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Determination of the Phytochemical, Antioxidant, and Antimicrobial Properties of *Smilax excelsa* L. Extracted with Different Solvents

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Research Article

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Abstract

Smilax excelsa L., an edible wild plant whose leaves and shoots are frequently consumed in the daily diet and used to treat various diseases in traditional medicine, grows in certain regions of Türkiye, especially in the Black Sea Region. This study aims to determine the phytochemical properties, antioxidant and antimicrobial activities of *S. excelsa* with different solvents. In this study, the shoots of *S. excelsa* were extracted with water, 70% ethanol, and 80% methanol solvents and an ultrasound-assisted extraction method was used. It was found that the total phenolic contents of the extracts obtained from the plant were higher in the ethanol (75.09 mg/g) and methanol (74.72 mg/g) extracts compared to the water extract (49.62 mg/g). While the water extract had the highest amount of flavonoids (22.96 mg/g), the 80% methanol extract had the highest amount of total anthocyanins (0.52 mg/g). The efficacy of the extracts against various pathogens was also investigated. It was determined that in the Petri dish in which *Enterococcus faecalis* ATCC 29212 pathogen was cultivated, a clear zone was formed around the disc into which only the water extract was impregnated. The zone diameter was 2.10 ± 0.08 cm. As a result of the study, it can be said that especially the ethanol and methanol extracts were effective in terms of both the phytochemical properties and antioxidant activities and therefore they exhibited high antioxidant properties.

Keywords: *Smilax excelsa*, edible wild plants, phenolic compound, antioxidant activity

Farklı Çözücülerle Ekstrakte Edilen *Smilax excelsa* L.'nin Fitokimyasal, Antioksidan ve Antimikrobiyal Özelliklerinin Belirlenmesi

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Öz

Yaprak ve sürgünleri günlük diyette sıklıkla tüketilen ve geleneksel tıpta çeşitli hastalıkların tedavisinde kullanılan, yenilebilir yabani bir bitki olan *Smilax excelsa* L. başta Karadeniz Bölgesi olmak üzere Türkiye'nin belirli bölgelerinde yetişmektedir. Bu çalışmanın amacı, farklı çözücülerle hazırlanan *S. excelsa* ekstraktlarının fitokimyasal özelliklerini, antioksidan ve antimikrobiyal aktivitelerini belirlemektir. Çalışmada, *S. excelsa* sürgünleri su, %70 etanol ve %80 metanol çözücüleriyle ekstrakte edilmiş ve ultrason destekli ekstraksiyon metodu uygulanmıştır. Bitkiden elde edilen ekstraktların toplam fenolik madde miktarının etanol (75.09 mg/g) ve metanol (74.72 mg/g) ekstraktlarında su ekstraktına (49.62

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mg/g) kıyasla yüksek olduğu bulunmuştur. Su ekstraktı en yüksek flavonoid (22.96 mg/g) miktarına sahipken, %80'lik metanol ekstraktı en yüksek toplam antosiyanin miktarına (0.52 mg/g) sahiptir. *S. excelsa* ekstraktlarının çeşitli patojenlere karşı etkinliği de araştırılmıştır. *Enterococcus faecalis* ATCC 29212 patojeninin ekili olduğu Petride, yalnızca su ekstraktının emdirildiği diskin etrafında açık zon oluşumu meydana geldiği belirlenmiştir. Zon çapı 2.10 ± 0.08 cm'idi. Çalışma sonucunda, özellikle etanol ve metanol ekstraktlarının hem fitokimyasal özellikler hem de antioksidan aktiviteler bakımından etkin olduğu ve bu nedenle yüksek antioksidan özellikler sergiledikleri söylenebilir.

Anahtar Kelimeler: *Smilax excelsa*, yenilebilir yabani bitkiler, fenolik bileşik, antioksidan aktivite

Introduction

Wild edible plants (WEPs) are found in various agroecosystems and offer both direct and indirect resources for human nutrition and health [1]. In addition to being a potential source of income, these plants are crucial for the traditional food, fodder, medicine, socio-cultural, and general well-being of rural dwellers, especially in mountainous areas [2, 3]. WEPs are an inexpensive source of minerals, vitamins, and antioxidants and a cultural heritage that should be protected worldwide [4]. In addition to their nutritional content, these plants are increasingly attracting people's attention due to diversifying dietary habits and promoting ecological and biodiversity sustainability [5]. Recently, a new trend in some developed countries and Europe is using local edible wild plants in modern cuisines, both for their health benefits and as an element of cultural identity [6, 4]. WEPs are an alternative raw material for clean-label food production in the food industry thanks to their bioactive compounds [7]. Due to their medicinal functions, many edible wild plants are believed to have positive effects on human health. Due to their medicinal functions, many edible wild plants are believed to have positive effects on human health. These plants are also known as "nutraceutical plants" because they offer nutritional and pharmaceutical benefits [8]. WEPs, which have many properties such as antioxidants, antimicrobial, anticarcinogenic, antidiabetic, anti-inflammatory, and anti-obesity effects, are the focus of studies in pharmacology and food science [7]. *Smilax* (Smilacaceae), including edible wild plants, is a genus of about 350 species in tropical, temperate, and subtropical regions worldwide. This genus contains plants in America, Europe, Asia, and Oceania. Species of the genus *Smilax*, commonly called sarsaparilla, have long, slender spiny stems and are characterized as climbers. They climb by clinging to other plants or objects with their tendrils, which grow continuously upwards [9]. The leaves, stems, roots, and rhizomes of sarsaparilla are consumed as food and used in traditional medicine. The genus *Smilax* has pharmacological properties with antioxidant, antibacterial, antifungal, and immunomodulatory effects. It is used in the treatment of diabetes, different types of cancer, ulcers, and various skin diseases as well as gout, fever, and eye diseases [10]. These plants, which have saponins in their roots, are also utilized as diuretics and tranquilizers [11]. *S. excelsa*, a species belonging to the genus *Smilax*, is a climbing, perennial and shrubby wild plant. It grows up to 800 m in height in shrubby and forested areas [12] and grows in temperate regions, especially in North and South America, and East Asia [13]. In Türkiye, it

is distributed in the Black Sea, Mediterranean, Aegean, and western Marmara regions including Samsun, Sinop, Artvin, Zonguldak, Trabzon, Tekirdağ, İstanbul, Bolu, Antalya, Muğla, Aydın, and Hatay provinces [14-17]. Locally known as "kırçan, dikenucu, melocan, saparna, merülcen, and melevcan" [18], the leaves and shoots of this plant are frequently consumed in the daily diet. Due to its antioxidant, antimutagenic, antiviral, and antimicrobial properties [19], this plant is used in traditional medicine in Türkiye to treat stomach pain, breast cancer, and indigestion [9]. In addition, it is of great importance in the field of pharmacology as it is actively used in treating rheumatism, diabetes, ulcers, and various skin diseases [20, 13]. In this study, it was aimed to determine the phytochemical properties (leaf properties, the total phenolic, flavonoid, and anthocyanin contents) of shoots of *S. excelsa*, which is frequently consumed in Türkiye, extracted with different solvents (water, ethanol, and methanol) and prepared by ultrasound-assisted extraction. In addition, the antioxidant [(DPPH (1,1-diphenyl-2-picrylhydrazyl), FRAP (ferric-reducing antioxidant power), and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] and antimicrobial activities of the *S. excelsa* extracts were also determined.

Materials and Methods

Sampling

The shoots of *S. excelsa* were collected from the Dikbıyık District of Çarşamba, in 2024 (41.221082° N 36.608975° E). Species identification was made by Prof. Dr. Hamdi Güray Kutbay from the Department of Botany, Department of Biology, Ondokuz Mayıs University. The weeds were removed from *S. excelsa* in the laboratory (Figure 1).

Figure 1. *The shoots of Smilax excelsa*

The shoots were washed with distilled water, and the water was removed. Some of the fresh shoots were separated for color determination. The remaining shoot samples were kept in a lyophilizer (Labconco Freezone 12 plus, USA) at 85°C and 0.020 mbar pressure for 24 hours to remove the water. After 24

hours, the samples taken from the device were pulverized with the help of a grinder (Sinbo, Scm 2934, Istanbul, Türkiye).

Preparation of the Extracts of *Smilax excelsa*

The method reported by Raghunath et al. [21] was modified and used to prepare extracts. A certain amount of the lyophilized *S. excelsa* powder was weighed and pure water, 70% ethyl alcohol, and 80% methyl alcohol were added as three different solvent with a solid: solvent ratio of 1:25. First, it was left to macerate in an ultrasonic water bath at 20°C for 30 minutes, then at room temperature for 2 hours. Then, it was centrifuged at 4000 rpm for 15 minutes and the obtained extracts were analyzed for antioxidant and phenolic compound composition by passing through a 0.45 μm pore teflon filter.

Color Measurement

Color measurement of *S. excelsa* leaves was made with the MiniScan EZ 4500 (Reston, Virginia, USA) color measuring device. L* (light/darkness), a* (redness/greenness), and b* (yellowness/blueness) values were measured.

Determination of Dry Matter

The fresh samples were weighed in a certain amount in tarred nickel containers. Then, they were dried in a vacuum oven at 70°C and 100 mm Hg pressure until they reached constant weight [22].

Total Phenolic Compound Analysis

The method reported by Singleton and Rossi [23] was modified and used to determine the total phenolic compound content. The total phenolic content of the *S. excelsa* was determined using the Folin-Ciocalteu reagent. For this, 150 μl of the diluted sample was mixed with 750 μl of 10% Folin-Ciocalteu reagent (5 minutes) and 600 μl of 7.5% Na_2CO_3. The mixture was kept in the dark for 2 hours, and then its absorbance was determined at 760 nm. Results are expressed as mg gallic acid equivalent/g (mg GAE/g).

Determination of Total Flavonoid Content (TFC)

1 ml of the diluted sample was taken, 0.3 ml of 5% $NaNO_2$ was added and then the mixture was left for 5 minutes. Then, 0.5 ml of 5% $AlCl_3$ was added. After the mixture was left for 6 minutes, 0.5 ml of 1 M NaOH was added. After 10 minutes, the absorbance was read at 510 nm. TFC was calculated based on a calibration curve created using the epicatechin standard. Results are given as mg epicatechin equivalent (ECE)/g dry weight [24].

Total Anthocyanin Analysis

The total anthocyanin contents of the extracts were determined by the pH differential method. The absorbances of the extracts mixed with pH 1 and 4.5 buffers were determined at 510 and 700 nm. Expressed as mg cyanidin-3-glucoside equivalent (mg CGE/g) per g of total anthocyanin [25].

Determination of Individual Phenolic Compounds

The samples were extracted with different solvents and the phenolic compound compositions were determined through LC-MS/MS. Liquid chromatography and Tandem MS mass spectroscopy were used in the study. MS/MS conditions, operating conditions, and Gradient program are given in Tables 1 and 2. A total of 10 phenolic compounds (prigallol, procateutic acid, procateutic aldehyde, catechin, chlorogenic acid, epicatechin, caffeic acid, taxifolin, luteolin-7-glycoside, and rutin) could be identified in *S. excelsa*.

Table 1. *MS/MS instrument conditions*

Capillary temperature	300 °C
Vaporizer temperature	350 °C
Sheat gas pressure (Arb)	30
Aux gas pressure (Arb)	13
Spray voltage (V) (Positive polarity)	4000
Spray voltage (V) (Negative polarity)	2500
Discharge current (µA)	4.0

Table 2. *Sample information and analysis test conditions.*

	Minute	**%A (0.1% Formic acid water)**	**%B (Methanol)**
Solvent Program	0	100	0
	1	100	0
	22	5	95
	25	5	95
	30	0	100
Solvent flow rate	0.7 ml/minute		
Column oven temperature	30 °C		
Column properties	ODS HYPERSIL 4,6*250 mm 5 µm colon		
Injection volume	20 µl		
Analysis time	34 Minutes		

Determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Effect

The method reported by Tural and Koca [26] was modified to determine the scavenging effect of DPPH radical (2,2-diphenyl-1 picrylhydrazyl). A solution of 50 µl of the extract was prepared and prepared and then mixed with 1 ml of a solution of 0.06 mM DPPH. The mixture was shaken and left in the dark for 2 hours until the reaction was complete. At the end of the reaction, the absorbance at 517 nm was recorded. The same process was repeated without extract and only solvents, and the DPPH free radical scavenging effect was determined using the following formula:

$$\text{Reduction (\%)} = \left(\frac{\text{A control sample} - \text{A sample}}{\text{A control sample}}\right) \text{x } 100$$

The DPPH radical scavenging effect was calculated by a calibration curve using Trolox as the standard. Results are given in mmol Trolox equivalent (TE)/g.

Determination of Iron Reducing Antioxidant Power (FRAP)

50 µl of the extract was mixed with 950 µl of FRAP solution containing 100 mM acetate buffer, 10 mM $FeCl_3$, and 10 mM TPTZ (2,4,6-tri-pyridyl-s-triazine). The mixture was shaken for approximately 5 minutes and the absorbance was read against the blank solution at 593 nm. FRAP values of the extracts were calculated with the help of a calibration curve prepared using Trolox as a standard. Results are given as mmol Trolox equivalents (mmol TE/g dry weight) [27].

ABTS Radical Scavenging Capacity

0.2 ml of the extract was mixed with 2 ml of $ABTS^+$ radical cation solution. Then, the mixture was left in the dark for 2 hours and measurements were made using a spectrophotometer at 734 nm. Results are expressed as mmol of Trolox equivalents per gram (mmol TE/g) [28].

Disc Diffusion Test Method

The antimicrobial activities of the extracts were determined by the disc diffusion method by Bauer et al. [29]. Five of the human and plant pathogens kept at -80°C in the Microbiology Laboratory at Ondokuz Mayis University were selected and cultivated with the help of a loop. Nutrient agar (NA) and Sabouraud dextrose agar (SDA) [30] media were used for incubation of pathogenic bacteria and fungus, respectively. The pathogen strains used in the study are given in Table 3.

Table 3. *Pathogenic strains were used in the study.*

Pathogenic strain	Group/Host	Medium	Incubation temperature
Aspergillus niger ATCC 16404	Fungus/Plant	SDA	30
Bacillus subtilis ATCC 6633	Bacteria/Human	Nutrient Agar	37
Enterococcus faecalis ATCC 29212	Bacteria/Human	Nutrient Agar	37
Klebsiella pneumoniae ATCC 700603	Bacteria/Human	Nutrient Agar	37
Staphylococcus aureus ATCC 25923	Bacteria/Human	Nutrient Agar	37

The Petri dishes were allowed to briefly dry at room temperature, then bagged and inverted, and incubated at 37°C for *A. niger* and 30°C for bacteria with an incubation period of 48 hours. At the end of 48 hours, the microbial growth and purity of the Petri dishes were checked. Cotton wool was placed in the mouth of the flasks and the cotton wool was covered and wrapped with aluminium foil. The flasks were autoclaved to sterilize the media before the bacterial and fungal inoculation. At the end of autoclaving, each of the pathogens was transferred to 20 ml Nutrient broth medium in 50 ml Erlenmeyer flasks. Then, each flask was placed in a shaking incubator running at 150 rpm and incubated at the specified temperature given above for both fungus and bacteria for 48 hours. After incubation period, the turbidity was checked in the media. Microbial suspensions were prepared as equivalent to 0.5 McFarland standard (approximately 1.5×10^8 cfu/ml for bacteria and 1.0×10^6 cfu/ml for fungi) within glass tubes containing 10 ml Nutrient and Sabouraud dextrose broth using a spectrophotometer. Then,

200 µl of each solution was taken and the pathogens were cultivated on a Nutrient agar and Sabouraud dextrose media by spreading plate method. Petri dishes were allowed to dry in a sterile cabinet for 30 minutes. Meanwhile, the empty discs were held with a sterile clamp and 20 µl of the plant extracts to be tested for antimicrobial activity and the solutions to be used as negative controls were drawn with a pipette and impregnated on these discs. Then, the discs were carefully placed on the Petri dishes where the pathogens were cultivated with the help of forceps. As negative controls, 70% ethanol, 80% methanol or water was used as appropriate for each extract. Discs containing the appropriate antibiotic to be used as a positive control were also placed in the Petri dishes (Table 4). For each pathogen, the study was performed in 3 replicates. A total of 7 discs were placed on each Petri dish. After placing the discs, the Petri dishes were bagged and inverted and incubated at 30°C and 37°C for 48 hours. At the end of the incubation period, each Petri dish was examined to see whether a clear zone formed around the discs. The diameter of the zone was measured with a ruler in the Petri dishes in which zone formation was observed and recorded [31].

Table 4. *Antibiotics used according to pathogenic strains in the disc diffusion method*

Pathogenic strain	**Antibiotics as positive control**
Aspergillus niger ATCC 16404	Amphotericin B (10 µg/disc)
Bacillus subtilis ATCC 6633	Amoxycillin (10 µg/disc)
Enterococcus faecalis ATCC 29212	Penicillin G (10 µg/disc)
Klebsiella pneumoniae ATCC 700603	Amoxycillin (10 µg/disc)
Staphylococcus aureus ATCC 25923	Penicillin G (10 µg/disc)

Minimum Inhibition Concentration (MIC) Test

MIC test was performed to determine the lowest concentration at which the inhibitory effect of the water extract, which gave positive results in the disc diffusion test, on E. faecalis ATCC 29212. For this purpose, a 96-well plate was used. Cells of the pathogen previously grown on Nutrient agar were removed with a toothpick and transferred to glass tubes containing 10 ml Nutrient Broth. Then, a Nutrient Broth solution with a McFarland value of 0.5 was obtained by diluting the solution in this glass tube. Then, 500 µl of distilled water was aseptically added to each of the 9 sterile centrifuge tubes. Using a pipette, 500 µl of the water extract in the capped glass tube was taken and transferred to the centrifuge tube containing 500 µl of distilled water. Thus, the concentration of the main extract was halved. Then, 500 µl was taken from this tube and transferred to the next tube in the same way and the process was continued until the 9th centrifuge tube. 200 µl of sterile Nutrient broth was added to the 12th well of the 96-well plate and 200 µl of bacterial solution was added to the 11th well. 100 µl of the bacterial solution was added to wells 1-10. 100 µl of the master stock of the water extract (undiluted) was added at the 1st well. In the 2nd-10th wells, 100 µl of the extracts diluted from high concentrated to low concentrated were added. The study was carried out in 3 replicates in each well. Then, the plate was covered with a

sterile lid and incubated at 37°C for 24 hours. At the end of the incubation, the turbidity in the wells was evaluated with the naked eye.

Statistical Analysis

The data were evaluated according to one-way analysis of variance (ANOVA) in the SPSS 22.0 package program (licensed by Ondokuz Mayıs University). The differences between the averages were determined by Duncan's multiple comparison. All measurements were performed in triplicate.

Results and Discussion

Phytochemical Contents

In this study, the shoots as edible parts of *S. excelsa* were extracted with different solvents and the phytochemical properties, and the antioxidant and antimicrobial activities of the extracts prepared by ultrasound-assisted extraction were determined. In the study, it was determined that the water content of fresh plants was 88.57±0.67% and the water content of plant powder was 8.93%. The mean *L** (brightness) value was 24.83± 4.84, *a** (redness) value was 5.41±1.03, and *b** (yellowness) value was 31.85±1.85. Topdas et al. [32] measured the color of lyophilized green (LY) and lyophilized reddish (LK) *S. excelsa* samples. As a result of their study, they determined the brightness (*L**) value as 50.01 in the LY sample and 40.93 in the LK sample; redness (*a**) value as -7.75 in the LY sample and 7.17 in the LK sample; yellowness (*b**) value as 30.03 in the LY sample and 14.00 in the LK sample. The brightness and redness values determined by the researchers are higher than our findings, while the yellowness value is lower. This situation indicates that our samples have a more intense yellowness tone. The main reason for the difference in color values from literature is the difference in water content between the samples [33]. The total phenolic, flavonoid, and anthocyanin contents and the antioxidant activities (DPPH, FRAP, and ABTS) of *S. excelsa* extracts are given in Table 5.

Table 5. *The phytochemical and antioxidant properties of the extracts from different solvents*

Solvent	Total phenolic content, mg/g	Total flavonoid content, mg/g	Total antosiyanin content, mg/g	DPPH, mmol/g	FRAP, mmol/g	ABTS, mmol/g
Water	49.62±0.53^{b}	22.96±0.81^{a}	0.19±0.01^{b}	807.54±198.07^{b}	918.05±226.11^{b}	322.17±38.10^{b}
Ethanol	75.09±0.73^{a}	22.50±0.59^{a}	0.11±0.07^{c}	1868.38±282.35^{a}	2000.46±92.70^{a}	326.66±39.38^{b}
Methanol	74.72±2.51^{a}	21.40±0.96^{a}	0.52±0.07^{a}	1701.51±63.21^{a}	2030.84±67.83^{a}	684.10±120.66^{a}

There is no statistical difference between the averages indicated with the same letter in the same column (P>0.05)

The total phenolic content of the *S. excelsa* varied significantly according to the solvent (P<0.05). The total phenolic content of the extract prepared with water was lower than the others. The extracts prepared with 70% ethanol and 80% methanol had higher phenolic contents (75.09 mg/g and 74.72 mg/g, respectively). There was no statistically significant difference between the ethanol and methanol extract

groups (P>0.05). Different results were obtained in various studies. For example, Miser-Salihoglu et al. [34] found the total phenolic content of the extract obtained from shoots and roots of *S. excelsa* to be 645.38 µg/ml gallic acid. Yılmaz-Sarıaltın et al. [18] investigated the total phenolic content of *S. excelsa* extracts prepared with different solvents by HPLC. The authors determined phenolic amounts as 402.94 mg GAE/g in the methanol extract and 366.46 mg GAE/g in the water extract of *S. excelsa*. As can be seen, the phenolic content obtained from the methanol extract was higher than that of the water extract. These findings are compatible with the results obtained in our study. However, when analyzed quantitatively, it was determined that the total phenolic content obtained from our study was lower than that determined by Yılmaz-Sarıaltın et al. [18]. Demir [12] determined the total phenolic contents in the ethanol, methanol, and water extracts of fresh shoots of *S. excelsa* as 4.96, 4.94, and 4.71 mg/g, respectively, which were considerably lower than the results obtained from our study. Şahin [35] proved that the total phenolic contents in the ethanol extract of *S. excelsa* leaves varied between 53.57-61.68 mg gallic acid/g depending on the solvent concentration and time, which was close to the results of our study. According to our results, the total flavonoid content did not change much depending on the solvent. The extracts prepared with water (22.96 mg/g) and ethanol (22.50 mg/g) solvents had the highest flavonoid contents, but there was no statistically significant difference (P>0.05). Yılmaz-Sarıaltın et al. [18] determined the total flavonoid content as 191.42 mg QE/g in the methanol extract and 109.96 mg QE/g in the water extract of *S. excelsa*. The authors also noted the high flavonoid contents in parallel with the high total phenolic contents, which are higher than our findings. When the anthocyanin contents were compared, it was determined that the extract prepared with 80% methanol gave higher results (0.52 mg/g) than the other extracts. Ozsoy et al. [19] found that the passage of antioxidant compounds was different depending on the solvent and preparation technique in the extraction of water, ethanol, and ethyl acetate of the leaves of *S. excelsa*. The researchers found that the total phenolic contents of the water and ethanol extracts (30.6 mg/g and 30.1 mg/g, respectively) were higher than that of ethyl acetate (8.8 mg/g). They found the highest flavonoid content in the ethanol extract (28.7 mg/g), which is almost similar to the results obtained in our study. Ozsoy et al. [19] determined the total anthocyanin content in leaves as 0.32 mg/g, which is much higher than the total anthocyanin content obtained in the methanol extract in our study. Al Yassine et al. [36] determined that the total phenolic content in the water extract of stems and leaves of *S. excelsa* was significantly higher than the ethanol extract. The results of our study do not coincide with the results of this study because the water extract gave the lowest result. Significant differences were found in the phenolic compound compositions of the *S. excelsa* extracted in different solvents (Table 6).

Table 6. *Phenolic compound composition of Smilax excelsa (µg/g).*

	Parent	Product	Collision energy	Polarity	Water	Methanol	Ethanol
Pyrogallol	124.86	69.31 79.28	20 23	− −	7.93±0.00^{b}	11.80±8.35^{a}	5.62±0.00^{c}
Procatechuic acid	155.01	65.40 93.20	22 13	+ +	2.75±0.00^{b}	Nd	5.75±0.66^{a}
Procatechuic aldehyde	136.9	92.25 108.20	25 25	− −	nd	Nd	3.04±1.25
Catechin	289.2	203.90 245.70	22 17	− −	nd	1250.77±31.75^{a}	1116.14±20.87^{b}
Chlorogenic acid	353.4	86.50 192.10	43 21	− −	nd	2246.13±0.86	2253.17±34.62
Epicatechin	291.5	123.30 139.30	15 16	+ +	3.12±2.21^{b}	124.38±4.63^{a}	106.26±12.83^{a}
Caffeic acid	179.7	135.20 136.20	27 18	− −	nd	0.42±0.04^{a}	0.21±0.15^{b}
Taxifolin	303	126.20 285.50	23 15	− −	0.21±0.00^{c}	3.31±0.09^{a}	2.95±0.06^{b}
Luteolin-7-glucoside	446.89	284.00 285.00	45 40	− −	0.14±0.05	0.12±0.01	0.17±0.06
Rutin	609.37	300.60 301.70	38 34	− −	4.95±0.50^{b}	494.06±26.53^{a}	500.31±3.07^{a}

nd: not detected. There is no statistical difference between the means indicated with the same letter on the same line (P>0.05).

Among all compounds, the highest values were found for chlorogenic acid in the methanol and ethanol extracts (2246.13 µg/g and 2253.17 µg/g, respectively). This situation indicates that both solvents provide high yields in the extraction of chlorogenic acid. Chlorogenic acid is an important compound with multiple pharmacological properties such as antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic [37], anti-hypertensive, anti-viral, neuroprotective, hepatoprotective, central nervous system stimulant, anti-proliferative, cardioprotective, and anti-obesity. Al Yassine et al. [36] examined the phenolic compound profile of *S. excelsa* and found protocatechuic acid (5.35 mg/100 g), chlorogenic acid (5.91 mg/100 g), and chlorogenic acid derivatives (9.76 mg/100 g) as the main phenolic acids. In particular, chlorogenic acid and its derivatives are known as compounds that increase the antioxidant capacity of the plant. Compounds such as protocatechuic acid and chlorogenic acid contribute significantly to the antioxidant potential of the plant since they contribute to cell protection with their free radical scavenging activities. In our study, especially chlorogenic acid is dominant. In this respect, the results of our study are consistent with the results of Al Yassine et al. [36]. In the present study, catechin, a flavonoid, was found at high levels in the methanol and ethanol extracts (1250.77 µg/g and 1116.14 µg/g, respectively). Catechin prevents cell damage and reduces oxidative stress by fighting free

radicals [38]. Rutin is an important flavonoid that supports the antioxidant capacity. The amount of rutin was found to be 500.31 µg/g in the ethanol extract and 494.06 µg/g in the methanol extract. It was reported that rutin can effectively eliminate free radicals and inhibit lipid peroxidation [39]. Cellat et al. [40] determined that protocatechuic acid was 0.416 mg/g, protocatechuic aldehyde was 0.072 mg/g, catechin was 2.203 mg/g, chlorogenic acid was 3.218 mg/g, epicatechin was 1.720 mg/g, caffeic acid was 0.043 mg/g, and luteolin was 0.15 mg/g in the ethanol extract obtained from the leaves of *S. excelsa*, but they could not detect rutin. Yılmaz-Sarıaltın et al. [18] showed that chlorogenic acid (2.78 mg/g) and caffeic acid (0.22 mg/g) were particularly high in the methanol extract of S. excelsa. The researchers also detected rutin (0.126 mg/g) in the *S. excelsa* extract. In the present study, it was also found that *S. excelsa* contains rutin and is rich in chlorogenic acid. On the other hand, luteolin-7 glycoside and taxifolin were found at the lowest amounts. Taxifolin is one of the flavonols, a subclass of flavonoids. They are potent phenolic compounds and are a food and dietary supplement. Taxifolin is a powerful antioxidant and fights against antiradical activities. It is used to minimize or prevent lipid oxidation in food products, delay the formation of toxic oxidation products and maintain nutritional quality [41]. Furthermore, this compound has been reported to show promising inhibitory activity against oxidative stress, microbial infection, inflammation, cardiovascular disease, and liver disease [42]. As a result of our study, it was recorded that the amounts of taxifolin varied between 0.21-3.31 µg/g. Luteolin-7-glycoside was very low in all extracts, 0.14 µg/g in the water extract, 0.12 µg/g in the methanol extract, and 0.17 µg/g in the ethanol extract. Similarly, the amounts of caffeic acid were also found at very low levels in the methanol and ethanol extracts (0.42 µg/g and 0.21 µg/g, respectively). These results showed that methanol and ethanol were more effective in the extraction of some phenolic compounds, while water provided low extraction efficiency, especially for compounds other than chlorogenic acid and catechin. Different information on the phenolic compound composition of *S. excelsa* was found in literature. For example, Demir [12] investigated the phenolic compound composition of S. excelsa and identified six phenolic acids. These were gallic acid (117.33 µg/g), vanillic acid (33.89 µg/g), caffeic acid (4.55 µg/g), ferulic acid (93.78 µg/g), rosmarinic acid (0.33 µg/g), and hydroxycinnamic acid (0.33 µg/g). The researcher reported that gallic acid, vanillic acid, and ferulic acid were the dominant phenolic acids in *S. excelsa*. The phenolic compound composition determined in the present study is different from the findings of Demir [12]. In another study, Khaligh et al. [13] reported the presence of five major compounds in *S. excelsa*, namely solanesol, violasterol A, trans-resveratrol, 5-O-caffeoylchikimic acid, and 6-O-caffeoyl-β-d-fructofuranosyl-(2-1)-α-d-glucopyranoside. Cellat et al. [40] reported the presence of twenty-four different phytochemicals, including phenolic acids and flavonoids, in the ethanol extract of *S. excelsa*. The phenolic compound composition determined by the researchers overlaps with the compounds detected in our study. The results obtained from both our study and other studies explain the differences in the total phenolic, flavonoid, and anthocyanin contents as well as the differences in the phenolic compounds composition as affected by many factors such as growing

conditions, soil structure and geographical region, collection time, climatic conditions, solvent type, and genetics.

Antioxidant Activity

According to the antioxidant activity (DPPH, FRAP, and ABTS) results, it was determined that the extracts prepared with 70% ethanol and 80% methanol solvents showed high antioxidant activities, especially in the DPPH and FRAP tests. The 70% ethanol extract had the highest value (1868.38 mmol/g) in the DPPH test, while the 80% methanol extract gave the highest value (2030.84 mmol/g) in the FRAP test. At the ABTS value, the 80% methanol extract exhibited a higher antioxidant capacity (684.10 mmol/g) compared to the others. In various studies, the antioxidant activities in the *S. excelsa* extracts prepared with different solvents were investigated. Yılmaz-Sarıaltın et al. [18] determined that in the DPPH test, the methanol extract of *S. excelsa* showed the strongest effect with an IC_{50} value of 45.81 µg/ml, while the water extract had a lower effect with an IC_{50} value of 57.49 µg/ml. These results reveal that the methanol extract is stronger than the water extract in terms of antioxidant capacity. In the ABTS test, the authors recorded the IC50 value of the methanol extract as 17.16 µg/ml and the value of the water extract as 21.31 µg/ml [17]. These results are consistent with the findings of our study, where we found that both the DPPH and FRAP values of the methanol extract were higher than those of the water extract. Ozsoy et al. [19] noted that the DPPH free radical scavenging value of the water extract of S. excelsa was higher than that of the ethanol extract, which does not coincide with the findings of our study. These researchers attributed the antioxidant activity of Smilax rhizomes to different compounds such as proanthocyanidins, trans-resveratrol, naringenin, 1-O-trans-p-coumarolglycerol, etc. [19]. In the current study, it has been proven that the most effective extract in terms of DPPH free radical scavenging effect was obtained with ethanol, and in terms of FRAP and ABTS-reducing activities, the most effective extract was obtained with methanol. Oğuz Akın et al. [43] found that the fruit extract of *S. aspera* exhibited 61% DPPH radical scavenging activity. Yıldız et al. [44] examined the characteristics of two different Smilax species growing in Hatay and its surroundings, and they found that the FRAP value of *S. excelsa* was 62.28-64.07 mmol Fe+2/kg in the leaves, which are much lower than our findings. As the researchers reported, these differences may result from climatic conditions or genotypes. Demir [12] prepared the extracts with different solvents from the fresh shoots of *S. excelsa* growing in Düzce and compared the antioxidant properties of the extracts. As a result of the study, the DPPH activities were listed as methanol>ethanol>water, while the ABTS activities were listed as ethanol≥ methanol>water. The fact that the water extract showed the lowest activities in both cases coincides with our study. The shoots and leaves of *S. excelsa* are sources of biologically active phytochemicals, including various phenolic compounds [45], and it has been proven that the main chemical components responsible for antioxidant activity consist of flavonoids and anthocyanins [19]. In our study, it has been proven that the extracts are rich in phenolic and flavonoid contents, and

especially the ethanol and methanol extracts with high phenolic amounts show high DPPH and FRAP values, which is attributed to the fact that phenolic compounds may have a strong antioxidant effect.

Results of Antimicrobial Activity and Minimum Inhibition Concentration (MIC)

In this study, the efficacy of different solvent extracts of S. *excelsa* against one fungus, three gram (+) bacteria, and one gram (-) bacteria was investigated. As a result of the disc diffusion test, a clear zone was observed around all discs used as positive controls. In the Petri dish in which the *E. faecalis* ATCC 29212 pathogen was cultivated, a clear zone formation was observed around the disc in which only the water extract was impregnated (Figure 2). The zone diameter for this Petri dish was measured with the help of a ruler (Table 7). Apart from this, no zones were formed in the other Petri dishes.

Figure 2. *Antibacterial activity of the Smilax excelsa extracts against Enterococcus faecalis* ATCC 29212. *1, methanol extract (80%); 2, ethanol extract (70%); 3, water; 4-6, negative control for methanol extract (80%), ethanol extract (70%) and water, respectively; 7, positive control*

Table 7. *Zone diameters of the water extract giving positive results in the disc diffusion test.*

Extract	Pathogen	Average zone diameter in positive control (cm) ± SD	Average zone diameter in extract (cm) ± SD
Water extract	*Enterococcus faecalis* ATCC 29212	2.10 ± 0.08	1.17 ± 0.05

SD: standard deviation

MIC test was performed to determine the lowest concentration at which the inhibitory effect of the water extract, which gave positive results in the disc diffusion test, on *E. faecalis* ATCC 29212 was achieved. At the end of incubation, the turbidity in the wells was evaluated with the naked eye. Accordingly, it was observed that there was no turbidity in the 12th well containing sterile medium, and therefore the study was carried out aseptically. In the wells containing the extract, an increased turbidity was observed in the 4th-11th wells compared to the previous 24 hours, but no such change was detected in the first 3 wells. As a result, it was found that the concentration of the extract in the 3rd well was the lowest concentration required for the inhibition of the pathogen. The minimum inhibitory concentration (MIC) of the water extract against *E. faecalis* ATCC 29212 was determined to be 10 mg/mL (Figure 3).

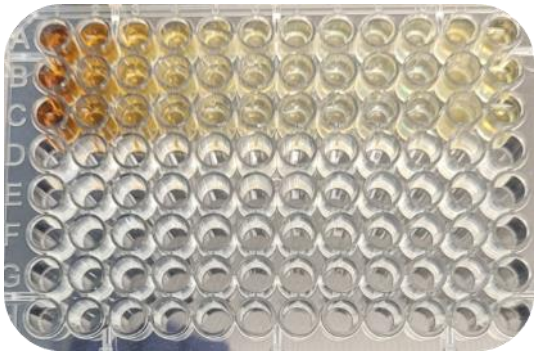

Figure 3. *Minimum inhibition concentration tests of the water extract against Enterococcus faecalis using a 96-well plate assay (conducted in triplicate)*

It was documented in a study that the extracts made from the fresh shoots of *S. excelsa* exhibited the antibacterial and antifungal activities [46]. Efe et al. [47] proved that the fruit extract of *S. excelsa* has an inhibitory effect against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus epidermidis, Candida krusei, Candida albicans*, and *Streptococcus mutans*. Researchers have determined different antimicrobial activities against all tested microorganisms; the maximum antimicrobial effect was recorded with a 16 mm inhibition zone against *E. coli*, while the lowest antimicrobial effect was reported to be obtained with an 11 mm inhibition zone against *S. aureus*. Khaligh et al. [13] reported that the compounds violasterol A and trans-resveratrol (3), obtained from the ethyl acetate extract of S. excelsa, showed activity against *S. aureus* with MIC values of 142.5 and 136.9 μM, respectively. Oğuz Akın et al. [43] found the MIC values of the ethanol extracts of *S. aspera* fruits against *E. coli* and *C. albicans* as 31.25 μg/ml. On the other hand, no antimicrobial activity of the methanol and ethanol extracts of *S. excelsa* was observed in this study, and the activity against *E. faecalis* ATCC 29212 strain was only found in the water extract. This situation reveals the effect of the choice of solvent. In addition, it is thought that the necessity of a synergistic effect in the antimicrobial activities of the *S. excelsa* extracts or the lack of stability of the compounds enabled these results to be obtained.

Conclusion

As a result of this study, it was determined that different solvent extracts of shoots, which are the edible parts of *S. excelsa*, differ in terms of phytochemical properties, and antioxidant and antimicrobial activities. In this context, it can be said that the ethanol and methanol extracts are more effective in terms of both phytochemical properties and antioxidant activities compared to the water extract. In particular, the high amounts of phenolic compounds in the ethanol and methanol extracts brought about high antioxidant activities. According to this result, it can be concluded that the edible parts of *S. excelsa* have an essential potential in terms of health and food. Plants are used in the treatment of various diseases. In addition, secondary compounds obtained from plants also have deterrent or toxic effects on pests. For this reason, it is recommended to determine the effects of the extracts that can be obtained from the edible wild plant or the secondary compounds that can be isolated from the plant on different

organisms. In line with the results obtained, it is suggested that, due to the antioxidant properties exhibited by *S. excelsa*, this plant can be an important source for different medicines and can be integrated into many treatment methods in modern medicine.

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Authors Contribution All authors read and approved the final manuscript.

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