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ORIGINAL ARTICLE

Biochemical components, enzyme inhibitory, antioxidant and antimicrobial activities in endemic plant *Scilla mesopotamica speta*

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Abstract

In this study, in vitro antioxidant, antimicrobial, anticholinesterase and phenolic profile of different solvent extracts of *Scilla mesopotamica speta* were determined in detail. In vitro antioxidant activities and total phenolic and flavonoid contents of plant extracts obtained with different solvents were tested in terms of 2.2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities. The highest total phenolic and total flavonoid contents were determined in the ethyl acetate extract (62.24 μg GAE/mg) and chloroform extract (87.72 μg QE/mg) respectively. The highest DPPH radical scavenging activity was detected in ethyl acetate extracts. Antimicrobial and antifungal activities were investigated by MIC method. The inhibitory activities of the extracts on the acetyl cholinesterase enzyme were investigated. Liquid chromatography (LC) tandem mass spectrometry LC-MS/MS was used to determine the phenolic component content of extracts. Thirty-one different components were identified in the analyses and their amounts were measured.

Practical applications

Scilla mesopotamica speta is an endemic and medicinal plant. It was determined that the extracts of this plant had a very rich content in terms of phenolic compounds, especially caffeic and ferulic acids. However, this plant was remarkable for its antioxidant, anticholinesterase, and antimicrobial activities. Considering the strong antioxidant, antimicrobial, and enzyme inhibition activities of the Scilla mesopotamica speta it can be suggested as a source of anticancer, antimicrobial, and antiviral drugs.

1 | INTRODUCTION

Free radicals are molecules that usually contain an oxygen atom that has lost an electron. This makes them unstable. This structure causes them to covet the electrons of neighboring molecules. Due to these properties, free radicals cause various cell damages in the human and animal body, causing many diseases, from gastrointestinal diseases to infertility, from cardiovascular diseases to respiratory and excretory system disorders, and neurodegenerative diseases. In order to prevent these diseases, which are associated with the level of free radicals, this oxidative damage should be kept in balance with antioxidants (Alkadi, 2020).

Since the antioxidant effect of phenolic compounds reduces the oxidative process, they play an important role in controlling oxidative changes in both the human body and food systems (İnan et al., 2018). In recent years, interest in antioxidant and antimicrobial phenolic compounds has increased due to the indiscriminate use of antibiotics and the resistance of human pathogenic bacteria to drugs. It has been determined in different studies that these compounds prevent the development of various bacteria and fungi

species in the environment and can prevent the spread of various infectious diseases that may arise from these microorganisms and are effective in the control of pathogens (Şahin et al., 2018).

It is reported that phenolic compounds found in all parts of plants at different levels inhibit different enzymes and have antiallergen, antimutagen, anticarcinogen, antiglycemic, anticholesterol, anti-inflammatory, antithrombotic, vasodilator, and calming properties (Tungmunnithum et al., 2018).

Acetyl cholinesterase (AChE) inhibitors inhibit the degradation of acetylcholine (Ach) by inhibiting the AChE enzyme (Emir et al., 2020). In the early stages of the disease, it increases the concentration and duration of function of the neurotransmitter ACh and may provide benefits in such patients (Pohanka, 2011). Polyphenols also have functions such as strengthening the human immune system, functioning as an antimicrobial, inhibiting viral replication, and reducing viral transmission which is through the host target cell membrane (Adem et al., 2020; Wang et al., 2021). It has been proven that natural phenolic compounds have a very high antimicrobial effect, interact synergistically with antibiotics, and can offer an alternative treatment approach in case of drug resistance of bacteria.

The Liliaceae family is a plant that is rich in natural bioactive components and is widely found all over the world. Some family types are often used in folk medicine to treat diseases traditionally related to inflammation and pain. However, available studies for this family are few and far between regarding its secondary metabolites (Yang & He, 2020; Zhou et al., 2021). *S. mesopotamica speta is in the Liliaceae family and is an endemic plant that grows in the Halfeti (Şanlıurfa) region. S. mesopotamica* speta has been used by local people for various diseases (Eker & Akan, 2010). It has been reported that *Scilla* species are used as antidote, blood thinner, anti-inflammatory, anti-tumor, and antioxidant in the treatment of thrombolytic, rheumatic fever, pain, bacterial, and fungal skin diseases (Munafo & Gianfagna, 2015). However, through the literature review done, it was determined that the biological activities of *S. mesopotamica speta* have not been studied yet.

This study aims to investigate the antioxidant, antimicrobial, and anticholinesterase enzyme activities of the extracts obtained from *S. mesopotamica speta*, which grows naturally in our habitat and the phenolic compounds responsible for these activities.

2 | MATERIALS AND METHODS

2.1 | Material

S. mesopotamica speta is a perennial, endemic, and herbaceous plant belonging to the *Liliaceae* family. It blooms in March and is frost tolerant (Munafo & Gianfagna, 2015). During the blooming period (March–April), immature tubers of *S. mesopotamica speta* were collected from Halfeti (rocky slopes). In the experimental studies, the subterranean parts of the plant (tuberous stem) were used. Plant samples were stored in the herbarium of Mardin Artuklu University (2020-5-MAU). The plant samples used in the study were collected by Dr Şükrü Akmeşe and identified by Dr Cumali Keskin in the same unit.

2.2 | Obtaining of plant extracts

The collected plant samples were dried at room temperature to constant weight and then pulverized. Thirty grams of ground herb sample was placed in Soxhlet's cartridge for extraction. Apolar and polar solvents were used in the extraction process. The obtained extracts were filtered with Whatman filter paper. Extracts kept under low temperature and vacuum were removed from their solvents. The crude extract amounts were determined as 0.520 g in the chloroform phase, 0.820 g in the ethyl acetate phase, and 1.200 g in the methanol phase. The obtained extracts were stored in a deep freezer at −20°C for later use.

2.3 | Preparation of plant stocks solutions

The concentrations of the stock solutions, which were prepared to be used in DPPH radical scavenging activity and antimicrobial activity studies, in ethyl alcohol in all extracts were 1 mg/ml for the quantitation of the total phenolic component in chloroform, ethyl acetate, and methanol extracts obtained from the *S. mesopotamica speta* plant, and 1, 2, and 5 mg/ml for the quantitation of the total flavonoid component.

2.4 | Quantitation of total phenolic content

In biological systems, phenolic compounds have functions in free radical inhibition, peroxide decomposition, metal inactivation, or oxygen scavenging and they prevent oxidative damage load (Heleno et al., 2015). The total amount of phenolic contents of extracts were determined according to the Folin-Ciocalteu method (Singleton & Rossi, 1965). A 5 mg/ml stock solution of gallic acid was prepared, and dilutions were made so that concentrations remained in the range of 50–500 μg/ml. Briefly, 1,160 μl of pure water and 200 μl of Folin-Ciocalteu's phenol reagent (2.0 N) were added to 40 μl of the prepared gallic acid and extract solutions, and they were mixed. It was kept at room temperature for 5 min and then 600 μl of 20% concentrated sodium carbonate solution was added and shaken in a shaking water bath for 2 hr at room temperature. Then, absorbance values at 765 nm were read in a spectrophotometer device (T80+UV/VIS Spectrometer).

Absorbance values corresponding to concentrations in the range of 50–500 μg/ml of gallic acid used as a standard were plotted. Plotting the standard curve yielded the value of $R^2 = 0.997$ and the following equation:

$$y = 0.0021x - 0.0347$$

2.5 | Quantitation of total flavonoid component

Flavonoids also cause complexation with ions that give color to flowers, vegetables, and fruits. Quantitation of the total flavonoid contents of extracts were determined by the modified method of Moreno (Popova et al., 2005). The absorbance was measured at a wavelength of 415 nm in the UV-VIS spectrophotometer. Pure water and ethanol were used as blanks (Zhishen et al., 1999). The absorbance values, corresponding to the concentration range of 5–100 μg/ml, of quercetin used as a standard were plotted ($y = 0.0152x - 0.0052$, $R^2 = 0.9992$).

2.6 | Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity was investigated in the concentration range of 5–500 μg/ml (Shekhar & Anju, 2014). The percent inhibition values of the DPPH radical scavenging activities of the BHT/BHA and extracts of *S. mesopotamica speta* species prepared in this concentration range with solvents of different polarity were found. As a result, the percent inhibition value due to increasing extract concentration was plotted and calculated in mg/mL. Percent inhibition values were calculated using the following formula and were tabularized.

$$\text{DPPH Scavenging Activity}(\%) = \left[\left(A_0 - A_1/A_0\right) \times 100\right]$$

A_0 = Absorbance of negative control sample (DPPH), A_1 = Absorbance of sample solution.

2.7 | Determination of phenolic component by LC-MS/MS

2.7.1 | Material and reagents

Analytical grade phenolic standards, formic acid, ammonium formate, and LC grade acetonitrile and methanol were provided commercially from Sigma-Aldrich (Germany). Pure water for chromatographic purposes was obtained by using the Milli Q water purification system (Millipore). All solutions were filtered using a captiva premium syringe filter that is polypropylene encased and has nylon membrane, 25 mm diameter and 0.45 μm pore size before LC-MS analysis.

2.8 | Preparation of compound solutions

All commercially provided phenolic compounds were used to develop the analytical method. The 1,000 mg/L master stock solution was prepared by dissolving standard compounds in solid form in methanol. The prepared stock solution was diluted with methanol: water (1:1). The prepared solutions were analyzed by LC-ESI-MS/MS.

2.9 | Mass spectrometry and chromatography conditions

The 1260 Infinity II LC System, which is high-performance liquid chromatography (HPLC) combined with a tandem mass spectrometer, was used to perform the qualitative evaluation of the compound. The reverse-phase HPLC is equipped with a column oven (1260 TCC), dual pumps (1260 Thousand Pumps), and a degasser (1260 Degasser). Chromatographic conditions were optimized to get optimum separation for the compound and to overcome suppression effects. Thus, chromatographic separation was carried out on an analytical column of a reverse-phase Agilent Poroshell 120 EC-C18 (100 × 3.0 mm, 2.7 μm). The column temperature was set to 25°C. The elution gradient was adjusted so that selective A was 5 mM ammonium formate in water, selective B was formic acid at 0.1% concentration in acetonitrile, the solvent flow rate was 0.250 ml/min, and the injection volume was 5 μl. The following gradient elution profile was used: 10% B (0–1 min), 40% B (1–3 min), 70% B (3–5 min), 40% B (5–6 min), and 10% B (6–8 min). Mass spectrometric detection was performed using an Agilent 6460 Triple Quadruple Mass Spectrometer System equipped with electrospray ionization (ESI) source operating in both negative and positive ionization modes. Acquisition and processing of LC-ESI-MS/MS data was done by Agilent Mass Hunter Software. The MRM method was optimized to selectively detect and quantify phytochemical compounds based on screening of ion transitions from the identified precursor phytochemical to the moiety. Collision energies (CE) were optimized to ensure optimal phytochemical degradation and maximum delivery of desired product ions. MS operating conditions were set so that the drying gas (N_2) flow was 15 L/min, the nebulizing gas (N_2) flow was 11 L/min, the capillary (V) was 4,000, and the gas temperature was 350°C.

2.10 | Determination of anticholinesterase (AChE) enzyme inhibition

The AChE inhibitory activities of extracts obtained from *S. mesopotamica speta* with different solvents (chloroform, ethyl acetate, and methanol) were determined by the modified spectrophotometric method (Ellman et al., 1961). A commercial form of AChE isolated from *Electrophorus electricus* (electric eel) was used as the enzyme source (EC 3.1.1.7, Sigma). Acetylthiocholine iodide (AChI) was used as the substrate for cholinergic reactions. Some Tris/HCl buffer (1.0 M, pH 8.0) and extract solutions of *S. mesopotamica speta* (10–30 μg/ml) prepared at different concentrations were added to 50 μl of AChE enzyme solution (5.32×10^{-3}) to initiate the reactions. After the solution was incubated at 20°C for 10 min, equal amounts of DTNB (5.50-dithiol-bis 2-nitro-benzoic acid) and AChE (50 μl, 0.5 mM) were added to the mixture and enzymatic reactions were initiated. AChE activity was measured spectrophotometrically as 412 nm (Öztaşkın et al., 2015). Tacrine was used as a positive control in enzyme activity measurements of AChE.

2.11 | Determination of antimicrobial properties

Extracts from *S. mesopotamica speta* were adjusted to an initial concentration of 1 mg/ml. The inhibition effects of the extracts on the growth of pathogenic microorganisms in humans were determined using the minimum inhibition concentration (MIC) method. For this purpose, bacterial strains of gram-negative *(Escherichia coli* ATCC 25922) and gram-positive (*Staphylococcus aureus* ATCC 29213) bacteria and yeast of *Candida albicans* (ATCC 10231) were used. Vancomycin, fluconazole, and colistin were used as standard antibiotics for *S. aureus*, *C. albicans*, and *E. coli* (colistin), respectively.

2.12 | Statistical analysis

All the experimental data were replicated three times. The test data were expressed as means ± standard deviation (*SD*). Statistical analyses were performed using the statistical package SPSS 15.0.

3 | RESULTS

3.1 | Total phenolic contents

The total phenolic component amounts of the chloroform, ethyl acetate, and methanol extracts of *S. mesopotamica speta* were calculated as 59.86 ± 0.28, 62.24 ± 0.32, 35.89 ± 0.12 µg GAE/mg extract, respectively.

3.2 | Total flavonoid contents

Flavonoids, having a polyphenolic structure generally found in medicinal plants, are another important secondary metabolite that is commonly found in fruits, vegetables, tubers, and certain beverages. Total flavonoid contents in chloroform, ethyl acetate, and methanol extracts of S. *mesopotamica speta* were measured spectrophotometrically. The amount of total flavonoid component was measured in terms of quercetin equivalent (QE; Table 1).

In *S. mesopotamica speta*, it was determined that the chloroform extract contained a higher rate of flavonoids compared to other solvents.

3.3 | LC-MS/MS profile of extracts

Agilent 6430 Triple Quadrupole HPLC with mass spectroscopy (LC-MS/MS) was used to determine the phenolic profiles of *S. mesopotamica speta*. The standard chromatogram obtained is given in Figure 1. LC-MS/MS analysis results of standard compounds and extracts used for the determination of phenolic compounds are given in Table 2.

TABLE 1 Total flavonoid contents of *Scilla mesopotamica speta*

	1 mg/ml	2 mg/ml	5 mg/ml
Solution	**µg QE/mg extract**	**µg QE/mg extract**	**µg QE/mg extract**
Chloroform	17.54 ± 0.8[a]	35.09 ± 1.2	87.72 ± 1.8
Ethyl acetate	14.32 ± 0.6	28.64 ± 0.9	71.60 ± 1.4
Methanol	12.94 ± 0.5	25.88 ± 0.7	64.69 ± 1.3

[a]Data are presented as mean values; ±standard deviation (*SD*) of triplicate values.

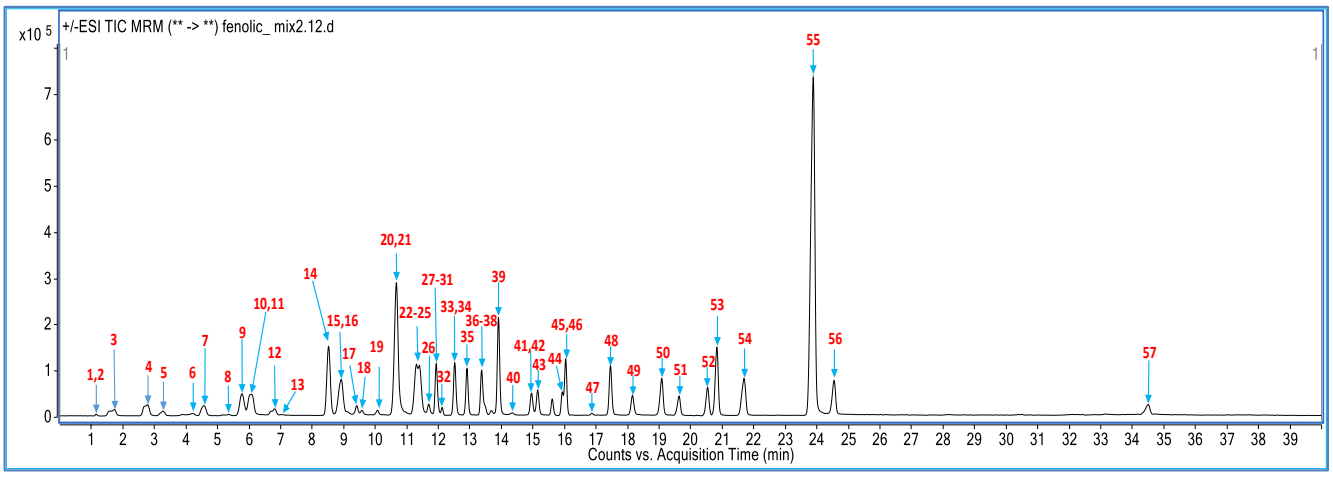

FIGURE 1 Standard chromatogram of LCMS/MS analysis. 1-2: Ascorbic acid, Shikimic acid, 3: Gallic acid, 4: Protocatechuic acid, 5: Genrretisic acid, 6: Catechin, 7: 4- Hydroxybenzoic acid, 8: Chlorogenic acid, 9: 4- Hydroxybenzaldehyde,10-11: Vanillic acid, caffeic acid, 12: Epicatechin, 13: Syringic acid,14: p- coumaric acid, 15-16: Salicylic acid, Taxifolin,17: Polydatin, 18: t- ferulic acid, 19: Sinapic acid, 20-21: Queersmethrin, Coumarin, 22-25: Scutellarin, o- coumaric acid, Cynarine, Protocatechuic ethyl ester, 26: Hyperoside, 27-31: Quercetın, 3-Glycoside, Rutin, Iso Quercetin, Resveratrol, Naringin, 32: Rosmarinic acid, 33-34: Quercetın, -3-o- Xyloside, Hesperidin 35: Neohesperidin, 36-38: Kaempferol-3-glycoside, Fisetin, Oleuropein, 39: Baicalin, 40: t- cinnamic acid, 41-42: Ellagic Acid, Quercetin, 43: Naringenin, 44: Silibinin, 45-46: Hesperetin, Morin, 47: Campherol, 48: Tamarixetine, 49: Baikalein, 50: 7-Hydroxyflavone, 51: 6- Hydroxyflavone, 52: Biokanin A, 53: Chrysin, 54: Flavone, 55: 5-Hydroxyflavone, 56: 6'2'4 trimethoxyflavone, 57: Diosgenin

TABLE 2 LC-MS/MS analysis results of standard compounds and *S. mesopotamica speta* extracts

Quantitative results											
Compounds	RT	Chloroform (μg/ml)	Ethyl acetate extract (μg/ml)	Methanol extract (μg/ml)	Ion Transitions (m/z)	Ion Mode	R^2	Linear (μg/ml)	LOD (μg/ml)	LOQ (μg/ml)	Recovery (%)
Tartaric acid	1.696	0.041	0.0340	ND	149–87	Neg	0.999	0.063–2	0.012	0.037	98.913
Citric acid	1.793	0.085	0.106	0.111	191.1–111	Neg	0.999	0.125–2	0.006	0.019	98.350
Ascorbic acid	1.802	0.051	0.059	0.080	175.1–114.9	Neg	0.999	0.063–2	0.008	0.024	96.700
Fumaric acid	1.821	0.016	0.017	0.037	115–71.1	Neg	0.999	0.031–2	0.006	0.019	99.757
Chicoric acid	1.989	0.564	ND	0.560	472.8–310.5	Neg	0.999	0.250–2	0.050	0.152	88.733
Gallic acid	2.605	ND	ND	ND	169–125	Neg	0.998	0.063–2	0.009	0.055	101.133
Chlorogenic acid	5.526	0.158	ND	0.159	353–191	Neg	0.999	0.250–2	0.065	0.196	88.833
4-Hydroxyibenzoic acid	6.531	0.044	0.043	0.052	137–93.1	Neg	0.999	0.063–2	0.002	0.007	94.667
Catechin	6.660	0.028	0.037	0.036	288.9245.1	Neg	0.999	0.063–2	0.003	0.008	98.033
Epicatechin	6.666	0.049	0.057	0.048	353–191	Pos	0.998	0.063–2	0.003	0.009	93.700
Hesperidin	6.674	ND	0.002	ND	611.3–357	Pos	0.999	0.125–2	0.033	0.099	102.767
Rutin	6.675	ND	ND	ND	608.9–299.4	Neg	0.997	0.125–2	0.029	0.085	101.333
Vanillic acid	6.687	0.115	0.083	0.228	167–151.8	Neg	0.998	0.063–2	0.003	0.008	95.600
Syringic acid	6.703	ND	ND	ND	197.1–181.8	Neg	0.999	0.063–2	0.004	0.013	101.900
Caffeic acid	6.703	0.168	1.933	0.005	178.9–135.1	Neg	0.999	0.125–2	0.026	0.078	100.700
Luteolin-7-glucoside	6.740	ND	0.021	ND	449–286.9	Pos	0.997	0.063–2	0.017	0.050	104.333
Apigenin-7-O-Glikozid	6.808	ND	ND	ND	430.8–267.4	Neg	0.998	0.125–1	0.018	0.055	96.533
Quercetin-3-Ksilozid	6.816	ND	ND	ND	432.7–299.5	Neg	0.995	0.063–2	0.010	0.030	103.767
Oleuropein	6.849	ND	ND	ND	539.1–275.1	Neg	0.999	0.063–2	0.017	0.053	102.367
Rosmarinic acid	6.875	0.023	0.030	0.017	358.9–160.7	Neg	0.998	0.063–2	0.016	0.048	96.067
P-coumaric acid	6.919	0.057	0.300	0.021	163–119	Neg	0.999	0.063–2	0.003	0.009	100.033
4-Hydroxybenzaldehyde	6.929	0.105	0.041	0.009	121–92	Neg	0.999	0.031–2	0.002	0.006	99.833
Trans-ferulic acid	6.968	0.501	0.295	0.041	193.1–133.9	Neg	0.998	0.031–2	0.007	0.022	95.900
Gentisic acid	7.243	ND	0.162	ND	153–109	Neg	0.999	0.250–2	0.045	0.135	98.267
Protocatechuic acid	7.243	0.056	0.030	0.027	152.9–108.9	Neg	0.999	0.063–2	0.015	0.047	96.853
Quercetin	7.306	ND	0.017	ND	300.7–150.9	Neg	0.997	0.063–1	0.015	0.045	101.667
Apigenin	7.555	0.027	0.056	ND	269–117	Neg	0.999	0.125–2,000	0.018	0.054	102.400
Naringenin	7.588	0.021	0.008	ND	270.9–119.1	Neg	0.999	0.125–2,000	0.024	0.074	100.267
Trans-cinnamic acid	7.591	0.041	0.027	0.029	148.8–104.8	Neg	0.999	0.063–2	0.014	0.041	101.333
Kaempferol	7.613	ND	0.229	ND	284.9–116.9	Neg	0.998	0.063–2	0.014	0.038	100.927

Abbreviations: LC-MS/MS, liquid chromatography tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; ND, not determined; RT, retention time.

The chromatogram of the chloroform, ethyl acetate, and methanol extracts of *S. mesopotamica speta* for the phenolic compound is given in Figure 2.

3.4 | Determination of DPPH radical scavenging activity

Their DPPH radical scavenging activity was measured to see the antioxidant properties of extracts of *S. mesopotamica speta* obtained through solvents with different polarity (Table 3). The radical scavenging level of a sample indicates the antioxidant potential that prevents the initiation of the oxidation chain. Decreasing sample absorption indicates a radical scavenging activity.

3.5 | Anticholinesterase (AChe) enzyme inhibition activity

Enzyme inhibition activities, in the concentration range of 0–0.25 μg/ml, of chloroform, ethyl acetate, and methanol extracts obtained from *S. mesopotamica speta* plant were measured spectroscopically (412 nm). Obtained results were plotted Figure 3a–c. The IC_{50} values of the extracts (chloroform, ethyl acetate, and methanol) and the standard inhibitor (tachrin) were measured as 40.06, 43.46, 38.69, and 98.5, respectively. Evaluating the results, it was observed that there was a parallelism between the increase in concentration and the level of inhibition in all extracts.

3.6 | Evaluation of antimicrobial properties

In the study, antimicrobial activity was determined by the microdilution method (MIC) on gram-positive *S. aureus*, gram-negative *E. coli* bacteria, and *C. albicans* yeast, which are nosocomial pathogens.

The highest inhibition level against bacterial strains of gram-positive and gram-negative bacteria and *C. albicans* yeast was that of ethyl acetate extract (Table 4).

4 | DISCUSSIONS

In this study, in vitro antioxidant, anticholinesterase, and antimicrobial properties and phenolic profile of extracts obtained from the

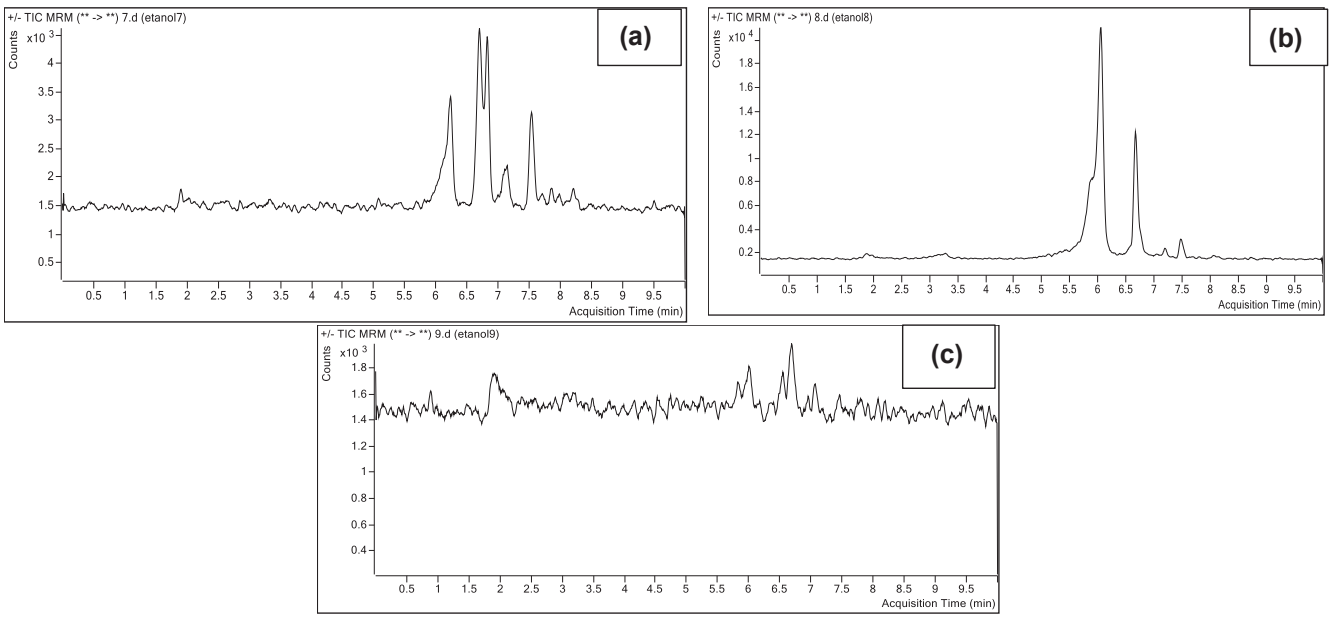

FIGURE 2 Phenolic compound chromatogram of Scilla mesopotamica speta plant (a) Chloroform (b) Ethyl Acetate (c) Methanol Extract

TABLE 3 DPPH radical scavenging activity of *S. mesopotamica speta* extract

% Activity (μg/ml)							
	5	25	50	100	250	350	500
Chloroform	4.15 ± 0.001[a]	10.80 ± 0.001	16.72 ± 0.002	29.39 ± 0.001	54.93 ± 0.001	70.20 ± 0.020	84.53 ± 0.001
Ethyl acetate	9.14 ± 0.001	15.16 ± 0.001	21.50 ± 0.001	36.86 ± 0.002	21.60 ± 0.002	78.40 ± 0.001	88.79 ± 0.001
Methanol	15.78 ± 0.001	20.98 ± 0.001	26.79 ± 0.001	31.36 ± 0.001	48.81 ± 0.001	65,63 ± 0.001	76.43 ± 0.020
BHA	40.24 ± 0.002	87.70 ± 0.001	90.78 ± 0.001	95.57 ± 0.001	98.46 ± 0.001	98.46 ± 0.001	98.57 ± 0.001
BHT	19.98 ± 0.005	40.51 ± 0.001	66.00 ± 0.001	81.92 ± 0001	91.59 ± 0.001	93.58 ± 0.001	93.67 ± 0.001

Abbreviations: BHA, butylated hydroxyl anisole; BHT, butylated hydroxyl toluene; DPPH, 2.2-diphenyl-1-picrylhydrazyl.

[a]Data are presented as mean values ± standard deviation (*SD*) of triplicate values.

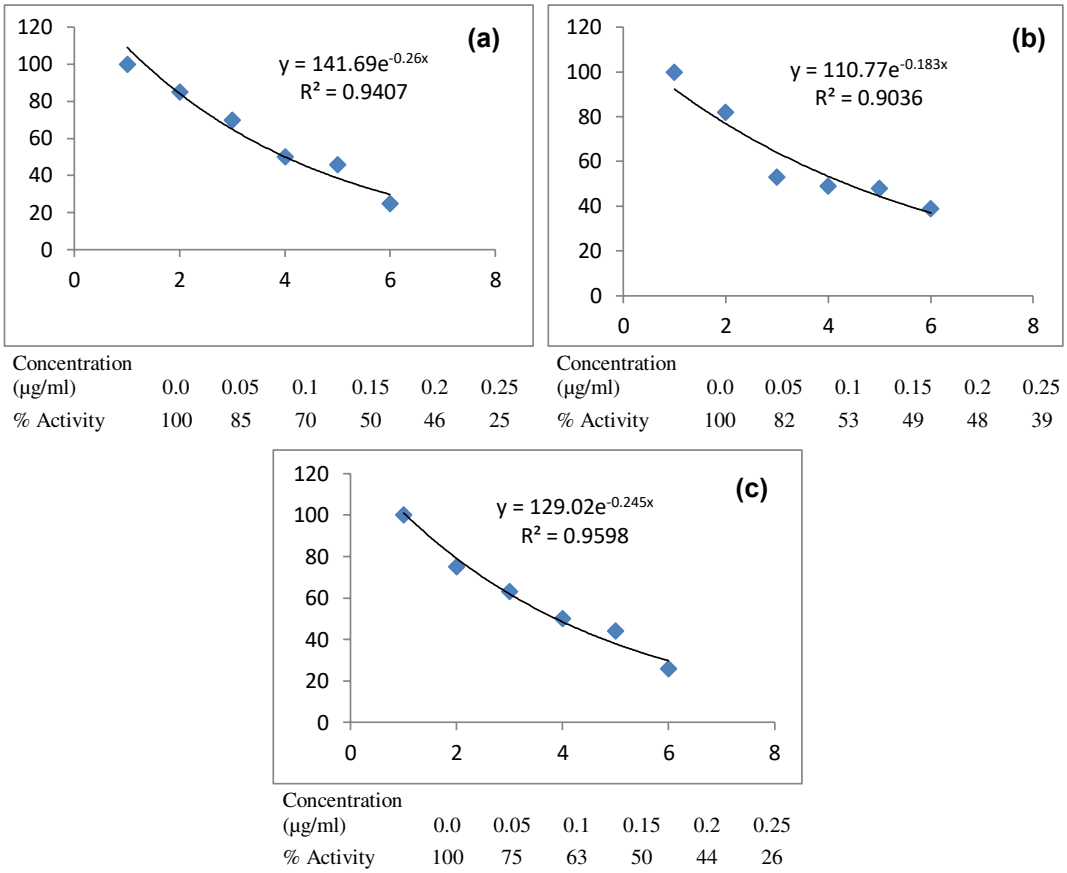

FIGURE 3 Anticholineesterase activity of Scilla mesopotamica speta (a) Chloroform (b) Ethyl Acetate (c) Methanol Extract

TABLE 4 Inhibition values of extracts from *Scilla mesopotamica speta* and standard antibiotics on *S. aureus* (vancomycin), *C. albicans* (fluconazole), and *E. coli* (colistin)

	Tested microorganisms	Chloroform [mg/ml]	Standard antibiotic [mg/ml]	Ethyl acetate [mg/ml]	Standard antibiotic [mg/ml]	Methanol [mg/ml]	Standard antibiotic [mg/ml]
Gram (+) bacteria strain	*S. aureus* (ATCC 2921)	0.0312	1.000	0.0156	1.000	0.125	1.000
Gram (−) bacteria strain	*E. coli* (ATCC25922)	0.0625	2.000	0.0312	2.000	0.0625	2.000
Fungi	*C. albicans* (ATCC 10231)	0.125	2.000	0.0156	2.000	0.125	2.000

endemic *S. mesopotamica speta* plant were investigated. It was determined that the chloroform extract contained highest phenolic components (62.24 ± 0.32 µg GAE/mg). In addition, as a result of LC-MS/MS analysis, Chicoric acid (0.564 µg/ml), Trans-ferulic acid (0.501 µg/ml), caffeic acid (0.165 µg/ml), and chlorogenic acid (0.158 µg/ml) amounts in the chloroform extract were again determined to be considerably high when compared with other extracts.

Chicoric acid, which is a hydroxycinnamic acid belonging to the phenylpropanoid family, is found in the roots of many plants grown in the Mediterranean Region. Many of these plants are used as alternative treatments or nutritional supplements (Peng et al., 2019). Chicoric acid is a phenolic compound that promises a wide range of biological activities due to its antioxidant, anticancer, immune-modulatory, anti-obesity, anti-diabetic, and antiviral properties (Peng et al., 2019). Awwad et al. (2020) investigated chicoric acid within the scope of many species that belong to the *Asteraceae* family, detected it at a high rate, and determined its curative effects in metabolic syndrome. McDougall et al. (1998) showed that varying concentrations of it inhibit the integration and replication of HIV. Ferulic acid is among the metabolites in the biosynthesis of lignins and is a phenolic compound that is one of the compounds of plant tissues, especially plant cell wall. It is an anti-oxidant, anti-microbial, anti-inflammatory, anti-diabetic compound, and it is effective against neurodegenerative diseases (Batista, 2014). It has been shown that caffeic acid has the same effects as other phenolic compounds and also has a high anticarcinogenic effect, and this anticarcinogenic

effect is related to the chemical structure with free phenolic hydroxyls, the number and position of OH in the catechol group, and the antioxidant and prooxidant capacity related to the double bond in the carbonic chain (Espíndola et al., 2019). Caffeic acid has also been shown to have a wide range of properties in terms of biological activity, including antimicrobial activity (Kępa et al., 2018). Adem et al. investigated the effectiveness of caffeic acid in the context of viral therapy in their study on the treatment of SARS-CoV-2, a newly emerging coronavirus type, and showed that the virus has strong molecular interactions with all proteins except the spike glycoprotein off state. In their study, they concluded that caffeic acid would reduce the efficiency and duration of viral destruction of viruses such as coronavirus and HIV, promote the production of antibodies against the virus and prevent inflammation. Moreover, in addition to the common ones with other phenolics, vanillic acid (Brimson et al., 2019), which is effective in pain, inflammatory diseases, and neurodegenerative disorders, citric acid with properties such as antiobesity, hepatoprotective, antilipemic, and immunomodulatory (Izquierdo-Vega et al., 2020), and epicatechin, which can prevent oxidative damage and endothelial dysfunction associated with hypertension as well as certain brain disorders, was found in considerable amount (0.115, 0.852, 0.493 μg/ml, respectively) in the chloroform extract as a result of LC-MS/MS analysis.

According to LC-MS/MS results, the caffeic acid, p-coumaric acid, trans-ferulic acid, kaempferol, and gentisic acid amounts of ethyl acetate extract were found very high (1.933 μg/ml, 0.301 ng/ml, 0.295 μg/ml, 0.229 μg/ml and 0.162 μg/ml, respectively). Caffeic acid is one of the very powerful antioxidant phenylpropanoids, it also has many other pharmacological properties such as anti-inflammatory, anticarcinogenic, antimicrobial effects. It has been reported that the level of its anticarcinogenic property is related to its chemical structure with free phenolic hydroxyls, the number and position of the OH in the catechol group, and the double bond in the carbonic chain (Espíndola et al., 2019). P-coumaric acid plays a very important role in the secondary metabolite in plants by transforming into phenolic compounds such as ferulic acid, caffeic acid, sinapic acid, and chlorogenic. It is found in plants in free form or compound form. Besides many antioxidants, anti-cancer, anti-inflammatory, antiplatelet aggregation, antimicrobial, antivirus, analgesic, antipyretic, anxiolytic, and anti-arthritis biological activities, it also has a mitigating effect in cases of obesity, diabetes, gout, and hyperlipidemia (Pei et al., 2016). Some studies on kaempferol have shown that some glycosides of kaempferol have a wide spectrum of pharmacological activities such as antioxidant, anticancer, antimicrobial, anti-inflammatory, cardioprotective, anti-osteoporotic, neuroprotective, antidiabetic, anxiolytic, estrogenic/antiestrogenic, and analgesic effects. The glycosides of kaempferol have looked very promising in the development of anti-coronaviral agents (Calderón-Montaño et al., 2011). Schwarz et al. (2014) emphasized in their experimental research that kaempferol may be hope for the development of new antiviral drugs with high bioavailability. Gentisic acid is a siderophore, a metabolite of a redox-active quinonoid acetylsalicylic acid. It is a phenolic compound with powerful antioxidant effects and anti-inflammatory molecular properties (Altinoz & Ozpinar, 2019). Studies have shown that gentisic acid palliates excessive pressure-induced cardiac hypertrophy and fibrosis (Sun et al., 2018), and promotes high cell proliferation activity in skin injuries and has a healing effect (Kim et al., 2020). Phenolics of citric acid, ascorbic acid, epicatechin, 4-hydroxybenzoic acid and, catechin were moderately high (0.103, 0.059, 0.057, 0.043, and 0.038 μg/ml, respectively; Table 2).

In the LC-MS/MS results of the methanol extract of *S. mesopotamica speta*, chicoric acid, vanillic acid, chlorogenic acid, citric acid were found at high levels (0.559, 0.228, 0.159, and 0.111 μg/ml, respectively), while the amounts of ascorbic acid, 4-hydroxybenzoic acid, epicatechin, trans-ferulic acid, fumaric acid, and catechin were lower (0.08, 0.052, 0.047, 0.041, 0.037, and 0.036 μg/ml, respectively; Table 2). Chlorogenic acid is one of the most abundant acids in the phenolic acid compounds that can be found naturally in tea and green coffee. It is an important and biologically active polyphenol with many antioxidant, anti-inflammatory, antibacterial, cardioprotective, hepatoprotective, anti-obesity, neuroprotective, and antipyretic activities (Naveed et al., 2018). Therefore, chlorogenic acid is a bioactive compound that may play a role in important studies in maintaining human health. Vanillic acid is a phenolic compound with cardioprotective, antimicrobial, antioxidant, neuroprotective, and memory-enhancing properties. It has also been reported to suppress hepatotoxin-induced liver fibrosis by reducing hydroxyproline. Accumulation of collagen fibril is one of the causes of stroke and myocardial infarction. Rasheeda et al. (2018) reported that the vanillic acid has exceptionally robust aromatic properties to inhibit the spontaneous association of Type I collagen in vitro. At the same time, vanillic acid reduces alveolar bone loss and collagen destruction, increases periodontal inflammation and osteoblastic activity, and cures periodontitis successfully (Karatas et al., 2019).

The antioxidant activity of chloroform, ethyl acetate, and methanol extracts of *S. mesopotamica speta* was initially determined using the DPPH radical scavenging activity (Table 3). DPPH is one of the most acceptable and spectrophotometric methods used to determine the radical scavenging and antioxidant capacity of plants. In this test, antioxidant compounds and plant extracts were able to lower a stable radical DPPH. The significant reduction of DPPH is related to the high scavenging activity performed by a particular sample (Molyneux, 2004). In parallel with increasing concentrations in the range of 5–500 μg/ml, an increase in activities was also observed.

AChE is a cholinergic system enzyme that can hydrolyze ACh in the peripheral nervous system. Studies have reported an increase in AChE activity in the early stage of Alzheimer's disease (AD). Therefore, it is thought that AChE may have an important role in improving cholinergic deficiency AChE inhibitors are among the drugs preferred for the treatment of neurodegenerative diseases due to their low side effects and beneficial results (Pohanka, 2011). In our study, the inhibition effect of ethyl acetate, chloroform, and methanol extracts of *S. mesopotamica speta* on the AChE enzyme were investigated (Ellman et al., 1961). IC_{50} values of chloroform, ethyl acetate, and methanol extracts were measured as 40.06 μg/ml (R^2: 0.9407), 43.46 μg/ml (R^2: 0.9036), and 38.69 μg/ml (R^2: 0.9598), respectively (Figure 3). Also, the

IC_{50} value of standard inhibitors (tacrine) was measured as 98.4 μg/ml (R^2: 0.9904). As seen in Figure 3, all extracts of *S. mesopotamica speta* have effective AChE inhibition capacities. We think that the major phenolics identified in *S. mesopotamica speta* extracts function as AChE inhibitors. It is also known that phenolic compounds have cholinergic enzyme inhibitors (Keskin et al., 2017). Hydrolysis of AChE breaks down ACh into choline and acetate. ACh levels decrease with aging, leading to the progression of neurological disorders such as AD. AChE inhibition increases ACh levels. Therefore, AChE inhibition has been recognized as a useful therapeutic approach for the treatment of neurological disorders, including AD (Talesa, 2001).

The extracts of *S. mesopotamica speta* were treated to the pathogen strains by MIC to determine their antimicrobial activities (Table 4). The extract obtained from ethyl acetate had a greater effect on microorganisms than the other extracts (0.016, 0.031, 0.016 mg/ml, respectively). Especially its effect on gram-positive *S. aureus* and *C. albicans* was remarkable. It is seen in the table that the extract of ethyl acetate has a stronger activity as a solvent. It is seen that ethyl acetate solvent is also more effective in *E. coli* bacteria. From the data in the table obtained by this microdilution analysis, it is understood that the extracts exert a stronger suppression than antibiotics. When we look at the LC-MS/MS results of the ethyl acetate extract of the *S. mesopotamica speta* plant in Table 2, we see that 1.933 μg/ml values was obtained in caffeic acid, and this is the highest amount. We think that caffeic acid has an important role in antimicrobial activity here. Studies confirm our claim. Kępa et al. (2018) in their study, determined that caffeic acid has a high antibacterial effect against *S. aureus* strains. Ethyl acetate extract was equally effective on *C. albicans* and showed a strong antifungal effect (0.016 mg/ml). Studies claim that phenolic compounds would be a good alternative to candidiasis, which has recently started to be a serious problem, become prevalent, and gain resistance to synthetic drugs, at the same time (Lima et al., 2016). Our study also shows parallelism with these studies. They were also quite effective in *E. coli*, a gram-negative bacterium (0.031 ml/Ml). In the study of Rodríguez-Pérez et al., phenolic compounds showed a quite good antibacterial effect on *E. coli*, and this study confirms our study, with remarkable results in the effect of caffeic acid and gallic acid, in particular (Lima et al., 2016; Rodríguez-Pérez et al., 2016).

The *S. mesopotamica speta* plant has a very rich character in terms of phenolic compounds. Phenolic compounds, which have been studied extensively recently, are compounds that are important for human health. The results show that the active phytochemical compounds found in the root of this plant are capable of healing some degenerative, bacterial, and viral diseases. Considering that they have few natural side effects, we believe that more studies are needed on these for the sake of human health.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Necmettin Aktepe: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Writing-original draft; Writing-review & editing. **Cumali KESKİN:** Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Resources; Visualization; Writing-review & editing. **Ayse Baran:** Formal analysis; Methodology; Resources; Supervision; Writing-original draft. **Mehmet Nuri Atalar:** Conceptualization; Methodology; Validation; Visualization; Writing-original draft. **Mehmet Fırat Baran:** Conceptualization; Formal analysis; Resources; Software; Supervision. **Şükrü Akmeşe:** Resources; Software; Validation; Writing-original draft.

ETHICAL GUIDELINES

Ethics approval was not required for this research.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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