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Antioxidant, antimicrobial, anti-urease and cytotoxic activities of various extracts from *Scutellaria sibthorpii* endemic to Cyprus

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Lamiaceae is one of the largest families in the plant kingdom. Including the genus *Scutellaria* whose species are used in traditional medicine in various countries for prevention and also the treatment of several disorders. *In vitro* biological activities of the various extracts of the aerial parts from *Scutellaria sibthorpii* were investigated in the present study. Antioxidant activity of the extracts was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS assays and ferric reducing antioxidant power (FRAP) assays. Cytotoxic potential and anti-urease activities of the extracts were also determined. Spectrophotometric analysis was used to establish the total phenol content of the extracts. The antibacterial activities of extracts were assessed by minimal inhibitory concentration (MIC) against seven bacteria and *Candida albicans*. Regarding the results, infusion showed considerable antioxidant properties in three method (IC_{50} value $0.133\pm0.0005_{DPPH}$, 26.54 ± 0.05 mM trolox/mg $extract_{ABTS}$, 8.76 ± 0.1 mM Fe+2/mg $extract_{FRAP}$). Other extracts exhibited high-to-moderate antioxidant effects. Among the studied samples, the infusion had the highest total phenolic content (0.0295 ± 0.0002 mg GAE/mg extract). According to cytotoxicity results, *n*-hexane extract had strong cytotoxic effects against PC-3 and NIH/3T3 cell line with IC_{50} values of 330.40 µg/mL and 340.85 µg/mL, respectively. In the antibacterial activity screening, the ethyl acetate extract showed higher activity against *Enterecocus facecalis*, *Klebsiella pneumoniae*, *Proteus mirabilis* with 625 µg/mL MIC value. According to the research carried out, it would seem that this is the first study on screening biological activities of extracts from *Scutellaria sibthorpii*. These findings indicate that these endemic species from Cyprus could be used in phytopharmaceutical preparation.

Keywords: Antimicrobial, Antioxidant, Anti-urease, Cytotoxic activity, *Scutellaria*, Traditional medicine

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Traditional medicines, especially produced from plant derivatives formed the basis of modern medicine. With the development of chemistry, new drugs have been produced based on substances isolated from plants[1]. However, herbal medicine is still an important part of the treatment in many countries notably those which have limited access to medication[2]. The genus *Scutellaria* is one of the important members of the Lamiaceae (Labiatae) family of which there are approximately 350 species called skullcaps all around the world[3]. Numerous species of the genus have long been used as a folk medicine for the treatment of several diseases due to their anticancer, antioxidant, antiviral, antibacterial, antipyretic, neuroprotective, and anti-inflammatory properties[3,4]. Some of these species are well-known including *S. lateriflora*, *S. baicalensis*, and *S. barbata*. The former, known as 'American skullcap' is sold as tea or supplement in health or food stores[5]. The most popular and well-studied species is *S. baicalensis* known as ''Huangqin'' in traditional Chinese medicine and ''Ogon'' in Japanese folk medicine[6]. It has been extensively studied because its roots are effective for the treatment of variety of ailments such as cancer, hepatitis atherosclerosis, cardiovascular problems, respiratory and gastrointestinal infections[7-9].

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S. barbata is the other well-known species and gained attention lately due to its demonstrated anticancer properties[4]. *Scutellaria* species are also known as 'kaside' in Turkish, one of these species *S. orientalis*, was used in Anatolian traditional medicine for constipation, as a hemostatic, tonic and wound healing agent[10].

In the investigation on phytoconstituents from the genus, over 300 compounds were obtained and that showed the presence of different kind of components such as flavonoids, polysaccharides, terpenes, iridoid and phenylethanoid glycosides, alkaloids, phytosterols, and essential oil[7,11-13]. *Scutellaria* extracts showed a wide range of biological activities and were largely composed of flavonoids and their glycosides, such as baicalein, baicalin, wogonoside and wogonin[3]. Given that previous studies found these flavonoids to be responsible for the majority of *Scutellaria*'s pharmacological effects, they have been investigated as potential bioactive molecules[14]. As a result of investigations on biological activities of *Scutellaria s*pecies, some traditional usage such as antimicrobial, antioxidant, anti-angiogenesis, anticancer, hepatoprotective, anticonvulsant and neuroprotective activities have been approved and responsible metabolites have been isolated[3,7,14-16]. Several investigations have revealed that the bioactivities of secondary metabolites particularly phenols and flavonoid compounds show variety, depending on their functional groups. Many studies confirm that many *Scutellaria s*pecies exhibited significant antioxidant effects. It is well known that the deficiency of antioxidant mechanisms in the body may cause different types of illness. Therefore, it is valuable to collect data for evaluating the biological potential of different *Scutellaria* species particularly on species that have limited studies.

Oxidative stress causes a slew of cell destruction, which leads to a variety of degenerative diseases such as cancer, neurodegenerative disorders, skin problems, and coronary diseases[17]. In the oxidation phase, antioxidants may postpone or inhibit oxidation or the proliferation of oxidizing chain reactions. Antioxidants gained attention in the last few years, but many studies demonstrated that synthetic antioxidants possess harmful effects that limit their usage in the body[18]. Therefore, researchers have focused to find natural antioxidants. During the last few years, several methods have been developed to reveal the antioxidant capacity of natural substances[17,18]. Many plants have much potential to prevent oxidation and also control microbial growth and that has oriented researchers to investigate antioxidant and antimicrobial activities of plants[1]. *Scutellaria* species are valuable for these investigations due to being rich in phenolic compounds, particularly flavonoids, and have recently received considerable attention for their anti-cancer properties[16]. The antioxidant capabilities of plants are based on the phenolic functional groups, which serve to protect cell macromolecules from oxidative damage and, therefore, help prevent degenerative diseases caused by oxidative stress.

So far as discovering prospective sources of herbal medications is concerned, it appears that phytochemical and biological investigations into plants are extremely significant. The activities of the fractionated extracts prepared with different solvents show a wide range of effects due to the differences in secondary metabolites. The genus *Scutellaria* is represented by five species in the flora of Cyprus while *Scutellaria sibthorpii* is an endemic species among them[19]. Since the lack of studies on the biological activities of *S. sibthorpii*, it is aimed to assess the beneficial effects of its different extracts.

Hence, to understand the biological potential of *S. sibthorpii* properly, different solvent extracts (*n*-hexane, ethyl acetate, methanol extracts, and infusion) from its aerial parts were prepared in the present work. Each extract was screened for its antioxidant, anti-urease, antimicrobial and cytotoxic effects.

Material and Methods

Plant material

Scutellaria sibthorpii aerial parts were obtained in April 2016 at the flowering stage from St. Hilarion, 8 km to Lapta, 700 m altitude, Girne, Northern Cyprus. The plant was identified by Prof Mehmet Koyuncu. Voucher specimens are kept at the Scientific and Technical Research Center of Traditional Medicine, Istanbul University, Istanbul, Turkey (GIMnumber: 687).

Preparation of extracts

Air-dried and powdered whole plant of *S. sibthorpii* was extracted consecutively with hexane, ethyl acetate and methanol using the Soxhlet apparatus[20]. The water extract was also prepared via., maceration procedure. Firstly, water extract was filtered, then the filtrates were frozen at 80°C in an ultra-low temperature freezer, lyophilized and stored at -20°C until analysis. A rotating evaporator at a

maximum temperature of 50°C evaporated solvents to dryness under decreased pressure. The crude extracts produced after solvent evaporation were kept at +4°C until analysis and utilized in all experiments.

Determination of total phenolic contents in extracts

4.5 mL water was added to each 0.1 mL tube and various concentrations of extracts (1-5 mg/mL) were mixed in. The Folin-Ciocalteu reagent (diluted 1/3 with distilled water) and 0.3 mL of 2% sodium carbonate solution were then added to the mixture. The absorbance was measured at 760 mm after the mixture was left at room temperature for two hours[21]. The total phenolic components in the extracts were measured in milligrams of gallic acid equivalents per milligram of extract.

Antioxidant activity

Determination of DPPH radical scavenging activity

The DPPH radical was used to test the extracts' free radical scavenging activity[22]. In a nutshell, 240 µL DPPH solution (0.1 mM) was added to 10 µL extracts made at various doses (50-200 µg/mL). The constituents were mixed for 1 min before being incubated at 25°C for 30 min. The absorbances of the mixes were measured at 517 nm on a regular basis. The absorbance of the control sample was determined under the identical circumstances as the extract, but with 10 µL of methanol instead of the extract. BHT solutions produced at various doses (0.5-0.05 mg/mL) were also utilized as a standard in DPPH radical scavenging tests. The formula for calculating the percent DPPH radical scavenging activity was:

Inhibition of the DPPH radical in percent: = $((A_0 - A_1)/A_0) \times 100$

A_0 represents the absorbance of the control solution, whereas A_1 represents the absorbance of plant extract or standard solutions.

The extracts' findings were expressed as IC_{50} mg/mL.

Determination of ABTS cation radical scavenging activity

7.45 mM potassium peroxodisulphate reacted with 7 mM ABTS ammonium salt dissolved in water. To create a navy blue tint, the ABTS + stock solution was left at room temperature for 12-16 h. Dilute the ABTS + stock solution with distilled water until it has an absorbance of 0.70 (0.02) at 734 nm. 40 µL of extracts at various concentrations (1-5 mg/mL) were mixed with 3960 µL of ABTS + working solution. The absorbance of the mixture was measured for six minutes against a reference at 734 nm[23]. The results of this study were expressed in millimolar of Trolox per milligram of extract.

Assay for ferric reducing antioxidant power (FRAP).

The ferric reducing ability of extract (FRAP) assay works on the premise of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex being reduced to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by antioxidants in a sample at low pH. The final product (Fe^{2+}-TPTZ) exhibits a blue tint with a maximum absorption at 593 nm and the change in absorbance is proportional to the extract's antioxidant capacity[24]. 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of TPTZ solution (10 mM TPTZ in 40 mM HCI), and 2.5 mL of 20 mM FeCl3.6H2O were combined to make the FRAP reagent. The FRAP reagent was then maintained in an incubator for 30 minutes at 37°C. Using 3.8 mL of the FRAP reagent and 0.2 mL of extract, the absorbance of the combination was measured against a reference at 593 nm after 4 min[24]. The samples' FRAP levels were given as mM Fe^{+2}/mg extract.

Anti-urease activity

Stock solutions with concentrations of 5 mg/mL were made from different extracts and infusions and working solutions with concentrations of 2 mg/mL were made by diluting these solutions. In the incubator, 500 µL of urease enzyme were added to 100 µL of working solutions and incubated for 30 min at 37°C. The mixture was then added to 1100 µL of urea and incubated for 30 min at 37°C in the incubator. The reagents R1 (1% phenol, 0.005% sodium nitroprusside) and R2 (0.5% NaOH, 0.1% sodium hypochlorite) were added to the mixture, respectively. The mixture's absorbance at 635 nm was measured after two hours of incubation at 37°C[25].

The formula was used to calculate the percent inhibition of the urease enzyme: % inhibition of an enzyme = $[(A_0 - A_1) / A_0] \times 100]$.

A_0 represents the absorbance of the control solution; A_1 represents the absorbance of the samples and standard solutions.

Cytotoxicity of the extracts

Cytotoxicity of samples was measured by using human prostate adenocarcinoma (PC-3) and mouse embryo fibroblast (NIH/3T3) cell lines. The IC_{50} values obtained from cancer and a normal cell line were compared to evaluate the anticancer potentials of the extracts and infusion. The PC-3 (CRL-1435) and

NIH/3T3 (CRL-1658) cell lines were obtained from the American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle medium: Nutrient Mixture F-12 (DMEM F-12) media supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin (100 U-100 µg/mL) at 37°C in a humidified environment with 5% subculturing was done every 3-4 days when the cells had reached 70-80% confluence. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) is a test that uses living cells to convert MTT to formazan crystals. Mitochondrial activity is measured using this conversion. The total mitochondrial activity of majority of cells is related to the number of viable cells[26]. 10^4 cells/100 µL were cultivated into each well of 96-well plates and incubated overnight for cell attachment. Cells were cultured for a total of 24 h after being treated with several dilutions of extracts. Growth, negative and positive controls were used, respectively, with cell culture medium, 1% DMSO and 1% SDS. After 3 h of incubation, each well was filled with 20 µL of MTT solution (5 mg/mL in PBS). The supernatant was then collected and 100 µL DMSO was added to each well for formazan crystal dissolution. The optical densities (ODs) of each well were measured at 590 nm (against a reference wavelength of 670 nm) using a microplate spectrophotometer system (Epoch, Germany) and the half-maximal inhibition of enzyme activity (IC_{50}) was calculated in contrast to the solvent control.

Antimicrobial activity

The extracts and the infusion of *S. sibthorpii* were studied for their antimicrobial potential against *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 12228, *Klebsiella pneumonia* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231[27,28].

The test mediums were Mueller-Hinton broth for bacteria and RPMI-1640 medium for yeast strains. Samples were prepared with serial dilutions at concentrations of 5000 mg/L to 4.8 mg/L. The inoculum was made by diluting a 4-6 h broth culture of each bacterium type and a 24 h culture of yeast strains adjusted to a turbidity comparable to 0.5 McFarland standard in broth media to generate a final concentration of 5×10^5 CFU/mL for bacteria and 5×10^3 CFU/mL for yeast in the test tray. To avoid evaporation, the trays were covered and stored in plastic bags. Mueller-Hinton broth trays were incubated for 18-20 h at 35°C, whereas RPMI-1640 medium trays were incubated for 24-48 h at 35°C. The minimum inhibitory concentration (MIC) is the lowest concentration of a substance that fully suppresses visible growth. As a control, the antibacterial capabilities of the solvents were tested using test microorganisms. The results were examined and compared to the control group's values.

Statistical analysis

All data is the average of three independent assessments. The data was analyzed using the Graphpad Prism 5 Demo and reported as mean and standard deviation. Tukey's Multiple Comparison Test ($p<0.05$) was used to examine the significance of the difference in means.

Results

The yield of extracts and infusion with total phenolic contents

The extract yields of *n*-hexane, ethylacetate, methanol and infusion were found as 55.3 mg/g, 88.9 mg/g, 54.4 mg/g, and 60.4 mg/g of dry weight, respectively. Among all the extracts, the methanol extract had the highest extract yield percentage. The total phenolic content of extracts is given in Table 1.

Table 1 — Total phenolic content, the antioxidant, and anti-ureaseactivities of *Scutellaria sibthorpii* extracts

Samples	Total phenolics (mg GAE/mg extract)	DPPH (IC_{50}: mg/mL)	ABTS (mM trolox/mg extract)	FRAP assay (mM Fe^{+2}/mg extract)	Urease inhibition (%) (12.5 µg/mL)
n-Hexane	0.023±0.0002*	0.549±0.135	6.78±0.04	5.58±0.05*	6.24±0.76*
Ethyl acetate	0.018±0.0001*	0.985±0.4	16.28±0.3*	5.70±0.06*	3.46±0.4
Infusion	0.0295±0.0002*	0.133±0.0005*	26.54±0.05*	8.76±0.1*	22.13±0.3*
Methanol	0.029±0.05*	0.138±0.008*	17.94±0.15*	8.66±0.04*	11.28±0.2*
BHT		0.087±0.0001	46.51±0.14	14.12±0.07	
BHA			52.63±0.008	16.91±0.02	
Thiourea					73.44±0.9

Values are mean of triplicate determination (n = 3) ± standard deviation Statistically significant at $p<0.05$

***In vitro* antioxidant activity**

Three methods were employed to determine the antioxidant capacity of different extracts from *S. sibthorpii* growing naturally in Northern Cyprus. The results of free radical scavenging, ABTS cation radical scavenging activity and ferric reducing antioxidant power of samples are given in Table 1.

Determination of DPPH radical scavenging activity

DPPH is the simplest, most straightforward, and cheapest process. As a result, it is commonly used to determine the antioxidant capacity of samples. In this work, one of the synthetic antioxidants, BHT, was utilized as a control sample, and the antioxidant potential of extracts and infusions was compared to that of the synthetic antioxidant. Nevertheless, all extracts showed lower activity comparing the standard, BHT. The antioxidant capacity of infusion was determined with IC_{50} value as 0.133±0.0005 mg/mL which was found to be the most potent free radical scavenger. Besides, in the methanol extracts, the DPPH assay IC_{50} value was determined as 0.138±0.008 mg/mL which is a quite similar activity with the infusion. As to the results, *n*-hexane and ethylacetate extracts showed weak DPPH radical scavenging activity with IC_{50} value 0.549±0.135 mg/mL and 0.985±0.4 mg/mL, respectively.

Determination of ABTS cation radical scavenging activity

ABTS radical cation scavenging activity of extracts and infusion was found as *n*-hexane (6.78±0.04 mM trolox/mg extract), ethyl acetate (16.28±0.3 mM trolox/mg extract), methanol (17.94±0.15 mM trolox/mg extract) extracts and infusion (26.54±0.05 mM trolox/mg extract). BHT and BHA were used as a positive control in this assay and their TEACvalues were determined as 46.51±0.14 mM trolox/mg extract and 52.63±0.008 mM trolox/mg extract, respectively. According to the results, infusion of *S. sibthorpii* demonstrated significant activity when compared to the other extracts. In all extracts, a positive correlation was detected between ABTS cation radical scavenging activity and the amount of total phenolic compounds. As well-known from the literature, there is a correlation between antioxidant capacity and the amount of total phenolic compounds in the extracts[29,30]. These results are also confirmed by our investigation.

Ferric reducing antioxidant power (FRAP) assay

The FRAP method is one of the popular antioxidant assays that measured the capacity of the extracts to reduce ferric ion to ferrous ion. The result values from the FRAP assay stated that the concentration of extracts with the reduction in the ferric ions (Fe^{3+}) to the ferrous ion (Fe^{2+})[18]. According to the results obtained from the FRAP assay, the power of infusion and methanol extract were found as 8.76±0.1 mM and 8.66±0.04 mM, respectively. These extracts showed stronger ferric-reducing antioxidant power activity while *n*-hexane (5.58±0.05 mM) and ethyl acetate (5.70±0.06 mM) extracts showed moderate activity.

Anti-urease activity

The results of the anti-urease activity of different extracts obtained from *S. sibthorpii* have been summarized in Table 1. As to the anti-urease activity, infusion showed the most active urease inhibition among the other extracts. The weakest activity was seen in ethylacetate extract. None of the fractions, were as active as the positive controls, thiourea.

Cytotoxic Activity

The results of the studied extracts of *S. sibthorpii* on cytotoxic activity are displayed in Table 2. On the cytotoxic test side, the potentials of three extracts and infusion of *S. sibthorpii* PC-3 and NIH/3T3 cell lines were similar compared to each other as following; Hexane Extract>Ethylacetate Extract >Methanol Extract >Infusion. Methanol and ethyl acetate extracts were approximately 3-fold and 2-fold, respectively, more cytotoxic to the PC-3 cell line. However, cytotoxicity of hexane extract and infusion were almost identical in PC-3 and NIH/3T3 cell lines.

Antimicrobial Activity

As shown in Table 3, ethyl acetate extract had greater antimicrobial efficacy against the measured microorganisms than other extracts. The antimicrobial tests showed that all of the extracts and the infusion showed antimicrobial activity against *S. aureus* and *S. epidermidis* with MIC values of 1250 µg/mL along with MIC values of 625 µg/mL against *E. faecalis*. The *n*-hexane extract showed antimicrobial activity with MIC values of 625 µg/mL against *K. pneumonie* and *P. mirabilis*, while the other ones had no activity.

Table 2 — The cytotoxicity of examined *Scutellaria sibthorpii* extracts

Extracts	IC_{50} values (µg/mL)	
n-Hexane	330.40	340.85
Ethyl acetate	631.77	1413.72
Methanol	2439.634	6514.85
Infusion	12064.59	11427.23

Table 3 — The antimicrobial activity of studied *Scutellaria sibthorpii* extracts

Microorganism	MIC Values (µg/mL)			
	n-Hexane extract	Ethylacetate extract	Methanol extract	Infusion
Staphylococcus aureus ATCC 29213	1250	1250	1250	1250
Staphylococcus epidermidis ATCC 12228	1250	1250	1250	1250
Enterococcus faecalis ATCC 29212	625	625	625	625
Escherichia coli ATCC 25922	-	-	-	-
Klebsiella pneumoniae ATCC 4352	-	625	-	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	-
Proteus mirabilis ATCC 14153	-	625	-	-
Candida albicans ATCC 10231	-	-	-	-

Antibacterial activity against *E. coli* and *P. aeruginosa* was not seen in any of the *S. sibthorpii* extracts.

Discussion

In this study, *in vitro* antioxidant, cytotoxic, anti-urease and antimicrobial activities of the aerial parts from *S. sibthorpii* were assessed as well as for its total phenolic content in different solvent extracts. The infusion of aerial parts from *S. sibthorpii* had considerably more phenolic compounds than the other samples studied. Furthermore, the amount of phenolic content in methanol extract was approximate to the infusion. The *n*-hexane and ethyl acetate extract contain a moderate amount of phenolic compounds. In addition, the total phenolic content of extracts was compared with their antioxidant capacity. As a result, there was a positive correlation between methanol extract and infusion. On the other hand, there is no relation between the activity and total phenolic compounds in *n*-hexane and ethyl acetate extracts. Based on works of literature, phenolics possess a great antioxidant potential and most of the extracts showed remarkable antioxidant activity due to containing high amounts and also the synergy of them[29].

Plenty of studies demonstrated that *Scutellaria* species show strong to moderate antioxidant activity[29-31]. These species are well known for their rich flavonoid content, which is possibly connected to strong antioxidant activity[3,30]. Şenol *et al.*[32] investigated acetylcholinesterase, butyryl–cholinesterase, and tyrosinase inhibition, and antioxidant activity of the ethyl acetate and methanol extracts prepared from 33 *Scutellaria* taxa growing in Turkey. According to their results, the extracts displayed a quite strong DDPH radical scavenging action and a mild antioxidant activity in ferrous ion-chelating and FRAP tests. Furthermore, methanol extracts from a few subspecies of *S. orientalis* showed high radical scavenging capacity, which was nearly equivalent to a positive control[32]. Additionally, Zengin *et al.*[33] reported the antioxidant capacity of two *Scutellaria* species using different assays. The ethyl acetate, methanol, and water extracts were studied and it was demonstrated that the water extracts showed the strongest antioxidant activity with a high amount of total phenolic content in all tests[33]. According to earlier studies, the presence of unique flavonoids in *Scutellaria* is considered to impose on antioxidant activity along with other phenolic compounds[8]. Currently, in the quest for natural and efficient antioxidants to combat free radical-related pathological problems, there is an increasing interest in screening and quantifying antioxidants from biological samples[33]. Researchers are increasingly interested in discovering reliable, nontoxic natural antioxidants derived from plants[18]. Previous investigations indicated that phenolic compounds particularly flavonoids are important to protect cells against oxidative damage caused by free radicals[29]. Thus, species like *Scutellaria* are considered valuable due to their rich possession of these compounds in the extracts.

Urease is an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia[34]. Since urease is the most important colonization and a virulence factor for *Helicobacter pylori*, urease inhibition strategies are now critical lines of treatment for *Helicobacter pylori* infection. Although it has been reported that *S. baicalensis* showed considerable anti-*Helicobacter pylori* activity, other *Scutellaria* species have not been examined so far[35]. In the present work, the anti-urease effects of the different extracts obtained from *S. sibthorpii* were examined by *in vitro* method. Although moderate urease inhibition was observed in the infusion it was not as effective as the positive control. However, further studies need to be lightened the responsible components in the

infusion and also evaluate the utilization of *S. sibthorpii* against *Helicobacter pylori*-related problems.

Consideration of ancient ethnomedicinal knowledge and investigation of locally accessible natural resources is one path to discover novel lead compounds against cancer[36]. Earlier studies have confirmed the anticancer properties of *Scutellaria* species and this effect was attributed to the presence of phenolic compounds particularly flavonoids and terpenes[4]. Owing to the promising preclinical evidence, clinical trials have been conducted with their extracts including significant secondary metabolites. It is demonstrated by several studies that baicalein decreased the growth of pancreatic, colorectal, and gastric cancer cell lines[6,8]. According to our results, methanol and ethyl acetate extracts of *S. sibthorpii* might have mild anticancer effects on human prostate cancer. It can be suggested that the extracts of *S. sibthorpii* be screened against different cancer cell lines.

According to the antimicrobial results, the extracts demonstrated mild to moderate inhibitory effects on tested microorganisms between the concentrations of 625 to 1250 µg/mL. Among the strains, *Enterococcus faecalis* were more susceptible to all extracts. The ethyl acetate extract was the most effective extract against the studied different strains. On the other hand, investigated extracts showed no anticandidal activity against *Candida albicans*. Previously, the essential oil composition with antimicrobial activity of the aerial parts from *S. sibthorpii* collected in Cyprus was examined by Dereboylu *et al*.[37], According to their results, the most susceptible strain was demonstrated as *S. aureus* whilst the most resistant yeast were found to be *C. albicans* with minimum inhibitory concentration values ranging from 10 to 20 mg/mL. The ethyl acetate extract of *S. litwinowii* was observed most effective to *B. cereus* and *P. aeruginosa* with a MIC value of 1.25 µg/mL[38]. In another study, the antimicrobial activities of methanol extracts prepared from 15 subspecies of *S. orientalis* collected in Turkey were studied using the broth microdilution method and their MIC values were varied from 62.5 µg/mL to 250 µg/mL[39]. Arıtuluk *et al*.[40], investigated the antimicrobial effectiveness of the different solvent extracts obtained from *S. salviifolia, S. pontica, and S. diffusa.* Their results indicated that the extracts possess low to moderate antibacterial activity with MIC values in the range from 256 to 1024 µg/mL. In addition, *n*-hexane and chloroform extracts of *S. salviifolia* and also chloroform and aqueous extracts of *S. pontica* showed strong antifungal effects against with MIC values ranging from 32 to 64 µg/mL[40].

Lately, the increased bacterial resistance has been a real concern in the health care system[41]. Random and misusage of antibiotics have brought huge problems for the treatment of some infections[42]. Aromatic plants are getting more popular because they contain different types of components. It is well-known that these compounds create synergy against microbes and reduce their resistance capability. Several investigations have shown that *Scutellaria* species have antimicrobial activity, however the efficiency rate varies depending on the solvent used and the extract composition[43-45]. It can be suggested that further investigation needs to be conducted on the antimicrobial potential of *S. sibthorpii* to explore the responsible components.

Conclusion

Plants are utilized in multitude of sectors such as cosmetics, food and healthcare[2,46]. For decades, manufacturers have used industrial natural preservatives to resist the degradation and microbial infection of packaged products and drugs. *Scutellaria* is one of the most investigated genus due to the existence of valuable therapeutic potential[45]. Along with the significance of these species which have been proven by researchers in therapy, investigations are focused to establish their phytochemical composition and pharmacological activities. Phytochemical constituents in drugs can differ not only from one plant to the next but also from different samples of the same species. Therefore, investigations on the member of the genus appear tremendously worthwhile for finding natural medicine sources. In the present work, a comprehensive study has been conducted on *S. sibthorpii* to explore its biological potential. Hence, antioxidant, antimicrobial, anticancer, anti-urease activities of different extracts prepared from aerial parts were investigated using several methods. The antioxidant ability of the samples ranged from moderate to high, with infusion having a high total phenolic content exhibiting significant action. However, ethylacetate extract exhibited remarkable antimicrobial activity with a less toxic effect, so it might be evaluated as a harmless agent. When compared to other species, *S. sibthorpii*

is found to be of great potential which can be assessed in pharmaceutical science.

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Conflict of Interest

The authors do not have any conflicts of interest to declare.

Authors' Contributions

GGT: Writing, Editing, Data curation, Investigation, TT: Writing, Editing, Investigation, EO: Methodology, Investigation, MH: Methodology, Investigation, ST: Investigation, GS: Editing, Supervision, and AM: Editing, Supervision.

References

1 Verpoorte R, Pharmacognosy in the new millennium: lead finding and biotechnology, *J Pharm Pharmacol*, 52 (3) (2000) 253-256.

2 David B, Wolfender J L & Dias D A, The pharmaceutical industry and natural products: Historical status and new trends, *Phytochem Rev*, 14 (2) (2015) 299-315.

3 Shang X, He X, He X, Li M, Zhang R, *et al*., The genus Scutellaria an ethnopharmacological and phytochemical review, *J Ethnopharmacol*, 128 (2) (2010) 279-313.

4 Tao G & Balunas M J, Current therapeutic role and medicinal potential of Scutellaria barbata in Traditional Chinese Medicine and Western research, *J Ethnopharmacol*, 182 (2016) 170-180.

5 Bardakci H, Skaltsa H, Milosevic-Ifantis T, Lazari D, Hadjipavlou-Litina D J, *et al*., Antioxidant activities of several Scutellaria taxa and bioactive phytoconstituents from Scutellaria hastifolia L., *Ind Crops Prod*, 77 (2015) 196-203.

6 Takahashi H, Chen M C, Pham H, Angst E, King J C, *et al*., Baicalein, a component of Scutellaria baicalensis, induces apoptosis by Mcl-1 down-regulation in human pancreatic cancer cells, *Biochim Biophys Acta Mol Cell Res*, 1813 (8) (2011) 1465-1474.

7 Zhao T, Tang H, Xie L, Zheng Y, Ma Z, *et al*., *Scutellaria baicalensis* Georgi. (Lamiaceae): A review of its traditional uses, botany, phytochemistry, pharmacology and toxicology, *J Pharm Pharmacol*, 71 (9) (2019) 1353-1369.

8 Wang Z L, Wang S, Kuang Y, Hu Z M, Qiao X, *et al*., A comprehensive review on phytochemistry, pharmacology and flavonoid biosynthesis of Scutellaria baicalensis, *Pharm Biol*, 56 (1) (2018) 465-484.

9 Zhao Q, Chen X Y & Martin C, Scutellaria baicalensis, the golden herb from the garden of Chinese medicinal plants, *Sci Bull*, 61 (18) (2016) 1391-1398.

10 Baytop T, Türkiye'de Bitkilerle Tedavi, (Nobel Tıp Kitapevleri Yayını, İstanbul), 1999, p. 480.

11 Kurkcuoglu M, Yildiz G & Kose Y B, Essential oil composition of two *Scutellaria* from Tokat province of Turkey, *J Turkish Chem Soc*, 6 (2019) 115-118.

12 Doğan Z, Ishiuchi K I, Makino T & Saraçoğlu İ, Alpha-pyrone glycosides from *Scutellaria salviifolia* Benth, *Turk J Chem*, 43 (3) (2019) 972-981.

13 Nie X P, Qu G W, Yue X D, Li G S & Dai S J, Scutelinquanines A–C, three new cytotoxic neo-clerodane diterpenoid from *Scutellaria barbata*, *Phytochem Lett*, 3 (4) (2010) 190-193.

14 Zhou X, Choi P S, Yang J M, Or P M, Hoi P M, *et al*., Chemical and pharmacological evaluations on the extract of Scutellaria baicalensis Georgi (Huang-Qin) prepared by various extraction methods, *Springer Plus*, 5 (1) (2016) 1-10.

15 Khumbongmayum A D, Khan M L & Tripathi R S, Ethnomedicinal plants in the sacred groves of Manipur, *Indian J Tradit Know*, 4 (1) (2005) 21-32.

16 Zhang L, Ren B, Zhang J, Liu L, Liu J, *et al*., Anti-tumor effect of *Scutellaria barbata* D. Don extracts on ovarian cancer and its phytochemicals characterization, *J Ethnopharmacol*, 206 (2017) 184-192

17 Bibi Sadeer N, Montesano D, Albrizio S, Zengin G & Mahomoodally M F, The versatility of antioxidant assays in food science and safety—Chemistry, applications, strengths, and limitations, *Antioxidants*, 9 (8) (2020) 709.

18 Koşar M , Göger F & Can Başer K H , In vitro antioxidant properties and phenolic composition of *Salvia virgata* Jacq. from Turkey, *J Agric Food Chem*, 56 (7) (2008) 2369-2374.

19 Meikle R D, Flora of Cyprus, Vol. 2. (The Bentham-Moxon Trust Royal Botanic Gardens, Kew, London), 1977, p. 1294-1295.

20 Li H B & Chen F, Isolation and purification of baicalein, wogonin and oroxylin A from the medicinal plant *Scutellaria baicalensis* by high-speed counter-current chromatography, *J Chromatogr A*, 1074 (1-2) (2005) 107-110.

21 Ozsoy N, Can A, Yanardağ R & Akev N, Antioxidant activity of *Smilax excelsa* L. leaf extracts, *Food Chem*, 110 (3) (2008) 571-583.

22 Wei F, Jinglou C, Yaling C, Yongfang L, Liming C, *et al*., Antioxidant, free radical scavenging, anti-inflammatoryand hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch.etSav.) Ching, *J Ethnopharmacol*, 130 (2010) 521-28.

23 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, *et al*., Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic Biol Med*, 26 (9-10) (1999) 1231-1237.

24 Benzie I F & Strain J J, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, *Anal Biochem*, 239 (1996) 70-76.

25 Taşkın D, Taşkın T & Rayaman E, Phenolic composition and biological properties of *Achillea nobilis* L. subsp. *neilreichii* (Kerner) Formanek, *Ind Crops Prod*, 111 (2018) 555-562.

26 Van Meerloo J, *et al.*, *Cell sensitivity assays: the MTT assay*, *Methods Mol Biol*, 731 (2011) 237-45.

27 Clinical and Laboratory Standards Institute (CLSI), Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard 7th Edition, M7-A7, Wayne, PA, USA, 2006.

28 Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard 3th Edition. M27-A3. Wayne, PA, USA: 2008.

29 Grzegorczyk-Karolak I, Kuźma Ł & Wysokińska H, Study on the chemical composition and antioxidant activity of extracts from shoot culture and regenerated plants of *Scutellaria altissima* L., *Acta Physiol Plant*, 37 (1) (2015) 1-9.

30 Golmakani E, Mohammadi A, Sani T A & Kamali H, Phenolic and flavonoid content and antioxidants capacity of pressurized liquid extraction and perculation method from roots of *Scutellaria pinnatifida* A. Hamilt. subsp *alpina* (Bornm) Rech. f., *J Supercrit Fluids*, 95 (2014) 318-324.

31 Gohari A R, Hajimehdipoor H, Saeidnia S, Ajani Y & Hadjiakhoondi A, Antioxidant activity of some medicinal species using FRAP assay, *J Med Plant*, 10 (37) (2011) 54-58.

32 Şenol F S, Orhan I, Yilmaz G, Cicek M & Şener B, Acetylcholinesterase, butyrylcholinesterase, and tyrosinase inhibition studies and antioxidant activities of 33 Scutellaria L. taxa from Turkey, *Food Chem Toxicol*, 48 (3) (2010) 781-788.

33 Zengin G, Llorent- Martínez E J, Molina- García L, Fernández- de Córdova M L, Aktumsek A, *et al*., Chemical profile, antioxidant, and enzyme inhibitory properties of two *Scutellaria* species: *S. orientalis* L. and *S. salviifolia* Benth, *J Pharm Pharmacol*, 71 (2) (2019) 270-280.

34 Krajewska B, Zaborska W & Leszko M, Inhibition of chitosan-immobilized urease by slow-binding inhibitors: $Ni^{2+,}$ F^{-} and acetohydroxamic acid, *J Mol Catal B Enzym*, 14 (4-6) (2001) 101-109.

35 Yu X D, Zheng R B, Xie J H, Su J Y, Huang X Q, *et al*., Biological evaluation and molecular docking of baicalin and scutellarin as Helicobacter pylori urease inhibitors, *J Ethnopharmacol*, 162 (2015) 69-78.

36 Özmen A, Madlener S, Bauer S, Krasteva S, Vonach C, *et al*., *In vitro* anti-leukemic activity of the ethno-pharmacological plant *Scutellaria orientalis* ssp. *carica* endemic to western Turkey, *Phytomedicine*, 17 (1) (2010) 55-62.

37 Dereboylu A E, Sarikahya N B, Sengonca N, Kirmizigul S, Yasa I, *et al*., Glandular trichomes morphology, chemical composition and antimicrobial activity of the essential oil of three endemic *Scutellaria taxa* (Lamiaceae), *Asian J Chem*, 24 (11) (2012) 4911.

38 Bazzaz B S F, Khayat M H, Emami S A, Asili J, Sahebkar A, *et al*., Antioxidant and antimicrobial activity of methanol, dichloromethane and ethyl acetate extracts of *Scutellaria litwinowii*, *Sci Asia*, 37 (4) (2011) 327-334.

39 Yılmaz G, Şimşek D, Altanlar N & Çiçek M, Comparison of the antimicrobial activity of some *Scutellaria orientalis* L. taxa growing in Turkey, *Int J Second Metab*, 7 (3) (2020) 192-199.

40 Arıtuluk Z C, Özkul Koçak C, Renda G, Ekizoğlu M & Ezer N, Antimicrobial activity of three *Scutellaria* L. species from Turkey, *J Res Pharm*, 23 (3) (2019) 552-558.

41 Skaltsa H D, Lazari D M, Mavromati A S, Tiligada E A & Constantinidis T A, Composition and antimicrobial activity of the essential oil of *Scutellaria albida* ssp. *albida* from Greece, *Planta Med*, 66 (07) (2000) 672-674.

42 Chandra H, Bishnoi P, Yadav A, Patni B, Mishra A P, *et al*., Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials—a review, *Plants*, 6 (2) (2017) 16.

43 Sato Y, Suzaki S, Nishikawa T, Kihara M, Shibata H, *et al*., Phytochemical flavones isolated from *Scutellaria barbata* and antibacterial activity against methicillin-resistant *Staphylococcus aureus*, *J Ethnopharmacol*, 72 (3) (2000) 483-488.

44 Kwapong A A, Soares S, Teo S P, Stapleton P & Gibbons S, *Myristica lowiana* phytochemicals as inhibitor of plasmid conjugation in *Escherichia coli*, *Evid Based Complement Alternat Med*, 2020 (2020) 1604638.

45 Rajendran N, Subramaniam S, Christena L R, Muthuraman M S, Subramanian N S, *et al*., Antimicrobial flavonoids isolated from Indian medicinal plant *Scutellaria oblonga* inhibit biofilms formed by common food pathogens, *Nat Prod Res*, 30 (17) (2016) 2002-2006.

46 Kilic M, Yıldız K & Kılıç F M, Traditional uses of wild plants in Mardin central district and attached villages (Turkey), *Indian J Tradit Know*, 20 (3) (2021) 784-798.