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Antioxidant and antimicrobial activities of plants grown in the Mediterranean region

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Abstract

Background: The main objective of this research was to identify plant species with possible bioactivities based on their total phenol content, antioxidant, and antimicrobial properties. Therefore, different parts of 42 plant species grown in the Mediterranean region were extracted with aqueous ethanol solutions to prepare extracts with antioxidant and antimicrobial activities, mainly resulting from their total phenol contents. No detailed laboratory data on the flora of this area exists regarding their total phenol contents and total antioxidant activities.

Results: Yields of extraction for each plant material were determined. Extracts were characterized based on their total phenol contents, total antioxidant (both hydrophilic and lipophilic), and antimicrobial activities using Folin–Ciocalteu, Photochemiluminescence, disc diffusion, and microdilution methods, respectively. The extract of *Hypericum empetrifolium* had the relatively highest total water-soluble and lipid-soluble antioxidant activities. *Sarcopoterium spinosum* extract had relatively high total phenol content. Preliminary screening study was conducted with the disc diffusion method to evaluate the extracts' antimicrobial activities. 26 out of 42 plant species showed significant antimicrobial activities against the growth of microorganisms. Microdilution assays were performed to evaluate the most active plant species with their minimum inhibition concentrations. *H. empetrifolium*, *Pistacia terebinthus*, *Arbutus unedo*, and *Cistus parviflorus* were the most antimicrobial plant species among those investigated.

CONCLUSION: The new potential sources for the isolation of bioactive natural compounds from specific plant species could be possible with the help of this present screening study. Isolated bioactive natural compounds can be utilized as raw materials in cosmetics, nutraceuticals, food supplements, and pharmaceutical industries.

KEYWORDS
antimicrobial activity, antioxidant activity, disc diffusion method, MIC medicinal plants, PCL method, total phenol content

INTRODUCTION

In many industrial applications, plant-derived natural compounds are preferred. Like natural resources, their usage in the pharmaceutical, nutritional, and cosmetic industries increases across the globe,[1] especially in the post-pandemic era.[2,3] Different disorders have been treated with medicinal and aromatic plants. Today, their clinical evidence is still being evaluated by discovering and isolating bioactive phytochemicals in these plant materials. Phytochemicals are bioactive natural compounds that are secondary plant metabolites belonging to

several subgroups.[4] A wide range of these compounds has antioxidant, antimicrobial, antiviral, and anticancer activities. Nowadays, plants have regained this attention primarily owing to their bioactivities that may protect or prevent the harmful damage of free radicals. The main reason for free radical production is the oxidation reaction occurring in food, medicine, and living systems. As by-products of aerobic metabolism, reactive oxygen species (O_2^-, H_2O_2, and OH^-) can destroy the membrane lipids, cellular proteins, and DNA are formed. The damage to these biomolecules results in specific diseases and aging.[4] To protect the significant biomolecules that are possibly attacked by free radicals, both enzymatic and non-enzymatic cellular defense systems are already available in living organisms.[5] Nutraceuticals, including dietary antioxidants, can help reduce oxidative stress effects. In plants, bioactive phenolic compounds act as natural antioxidants that inhibit free radicals by giving off one hydrogen atom from their structure.[6] Nowadays, plants have regained significant attention mainly owing to their antimicrobial activities that may delay or inhibit the hazardous effects of harmful microorganisms. The persistent emergence of multidrug-resistant microorganisms is a severe problem addressed by today's healthcare professionals. The widespread and sometimes improper use of antibiotics is a significant factor in developing antimicrobial-resistant strains. As antibiotic resistance has grown, there has been a research push and develop novel antimicrobial medicines based on plant-derived active principles.[7] For instance, plant-derived therapeutics based on the extraction of plant bioactives have been developed[8] for different uses, for example, against multidrug-resistant bacteria, which are pure compounds with far more potent pharmacological actions than their synthetic counterparts. Plant extracts are important materials that have potential antimicrobial agents that confer an antimicrobial defense against microbes in their own environment.[9] Exploration of the chemical constituents of plants and pharmacological screening are of great importance which leads to the development of novel agents.[10] The most important phytochemicals are alkaloids, flavanoids, tannins, terpenoids, and some other phenolic compounds which are abundantly found in plants.[11] In recent studies, the use of therapeutically valuable plant-derived extracts and their isolated bioactive compounds as antimicrobial agents has become important.[12–16] Plant-derived antimicrobial agents showed strong antibacterial activity against gram-negative and gram-positive bacteria.[17–19] Flavonoids and other polyphenols have also been suggested as potential inhibitors of SARS-CoV-2 infection.[8] As a result, plants serve as green factories, producing numerous components with medicinal potential. This implies that plants and their derivatives could be screened for their bioactivities, particularly their antioxidant and antibacterial properties.[20] Plant bioactive and polyphenolic extracts could be recovered with the final aim of fortifying foods and replacing synthetic additives.[21–23] Many different endemic species grown in Turkey can be considered as a significant part of the rich flora in Europe. The main objective of this research was first to prepare the extracts from the plant materials belonging to the 42 common plant species from the same location, and then identify their relative total phenol content, antioxidant and antimicrobial activities. This paper also provides a scientific evaluation of plant species that might have potential health-beneficial effects, forming the basis for more detailed investigations of particular plant species in the future.

MATERIALS AND METHODS

Reagents

Kits for measuring total antioxidant activities with a Photochem analyzer were purchased from Analytik Jena AG. Dimethyl sulfoxide (DMSO) was obtained from Ameresco. Methanol, ethanol, gallic acid, sodium carbonate anhydrous, and Folin–Ciocalteu reagent were all bought from Sigma-Aldrich.

Various growth mediums (broth and agar) were utilized to cultivate microorganisms. Nutrient Agar (70116, Fluka), Nutrient Broth (70122, Fluka), and Bacto Agar (214010, Fisher Scientific) were used as growth media. For diluting the microbial cultures, Mueller-Hinton Broth (A3751) and Bacto Peptone (211677) were purchased from Applichem and Thermo Fisher Scientific, respectively. The antibacterial activity of plant extracts in microdilution experiments was compared to that of gentamicin (15710, Invitrogen) and penicillin (Iecilline flacon 400,000 IU, local pharmacy store). The antibiotic discs including penicillin G (CTOO43B), gentamicin (CTOO24B), and vancomycin (CTOO58B) from OXOID were used in the disc diffusion experiments. Stock cultures were prepared with glycerol and stored in a refrigerator at −80°C (Revco, Thermo Scientific). Minimum inhibition concentration (MIC) values were observed visually with Iodonitrotetrazolium chloride (INT) as a drying reagent in 96 well plates.

Materials

Foty-two plant species were gathered from the Karaburun region (west of Turkey). Different parts of the plants, such as fruits, stems, and leaves, were used to prepare their extracts for further analysis corresponding to extracts of 47 plant materials. Voucher specimens of all the reported plants were taken after an authority identified their scientific names.

Preparation of plant extracts

All plant materials were subjected to the same extraction procedure. After grinding the dried plant materials, 10 g of powdered plant materials were extracted with 200 ml of aqueous ethanol solution (80% ethanol by volume) at 35°C and 180 rpm for 2 h. After centrifugation at 5000 rpm, the liquid ethanolic extract was further processed with a rotary vacuum evaporator to remove ethanol. The remaining aqueous liquid extracts were dried with a lyophilizer to obtain solid crude extracts. Extraction percent yield (wt/wt) was determined based on the amount of these solid crude extracts stored in a refrigerator for further use in all analyses.

Determination of antioxidant activity

Total water-soluble (ACW) and lipid-soluble (ACL) antioxidant activities were measured with a Photochem antioxidant analyzer (Analytik Jena AG, Germany), which was developed based on the photochemiluminescence method (PCL) method in the literature.[24] The PCL assay depends on photochemical free radical generation to be detected sensitively using chemiluminescence.[25] In this assay, the superoxide radical ($O_2^{\bullet -}$) is formed with a reaction that is induced by optical excitation ($h\upsilon$) of the photosensitizer (luminol) S (Equation 1). It is known to be the most dangerous ROS (Reactive Oxygen Species) found in the human body.[5]

$$S + h\upsilon + O_2 \rightarrow [S^* O_2] \rightarrow S^{\bullet +} + O_2{}^{\bullet -}. \quad (1)$$

In PCL assays, luminol is used as a photosensitizer, and another action of luminol is an oxygen radical detection reagent in the assay.[25] A measuring signal is obtained related to the free radical generation, and the quantification of the antioxidants is based on the changes in the measuring signal caused by them. The antioxidant activities are expressed in terms of an equivalent concentration of Ascorbic acid and Trolox for ACW (antioxidant capacity of water-soluble compounds) and ACL (antioxidant capacity of lipid-soluble compounds), respectively.[26] ACW measurements compare the time difference as lag time between the sample and the blank. ACL measurements are based on the area under the curve due to ROS inhibition for the blank sample.[27]

Determination of total phenol content

The crude extracts of plant materials were subjected to total phenol content analyses. The Folin–ciocalteu method was used for the analyses. After 10-fold dilution with deionized water, 2.5 ml Folin–Ciocalteu reagent was mixed with 500 μl of plant extract or standard (gallic acid) solutions. Then this mixture was kept at room temperature for 2.5 min before adding 2 ml of sodium carbonate (7.5%) and incubating (for 1 h in the dark) at room temperature. The absorbance was determined using a UV spectrophotometer (Perkin Elmer) at 725 nm. Finally, the content of total phenols for each extract was calculated as gallic acid equivalents (GAE)/g of the extract by using the calibration curve of gallic acid.

Determination of antibacterial activities of plant extracts

Preparation of stock cultures of strains

The overnight incubation of strains (lyophilized powders from suppliers) at optimum conditions (time, temperature, shaking, etc.) was performed using appropriate broths. In assays, optical density (OD) values of cultures inoculated into 96-well plates were fixed to obtain the same microbial load that reflects a certain number of bacteria (CFU/ml) measured using the classic colony counting method.

Disc diffusion assays

Disc diffusion assays determined the initial screening of the 47 plant extracts' relative antimicrobial effects. In this method, the antimicrobial agent impregnated into a filter disc was allowed to diffuse into the agar medium inoculated with a specific culture of interest to determine the susceptibility of a microorganism to an antimicrobial agent. Both gram-positive (*Enterococcus faecium*, *Bacillus subtilis*, NRRL-B-2354, and NRRL B-4378) and gram-negative (*Escherichia coli*, NRRL B-3008) bacteria were selected to monitor the antimicrobial properties of the plant extracts in the disc diffusion assays. All bacteria species were obtained from the Agricultural Research Service Culture Collection (USA).

Determination of MIC values

In the 2-fold micro-broth dilution method,[28] both gram-negative (*E. coli*, NRRL B-3008) and gram-positive (*Staphylococcus aureus*, ATCC 29213 and *Staphylococcus epidermidis*, ATCC 12228) bacteria were used to determine the MIC of the plant extracts. Plant extracts to be tested were dissolved in DMSO to receive stock solutions of 100 mg/ml. The final concentration of the plant extracts was adjusted to 50 mg/ml by two-fold serial dilutions using sterile deionized water. Then each well of the 96-well microplate was filled with 100 and 95 μl of extract solution and nutrient broth, respectively. Next, each well was inoculated with 5 μl of 6 incubated bacterial suspensions. The latest was standardized using a UV spectrophotometer (Perkin Elmer) by correcting their optical densities at 420 nm to 0.8–1.2 absorbance values corresponding to 10^7 CFU/ml. Serial dilutions of both DMSO (50%) and another control well, including 195 μl of NB (Nutrient broth) and 5 μl of the standard inoculum, were considered negative controls for each strain. Serial dilutions of both penicillin (400 U) and gentamicin (10 mg/ml) antibiotics were positive controls. The growth kinetics of each strain was determined by recording the turbidity of microplate wells at 37°C for 24 h with a microplate reader (Varioskan, Thermo) at 620 nm. MIC values (mg/ml) were obtained for each plant extract after the 24 h incubation. The reaction between the metabolites produced by the microorganisms and INT added into each test well caused the pink color to ensure growth. The absence of this visible color indicates the MICs.

RESULTS AND DISCUSSION

The extracts of plant materials from different parts of 42 plant species were prepared to determine extraction yields, their total phenol contents, and total antioxidant activities. The scientific and common names of these plants, along with their codes, are tabulated in

TABLE 1 Plant codes, scientific names, plant parts, and yields of their extraction (%), values of total phenol contents, water-soluble, and lipid-soluble antioxidant activities[29]

Plant codes	Scientific names of plant species	Type of plant parts	Common names	Yields of extraction (%)	Total phenol content (mg GAE/g extract)	ACW (water-soluble compounds) (μg ascorbic acid equivalent/mg extract)	ACL (lipid-soluble compounds) (μg Trolox equivalent/mg extract)
1	*Sarcopoterium spinosum*	L	Thorny burnet	28.74	635.2 ± 12.9	954.6 ± 48.4	789.9 ± 15.3
2	*Pistacia terebinthus*	L	Cyprus turpentine	29.59	567.6 ± 18.4	684.2 ± 3.9	711.1 ± 17.3
3	*Cistus parviflorus*	L	Rockrose	19.05	552.7 ± 17.2	728.9 ± 90.8	644.7 ± 29.7
4	*Arbutus unedo*	L	Strawberry tree	35.81	500.3 ± 34.4	517.1 ± 2.9	867.0 ± 4.4
5	*Quercus coccifera*	L	Kermes oak	7.62	489.6 ± 9.8	693.1 ± 19.9	1053.0 ± 23.7
6	*Hypericum empetrifolium*	L	St John's worts	37.17	483.9 ± 17.8	1866.4 ± 83.9	1889.5 ± 13.8
7	*Pistacia lentiscus*	L	Mastic tree	31.98	469.4 ± 3.3	403.9 ± 4.3	818.8 ± 23.6
8	*Helichrysum pallasii*	F	Pallas' everlasting	8.85	419.1 ± 0.8	649.6 ± 26.1	337.2 ± 31.3
9	*Cercis siliquastrum*	S	Judas tree	14.56	400.6 ± 11.5	138.5 ± 1.5	768.3 ± 1.8
10	*Rumex pulcher*	L	Fiddle dock	18.66	318.5 ± 6.4	144.9 ± 30.8	641.5 ± 12.1
11	*Teucrium chamaedrys*	L	Wall germander	27.8	300.9 ± 11.1	1249.9 ± 39.5	1020.2 ± 16.5
12	*Phillyrea latifolia*	L	Phillyrea	29.91	283.2 ± 26.7	736.3 ± 25.5	1852.6 ± 28.0
13	*Quercus infectoria*	L	Oak	2.79	260.6 ± 2.1	521.4 ± 16.6	676.1 ± 3.2
14	*Salvia virgata*	L	Meadow sage	1.24	258.8 ± 2.1	1212.2 ± 36.3	774.1 ± 29.0
15	*Stachys cretica*	L	Cretan hedge nettle	ND	254.6 ± 2.2	906.4 ± 28.9	937.7 ± 24.4
16	*Anthyllis hermanniae*	L	Kidney vetch	21.37	243.8 ± 0.5	102.6 ± 7.8	151.9 ± 3.9
17	*Lavandula stoechas*	F	French lavender	14.34	228.2 ± 10.9	722.2 ± 30.7	603.2 ± 3.8
18	*Origanum onites*	L	Pot marjoram	19.32	202.8 ± 1.6	820.6 ± 23.4	738.2 ± 19.3
19	*Lavandula stoechas*	L	French lavender	21.55	202.8 ± 14.1	739.8 ± 24.3	701.5 ± 9.3
20	*Vitex agnus-castus*	F	Chaste tree	32.04	192.1 ± 2.9	521.5 ± 22.6	1199.3 ± 33.1
21	*Vitex agnus-castus*	L	Chaste tree	23.34	175.1 ± 4.6	417.7 ± 12.9	1181.4 ± 11.8
22	*Anchusa azurea*	F	Anchusa	14.55	171.2 ± 0.4	128.4 ± 1.4	269.4 ± 1.3
23	*Teucrium polium*	L	Golden germander	11.01	167.8 ± 0.7	739.9 ± 7.2	715.6 ± 4.7
24	*Solanum nigrum*	L	Black nightshade	18.92	167.4 ± 2.5	302.5 ± 3.1	314.6 ± 15.3
25	*Chrysanthemum segetum*	F	Corn daisy	19.41	159.3 ± 4.9	255.5 ± 7.9	212.3 ± 8.4
26	*Smyrnium rotundifolium*	L	Perfoliate alexander	28.74	157.3 ± 9.2	30.3 ± 0.6	50.9 ± 3.3
27	*Verbascum lychnitis*	F	White mullein	1.74	152.3 ± 4.0	367.2 ± 26.5	547.1 ± 24.0

(Continues)

TABLE 1 (Continued)

Plant codes	Scientific names of plant species	Type of plant parts	Common names	Yields of extraction (%)	Total phenol content (mg GAE/g extract)	ACW (water-soluble compounds) (μg ascorbic acid equivalent/mg extract)	ACL (lipid-soluble compounds) (μg Trolox equivalent/mg extract)
28	*Rhamnus alaternus*	L	Italian buckthorn	31.34	151.2 ± 3.5	93.3 ± 1.2	158.5 ± 4.1
29	*Corydothymus capitatus*	L	Oregano Spanish	ND	148.6 ± 5.2	110.6 ± 12.2	573.3 ± 19.7
30	*Alkanna tinctoria*	L	alkanet	8.72	141.4 ± 6.4	613.1 ± 14.0	750.6 ± 29.3
31	*Stachys cretica*	F	Cretan hedge nettle	ND	129.2 ± 0.9	257.1 ± 7.9	374.4 ± 23.6
32	*Verbascum lydium*	L	-	ND	125.6 ± 1.4	319.7 ± 17.7	549.6 ± 15.4
33	*Capparis spinosa*	L	Spiny caper	19.72	122.5 ± 3.6	85.8 ± 8.9	224.9 ± 6.8
34	*Rhamnus alaternus*	S	Italian buckthorn	32,14	121.8 ± 3.0	33.7 ± 1.5	130.5 ± 2.1
35	*Chrozophora tinctoria*	L	Giradol	12,33	121.2 ± 14.7	91.6 ± 0.5	416.7 ± 7.2
36	*Genista acanthoclada*	L	-	15,12	109.9 ± 1.6	115.9 ± 7.9	267.5 ± 7.5
37	*Ballota acetabulosa*	L	Greek horehound	12.03	97.9 ± 1.8	387.4 ± 43.0	516.9 ± 25.2
38	*Rumex pulcher*	S	Fiddle dock	8.31	88.1 ± 2.3	43.6 ± 0.3	106.8 ± 5.2
39	*Aristolochia hirta*	L	-	25.05	83.8 ± 1.6	413.8 ± 8.9	587.8 ± 15.6
40	*Smyrnium rotundifolium*	S	Perfoliate alexander	7.18	81.1 ± 4.9	152.4 ± 2.0	508.3 ± 9.08
41	*Onopordum illyricum*	F	Illyrian cotton thistle	ND	68.5 ± 0.9	463.8 ± 16.4	350.5 ± 9.4
42	*Psoralea bituminosa*	L	Scurvy pea	21.89	67.7 ± 0.4	79.1 ± 3.2	118.4 ± 2.6
43	*Eryngium campestre*	L	Field eryngo	12.99	62.2 ± 2.2	217.2 ± 7.2	325.0 ± 28.9
44	*Allium ampeloprasum*	F	Broadleaf wild leek	ND	60.6 ± 2.6	19.7 ± 0.3	90.9 ± 1.3
45	*Echium plantagi neum*	L	Salvation Jane	ND	42.9 ± 2.6	45.1 ± 9.1	193.5 ± 10.7
46	*Carlina corymbosa*	F	-	16.04	32.2 ± 1.5	52.6 ± 2.1	327.7 ± 2.4
47	*Urtica dioica*	F	Great nettle	9.75	*27.3* ± 0.7	*38.5* ± 3.7	105.8 ± 1.8

Abbreviations: F, flower; L, leaf; ND, not determined; S, seed.

Table 1. The extraction yields varied from 1.74% to 37.17%. Among all plant materials, the highest extraction yields were obtained for *Hypericum empetrifolium* (leaf), *Arbutus unedo* (leaf), *Rhamnus alaternus* (seed), *Vitex agnus-castus* (flower), and *R. alaternus* (leaf).

Antioxidant activity with the PCL assay

Average values as the mean of triplicate measurements for lipid-soluble (ACL) and water-soluble (ACW) compounds antioxidant activities are summarized in Table 1. Antioxidant activities of lipid-soluble (ACL) and water-soluble (ACW) fractions are given as ascorbic acid and Trolox equivalents, respectively. Flavonoids, ascorbic, aminoacid, etc., are considered in water-soluble (ACW) antioxidant activity measurements. In contrast, tocopherols, tocotrienols, carotenoids, etc., are taken into account in lipid-soluble (ACL) antioxidant activity measurements. These fractional antioxidant activities depend on the polarities of natural compounds present in the crude extracts. The sum of the individual ACL and ACW values provides the integral antioxidant capacity.[30]

Almost all extracts showed antioxidant activity in varying degrees. *Hypericum empetrifolium* (leaf) extract had the highest water-soluble antioxidant activity of 1866.39 μg Ascorbic acid equivalent per gram of extract. Another extract with a significant water-soluble antioxidant activity was obtained from *Teucrium chamaedrys* (leaf), which possesses an inhibition effect against free radicals as reported earlier in the literature.[31] The extracts obtained from *Smyrnium rotundifolium* (leaf) and *Allium ampeloprasum* (flower) exhibited the lowest activities among all plant extracts listed in Table 1. In this study, extracts prepared from *A. ampeloprasum* had the lowest total phenol content and antioxidant activities. Similar findings indicating relatively weak bioactivities were reported in some screening studies.[32]

The highest antioxidant activity for the lipid-soluble fraction of the extract prepared from *H. empetrifolium* (leaf) was determined as 1889.51 μg Trolox equivalents per mg extract (Table 1). Among all extracts, relatively higher antioxidant activities were observed for *Phillyrea latifolia* (leaf) and *V. agnus-castus* (flower) extracts. The bioactive compounds present in *V. agnus-castus* were isolated and identified by several researchers in the literature.[33] *V. agnus-castus* was an important plant that was used for the treatment of diseases in folk medicine. The lowest activity for a lipid-soluble fraction of the extract was determined for *A. ampeloprasum* (flower).

Total phenol contents of extracts

The extracts of all plant materials had total phenol content of 635.2 to 27.3 mg GAE/g extract. The highest phenolic contents were observed for the extracts of *Sarcopoterium spinosum* (leaf), *Pistacia terebinthus* (leaf), and *Cistus parviflorus* (leaf). Extract of *S. spinosum* (leaf) had the highest phenolic content as 635.2 mg GAE/g extract. Among all other plant materials listed in Table 1, the lowest total phenol contents were recorded for the extracts of *Urtica dioica* (flower), *Carlina corymbosa* (flower), and *Echium plantagineum* (leaf).

Although extracts from several plant species were prepared and had significant antioxidant activities in the present study, the bioactivities of the extracts from these plant materials are still unexplored. Therefore, they need to be investigated with detailed laboratory studies. Among all plant materials in Table 1, our results revealed that extracts of *H. empetrifolium* (leaf) and *S. spinosum* (leaf), *P. latifolia* (leaf), and *C. parviflorus* (leaf) might have a potential for the isolation of bioactive phytochemicals in their contents. Therefore, identifying their phenolic content is necessary to use them in different applications. For example, inhibition of lipid oxidation, scavenging of free radicals, chelation of metal ions, activation of antioxidant enzymes, and inhibition of enzymes that cause oxidation reactions can be performed by phenolic compounds having antioxidant properties. Although researchers have highlighted the direct correlation between total polyphenol contents and antioxidant activities of plant extracts,[34] of our findings did not confirm any strong correlation (Figure S16). Nevertheless, this outcome provided concrete suggestions regarding phenolics, which are not the only vital compounds that cause antioxidant properties. In addition, the presence of considerable quantities of non-phenolic components such as carotenoids, alkaloids, vitamins, and terpenes that contribute to antioxidant activity[4] is also indicated.

Antibacterial activities of plant extracts

Disc diffusion assays

The existence of growth inhibition zones indicates the efficacy of specific antimicrobial agents. Around the disc, the inhibition zones are observed where the antimicrobial chemicals are diffused. Therefore, the agent's concentration and microbe sensitivity, the antimicrobial agent's diffusional rate, the culture media's density or viscosity, and the interaction between the medium and the antimicrobial agent can all influence the zone size. Twenty-six plant species were among the 47 plant extracts of 42 plant species subjected to disc diffusion tests to determine their antibacterial activities inhibiting one or more microorganisms. Table 2 summarizes the antimicrobial activities of 47 plant extracts of all species. No inhibition was observed in 16 plant species in the studied concentration range in the tests and plant extracts having no inhibition were shown with a line in Table 2. Significant antibacterial activities against all tested microorganisms were observed for the extracts of *A. unedo* (L), *C. parviflorus* (L), *Cercis siliquastrum* (S), *Rumex pulcher* (S), *H. empetrifolium* (L), *Pistacia lentiscus* (F), *Quercus coccifera* (L), and *Psoralea bituminosa* (L). In addition, gram-positive bacteria were more sensitive to tested plant extracts compared with gram-negative bacteria. This difference in sensitivity can be attributed to the different structural and inherited features of these two groups of bacteria. In addition, the antimicrobial activities of the plant extracts were compared with those of antibiotics used as controls. As seen in Table 2, robust antimicrobial activity against bacteria tested

TABLE 2 Results for disc diffusion testing of 47 plant extracts and antibiotics (inhibition zones expressed in mm)[29]

Plant codes	Scientific names of plant species	Type of plant parts	Disc diffusion zone (mm)		
			E. coli	*E. faecium*	*B. subtilis*
1	*Sarcopoterium spinosum*	L	8.23 ± 0.421	6.74 ± 1.046	7.33 ± 0.098
2	*Pistacia terebinthus*	L	-	7.75 ± 0.692	8.37 ± 0.077
3	*Cistus parviflorus*	L	7.56 ± 0.556	9.59 ± 0.586	9.19 ± 0.155
4	*Arbutus unedo*	L	6.57 ± 0.806	7.35 ± 0.021	9.75 ± 2.446
5	*Quercus coccifera*	L	7.48 ± 0.856	9.69 ± 0.933	11.35 ± 0.070
6	*Hypericum empetrifolium*	L	7.09 ± 0.042	7.75 ± 0.530	12.30 ± 1.477
7	*Pistacia lentiscus*	F	7.39 ± 0.042	8.345 ± 0.106	9.71 ± 0.657
8	*Helichrysum pallasii*	F	-	-	8.58 ± 0.615
9	*Cercis siliquastrum*	S	7.69 ± 0.509	9.73 ± 0.403	9.38 ± 0.070
10	*Rumex pulcher*	L	-	-	-
11	*Teucrium chamaedrys*	L	-	13.90 ± 0.700	7.83 ± 0.905
12	*Phillyrea latifolia*	L	-	-	6.13 ± 0.183
13	*Quercus infectoria*	L	8.36 ± 0.084	7.22 ± 0.247	7.2 ± 0.141
14	*Salvia virgata*	L	-	-	8.32 ± 0.106
15	*Stachys cretica*	L	-	7.06 ± 1.506	6.24 ± 0.34
16	*Anthyllis hermanniae*	L	-	9.71 ± 3.471	7.29 ± 0.042
17	*Lavandula stoechas*	F	-	6.62 ± 0.530	-
18	*Origanum onites*	L	-	-	7.24 ± 0.289
19	*Lavandula stoechas*	L	-	6.29 ± 0.098	-
20	*Vitex agnus-castus*	F	-	7.17 ± 0.169	9.31 ± 0.254
21	*Vitex agnus-castus*	L	-	6.59 ± 0.841	7.29 ± 0.007
22	*Anchusa azurea*	F	-	-	-
23	*Teucrium polium*	L	-	6.56 ± 0.791	6.3 ± 0.141
24	*Solanum nigrum*	L	-	-	-
25	*Chrysanthemum segetum*	F	7.67 ± 2.085	-	-
26	*Smyrnium rotundifolium*	L	-	-	-
27	*Verbascum lychnitis*	F	-	-	-
28	*Rhamnus alaternus*	L	-	7.06 ± 0.014	-
29	*Corydothymus capitatus*	L	-	-	8.92 ± 0.742
30	*Alkanna tinctoria*	L	-	-	-
31	*Stachys cretica*	F	-	7.64 ± 0.714	6.57 ± 0.601
32	*Verbascum lydium*	L	-	-	-
33	*Capparis spinosa*	L	-	-	-
34	*Rhamnus alaternus*	S	6.54 ± 0.763	6.57 ± 0.813	-
35	*Chrozophora tinctoria*	L	-	-	6.40 ± 0.063
36	*Genista acanthoclada*	L	-	-	-
37	*Ballota acetabulosa*	L	-	-	7.8 ± 0.876
38	*Rumex pulcher*	S	7.76 ± 0.466	9.30 ± 0.219	7.26 ± 0.141
39	*Aristolochia hirta*	L	-	-	-
40	*Smyrnium rotundifolium*	S	-	-	-
41	*Onopordum illyricum*	F	-	-	-
42	*Psoralea bituminosa*	L	6.59 ± 0.834	7.36 ± 0.113	16.21 ± 0.261
43	*Eryngium campestre*	L	-	-	-
44	*Allium ampeloprasum*	F	-	-	-

(Continues)

TABLE 2 (Continued)

Plant codes	Scientific names of plant species	Type of plant parts	Disc diffusion zone (mm)		
			E. coli	*E. faecium*	*B. subtilis*
45	*Echium plantagineum*	L	-	-	-
46	*Carlina corymbosa*	F	6.23 ± 0.332	-	-
47	*Urtica dioica*	F	-	-	-
Antibiotics	Gentamicin		22.24 ± 0.11	11.25 ± 1.08	25.1 ± 0.14
	Penicillin		10.15 ± 0.007	-	23.42 ± 0.01
	Vancomycin		-	-	19.66 ± 0.61

Abbreviations: F, flower; L, leaf; S, seed.

TABLE 3 MIC and disc diffusion assay results of selected plant extracts and antibiotic controls

Plant species	Type of plant parts	*E. coli*		*S. aureus*		*S. epidermidis*	
		Disc diff. Zone (mm)	MIC (mg/ml)	Disc diff. Zone (mm)	MIC (mg/ml)	Disc diff. Zone (mm)	MIC (mg/ml)
Arbutus unedo	L	6.57	>50	7.65	1.56	7.18	0.78
Cistus parviflorus	L	7.56	25	7.84	1.56	7.34	0.78
Hypericum empetrifolium	L	7.09	>50	11.05	0.78	13.20	0.78
Pistacia lentiscus	L	7.39	12.5	9	0.78	7.56	1.56
Pistacia terebinthus	L		>50	8.15	1.56	7.45	0.78
Psoralea bituminosa	L	6.59	25	8.27	>3.125		>1.56
Rumex pulcher	S	7.76	25	9.30	3.125	7.04	>1.56
Sarcopoterim spinosum	L	8.23	>50	10.10	>3.125	7.87	>1.56
Quercus coccifera	L	7.48	25	11.06	>3.125	10.12	3.125
Quercus infectoria	L	8.36	25	7.43	>3.125		>1.56
Vitex agnus castus	L		>50	7.26	>3.125	7.57	>1.56
Positive controls (antibiotics)							
Penicillin		7.02	2 (IU)	≫25	>0.02	≫25	>0.02
Gentamicin (μg/ml)		10.25	0.625	11.35	2.5	10.35	100

Abbreviations: L, leaf; S, seed.

was observed for gentamicin among the antibiotic discs used. Both *E. faecium* and *E. coli* were found to be more resistant to the plant extracts and antibiotic controls. Nevertheless, *B. subtilis* was highly sensitive to both antibiotic controls and plant extracts. The results of disc diffusion assays for the antimicrobial effects of antibiotic controls and some plant extracts against *E.coli* are demonstrated in Figure S1. For each species, disc diffusion tests were carried out in duplicate. The antimicrobial properties of several of the studied extract samples and their duplicates are shown in Figure S2. In disc diffusion tests, *B. subtilis* demonstrated modest resistance to extract samples and antibiotic controls.

Except for *S. spinosum* extract, all plant materials tested in the disc diffusion experiments demonstrated bactericidal activity against *S. aureus*. Bactericidal activity can be observed by visibly cleaned zones surrounding discs. On the other hand, bacteriostatic activity is indicated by cleared zones harboring microcolonies around the discs. With such micro-colonies, the extract of *S. spinosum* demonstrated bacteriostatic activity (Figure S3).

MIC values of plant extracts

In the preliminary experiments, the extracts of 11 antimicrobial (as confirmed by the disc diffusion tests) plant species were examined further for their MIC (Table 3). Thpotentialrial micro-broth dilution method was conducted using 96 well microtiter plates with the final aim of determining the MIC. According to the results, the most active plant species were *A. unedo*, *C. parviflorus*, *P. terebinthus*, *H. empetrifolium*, and *Pistacia lentiscus*. Some of the plant species mentioned above have already been reported for their antimicrobial potential.[35,36] The antimicrobial activities of *P. lentiscus* and *A. unedo* have been well represented in the literature. In contrast, the essential oils

of *C. parviflorus* have been examined for their antimicrobial activity. However, this is the first comprehensive study of the MIC and antimicrobial activities in general of *P. terebinthus* and *H. empetrifolium*.

Our study shows that DMSO used for dissolving extracts has no inhibitory effect on *S. epidermidis*, *S. aureus*, and *E. coli* (Figures S4–S7). Penicillin and Gentamicin (Figures S7–S9) have also been assayed to monitor the strains' resistance. According to test results, *E. coli* (NRRL B 3008) as a gram-negative bacteria was a more resistant strain against control antibiotics than the other two species. It is vital to remark that gentamicin showed a MIC value of 100 μg/ml (0.1 mg/ml) for *S. epidermidis*. At the same time, *P. terebinthus*, *C. parviflorus*, *H. empetrifolium*, and *A. unedo* extracts showed MIC of 0.78 mg/ml. This dose could be considered as an alternative concentration for gentamicin.

The negative and positive control figures were presented in Figures S4–S7. In the control tests, DMSO was shown to have no inhibitory effect on any assayed bacteria. Figures S10–S15 present the MIC values of some species together with their impacts on bacterial growth. A visible color indication for MIC was conducted using INT to confirm the test results. This dye reacts with the microorganisms' metabolic products developing a pink color in the wells that indicates the microbial growth presence. Figures S10–S15 present the visual confirmation of MIC values of the plant extracts. These figures show the antibacterial effects of the most active species (*C. Parviflorus*, *H. empetrifolium*, *P. terebinthus*, and *P. Lentiscus*). It is essential to evaluate these active plants with their chemical constituents in future studies. The phytochemical analysis of other studies has shown that *A. unedo* extracts contain significant amounts of tannins and flavonol glycosides.[37] For instance, its leaves contain 37% tannins, rutin arbutin (as glycoside), quercetin, and arbutoflavoneols.[38,39]

Pistacia leaves are known to contain high amounts of α-Tocopherol (Vitamin E) and, in folk medicine, are widely used to treat ulcers and throat infections.[40] Besides their anti-inflammatory and antioxidant properties, Pistacia species have antimicrobial potential attributed mainly to their phenolic constituents and flavonoids.[41] The antimicrobial effects of *H. empetrifolium* extracts have not been studied in the past except for a study referring to its antimicrobial potential using the disc diffusion method.[42] *C. parviflorus* has also been extensively investigated for the antimicrobial activities of its volatile constituents but is still not thoroughly researched for its leaf extracts.

CONCLUSIONS

Under certain experimental conditions, some potential species were identified for their antibacterial, antioxidant, and total phenol content. Although it is untested to compare quantitatively the results of this study with other screening investigations, a relative correlation confirms the results obtained for some species. Herein, *S. spinosum* exhibited the highest antibacterial and antioxidant properties, as reflected by its high phenolic content. These species have never been examined scientifically before and could be considered a new natural source for the food and nutraceutical industries. Therefore, the extracts of these plant species grown in the region of Karaburun-İzmir can find many potential applications in the food supplement, nutraceutical, and cosmetic industries.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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