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Biological Activity Evaluation of *Scorzonera tomentosa* L.

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Abstract: This study aimed to evaluate the phytochemical components, antimicrobial activity, and antioxidant activity of 80% ethanol extract of *Scorzonera tomentosa*, an endemic species. The chemical constituents of the ethanolic extract of *S. tomentosa* was primarily characterized by gas-chromatography-mass spectrometry analysis (GC-MS), ten components were identified. The major component was found as 2-pentanamine (35.68%). When the antioxidant capacity of *S. tomentosa* was examined, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities were determined to be quite high compared to the reference drug (IC_{50} values; DPPH: 517.0 ± 1.86 µg mL^{-1}; ABTS: 244.8 ± 0.94 µg mL^{-1}; reference drug: 1.313 ± 0.24 µg mL^{-1}). In addition, according to total phenol content and total flavonoid content analyses, it was determined that the plant is richer in flavonoids. The antimicrobial activity of this species is not at an effective level. More extensive studies with *S. tomentosa* may allow the plant to be used as a natural antioxidant.

Keywords: *Scorzonera tomentosa*, antioxidant capacity, chemical content, antimicrobial activity

1. Introduction

The plant genus *Scorzonera* is a member of the Asteraceae family, which is widespread throughout Asia, Europe, and North Africa. There are 54 records of this genus in Türkiye, which is a country rich in terms of medicinal and aromatic plants (Duran and Hamzaoğlu, 2004; Sarı et al., 2019; Ak and Zengin, 2021). These plants, which are used as flavorings and spices or consumed as a tea, have extremely high medical and economic value. Previous research has shown that plants of the *Scorzonera* genus show analgesic, anti-rheumatismal, diuretic, and wound healing properties in traditional medicine, and form an *in vitro* response to the treatment of various diseases such as hypertension, infertility, gout, pulmonary oedema, diarrhea, stomach ulcer, and cancer (Csupor-Löffler et al., 2009; Harkati et al., 2010; Milella et al., 2014; Bahadır-Acıkara et al., 2018; Petkova, 2018; Akkol et al., 2019; Lendzion et al., 2021).

The *Scorzonera tomentosa* used in the study is widespread throughout Türkiye and actively used in traditional medicine. Previous studies have shown that the latex produced from this plant exhibits wound healing, analgesic, anti-rheumatismal, and anti-helminthic properties (Bahadır Acıkara et al., 2013a, b; Lendzion et al., 2021).

The differences seen in the plant components can be attributed to the preparation of plant extracts

with different solvents or working with certain sections of the plant organs, also genotypic differences. Components such as lupeol acetate, taraxasteryl acetate, and lupeol have been previously determined in the extract prepared with n-hexane from the stem of *Scorzonera tomentosa* (Bahadır-Acıkara et al., 2018). Components such as chlorogenic acid, (±)-scorzotomentosin have been determined from the methanol extract (Sarı et al., 2007).

For many years, medicinal plants have been actively used in the therapy of infectious diseases caused by micro-organisms. Due to the antibiotic resistance of bacteria, studies with these plant species are promising. Studies have shown that many species of the genus *Scorzonera* have antimicrobial effects. For example, it has been reported that *Scorzonera undulata* ssp. *deliciosa* has no antimicrobial effect on gram-negative bacteria but is effective on some gram-positive bacteria (Boussaada et al., 2008).

In a study that evaluated the antimicrobial activity of petrol ether/diethyl ether/methanol extract of *Scorzonera divaricata*, it was determined that the Sulfoscorzonin-D alkaloid obtained from the plant showed stronger activity against *Clostridium perfingens* than ampicillin (Wu et al., 2018). When the antimicrobial activity of water and ethanol extract *of Scorzonera mackmeliana* was examined, the extract of water was found to be effectual against *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Escherichia coli* whereas the same rate of activity was not determined for the ethanol extract (Sweidan et al., 2020).

Antioxidants are extremely important in the protection of homeostasis. The negative effects on the body created by free radicals cause the development of several diseases. In addition, nowadays the harmful effects of synthetically produced antioxidants are better known (Olszowy, 2019). Therefore, the importance of medicinal plants with antioxidant properties is currently increasing. Several types of antioxidant activity have been shown in previous studies of the *Scorzonera* genus. In a study conducted with the methanol extract of *Scorzonera divaricata* and *Scorzonera pseudo divaricata*, it was reported that much higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity was seen compared to chlorogenic acid, which was the reference component (Tsevegsuren et al., 2007). In a 2013 study of three different *Scozonera* species (*S. latifolia*, *S. laciniata* and *S. suberosa*), it was suggested that these plants had high antioxidant capacity (Erden et al., 2012).

Taking all these findings into consideration, this study investigated for the first time the antioxidant and antimicrobial activities of the chemical components of 80% ethanol extract of the endemic plant *Scorzonera tomentosa*, which is important in medicinal respect.

2. Materials and Methods

2.1. Plant material collection and extraction

Scorzonera tomentosa plant materials, an endemic region in terms of wild plants in Türkiye, which were collected from Hafik, Sivas (B6 Sivas-Hafik west of Lota lakes, 1290-1295 m, Akpulat 6124) on 27.06.2017. The plant was identified by Prof.Dr. Hüseyin Aşkın Akpulat at Sivas Cumhuriyet University, Faculty of Science, Department of Biology. After the leaves of the plant were dried and ground, 10 g of this sample was taken and dissolved in 80% ethanol. The chemical composition of obtained extract was identified using gas-chromatography-mass spectrometry (GC-MS) (Shimadzu, GCMS-QP2010 Ultra). Helium gas was handled as carrier gas at a constant flow rate of 1.5 ml per minute. In splitless mode, the injection volume of 1 µl was designed to be 5 per minute between 35-550 and was set at 280 °C for 2 minutes after the run. The total running time was 1 hour. The chemical content of the extract was researched through different libraries (NIST05a.L, Wiley7n.I, and W9N11.L).

2.2. DPPH assay

The antioxidant activity of the extract was prepared in accordance with the procedure of Eruygur et al. (2019). The activity value is expressed as a percentage. The DPPH solution was freshly prepared by dissolving it in ethanol. 20 µL of the plant extracts dissolved in dimethyl sulfoxide (DMSO) were mixed with 180 µL of DPPH solution (40 µg mL^{-1}) in a 96-well plate. After the well plates were left in the dark for fifteen minutes, absorbance levels were measured at 540 nm on a spectrophotometer. In the experiment, DMSO was accepted as the control and Trolox was accepted as the standard. The standard deviation of the results of the experiment repeated three times was calculated and evaluated accordingly.

2.3. 2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The decolourisation activity of the ABTS cation radicals of the extracts was determined according to the method of Re et al. (1999) with minor modifications. The test sample and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

radical stock solution were prepared following the DPPH method and diluted (7 mM ABTS, 140 mM potassium persulfate) just before analysis. The ABTS working solution was diluted with ethanol and the absorbance value was measured at 734 nm. According to the data obtained, the value was taken as 0.70 ± 0.02. In the experiment, 50 µL of sample solution with 0-1 mg mL^{-1} concentration was mixed and added to 100 µl ABTS solution on a microplate. 734 nm absorbance value was measured after 10 minutes at room temperature. ABTS scavenging activity was evaluated using Trolox as an antioxidant standard.

2.4. Specification of total phenolic (TPC) and flavonoid contents (TFC)

Determination of TPC was performed with FolinCiocalteu (F-C) reagent (Clarke et al., 2013). After the extract was diluted with DMSO, F-C reagent and distilled water were added to the mixture. After waiting for 5 minutes, 7.5% Na_2CO_3 was suffixed and incubated for one hour, and finally, absorbance was measured at 650 nm. Gallic acid with added DMSO was run as a reference and DMSO was run in parallel with blank.

TFC in the extract was defined through the aluminum chloride colorimetric assay (Molan and Mahdy, 2014). It was calibrated by preparing serial dilution solutions. The reagent was prepared with ethanol (150 µL, 0.3 mg mL^{-1}) and mixed with 2% $AlCl_3$, added to the microplate. The absorbance value of the solution, which was kept at 22 °C for 15 min, was measured at 435 nm. Then, the total flavonoid contents of the extract were expressed as mg of quercetin equivalent to their dry weight.

2.5. Antimicrobial activity

The Minimum Inhibitory Concentration (MIC) [using Eloff's method (Eloff, 1998)] values of the ethanol extract used in the study on predetermined bacteria and fungi were determined. *Candida albicans*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* were used as microbial agents in the study. The extract dissolved with 8% DMSO was added to the microtiter plate diluted with 90 µl broth in 10 µl volume. In the second line, 50 µL of the sample was added and two-fold serial dilution was made with the broth. The concentration of the plant extract in the well after the application was 5.00-0.002 mg mL^{-1}. The final inoculum size was 0.5-2.5×10^3 CFU mL^{-1} in Candida and 5×10^5 CFU mL^{-1} in bacteria per well. The bacterial culture was diluted with Sabouraud Dextrose Broth (Himedia ME033) and the Candida culture was diluted with Mueller Hinton Broth (Accumix®). 50 µL of fungal and bacterial suspension were added to the prepared samples and incubated for 16-24 hours at 35 °C and 37 °C, respectively. To detect growths in the well, 2,3,5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) was added in a volume of 50µL (2 mg mL^{-1}) and incubated at 37 °C for 2 hours. The decrease in the intensity of the red color of formazan at the end of this period was accepted as the MIC value. The study, in which the standard deviation was accepted as zero, was performed in two replicates.

2.6. Statistical analysis

The results of the biological activity analysis repeated three times were expressed as mean ± standard deviation values. Statistical evaluation of the obtained data was performed with Graphpad 6.0 software.

3. Results and Discussion

3.1. GC-MS analysis

In this study, the chemical components of 80% ethanol extracts of *Scorzonera tomentosa* were evaluated using GS-MS analysis (Table 1).

Table 1. Chemical components of *S. tomentosa* 80% ethanol extract

No	Retention time	Components	Area (%)
1	2.035	1,4-Dioxane-2,6-dione	1.71
2	2.085	Dimethylhydrazone	5.83
3	2.161	Propiolic acid	19.00
4	2.235	2-Pentanamine	35.68
5	2.267	Methyl hydrogen disulfide	31.79
6	2.43	Acetic acid	0.25
7	3.843	1,1-Diethoxypropanal	1.25
8	35.732	Ethyl Oleate	0.11
9	45.816	(9Z)-9-Octadecenamide	0.41
10	47.458	Hexatriacontane	0.05
Total			96.08

The chemical contents of the ethanol 80% extract of *Scorzonera tomentosa* was determined by GC-MS. Plants components and their amounts may differences according to species type and these differences may cause changes in biological activities. According to the data obtained, "2-Pentanamine" (35.68%) was determined as a major component. Followed by "Methyl hydrogen disulfide" with a value of 31.79% and "Propiolic acid" with a value of 19.00% (Table 1). In previous study, aerial part n-hexane extract of *S. tomentosa* is reported to contain taraxasteryl acetate, lupeol acetate, and lupeol (Bahadır-Acıkara et al., 2018) In the aqueous ethanol extract, cyranoside and chlorogenic acid were detected (Küpeli Akkol et al., 2011). When compared with the major compounds obtained in this study, it was seen that the extracts prepared with different solvents can show changes

in terms of phytochemical content in different organs of the plant.

3.2. *In vitro* antioxidant activity

The DPPH and ABTS radical scavenging activity results are shown in Figure 1. Although the antioxidant activity value of the *S. tomentosa* was found to be higher than the ABTS method, the antioxidant activity value by both DPPH (IC_{50} value 517.0 ± 1.86 µg mL^{-1}) and ABTS (IC_{50} value 244.8 ± 0.94 µg mL^{-1}) method was found to be quite high when compared to the reference substance trolox (IC_{50} value 1.313 ± 0.24 µg mL^{-1}).

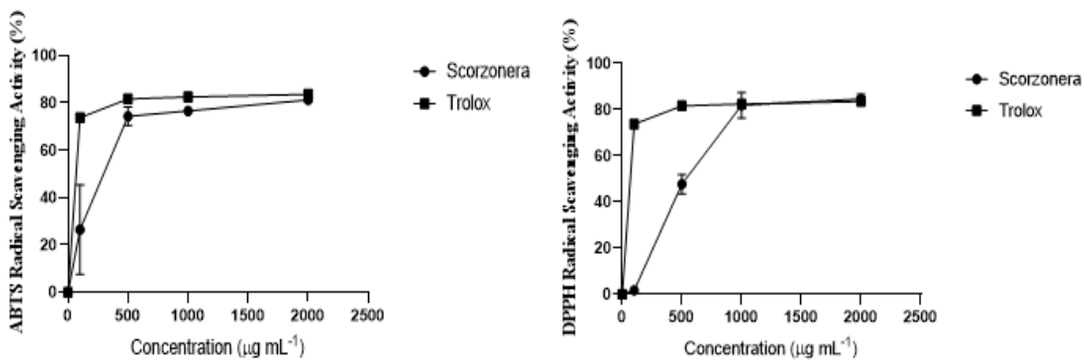

Figure 1. DPPH and ABTS radical scavenging activity of *S. tomentosa* 80% ethanol extract and positive drug trolox

In many studies with the antioxidant activity of *Scorzonera* species, ABTS and DPPH radical scavenging activities were found to be strong. Nasseri et al. (2015) investigated the DPPH activity of the ethanol/water extract obtained from the leaves and roots of *Scorzonera paradoxa* and found that the leaf extract showed stronger antioxidant activity than the root extract. In another study, the DPPH activity of the methanol extract of *Scorzonera radiata* was examined and it was reported that Scorzodihydrostilbenes A and E obtained from the extract showed much stronger antioxidant activity than the reference drug (Wang et al., 2009). In the study by Yang et al. (2016), the ABTS antioxidant capacity of the root ethanol extract of *Scorzonera divaricata* was investigated and the radical scavenging values of the two compounds obtained were found to be moderately active. In one of the recent studies, the antioxidant activity of *Scorzonera veratrifolia* was studied by preparing different extracts (n-heptane, chloroform and methanol). It was found that the activity of the methanol extract for scavenging DPPH radicals was higher than that of the other extracts (Taşkın et al., 2021).

When the data obtained from this study are compared with other studies, it can be said that the antioxidant activity of most of the plants belonging to the *Scorzonera* genus is quite high.

The phenolic and flavonoid compositions of the ethanol extract (80%) of *S. tomentosa* were evaluated (Figure 2). It was determined that the flavonoid content had the highest phenolic content (248.96±6.4 mg GAE g^{-1} and 274.8±14.5 mg QE g^{-1}, respectively). Studies investigating the phenolic content of plants belonging to the genus *Scorzonera* are limited. In a study reported in 2015, it was reported that the *S.paradoxa* species is rich in phenolic compounds (Nasseri et al., 2015).

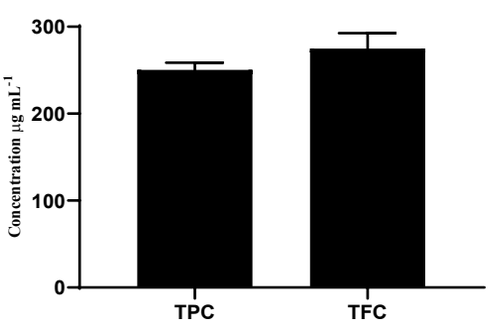

Figure 2. TPC and TFC of *S. tomentosa* 80% ethanol extract

3.3. Antibacterial activity

The antimicrobial activity of the ethanol 80% extracts of *Scorzonera tomentosa* against *C. albicans*, *B. cereus*, *P. aeruginosa*, *E. coli*, and *S. aureus* were determined using the broth microdilution analyses at the concentration range 0.312 to >2.5 mg mL^{-1} (Table 2). It was observed that the extract showed weak antimicrobial activity on these applied strains.

Table 2. Antimicrobial activity of ethanol extract of *S. tomentosa* against tested microorganisms

	Staphylococcus aureus	*Escherichia coli*	*Bacillius cereus*	*Candida albicans*	*Pseudomonas aeruginosa*
	ATCC 29213	ATCC 25922	ATCC11778	ATCC10231	ATCC 27853
Scorzonera tomentosa	5	>5	>5	>5	>5

Interestingly, Ak et al. (2022) reported that extracts prepared with different solvents (ethyl acetate, hexan, dichloromethane, methanol) on the aerial parts and roots of the same plant showed strong antimicrobial activity. In another study with the ethyl acetate extract of *Scorzonera undulata,* it was reported that the extract showed antimicrobial activity on *Candida freundei*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus and Proteus mirabilis* (Abdelkader et al., 2010). In a comprehensive study conducted with an endemic species, *Scorzonera mackmeliana* in 2020, the antimicrobial activity of the plant was investigated. Ethanol and water extracts were prepared from different parts of the plant (root, stem, leaf, flower) and it was reported that it showed very strong activity on gram positive and gram negative bacteria (Sweidan et al., 2020). The use of various parts of the plant and different solvents in the prepared extracts may explain this difference.

4. Conclusions

In this study investigating the biological activities of *Scorzonera tomentosa*, an endemic plant species, considering total phenolic and flavonoid content, DPPH, and ABTS radical scavenging activities, it can be said that it is a promising natural antioxidant source. However, more extensive studies are needed on this plant. In addition, thanks to the rich phytochemical content of the plant, it is thought that it can lead to phytotherapeutic studies being carried out with this plant in the future.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

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Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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