimum* different cultivars. BMC Complement Altern Med 17: 60-68. https://doi.org10.1186/s12906- 017-1587-5.

AYDİN E & TURKEZ H. 2011. Effects of Lichenic extracts (*Bryoria capillaris, Peltigera rufescens* and *Xanthoria elegans*) on human blood cells: a cytogenetic and biochemical study. Fresenius Environ Bull 20: 2992- 2998.

BAGHEL SS, SHRİVASTAVA S, BAGHEL RS, AGRAWAL P & RAJPUT S. 2012. A review of quercetin:antioxidant and anticancer properties. WJPPS 1(1): 146- 160.

BALAKRİSHNA M, KAKİ SS, KARUNA MSL, SARADA S, KUMAR CG & PRASADRBN. 2017. Synthesis and in vitro antioxidant and antimicrobial studies of novel structured

phosphatidylcholines with phenolic acids. Food Chem 221: 664-672. http://dx.doi.org/10.1016/j.foodchem.2016.11.121.

BALDEA I, OLTEANU DE, BOLFA P, TABARAN F, ION R-M & FILIP GA. 2016. Melanogenesis and DNA damage following photodynamic therapy in melanoma with two meso-substituted porphyrins. J Photochem Photobiol B: Biol 161: 402-410. https://doi.org/10.1016/j.jphotobiol.2016.06.012.

BARRETO-BERGTER E, PINTO MR & RODRIGUES ML. 2006. Structural and Functional Aspects of Fungal Glycosphingolipids. Stud Nat ProdChem 33: 1025-1055.

BORICK PM, BRATH M, WILSON AG, WIENTRAUB L & KURA M. 1959. Microbiological activity of certain saturated and unsaturated fatty acids salts of tetra decylamine and related compounds. Appl Microbiol 7(4): 248-251. https://doi.org/10.1128/am.7.4.248-251.1959.

BRÄSE S, GLÄSER F, KRAMER CS, LINDNER S, LINSENMEIER AM, MASTERS K-S, MEISER A-C, RUFF BM & ZHANG S. 2012. Skyrins. Progress in the chemistry of organic natural compounds. Chem Mycotox 2012: 139-151.

BRODO IM, SHARNOFF SDVE & SHARNOFF S. 2001. Lichens of North America. New Haven and London, USA: Yale University Press.

BURGOS EG & SERRANILOS MPG. 2012. Terpene Compounds in Nature: A Review of Their Potential Antioxidant Activity. Curr Med Chem 19: 5319-5341. https://doi.org/10.2174/092986712803833335.

BRUNET JM, DJILAS SM, CETKOVIC CS & TUMBAS VT. 2005. Free-radical scavenging activity of wormwood (*Artemisia absinthium* L) extracts. J Sci Food Agric 85: 265-272. https://doi.org/10.1002/jsfa.1950.

CHEN Q-X & KUBO I. 2002. Kinetics of Mushroom Tyrosinase Inhibition by Quercetin. J Agric Food Chem 50: 4108-4112. https://doi.org/10.1021/jf011378z.

CHEUNG LM, CHEUNG PCK & OOI VEC. 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem 81: 249-255. https://doi.org/10.1016/S0308-8146(02)00419-3.

CHIAN R, KASSIM NK, YEAP YS, LIAN GC, YAZAN SL & MUSA KH. 2018. Isolation of Carbazole Alkaloids and Coumarins from *Aegle marmelos* and *Murraya koenigii* and Their Antioxidant Properties. Sains Malays 47(8): 1749-1756. http://dx.doi.org/10.17576/jsm-2018-4708-14.

DALMARCO EM, BUDNİ P, PARİSOTT EB, FİLHO DW & FRÖDE TS. 2009. Antioxidant effects of mycophenolate mofetil in a murine pleurisy model. Transpl Immunol 22: 12-17. https://doi.org/10.1016/j.trim.2009.09.005.

DECKER E. 1997. Phenolics: prooxidants or antioxidants. Nutr Rev 55: 396-398. https://doi.org/10.1111/j.1753-4887.1997.tb01580.x.

DİMBERG LH, THEANDER O & LİNGNERT H. 1993. Avenanthramides: A group of phenolic antioxidants in oats. AACCI 70(6): 637-641.

EMSEN B. 2019. The antioxidant antigenotoxic potential of *Peltigera canina* and *Umblicaria nylanderiana* based on their phenolic profile. Farmacia 67(5): 912-921. https://doi.org/10.31925/farmacia.2019.5.24.

ESMAEİLİ A & KHODADADİ A. 2002. Antioxidant activity of a solution of thymol in ethanol. ZJRMS 14 (7): 14-18.

GUVEN ZB, SARACOGLU I, NAGATSU A, YILMAZ MA & BASARAN AA. 2023. Anti-tyrosinase and antimelanogenic effect of cinnamic acid derivatives from *Prunus mahaleb* L.: Phenolic composition, isolation, identification and inhibitory activity. J Ethnopharmacol 310: 116378. https://doi.org/10.1016/j.jep.2023.116378.

HE Y, SUYAMA TL, KİM H, GLUKHOV E & GERWİCK WH. 2022. Discovery of Novel Tyrosinase Inhibitors From Marine Cyanobacteria. Front Microbiol 13: 912621. https://doi.org/10.3389/fmicb.2022.912621.

HEO HJ, KİM YJ, CHUNG D & KİM D-O. 2007.Antioxidant capacities of individual and combined phenolics in a model system. Food Chem 104: 87-92. https://doi.org/10.1016/j.foodchem.2006.11.002

HILLIER SL, MOENCH TMP, SHATTACK R, REICHERDELFER P & VERONESE F. 2005. *In vitro* and *in vivo* the story of nonoxynol-9. J Acquir Immune Defic Syndr 39(1): 1-8. https://doi.org/10.1097/01.qai.0000159671.25950.74.

HOU T, NETALA VR, ZHANG H, XING Y, LI H & ZHANG Z. 2022. *Perilla frutescens*: A Rich Source of Pharmacological Active Compounds. Molecules 27: 3758. https://doi.org/10.3390/molecules27113578.

HUANG L, ZHU X, ZHO S, CHENG Z, SHI K, ZHANG C & SHAO H. 2021. Phthalic acid esters: Natural sources and biological activities. Toxins 13: 495. https://doi.org/10.3390/toxins13070495.

HUNECK S & YOSHİMURA I. 1996. Identification of Lichen Substances (1). Verlag Berlin Heidelberg: Springer.

ITTARAT W, SREEPIAN A, SRISARIN A & PATHEPCHOTIVANG K. 2003. Effect of dihidroartemisinin on the antioxidant capacity of *P. falciparum* infected erythrocytes. Southeast Asian J Trop Med Public Health 34(4): 744- 749.

IVANOVA D & IVANOV D. 2009. Ethnobotanical use of lichens: Lichens for food review. Scr Sci Med 41(1): 11- 16. http://dx.doi.org/10.14748/ssm.v41i1.456.

KARAOGLAN FS & KOCA M. 2020. Tyrosinase and cholinesterase inhibitory activities and molecular docking studies on apigenin and vitexin. Ist J Pharm 50(3): 268-271.

KANIPANDIAN N, KANNAN S, RAMESH R, SUBRAMANIAN P & THIRUMURUGAN R. 2014. Characterization, antioxidant and cytotoxic evaluation of green synthesized silver nanoparticles using *Cleistanthus collinus* extract as surface modifier. Mater Res Bull 49: 494-502. https://doi.org/10.1016/j.materresbull.2013.09.016.

KHALIL N, ELHADY SS, DIRI RM, FEKRY MI, BISHR M, SALAMA O & EL-ZALABANI SM. 2022. Salicylic Acid Spraying Affects Secondary Metabolites and Radical Scavenging Capacity of Drought- Stressed *Eriocephalus africanus* L. Agronomy 12: 2278. https://doi.org/10.3390/agronomy12102278.

KHAN M, MOHAN VK, KUMBALA D & KUPPUSORY P. 2007. Cardioprotection by sulphaphenazole, a cytochrome p 450 inhibitor: Mitigation of ischemia-reperfusion injury by scavenging of reactive oxygen species. J Pharm Experiment Ther 323(3): 813-821. https://doi.org/10.1124/jpet.107.129486.

KIM K. 2015. Effect of ginseng and ginsenosides on melanogenesis and their mechanism of action. J Ginseng Res 39: 1-6. https://doi.org/10.1016%2Fj.jgr.2014.10.006.

KIM SB, LIU Q, AHNJH, JO YH, TURK A, HONG IP, HAN SM, HWANG BY & LEE MK. 2018. Polyamine derivatives from the bee pollen of *Quercus mongolica* with tyrosinase inhibitory activity. Bioorg Chem 81: 127-133. https://doi.org/10.1016/j.bioorg.2018.08.014.

KIRKAN B, CEYLAN O, SARIKURKCU C & TEPE B. 2021. Phenolic profile, antioxidant and enzyme inhibitory activity of ethyl acetate, methanol and water extracts of *Capparis spinosa* L. Int J Second Metab 8(4): 337-351. https://doi.org/10.21448/ijsm.981149.

KOOTHEAT T, TEDASEN A, YAMASAKI K & CHATATIKUN M. 2023. Melanogenesis Inhibitory Activity, Chemical Components and Molecular Docking Studies of *Prunus cerasoides* Buch.-Ham. D. Don. Flowers. J Evid Based Immig Med 28: 1-20. https://doi.org/10.1177/2515690x231152928.

KORKAK FA, KESKIN ALKAÇ Z, TANYILDIZI S & DAGOGLU G. 2022. Doğal Kaynaklardan Yeni Antimikrobiyal Madde Tarama Yöntemleri. Fırat Uni Vet J Health Sci 36(3): 251-257.

LANFER UM, BARROS RMC & SINNECKER P. 2005. Antioxidant activity of chlorophylls and their derivatives. Food Res Int 38: 885-891. https://doi.org/10.1016/j.foodres.2005.02.012.

LAY-JING S, HOOI-KHENG B, PAZILAH I, AMIRIN S & MOHDZAINI A. 2012. Antimicrobial activity of *Gynura segetum*'s leaf extracts and its active fractions. Cellmed 2(2): 20.1-20.5. https://doi.org/10.5667/tang.2012.0010.

LEE I-K & AHN S-Y. 1985. The Antioxidant activity of gingerol. Korean J Food Sci Technol 17(2): 55-59. https://doi.org/10.1002/biof.552210157.

LEROUX P, LANEN C & FRITZ R. 1992. Similarities in the antifungal activities of fenpiclonil, iprodione and triclofos-methyl against *Bortyris cinerea* and *Fusarium nivale*. Pestic Sci 36: 255-261. https://doi.org/10.1002/PS.2780360312.

LI H-T, RUAN S-W, HUANG J-C, CHEN H-L & CHEN C-Y. 2012b. Antioxidant and tyrosinase inhibitor from *Leucaena leucocephala*. Afr J Biotechnol 11(77): 14182-14185. https://doi.org/10.5897/AJB12.1119.

LI J, JIANG S, HUANG C & YANG X. 2022. Atraric Acid Ameliorates Hyperpigmentation through the Downregulation of the PKA/CREB/MITF Signaling Pathway. Int J Mol Sci 23: 15952. https://doi.org/10.3390/ijms232415952.

LI W-J, LIN Y-C, WU P-F, WEN Z-H, LIU P-L, CHEN C-Y & WANG H-M. 2013. Biofunctional Constituents from *Liriodendron tulipifera* with Antioxidants and Anti-Melanogenic Properties. Int J Mol Sci 14: 1698-1712. https://doi.org/10.3390%2Fijms14011698.

LI X, LI J, DONG S, LI Y, WEI L, ZHAO C, LI J, LIU X & WANG Y. 2012a. Effects of germination on tocopherol, secoisolarlciresinol diglucoside, cyanogenic glycosides and antioxidant activities in flaxseed (*Linum usitatissimum* L.). Int J Food Sci Technol 54: 2346-2354. https://doi.org/10.1111/ijfs.14098.

LIN Y-S, CHEN S-H, HUANG W-J, CHIEN M-Y, LIN S-Y & HOU W-C. 2012. Effects of nicotinic acid derivatives on tyrosinase inhibitory and antioxidant activities. Food Chem 132: 2074-2080. https://doi.org/10.1016/j.foodchem.2011.12.052.

MAHAMUNI SV. 2015. Antifungal trait of *Burkholderia gladioli* strain VIMPO2 (JQ811557). Int J Sci Res 4(8): 2059-2064.

MALASPINA P ET AL. 2020. Depigmenting potential of lichen extracts evaluated by *in vitro* and *in vivo* tests. Peer J 8: e9150. https://doi.org/10.7717/ijfs.14098.

MASUDA T, YAMASHITA D, TAKEDA Y & YONEMORI S. 2005. Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. Biosci Biotechnol Biochem 69: 197-201. https://doi.org/10.1271/bbb.69.197.

MATOS JM, SALETA V-R ANDRE F, EUGENIA U, LAURDES S & FERNANDO B. 2017. Heterocyclic antioxidants in nature: Coumarins. Curr Org Chem 21(4): 311-324. http://dx.doi.org/10.2174/1385272820666161017170652.

MENEZES JCJMDS & DIEDERICH MF. 2019. Natural dimers of coumarin, chalcones, and resveratrol and the link between structure and pharmacology. Europ J Med Chem 182: 111637. https://doi.org/10.1016/j.ejmech.2019.111637.

MITROVIC T ET AL. 2014. *Platismatia glauca* and *Pseudovernia furfuracea* lichens as sources of antioxidant, antimicrobial and antibiofilm agents. EXCLI J 13 938-953.

MUNZI S, TRIGGIANI D, CECCARELLI D, CLIMATI E, TIEZZI A & PISANI T. 2014. Antiproliferative activity of three lichen species belonging to the genus *Peltigera*. Plant Biosyst 148(1): 83-87.

NARDEMIR G, YANMIŞ D, ALPSOY L, GULLUCE M & AGAR G. 2015. Genotoxic, antigenotoxic and antioxidant properties of methanol extracts obtained from *Peltigera horizontalis* and *Peltigera praetextata*. Toxicol Indust Health 31(7): 602-613.

NILE SH & PAR SW. 2015. Chromatographic analysis, antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds IndCrops Prod 70: 238-244. https://doi.org/10.1016/j.indcrop.2015.03.033.

OLATUNJI TL, SIEBERT F, ADETUNJI AE, HARVEY BH, GERICLE J, HAMMAN JH & VANDER KOOY F. 2021. *Sceletium tortuosum*: Areview on its phytochemistry, pharmacokinetics, biological and clinical activities. J Ethnopharmacol, 280: 114176. https://doi.org/10.1016/j.jep.2021.114711.

OLGUN FAO, UZER A, OZTURK BD & APAK R. 2018. A novel cerium oxide nanoparticles-based colorimetric sensor using tetramethyl benzidine reagent for antioxidant activity assay. Talanta 182: 55- 61. https://doi.org/10.1016/j.talanta.2018.01.047.

OLIVEIRA SDDS, SILVA AMDOE, BLANK AF, NOGUEIRA PCDL, NIZIO DADC, ALMEIDA-PEREIRA CS, PEREIRA RO, MENEZES-SA TSA, SANTANA MHDS & ARRIGONI-BLANK MDF. 2021. Radical scavenging activity of the essential oils from *Croton grewioides* Baill accessions and the major compounds eugenol, methyl eugenol and methyl chavicol. J Essent Oil Res 33(1): 94-103. https://doi.org/10.1080/10412905.2020.1779139.

PANDEY N & BARVE D. 2011. Phytochemical and Pharmacological Review on *Annona squamosa* Linn. Int J Res in Pharmaceut Biomed Sci 2(4): 1404- 1412.

PARK J-Y, CHOI H-J, PARK T, LEE M-J, LIM H-S, YANG W-S, HWANG C-W, PARK D & KIM C-H. 2021. Inhibitory Effect of Avenanthramides (Avn) on Tyrosinase Activity and Melanogenesis in _- MSH-Activated SK-MEL-2 Cells: *In Vitro* and *In Silico* Analysis. Int J Mol Sci 22: 7814. https://doi.org/10.3390/ijms22157814.

PATEL A, PATEL A & PATEL NM. 2010. Determination of polyphenols and free radical scavenging activity of *Tephrosia pupurea* linn leaves. Pharmacognosy Res 2: 152-158. https://doi.org/10.4103/0974- 8490.65509.

PATEL DK & PATEL K. 2022. Potential theuropeutic applications of eudesmin in medicine: An overview on medicinal importance, pharmacological activities and analytical prospects. Pharmacol Res- Modern Sci Med 5: 100-175. https://doi.org/10.1016/j.prmcm.2022.100175.

PATEL DK, PATEL K, RAHMAN M & CHAUDHARY S. 2020. Theurapeutic potential of “Aegline”, an important phytochemical of *Aegle marmelos*: Current health perspectives for the treatment of disease. Chapter Nanomed Bioact: 383- 392.

PAUDEL B, BHATTARAI HD, PANDEY DP, HUR JS & HONG SG. 2012. Antioxidant, antibacterial activity and Brine shrimp toxicity test of some mountainous lichens from Nepal. Biol Res 45: 387-391. https://doi.org/10.4067/s0716-97602012000400010.

PLAZA CM, DIAZ DE TORRES LE, LUCKING RK, VIZCAYA M & MEDINA GE. 2014. Antioxidant activity, total phenols and flavonoids of lichens from Venazuelan Andes. J Pharm Pharmacognosy Res 2(5): 138-147. https://doi.org/10.4067/s0716-97602012000400010.

RAMMALI S, HILALI L, DARI K, BENCHARKI B, RAHIM A, TIMINOUNI M, GOBOUNE F, ADLOUNI ME & KHATTABI A. 2012. Antimicrobial and antioxidant activities of *Streptomyces* species from soils of three different cold sites in the Fez-Melones region Moracco. Nature 12: 17233. https://doi.org/10.1038%2Fs41598-022-21644-z.

RANKOVIC B & KOSANIC M. 2015. Lichens as potential source of bioactive secondary metabolites. Lichen Secondary Metabolites. London: Springer.

RER A, PELLEGRINI N, PROTEGGENTE A, PANNOLA A, YONG M & RICE-EVANS C. 1999. Antioxidant activity applying an improved ABTS radical cotion decolorization assay. Free Rad Biol Med 26: 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

REYES-SOTO CY ET AL. 2022. Oleamide Reduces Mitochondrial Dysfunction and Toxicity in Rat Cortical Slices Through the Combined Action of Cannabinoid Receptors Activation and Induction of Antioxidant Activity. Neurotox Res 40: 2167-2178. https://doi.org/10.1007/s12640-022-00575-7.

SADASIVAM K & KUMERESAN R. 2011. A comperative DFT study on the antioxidant activity of apigenin and scutellarein flavonoid compounds. Mol Phys 109(6): 839-852. http://dx.doi.org/10.1080/00268976.2011.556576.

SAITO T & MITSUHASHI S. 1971. Antibacterial activity of megolamicin and its inducer activity for macrolid resistance in Staphylococci. The Antibiot 23(12): 850-854. https://doi.org/10.7164/antibiotics.24.850.

SALEEM H, ZENGIN G, LOCATELLI M, ABIDIN SAZ & AHEMAD N. 2021. Investigation of phytochemical composition and enzyme inhibitory potential of *Anagallis arvensis* L. Nat Prod Res 36 (14): 3750-3755. https://doi.org/10.1080/14786419.2021.1880404.

SI Y-X, YIN S-J, OH S, WANG Z-J, YE S, YAN L, YANG J-M, PARK Y-D, LEE J & QIAN GY. 2012. An Integrated Study of Tyrosinase Inhibition by Rutin: Progress using a Computational Simulation. J Biomol Struct Dyn 29(5): 999-1002. https://doi.org/10.1080/073911012010525028.

SIRHINDI G, MIR MA, ABD-ALLAH EH, AHMAD P & GUCEL S. 2016. Jasmonic Acid Modulates the Physio-Biochemical Attributes, Antioxidant Enzyme Activity, and Gene Expression in Glycine max under Nickel Toxicity. Front in Plant Sci 7: 591-603. https://doi.org/10.3389/fpls.2016.00591.

SIVAS HZ. 2019. Antigenotoxic effect of some lichen metabolites. Lichen Secondary Metabolites. Rankovic, B. London: Springer.

SMITH CW, APTROOT A, COPPINS BJ, FLETCHER A, GILBERT OL, JAMES PW & WOLSELEY PA. 2009. The Lichens of Great Britain and Ireland. London, UK: Natural History Museum Publications in association with The British Lichen Society.

SONOWAL H, SHUKLA K, KOTA S, SAXENA A & RAMANA KV. 2018. Vialinin A, an Edible Mushroom-Derived p-Terphenyl Antioxidant, Prevents VEGF-Induced Neovascularization *In Vitro* and *In Vivo*. Oxid Med Cell Longev 2018: 1-10. https://doi.org/10.1155/2018/1052102.

TADOKORO T, ROUZAUD F, ITAMI S, HEARING WJ & YOSHIKAWA K. 2003. The Inhibitory Effect of Androgen and Sex-Hormone-Binding Globulin on the Intracellular cAMP Level and Tyrosinase Activity of Normal Human Melanocytes. Pigment Cell Res 16: 190-197. https://doi.org/10.1034/j.1600- 0749.2003.00019.x.

TAMSIR NM, ESA NM, OMAR SNC & SHAFIE NH. 2020. *Manilkara zapota* (L.) P. Royen: Potential Source of Natural Antioxidants. Malaysian J Med Health Sci 16: 193- 201.

TANAKA JCA, SILVA CC, OLIVEIRA AJB, NOKAMURA CV & DIAS FILHA BF. 2006. Antibacterial activity of indole alkoloids from *Aspidormea ramiflorum*. Braz J Biol Res 39(3): 387-391. https://doi.org/10.1590/S0100-879X2006000300009.

TAO N, WANG R, XU X, DONG T, ZHANG S, LIANG M &WANG Q. 2021. Xanthosine is a novel anti-browning compound in potato identified by widely targeted metabolomic analysis and *in vitro* test. Postharvest Biol Technol 171: 111367. https://doi.org/10.1016/j.postharvbio.2020.111367.

UMUKORO S, ADEWOLE FA, EDUVIERE AT, ADERIBIGBE AO & ONWUCHEKWA C. 2014. Free Radical Scavenging Eff ect of Donepezil as the Possible Contribution to its Memory Enhancing Activity in Mice. Drus Res 64(5): 236-239. https://doi.org/10.1055/s-0033-1357126.

UNUVAR S, GÜRSOY Ş, BERK A, KAYMAZ B, İLHAN N & AKTAY G. 2021. Antioxidant Effect of a Dihydropyridine Calcium Antagonist Nitrendipine in Streptozotocin_Induced Diabetes. Experiment Papers 57(1): 126-133.

VIGGIANO S, PANZELLA L, REICHENBACH M, HANS J & NAPOLITANO A. 2023. The Effect of Cosmetic Ingredients of Phenol Type on Immediate Pigment Darkening and Their (Photo)Protective Action in Association with Melanin Pigmentation: A Model *In Vitro* Study. Cosmetics 10: 22-37. https://doi.org/10.3390/cosmetics10010022.

WANG W, SNOOKS HD & SANG S. 2020. The Chemistry and Health Benefits of Dietary Phenolamides. J Agric Food Biochem 68: 6248-6267. https://dx.doi.org/10.1021/acs.jafc.0c02605.

WATSON RR & SCHÖNLAU F. 2015. Nutraceutical and antioxidant effects of a delphinidin-rich maqui berry extract Delphinol: a review. Minerva Cardioangiol 63: 1-12.

WETCHAKUL P, CHANSUT P, PUNSAWAD C & SONPİNİT S. 2022. Characterization, antioxidant activity and *in-vitro* toxicity of medicinal plants from the Tri-Than-Thip Remedy. Evidence Based Complement Med 2022: 1-10. https://doi.org/10.1155/2022/4477003.

WIRTH W. 1995. Die Flechten Baden-Württembergs. Stutttgart, DEU: Ulmer.

YANG L, HAN H, NAKAMURA N, HATTORI M, WANG Z & XU WL. 2007. Bio-guided isolation of antioxidants from the stems of *Dendrobium aurantiacum* var. *denneanum*. Phytothe Res 21(7): 696-698. https://dx.doi.org/10.1002/ptr.2133.

ZAGOSKINA NV, NIKOLAEVA TN, LAPSHIN PV, ZAVARZIN AA & ZAVARZINA AG. 2013. Water-soluble phenolic compounds in lichens. Microbiol 82(4): 445-452. http://dx.doi.org/10.1134/S0026261713030132.

ZEISS DR, PIATER LA & DUBERY IA. 2021. Hydroxycinnamate amides: Intriguing conjugates of plant protective metabolites. Trends in Plant Sci 26(2): 184-195. https://doi.org/10.1016/j.tplants.2020.09.011.

ZHANG L, ZHAO X, TAO G-J, CHEN J & ZHENG Z-P. 2017. Investigating the inhibitory activity and mechanism differences between norartocarpetin and luteolin

for tyrosinase: A combinatory kinetic study and computational simulation analysis. Food Chem 223: 40-48. https://doi.org/10.1016/j.foodchem.2016.12.017.

ZHAO Y, WANG M & XU B. 2021. A comprehensive review on secondary metabolites and health- promoting effects of edible lichen. J Func Foods 80: 104283. https://doi.org/10.1016/j.jff.2020.104283.

## SUPPLEMENTARY MATERIAL

**Table SI.**

**How to cite**

TÜZÜN BS, KARADAĞ BT, ORAN S, ÖZTÜRK Ş, YILMAZ FF, FAFAL T & KIVÇAK B. 2024. Determination of phytochemical contents by LC/QTOF/MS and evaluation of *in-vitro* biological activities of 2 *Peltigera* lichens from Bursa. An Acad Bras Cienc 96: e20230657. DOI 10.1590/0001-3765202420230657.

*Manuscript received on June 19, 2023;*
*accepted for publication on February 3, 2024*

**BURCU S. TÜZÜN**[1]
https://orcid.org/0000-0002-9194-3447

**BAŞAK T. KARADAĞ**[1]
https://orcid.org/0000-0002-5731-5324

**SEYHAN ORAN**[2]
https://orcid.org/0000-0001-8196-2065

**ŞULE ÖZTÜRK**[2]
https://orcid.org/0000-0002-2161-7400

**FETHİYE F. YILMAZ**[3]
https://orcid.org/0000-0003-1102-7826

**TUĞÇE FAFAL**[1]
https://orcid.org/0000-0002-7445-5855

**BİJEN KIVÇAK**[1]
https://orcid.org/0000-0001-7645-1930

[1]Ege University, Department of Pharmacognosy, Faculty of Pharmacy, Erzene District, Bornova, 35040, Izmır, Turkıye

[2]Uludag University, Deparment of Botany, Faculty of Science, Görükle District, Nilüfer, 16285, Bursa, Turkıye

[3]Ege University, Deparment of Pharmaceutical Microbiology, Faculty of Pharmacy, Erzene District, Bornova, 35040, Izmır, Turkıye

Correspondence to: **Burcu Sümer Tüzün**
*E-mail: burcusmer@gmail.com / burcu.sumer@ege.edu.tr*

### Author contrubition

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by ŞULE ÖZTÜRK ,SEYHAN ORAN and BURCU SÜMER TÜZÜN; The first draft of the manuscript was written by [BURCU SÜMER TÜZÜN] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript. The authors have no relevant financial or non-financial interests to disclose. All data materials are availiable. All authors state there are no conflict of interests.