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Chemical and functional composition and biological activities of Anatolian *Hypericum scabrum* L. plant

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ABSTRACT

Hypericum scabrum L. plant has wide use in ethnopharmacology and is cultivated both naturally and technologically. This study was conducted to determine chemical and functional properties and in vitro biological activities as antimicrobial, antioxidant, anticancer composition of *H. scabrum* L. (Hypericaceae) from Anatolia. This plant was collected from Elazıg and Sivas province of Turkey. The ICP-OES method was used for the investigation of the heavy metal composition and GC–MS analysis was used for the defining of chemical components. Antimicrobial qualifications of plant extracts were investigated on 8 different microorganisms by minimum inhibition concentration (MIC) method. Antioxidant activities were investigated by using Diagnostic kits, which provide 99% reliable results with chemical reduction reactions. In vitro anticancer properties of the extracts were investigated on 5 different cell lines by using MTT method. It was observed that the flower and leaf parts of *Hypericum* plant have a very high antioxidant capacity and has mediocre antimicrobial activity only on 2 microorganisms. Also moderate cytotoxic activities on some cell lines were determined. It is thought that *H. scabrum* plant has functional bioactive compounds and might be an important natural antioxidant source in various sectors like cosmetics, drug and food industries.

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1. Introduction

For eons humans have used various plants as food sources and also for medications and benefits from such practices are known to be supported by many scientific studies. It has been proved that the primary and secondary metabolites, antioxidants and mineral compositions of the plants have a benefactory role in enhancing the body resistance [1–8]. It is also a known fact that the great majority of active ingredients present in drugs are obtained from plants. Phytotherapy is again in the spotlight due to the possible adverse effects posed by synthetic drugs and to the multiple beneficieries obtained from plant based medications [9] It is therefore important to investigate biological and chemical properties of various plant extracts and provide scientific results which would greatly support different sectors like pharmaceutics, cosmetics, and food industry. Revealing the properties of a very rich biodiversity we have would also be a great addition to scientific literature.

Hypericum species have been in traditional medicine since the antiquity. *Hypericum* (Guttiferae) naturally grows in temperate thickets and heatlands. *Hypericum* genus has almost 400 different specie worldwide and has 89 specie in Turkey of which 43 are endemic to Turkey [10]. Branches that have leaves, flowers and fruits of this plant are in use in folk medicine. In Turkey, traditionally *Hypericum* species are used for their antispazmolytic, antiseptic, tranquilisan, and wound binding properties [11]. Since *Hypericum* plants are effective in today's most prominent syndrome of depression, the studies regarding these plants and efforts to investigate compounds from such plants are increased [12]. Due to it's medicative properties, *Hypericum* species are cultived both naturally and with tissue culturing, and various researchers are known to studied hypericine and psuedohypericine content of *Hypericum* species [13,14]. It was determined that hypericin has a strong cytotoxic activity on different cancerous cell lines [15]. Additionally, xanthons present in *Hypericum* species are known to have antiinflammatory, antiviral, antimicrobial, and antitumor properties [16]. *H. scabrum* L. is a prennial herbaceous plant that yellow fancy flowers and red colored glands. It has a length of 40 to 50 cm and grows naturally in rough hillsides, forest clearances, and steppes. It has a flowering period between May and August. This plant is also used in folk medicine for various ailments of bladder, intestines, and heart and for rheumatism and cystitis [17]. In this way, *Hypericum* plant is widely used in complementary medicine ethnobotanically. It is intended to evaluate the therapeutic nature of the

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H. scabrum plant from Anatolia with chemical methods and biological experiments.

2. Material and methods

2.1. Collection of plant samples and extraction

H. scabrum plants were collected from Elazıg and Sivas province in Anatolia during the flowering period between June and July. Plant specie identification was done by Assos. Prof. Dr. Ismail Turkoglu and Assos. Prof. Dr. Mustafa Sevindik. Leaf and flower parts of the plant separated and dried at room temperature under shading. In ethanol extraction, plant samples were weighted and added into 70% ethanol and macerated at room temperature with occasional shaking (3 times for 24 h). After extraction, upper phase of solutions were filtered on Whatman filter papers and alcohol was vaporized at 40 °°C in a rotary evaporator device (Buchii). Extracts were saved at −20 °C until required.

2.2. GC–MS analysis

GC–MS analysis of leaf and flower parts of the plant was conducted in Shimadzu GC-MS QP 2010 ULTRA device by using RTX-5MS Capillary column (30 mm; 0.25 mm; 0.25um). Helium (0.7 mL/min.) chemical was applied as carrier gas. Temperature of column oven was adjusted to 40 °C and injection temperature was adjusted to 250 °C. The pressure was adjusted to 100 kPa and injection mode was selected as split. The split ratio was selected as 5. The injection volume was adjusted to 1.0 microliters. Oven temperature program was adjusted to following: 3 min at 40 °C and to 240 °C from 40 °C with 4 °C per min for a total of 78 min. Interface temperature was selected as 250 °C and ion source temperature was selected as 200 °C. Afterwards, dilution was done by 1/10 hexane.

2.3. Heavy metal and mineral analysis by ICP-OES method

For the microwave solubilization, 0.5 g sample was transferred into teflon solubilization vessel and 6 mL 65% nitric acid, and 2 mL 30% hydrogen peroxide were added, then teflon bombs placed inside microwave oven. Microwave oven was programmed to reach 200 °C in 15 min, and stay constant at 200 °C for 15 min. Following the combustion process, solubilized samples were poured into flasks, then adjusted to 50 mL with sterile and ultrapure water. Plasma of the ICP device blasted and ultrapure water was circulated in the system for device balancing. All the reference solutions were mixed as standard according to the type of elements to be analyzed and calibration graphs were assembled. Once the calibration graphs assembled, samples were fed to the system and reading conducted. Different calibration graphs in ppm and ppb resolutions were prepared for analysis outputs that had not fit into calibration graph and re-reading was conducted.

2.4. Determination of antimicrobial activities

Microdilution Broth method was used in the research to evaluate minimum inhibition concentration (MIC) of plant extracts on microorganisms [18]. A total of 8 microorganisms (*Staphylococcus aureus, Enterococcus faecalis, Bacillus cereus, Bacillus kochii, Pseudomonas aeruginosa*, *Escherichia coli, Bacillus thurigiensis*, and *Candida albicans*) were used for the analysis. These microorganisms were chosen based on their high prevalence in food contaminants and human infections. Plant extracts were prepared homogeneously in 40% Dimethyl sulfoxide (DMSO) and stock solutions were prepared. For bacteria Mueller Hinton broth and for fungi Saboraud Dextrose broth [19,20] were used. Microorganisms reproducing on blood agar were collected by using sterile loop and suspensions at McFarland 0.5 opacity were prepared. For bacteria, 5×10^5 CFU/mL and for fungi, $0.5–2.5 \times 10^3$ CFU/mL suspensions at 50 µL were added into each well. Plaques with bacteria suspensions were incubated at 37 °C, and plaques with *Candida* suspensions were incubated at 35 °C for 16–24 h. At the end of incubations, 50 µL 2 mg/mL 2,3,5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) were added into wells and incubated for 2 h at 37 °C to reveal growths. First wells where there was no color change were accepted as MIC values [21].

2.5. Determination of antioxidant activities

Total antioxidant level (TAS) and total oxidant substance level (TOS) of flower and leaf extracts of *H. scabrum* plant were assayed by using commercial Rel Assay Diagnostic kit [22]. For TAS analyses, Trollox standard, and for TOS analyses, hydrogen peroxide standard were used as references. Oxidative stress index (OSI) value which indicates the real oxidative load of the samples were defined with the following formula and antioxidant capacity comparison between leaf and flower parts was determined [23].

OSI (Arbitrary Unit) = TOS value (µmol H_2O_2 equiv./L) / [TAS value (mmol Trolox equiv./L) x10]

2.6. Cell culture and cell viability assay

In this study MCF-7 (Human breast adenocarcinoma cell), HT29 (Human colorectal adenocarcinoma cell), C6 (Glioma cell line from rats), PC-3 (Human prostate cancer cell), and HUVEC (Human umbilical vein endothelial cell) lines were choosed for cytotoxicity analyses. The cell lines were maintained in DMEM medium (Invitrogen) additioned with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin and kept at 37°C incubator with 5% CO_2 in air. To determine the effects on cell viability of plant extracts, cells were plated onto 96-well plates (1×10^4 cells/well). The in vitro cytotoxicity of the extract were evaluated in the colorimetric MTT (3-(4,5-dimethylthiazolyl-2)−2,5-diphenyltetrazolium bromide) assay. The different concentrations of the extracts (1, 10, 100, and 1000 μg/mL) prepared in 1% DMSO-growth medium was added on to the cell culture in 96 well plate during 24 h. Each experiments on every concentration were performed in triplicate. After 24 h, 10 µL of 12 mM MTT (Vybrant, Invitrogen) solution were added into wells and incubated inside a 5% CO_2 stove at 37 °C for 4 h. The value of absorbance of violet color occurred at the end of 4th hour was measured at 570 nm in an Elisa reader (Epoch, USA). At the end of the MTT testing, IC_{50} values were calculated by using GraphPad software.

2.7. Statistical analyses

Graphad Prism v8 software and SPSS v.22.0 (IBM Corp., Armonk, NY, USA) software were used for the analysis to calculate the IC50 values and demonstrate the data graphically. All data were analyzed at 95% confidence and $p<0.05$ was accepted as significance.

3. Results and discussion

Ethanol extracts were provided in 5.9% and 4.7% yield for leaf and flower parts respectively. The medicinal quality of *H. scabrum* plant was investigated as a whole by determining metal and chemical contents, antimicrobial, antioxidant, and anticancer properties. Chemical compositions of ethanol extracts of *H. scabrum* leaves obtained from GC–MS analysis were given in Figs. 1 and Table 1. The GC–MS chromatogram was redrawn using the Origin (Pro), 2021

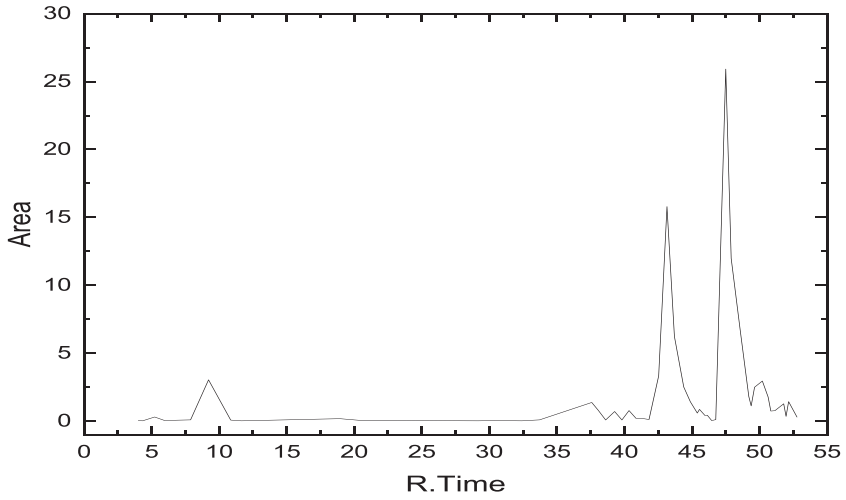

Fig. 1. GC–MS chromatogram of ethanolic extract of *H. scabrum* plant from Elazığ.

Table 1
Chemical composition of ethanolic extract of *H. scabrum* plant from Elazığ.

Peak#	R.Time	Area	Area%	Name
1	4.000	247,210	0.04	2-Propenoic acid, methyl ester (CAS)
2	4.335	190,645	0.03	2-Propanone, 1–hydroxy- (CAS)
3	5.231	1,754,243	0.31	1,1-Diethoxypropanal
4	5.961	291,378	0.05	1,1-Diethoxy-2-butene
5	6.115	272,187	0.05	Ethoxyacetaldehyde diethylacetal
6	7.891	589,717	0.10	2-D-2-PENTADECYL-1,3-DIOXOLANE
7	9.222	17,424,779	3.04	.ALPHA.-PINENE, (-)-
8	10.858	478,158	0.08	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-
9	11.646	190,541	0.03	Propionoin
10	12.927	261,220	0.05	dl-Limonene
11	18.940	1,037,156	0.18	Benzoic acid
12	20.500	279,985	0.05	2-Hexanol, 2,3-dimethyl- (CAS)
13	29.181	176,615	0.03	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-
14	30.386	213,743	0.04	.alpha.-Muurolene
15	32.418	196,536	0.03	Spathulenol
16	33.714	563,195	0.10	Citronellol epoxide (R or S)
17	37.588	7,919,160	1.38	Tetradecanoic acid
18	38.060	4,628,388	0.81	Tetradecanoic acid
19	38.610	515,238	0.09	
20	39.273	4,087,051	0.71	Pentadecanoic acid (CAS)
21	39.810	491,403	0.09	Phytone
22	40.346	4,482,559	0.78	Pentadecanoic acid
23	40.860	1,196,245	0.21	2-Decanone
24	41.320	1,123,784	0.20	
25	41.819	710,285	0.12	Hexadecanoic acid, methyl ester
26	42.540	19,092,464	3.33	Cis-9-Hexadecenoic acid
27	43.154	90,600,851	15.80	Palmitic acid
28	43.704	35,746,587	6.23	9-Octadecenoic acid (Z)- (CAS)
29	44.395	14,446,491	2.52	Oleic Acid
30	44.883	8,175,801	1.43	9-Hexadecenoic acid
31	45.397	3,467,993	0.60	Heptadecanoic acid (CAS)
32	45.560	4,996,694	0.87	Octadecanoic acid, 2-propenyl ester
33	45.962	2,426,068	0.42	Decanoic acid, 1-[[[(2-aminoethoxy)hydroxyphosphinyl]oxy]methyl]−1,2- ethanediy
34	46.136	2,538,147	0.44	11-Octadecenoic acid, methyl ester
35	46.460	237,074	0.04	
36	46.762	654,582	0.11	Methyl stearate
37	47.507	148,606,331	25.92	Cis-Vaccenic acid
38	47.904	68,679,411	11.98	Octadecanoic acid
39	48.585	37,945,863	6.62	Glutaric acid, di(3-(2-methoxyethyl)nonyl) ester
40	49.210	10,462,238	1.82	9-Octadecenal, (Z)-
41	49.385	6,439,565	1.12	
42	49.635	14,316,702	2.50	Decanoic acid, 1-[[[(2-aminoethoxy)hydroxyphosphinyl]oxy]methyl]−1,2-ethanediy
43	50.206	16,945,457	2.96	(E)−13-Docosenoic acid
44	50.632	10,222,732	1.78	Hexadecanoic acid, 1-(hydroxymethyl)−1,2-ethanediyl ester
45	50.832	4,309,674	0.75	
46	51.138	4,435,881	0.77	
47	51.786	7,349,197	1.28	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-
48	51.960	2,015,604	0.35	Isobutyl propane-1,3-diyl dicarbonate
49	52.150	8,260,657	1.44	9-Octadecenamide, (Z)- (CAS)
50	52.785	1,634,826	0.29	Cis, 6-Octadecenoic acid, trimethylsilyl ester
		573,328,311	100.00	

Table 2
Antimicrobial activities of ethanolic extract of *H. scabrum* plant (mg/mL).

Extracts	MIC values (mg/mL)								
	Locality	*E.coli*	*S.aureus*	*P.aeruginosa*	*B. thurigiensis*	*B.cereus*	*B. kochii*	*E.faecalis*	*C.albicans*
Flower extracts	**Sivas**	**0.3**	2.6	1.7	>5.8	1.7	>5.8	>5.8	2.7
	Elazığ	**0.5**	1.3	1.3	>5.4	1.3	>5.4	>5.4	>5.4
Leaf extracts	**Sivas**	**0.5**	**0.5**	3.7	>7.5	1.8	>5.8	>7.5	1.8
	Elazığ	**0.3**	**0.3**	2.6	>5.4	2.6	>5.4	>5.4	2.6

program for better quality (Fig. 1) [24]. It was seen that 50 different compounds are present in leaf extracts. Highest amounts among these compounds were determined as cis-vaccenic acid (25.92%), palmitic acid (15.80%), and octa-decanoic acid (11.98%). Other than these, effective substances such as alpha-pinene, propionin, oleic acid were also observed (Fig. 1; Table 1). Essential oil and fatty acid contents of *H. scabrum* plant were investigated in literature by GC–MS analysis and the essential oil composition was found to be having 28 to 80 different substances. In many different studies maden in different countries, the major component of essential oil content of *H. scabrum* plant was found as Alpha-Pinene (12.52 to 49.96%) [25]. It is also within acceptable range that the same plant would provide different chemical composition in GC–MS analysis in different studies due to the geographical differences and the polarity of extraction solvent [26]. It is therefore necessary to conduct comparative content analysis of plant extracts in terms of biological activities of extracts and extract or essential oil components.

Antimicrobial activities of leaf and flower extracts of *H. scabrum* samples were given in Table 2. Antimicrobial activities of studied samples were accepted as strong if equal to or less than 0.1 mg/mL, as mediocre if 0.1<MIC<0.625 mg/mL, and as mild if MIC higher than 0.625 mg/mL [27,28]. Both leaf and flower extracts of the plant were shown mediocre antimicrobial activity on *E. coli* (MIC values 0.3 and 0.5 mg/mL, respectively). Leaf extracts of the plant were shown mediocre antimicrobial activity on *S. aureus* (MIC value 0.5 mg/mL). Overall, neither leaf nor flower extracts of the plant have a significant antimicrobial activity on studied microorganisms. Antimicrobial activities of essential oils obtained from *H. scabrum* plant were investigated in different localizations by various researchers. Since the localizations where *H. scabrum* samples collected differ in their altitude, humidity, precipitation, temperature and similar climatic factors, both the essential oil components and antimicrobial activities also change. In a previous study on essential oils of *H. scabrum* plant, a wide antimicrobial spectrum was observed and higher activity compared to standard antibiotics against *B. cereus, E. coli, S. aureus*, and *P. aeruginosa* was determined [29]. Conversely, some studies were determined no significant effect of *H. scabrum* plant extracts on bacteria and fungi [30]. In another study conducted in 2008, ethanol, methanol extracts of *H. scabrum* species were used have shown to be effective on Gram positive bacteria [31]. In a study in Pakistan it was found that the methanolic extract and essential oils of the *H. scabrum* plant exhibited strong antimicrobial activity but no antimicrobial activity found in ethanol, water, acetone extracts [32]. In our study, ethanol extracts of *H. scabrum* plant were provided generally no favorable antimicrobial activity on studied microorganisms (MIC value > 0.625 mg/mL). Only mediocre antimicrobial activity was observed on *E. coli* and *S. aureus*. Precise antimicrobial potency of the plant might be revealed in further studies by challenging microorganisms that are more diverse.

Antioxidant potency of plants are resulting from their capabilities of eliminating or transforming free radicals. It can be postulated that higher the antioxidant capacity of a plant, so higher the therapeutic potential of the plant [8,21,33]. It was determined

Table 3
TAS, TOS and OSI values of *H. scabrum* plant extracts.

		TAS (mmol/L)	TOS (μmol/L)	OSI
H. scabrum **flower extract**	**Sivas**	16.2 ± 0.6	8.3 ± 1.0	0.05±0.2
	Elazığ	16.6 ± 0.5	9.0 ± 2.0	0.04±0.1
H. scabrum **leaf extract**	**Sivas**	17.1 ± 1.4	7.5 ± 0.9	0.04±0.2
	Elazığ	17.3 ± 0.5	8.2 ± 0.5	0.05±0.1

in the study that both all extracts of each parts of the plant have a potent antioxidant capacity and antioxidant activities of leaf and flower extracts are similar to each other (Table 3). In addition, oxidative stress index (OSI) which is important in evaluation of antioxidant to oxidant ratio of *H. scabrum* plant extracts was observed to be very low. In many studies on different plant species, TAS value of *Mentha* sp. was observed 3.628 mmol/L, TOS value was 4.046 μmol/L [34]. TAS value of *Salvia multicaulis* was obtained 6.434 mmol/L, TOS value was 22.441 μmol/L [35]. TAS value of *Rhus coriaria* var. *zebaria* was defined 7.342 mmol/L, TOS value was 5.170 μmol/L [36]. TAS value of *Allium calocephalum* was demonstrated 5.853 mmol/L, TOS value was 16.288 μmol/L [37]. TAS values of *Thermopsis turcica, Brassica rapa* and *Calendula officinalis* have been presented as 2.06 mmol/L, 1.25 mmol/L and 5.55 mmol/L, respectively [38–40]. Also TAS values of *Rumex crispus, Gundellia tournefortii, Glaucium grandiflorum* have been reported as 7.313 mmol/L, 6.831 mmol/L, 0,475 mmol/L, respectively [21,33]. Compared to these studies, TAS values of *H. scabrum* was found higher than all these plants. TOS values of *H. scabrum* was found also higher than these plants. Furthermore, OSI values of *H. scabrum* was found lower than all these plants. This difference in TAS, TOS and OSI values is due to the potential to provide endogenous antioxidants of plants. There are different studies in literature for assessment of antioxidant activities of *Hypericum* plants by different methods, and according to these studies antioxidant activities of plant extracts from *Hypericum* species are known to be very high [41]. Our study also supports the literature. In our study, determination of TAS, TOS, and OSI values of *H. scabrum* by using Diagnostic kit, which provides 99% reliable results, was conducted for the first time [21]. *H. scabrum* plant can be stated as a good source for antioxidants since its extracts have a high antioxidant capacity and low oxidative stress index. Therefore, it can be used in further studies where clearing up of ailments caused by free radicals will be studied.

Cytotoxic activities of both flower and leaf extracts of *H. scabrum* plant on 5 different cell lines in several concentrations (1–10 to 100–1000 μg/mL) after 24 h of incubation were examined. IC_{50} values of extracts, which indicates the extract effects on different cell lines were given in Table 4. It was determined that IC_{50} values of extracts were generally particularly higher than 100 μg/mL dosage. Consequently, it is obvious that extracts have no significant cytotoxic effect on cell lines (Table 4 and Fig. 2). Again, leaf extracts showed no cytotoxic effect on HUVEC, MCF-7, and HT29 lines but provided slight cytotoxicity on PC-3 and C6 lines (Fig. 2). Advanced anticancer analyses were not conducted since IC_{50} values of extracts on cell lines were higher than 100 μg/mL threshold. However, by using IC_{50} values, it is possible to compare

Table 4
IC_{50} levels in different cell lines of *H. scabrum* plant crude extracts (μg/mL).

		HUVEC	MCF-7	PC-3	C6	HT29
Flower extract	**Sivas**	405.4 ± 2.3	215.6 ± 2.1	296.1 ± 2.6	179.0 ± 3.4	365.8 ± 4.4
	Elazığ	358.7 ± 3.2	374.4 ± 4.5	361.8 ± 4.0	218.9 ± 4.2	340.6 ± 4.9
	Sivas	496.6 ± 4.4	318.7 ± 4.0	201.4 ± 2.8	205.9 ± 4.0	419.8 ± 5.7
Leaf extract	**Elazığ**	425.4 ± 4.6	333.9 ± 3.9	279.4 ± 4.7	217.4 ± 3.9	402.6 ± 6.0

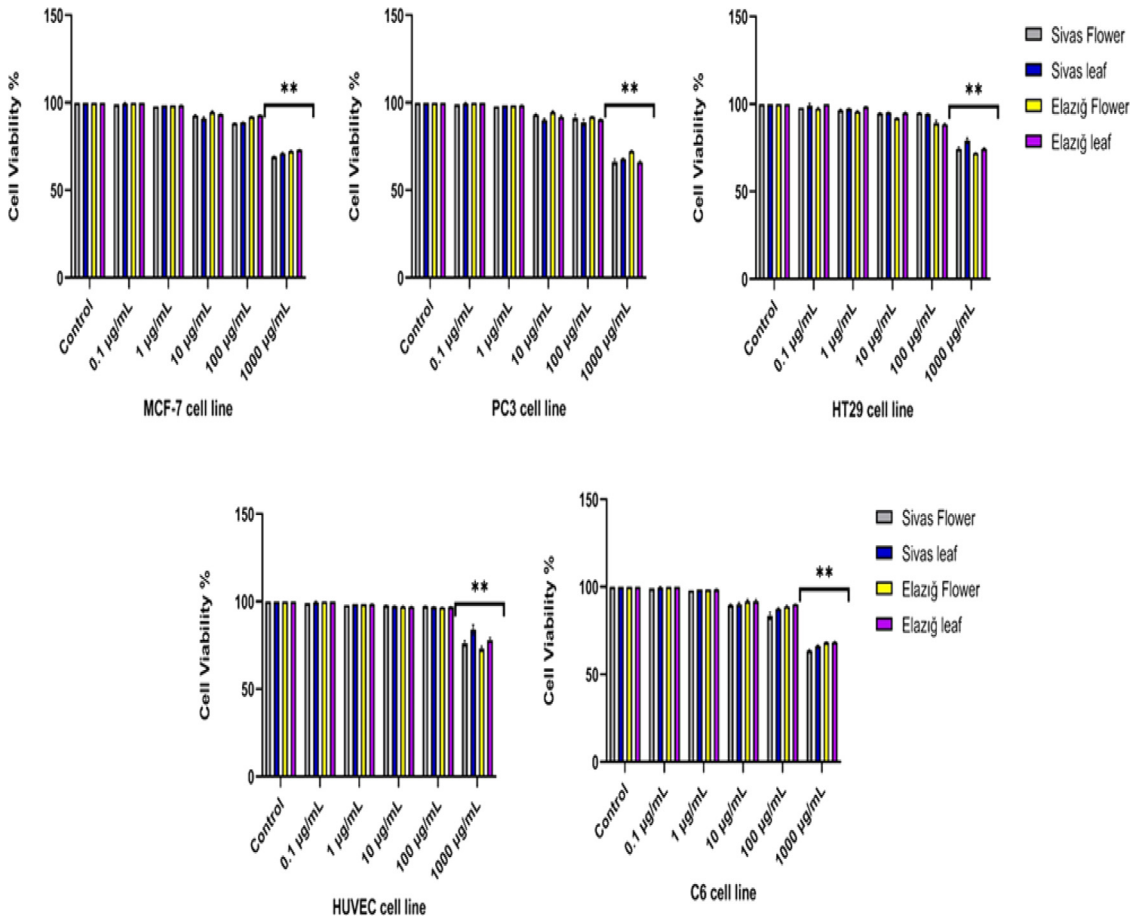

Fig. 2. Cell viability of plant extracts in different cells.

Table 5
The selectivity index (SI) of plant extracts at 24 h treatment.

Selectivity index		HUVEC/PC-3	HUVEC/MCF-7	HUVEC/HT29	HUVEC/C6
Flower Extract	**Sivas**	1.37	1.88	1.11	2.27
	Elazığ	0.99	0.96	1.05	1.64
Leaf extract	**Sivas**	2.47	1.56	1.18	2.41
	Elazığ	1.52	1.27	1.06	1.96

plant extracts on 5 cell lines to determine to provide which cell lines are more affected by using Selectivity Index (SI). The selectivity index (SI) of plant extracts were evaluated by obtaining the ratio of IC_{50} in the healty cell line/IC50 in the cancer line [42]. The SI value of flower extracts of plant were not higher than the leaf extracts in almost all cell lines. These results suggest that flower extracts are more cytotoxic against the almost all cancer cell lines in this study (Table 5). According to the literature, essential oil components of *Hypericum* species have been reported to exhibit antitumoral activity. Researchers found that the methanolic and water extracts of the root parts of the *H. scabrum* plant have cytotoxic activity on some cell lines such as A549, L929 [43]. In our study, the cytotoxic effects of crude ethanolic extracts collected from the aerial parts of the *H. scabrum* plant on the cell lines we tried were not detected. Since HUVEC cell line is composed of healthy epithelial cells, the observation of extracts not damaging the healthy cells is a promising result. Overall, it is expected in drug developments from natural crude extracts to not damage healthy cells while being cytotoxic to cancerous cells. However, such an observation is possible with using raw extracts where purification of components is not performed. Since plants have hundreds of compounds and only if one of these has a real anticancer activity, it would be difficult to obtain reliable activity values of such compounds in raw extracts.

Table 6
Some Mineral contents of *H. scabrum* plant extracts from Elazığ.

ICP-OES results	Elements	*Hypericum scabrum* Leaf Extract		*Hypericum scabrum* Flower Extract	
		Mean±SD	Min.-Max.	Mean±SD	Min.-Max.
Parts Per Billion (ppb)	Co	3.2 ± 0.1	0.317 - 120.0	4.1 ± 0.1	0.317 - 120.0
	Cu	69.1 ± 0.3	0.639 - 120.0	115.9 ± 1.1	0.639 - 120.0
	Cd	3.3 ± 0.1	0.063 - 90.0	2.6 ± 0.1	0.561 - 90.0
	Pb	10.9 ± 0.1	0.377 - 120.0	9.1 ± 0.4	0.870 - 120.0
	Ni	17.3 ± 0.2	0.171 - 120.0	15.7 ± 0.3	0.696 - 120.0
	Cr	7.6 ± 0.2	0.311 - 120.0	8.6 ± 0.1	0.251 - 120.0
	Mn	240.5 ± 0.8	0.203 - 120.0	206.4 ± 0.5	0.295 - 120.0
	Se	33.9 ± 0.3	1.821 - 120.0	32.0 ± 1.1	2.907 - 120.0
	Zn	118.8 ± 0.3	0.384 - 120.0	119.9 ± 1.0	0.793 - 120.0
	As	24.8 ± 0.4	1.509 - 120.0	23.6 ± 0.8	2.025 - 120.0
	Ba	77.8 ± 0.5	0.483 - 120.0	27.2 ± 0.1	0.752 - 120.0
Parts Per Million (ppm)	Na	0.3 ± 0.01	0.003 - 12.0	0.3 ± 0.01	0.003 - 12.0
	Ca	32.4 ± 0.01	0.002 - 12.0	32.5 ± 0.02	0.002 - 12.0
	Al	2.9 ± 0.01	0.001 - 6.0	3.1 ± 0.01	0.001 - 6.0
	Fe	2.1 ± 0.01	0.001 - 12.0	2.2 ± 0.01	0.001 - 12.0
	P	18.0 ± 0.03	0.007 - 12.0	28.5 ± 0.08	0.007 - 12.0
	Mg	39.2 ± 0.09	0.008 - 12.0	29.5 ± 0.05	0.008 - 12.0
	B	0.5 ± 0.01	0.007 - 12.0	0.3 ± 0.01	0.007 - 12.0
	Si	6.8 ± 0.03	0.035 - 12.0	5.9 ± 0.03	0.035 - 12.0

Mineral contents of raw extracts of both leaf and flower parts of the plant were observed by using Inductive Coupled Plasma Optical Emission Spectrophotometry (ICP-OES). In Table 6, concentrations of toxic minerals (Cd, Pb, Cr, Ba, As, Al, and Ni), micro minerals (Cu, Fe, Mn, B, Co, Se, and Zn), and macro minerals (Ca, P, Na, Si, and Mg) in plant extracts were given. Overall, amounts of minerals in leaf and flower parts were determined to be similar to each other. It was known that the vegetation diminishes by closing to the roadsides. Also, it was reported that heavy metal concentrations of plants and soil increase by being in residential and industrial regions [44]. It was also reported that different elements accumulate in varying amounts in different parts of plants [45]. Tolerable limits of heavy metals with high concentrations are differing in plant species. According to Allen (1989), concentrations in plants for Mn are 50–500 µg/g, Ni are 0.5–5 µg/g, Cr are 0.05–0.5 µg/g, Cu are 2.5–25 µg/g, Cd are 0.01–0.3 µg/g, Pb are 30–3000 µg/g, Zn are 10–100 µg/g [46]. Obtained element results in our study were close to these values.

4. Conclusion

H. scabrum has a very high potency in antioxidant capacity and shows mediocare antimicrobial activity on some bacteria. But it has no significant cytotoxicity on investigated cell lines. Nevertheless, this research, in which we try to reveal the bioactive pharmacological properties of the *H. scabrum* plant, will contribute to the available literature. Consequently, our study would be beneficial for the selection and designing of model molecules and for pharmacognosy projects. Since pharmacology studies on *H. scabrum* plant are not enough at present, further studies in industrial, agricultural, and pharmaceutical prospects are recommended to reveal exact medicinal potency of the plant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] Z. Selamoglu, The natural products and healthy life, J. Trad. Med. Clinic. Naturop. 7 (2) (2018) 1–2.
[2] Z. Selamoğlu, Polyphenolic compounds in human health with pharmacological properties, J. Trad. Med. Clinic. Naturop. 6 (4) (2017) 137.
[3] G. Akalin, Z. Selamoglu, Nutrition and foods for skin health, J. Pharm. Care 7 (2019) 31–33.
[4] U.M. Khan, M. Sevindik, A. Zarrabi, M. Nami, B. Ozdemir, D.N. Kaplan, Z. Selamoglu, M. Hasan, M. Kumar, M.M. Alshehri, J.S. Rad, Lycopene: food sources, biological activities, and human health benefits, Oxid. Med. Cell Longev. (2021) 2713511.
[5] B. Nageen, I. Sarfraz, A. Rasul, G. Hussain, F. Rukhsar, S. Irshad, A. Riaz, Z. Selamoğlu, M. Ali, Eupatilin: a natural pharmacologically active flavone compound with its wide range applications, J Asian Nat Prod Res 22 (1) (2020) 1–16.
[6] J. Nawaz, A. Rasul, M.A. Shah, G. Hussain, A. Riaz, I. Sarfraz, S. Zafar, M. Adnan, A.H. Khan, Z. Selamoğlu, Cardamonin: a new player to fight cancer via multiple cancer signaling pathways, Life Sci. 250 (2021) 117591.
[7] Z. Selamoğlu, biotechnological approaches on anticancer activity of flavonoids, Modern Approaches Drug Des. 1 (2) (2017) 510.
[8] Z. Selamoglu Zeliha, C. Düşgün, H. Akgul, M.F. Gülhan, In-vitro antioxidant activities of the ethanolic extracts of some contained-allantoin plants, Iran. J. Pharm. Res. 16 (5) (2017) 92–98.
[9] S. Türkoglu, S. Celik, I. Türkoglu, U. Cakılcıoglu, M. Bahsi, Determination of the antioxidant properties of ethanol and water extracts from different parts of *Tecrium parviflorum* Schreber, Afr. J. Biotechnol. 9 (40) (2010) 6797–6805.
[10] P.H. Davis, R.R. Mill, K. Tan, in: Flora of Turkey and East Aegean Islands, 10, Edinburgh University Press, Edinburgh, 1988, p. 590.
[11] T. Baytop, Therapy With Medicinal Plants in Turkey (Past and Present), 2nd ed., Nobel Press, Istanbul, Turkey, 1999.
[12] G. Laakmann, C. Schule, T. Baghai, M. Kieser, St. John’s wort in mild to modarate depression; the relevance of hyperforin for the clinical efficacy, Pharmacopsychiatry 31 (1998) 54–59.
[13] K.N. Schotz, Rytin is essential for the antideppressant activity of *Hypericum perforatum* extracs in the forced swimmingtest, Planta Med. 68 (2002) 577–580.
[14] J.F. Rodriguez-Landa, C.M. Contreras, A review of clinical and experimental observations about antidepressant actions and side effects produced by *Hypericum perforatum* extracts, Phytomedicine 10 (2003) 688–699.

[15] X. Xie, J.B. Hudson, E.S. Guns, Tumor-specific and photodependent cytotoxicity of hypericin in the human LNCaP prostate tumor model, Photochem. Photobiol. 74 (2001) 221–225.
[16] J.G. Bennet, H.H. Lee, Xsanthones from Guttiferae, Phytochemistry 28 (1989) 967–998.
[17] N. Tanaka, Y. Takaishi, Y. Shikishima, Y. Nakanishi, K. Bastow, K.H. Lee, Prenylated benzophenones, xanthones *H. scabrum*, J. Nat. Prod. 67 (2004) 1870–1875.
[18] J.N. Eloff, A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria, Planta Med. 64 (1998) 711–713.
[19] CLSIMethods For Dilution Antimicrobial Susceptibility Tests For Bacteria that Grow Aerobically, Approved Standard, 9th ed., Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012 CLSI document M07-A9.
[20] CLSIReference Reference Method For Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard, 2nd ed., CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087- 1898, USA, 2002 NCCLS document M27- A2.
[21] S. Durna Dastan, H. Durukan, A. Demirbas, E. Donmez, Bioactivity and therapeutic properties of evelik (*Rumex crispus*), a naturally growing and edible plant in Sivas province, Turk. J. Agric. Food Sci. Technol. 7 (2019) 67–71.
[22] O. Erel, A novel automated direct measurement method total antioxidant capacity using a new generation, stable ABTS radical cation, Clin. Biochem. 37 (4) (2004) 277–285.
[23] O. Erel, A new automated colorimetric method for measuring total oxidant status, Clin. Biochem. 38 (12) (2005) 1103–1111.
[24] Origin (Pro)Origin 2021, OriginLab Corporation, Northampton, MA, USA, 2021.
[25] F.S. Sharopov, I.S. Gulmurodov, W.N. Setzer, Essential oil composition of H. perforatum, and H. scabrum growing part of Tajikistan, J. Chem. Pharm. Res. 2 (6) (2010) 284–290.
[26] J. Jamilah, A.A. Sharifa, N.R.S.A. Sharifah, GC-MS analysis of various extracts from leaf of Plantago major used as traditional medicine, World Appl. Sci. J. 17 (2012) 67–70.
[27] V. Kuete, Potential of Cameroonian plants and derived products against microbial infections: a review, Planta Med. 76 (2010) 1479–1491.
[28] M.D. Awouafack, L.J. McGaw, S. Gottfried, R. Mbouangouere, P. Tane, M. Spiteller, J.N. Eloff, antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from Eriosema robustum (Faba-ceae), BMC Complement Altern Med 13 (2013) 289.
[29] G. Kizil, Z. Toker, H.C. Ozen, C. Aytekin, The antimicrobial activity of essential oils of *Hypericum scabrum, Hypericum scrabroides, Hypericum triquetrifolium*, Phytother. Res. 18 (2004) 339–341.
[30] P.A. Ghasemi, G.H. Rahnama, F. Malekpoor, B.H. Roohi, Variation in antibacterial activity and phenolic content of *Hypericum scabrum* L. populations, J. Medicin. Plants Res. 5 (17) (2011) 4119–4125.
[31] R. Duman, A. Sevimli, The determination of antibacterial activities of *H. perforatum, H. scabrum* and *H. kotschyanum* extracts, Suleyman Demirel Univ. J. Sci. Liter. Faculty 31 (2008) 27–33.
[32] A. Heshmati, M.Y. Alikhani, M.T. Godarzi, M.R. Sadeghimanesh, The evaluation of antioxidant and antimicrobial activities of essential oil and aqueous, methanol, ethanol, ethyl acetate and acetone extract of *Hypericum scabrum*, Int. J. Nutr. Food Eng. 12 (2) (2018) 1–10.
[33] H. Sarac, A. Demirbas, S. Durna Dastan, M. Atas, O. Çevik, N. Eruygur, Evaluation of nutrients and biological activities of kenger *(Gundellia tournefortii)* seeds cultivated in Sivas, Turk. J. Agric. Food Sci. Technol. 7 (2019) 52–58.
[34] M. Sevindik, H. Akgul, M. Pehlivan, Z. Selamoglu, Determination of therapeutic potential of *Mentha longifolia* ssp. *longifolia*, Fresenius Environ. Bull. 26 (2017) 4757–4763.
[35] M. Pehlivan, M. Sevindik, Antioxidant and antimicrobial activities of *Salvia multicaulis*, Turk. J. Agric. Food Sci. Technol. 6 (5) (2018) 628–631.
[36] F.S. Mohammed, H. Akgul, M. Sevindik, B.M.T. Khaled, Phenolic content and biological activities of *Rhus coriaria* v. *zebaria*, Fresenius Environ. Bull. 27 (8) (2018) 5694–5702.
[37] F.S. Mohammed, M. Karakas, H. Akgul, M. Sevindik, Medicinal properties of *Allium calocephalum* collected from Gara Mountain (Iraq), Fresenius Environ. Bull. 28 (10) (2019) 7419–7426.
[38] L. Aksoy, E. Kolay, Y. Agılonu, Z. Aslan, M. Kargıoglu, Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *T. turcica*, Saudi J. Biol. Sci. 20 (3) (2013) 235–239.
[39] S. Gul, S. Ahmed, H. Gul, K.F. Shad, M. Zia-Ul-Haq, D. Badiu, The antioxidant potential of *Brassica rapa* L. glutathione peroxidase, superoxide dismutase enzymes, antioxidant status, Roman. J. Lab. Med. 21 (2) (2013) 161–169.
[40] P.K. Verma, R. Raina, M. Sultana, M. Singh, P. Kumar, Total antioxidant and oxidant status of plasma and renal tissue of cisplatin-induced nephrotoxic rats: protection by floral extracts of *Calendula officinalis* Linn, Ren. Fail. 38 (1) (2016) 142–150.
[41] K. Shimada, K. Fujıkava, K. Yahara, T. Nakamura, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, J. Agric. Food Chem. 40 (1992) 945–948.
[42] M. Al-Qubaisi, R. Rozita, S.K. Yeap, A.R. Omar, A.M. Ali, N.B. Alitheen, Selective cytotoxicity of goniothalamin against hepatoblastoma HepG2 cells, Molecules 16 (2011) 2944–2959.
[43] G. Şeker Karatoprak, C. Yucel, H.C. Kaytan, S. Ilgun, E.K. Safak, Antioxidant and cytotoxic activities of aerial and underground parts of *H. scabrum*, Iran. J. Sci. Technol. 43 (2019) 2107–2113.
[44] S. Onder, S. Dursun, S. Gezgin, A. Demirbas, Determination of heavy metal pollution in gras and soil of city centre green areas (Konya, Turkey), Polish J. Environ. Stud. 16 (1) (2007) 145.
[45] G. Guleryuz, A. Arslan, B. Izgi, S. Gocer, Element content (Cu, Fe, Mn, Ni, Pb, and Zn) of the ruderal plant *Verbascum olympicum* Boiss from east mediteranean Z, Naturforsch 61 (2006) 357–362.
[46] S.E. Allen, Analysis of Ecological Materials, 2nd ed., Blackwell Scientific Publications, Oxford, 1989.