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DİDEM DELİORMAN ORHAN

BERRİN ÖZÇELİK

SANEM HOŞBAŞ

MECİT VURAL

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Assessment of antioxidant, antibacterial, antimycobacterial, and antifungal activities of some plants used as folk remedies in Turkey against dermatophytes and yeast-like fungi

Didem DELİORMAN ORHAN[1], Berrin ÖZÇELİK[2], Sanem HOŞBAŞ[1], Mecit VURAL[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Hipodrom, Ankara - TURKEY

[2]Department of Microbiology, Faculty of Pharmacy, Gazi University, 06330 Hipodrom, Ankara - TURKEY

[3]Department of Biology, Faculty of Science and Art, Gazi University, 06330 Hipodrom, Ankara - TURKEY

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Abstract: Twenty-seven aqueous extracts obtained from 21 plants used in the treatment of respiratory tract infections as folk remedies in Turkey were investigated for their relative total phenolic contents and antioxidant, antibacterial, antimycobacterial, and antifungal activities. Antibacterial and antifungal activities against dermatophytes and yeast-like fungi were determined using the microdilution method. Antibacterial activity of all extracts was more pronounced against gram-positive bacteria than against gram-negative bacteria. These extracts also exhibited higher antifungal activity against *Candida krusei* than ketaconazole. Six extracts displayed antidermatophytic activity against *Epidermophyton floccosum*, while only 3 extracts were highly active against *Trichophyton rubrum*. The extracts showed strong activity against both *Mycobacterium tuberculosis* and *Mycobacterium avium*, except for the *Teucrium polium*, *Urtica dioica*, *Salvia fruticosa*, *S. verticillata*, *Rosa canina* (flower, shoot, and root), *Rubus sanctus*, and *Crepis foetida* extracts. Additionally, all extracts exhibited antioxidant activity in DPPH assay except for the *Primula vulgaris*, *Ononis spinosa*, *Nepeta italica*, *Hedera helix*, *Lantana camara* (orange flowers), *U. dioica*, and *C. foetida* extracts. The highest total phenolic content was observed in *R. canina* root extract. No correlation was observed between biological activities and the amount of phenolic compounds. All plants used in this study could be potential sources of new antimicrobial agents.

Key words: Antioxidant activity, antimicrobial activity, total phenolic content

Introduction

The emergence of organisms resistant to nearly all classes of antimicrobial agents has become a serious public health concern in the past several years. The plants that exhibit great activity could be considered as a source of potential antimicrobial compounds. Currently, out of 80% of pharmaceuticals derived from plants, very few are being used as antimicrobials (1). Therefore, many screening studies have been conducted to find new antimicrobial agents from natural or synthetic compounds for a variety of novel active compounds with different molecular targets that control infections caused by microorganisms. Since not all people have access to the benefits of professional health services, particularly in rural areas of developing countries, crude plant extracts that were used in traditional folk medicine for their antimicrobial properties are still widely used to treat infections. Besides the usage of crude plant extracts by human beings for their antimicrobial activity, plants can also

produce antimicrobial compounds to protect themselves from biotic attacks that could be essential for microbial infection. Therefore, it is worthwhile to study plants and plant products for activity against microorganisms (2).

Many medicinal plants used in folk medicine against various diseases have been reported with the ethnobotanical field surveys carried out in Anatolia. Numerous scientific studies were designed for the plant species used as folk remedies. Most of the research results are in good agreement with the traditional utilizations of the tested plants. Therefore, the discovery of antimicrobial agents from plants based on the evaluation of traditional plant extracts is a very important research topic.

Free radical-induced oxidative stress is involved in the development of infection and chronic diseases. Antioxidants are considered to play an effective role in inhibiting and scavenging free radicals. The most commonly used synthetic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have side effects such as liver damage and carcinogenesis (3). Therefore, the discovery of natural antioxidants is currently a main topic of intensive research. It is believed that folk remedies are major sources of new materials for antimicrobial and antioxidant drugs. The plant species selected for the present study are popularly used in the treatment of cold, cough, catarrh, tonsillitis, and bronchitis in Anatolia (4-17). We gathered 21 plants for antimicrobial and antioxidant screenings: *Achillea biebersteinii* Afan., *Anthemis pseudocotula* Boiss., *A. tinctoria* var. *tinctoria* L., *Artemisia austriaca* L., *Crepis foetida* L., *Cydonia oblonga* L., *Hedera helix* L., *Lantana camara* L. (orange flowers, orange and pink flowers), *Nepeta italica* L., *Ononis spinosa* L., *Paliurus spina-christi* Mill., *Plantago lanceolata* L., *P. major* L., *Primula vulgaris* Huds., *Rosa canina* L., *Rubus sanctus* Schreb., *Salvia fruticosa* Mill., *S. verticillata* L., *Teucrium polium* L., and *Urtica dioica* L. For the first time, the antimicrobial activities of traditionally prepared decoctions or infusions of the above-mentioned plants used as folk remedies in Anatolia were tested in this study.

In this study, the decoctions or infusions of different parts of the plants were tested for their antimicrobial and antioxidant activities. The aim of this study was to evaluate the in vitro antibacterial, antimycobacterial, antifungal, antidermatophytic, and antioxidant activities of 27 aqueous extracts of 21 plants collected from Turkey, and to determine the relationship between the content of phenolic compounds and antioxidant activities.

Materials and methods

Plant material

All plants were collected from different regions of Turkey between April and August of 2010. All species were identified by Prof Dr Mecit Vural of the Department of Biology, Faculty of Science and Art, Gazi University, Ankara, Turkey. Voucher specimens are stored in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey (GUE). The collection sites and herbarium numbers of the plant materials are given in Table 1.

Preparation of plant extracts

For decoctions, 1 g of air-dried plant material was added to 100 mL of distilled water and boiled on slow heat for 30 min. Infusions were prepared by pouring 100 mL of boiling water onto 1 g of dried plant material. The extraction continued for 30 min while cooling. Aqueous extracts were then filtered. Extraction yields after freeze-drying are given in Table 2.

Microbiological studies

Extracts were dissolved in a mixture of dimethyl sulfoxide (DMSO) (30%) and H_2O (70%) at a final concentration of 512 µg/mL, sterilized by filtration using 0.22-µm Millipore filters, and used as the stock solutions. Ampicillin-clavulanate, meropenem, gentamicin, levofloxacin, ampicillin, ketoconazole, and fluconazole were used as the standard antibacterial and antifungal drugs. Reference agents were obtained from Sigma and dissolved in phosphate buffer solution (ampicillin-clavulanate, pH 6.0, 0.1 mol/mL), DMSO (ketoconazole), or water (meropenem, gentamicin, levofloxacin, and fluconazole). The stock solutions of the agents were prepared in medium according to the Clinical and Laboratory Standards Institute (CLSI) (18). Antibacterial and antidermatophytic activity tests were carried out against standard and

Table 1. Collection sites, herbarium numbers, families, and parts of the plants.

Plant name and herbarium no.	Family	Part used	Collection site
Achillea biebersteinii Afan. (AB) 2930	Asteraceae	Inf	Palandöken, Erzurum
Anthemis pseudocotula Boiss. (AP) 2966	Asteraceae	Inf	Akşehir, Konya
Anthemis tinctoria var. *tinctoria* L. (AT) 2967	Asteraceae	Inf	Akşehir, Konya
Artemisia austriaca L. (AA) 2945	Asteraceae	Inf	Palandöken, Erzurum
Crepis foetida L. (CF) 2946	Asteraceae	Inf	Işık Mountain, Ankara
Cydonia oblonga L. (CO) 2947	Rosaceae	L	Lalahan, Ankara
Hedera helix L. (HH) 2948	Araliaceae	L	Tennis Club, Ankara
Lantana camara L. 2949 (orange flowers) (LC.of)	Verbenaceae	AP	Çubuklu Cove, Mersin
Lantana camara L. 2950 (orange and pink flowers) (LC.opf)	Verbenaceae	AP	Çubuklu Cove, Mersin
Nepeta italica L. (NI) 2953	Lamiaceae	AP	Işık Mountain, Ankara
Ononis spinosa L. (OS) 2954	Fabaceae	R	Ayşantı Pass, Ankara
Paliurus spina-christi Mill. (PS) 2955	Rhamnaceae	F	Gülnar Plateau, Mersin
Plantago lanceolata L. (PL) 2956	Plantaginaceae	L	Eymir Lake, Ankara
Plantago major L. (PM) 2957	Plantaginaceae	L/FL	Işık Mountain, Ankara
Primula vulgaris Huds. (PV) 2958	Primulaceae	L	Işık Mountain, Ankara
Rosa canina L. (RC) 2959	Rosaceae	SH/L/FL/R	Eymir Lake, Ankara
Rubus sanctus Schreb. (RS) 2960	Rosaceae	SH/L/FL	Beypazarı, Ankara
Salvia fruticosa Mill. (SF) 2061	Lamiaceae	L	Kızılağaç Forest, Bodrum
Salvia verticillata L. (SV) 2963	Lamiaceae	L	Işık Mountain, Ankara
Teucrium polium L. (TP) 2965	Lamiaceae	AP	Burdur Road, Antalya
Urtica dioica L. (UD) 2928	Urticaceae	L	Işık Mountain, Ankara

Inf: inflorescence, AP: aerial part, L: leaf, SH: shoot, R: root, F: fruit, FL: flower.

isolated strains. As standards, gram-negative strains of *Klebsiella pneumoniae* RSKK 574, *Haemophilus influenzae* ATCC 49766, *Pseudomonas aeruginosa* ATCC 10145, and *Acinetobacter baumannii* RSKK 02026 and gram-positive strains of *Streptococcus pneumoniae* ATCC 19615, *Streptococcus pyogenes* ATCC 13615, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* ATCC 12228 were used for the determination of antibacterial activity. *Candida albicans* ATCC 10231, *C. tropicalis*, *C. parapsilosis* ATCC 22019, and *C. krusei* were used for the determination of antifungal activity. Mueller Hinton Broth (Difco) and Mueller Hinton Agar (Oxoid) were applied for the growing and diluting of the bacterial suspensions. The synthetic medium RPMI-1640 with L-glutamine was buffered to pH 7 with 3-[N-morpholino]-propansulfonic acid and culture suspensions were prepared as described previously (18,19). The bacterial suspensions used for inoculation were prepared at 10^5 colony forming units (cfu)/mL by diluting fresh cultures at McFarland 0.5 density (10^8 cfu/mL). The fungi suspension was prepared by the spectrophotometric method of inoculums. For antimycobacterial activity, the strains of *Mycobacterium tuberculosis* H37Rv (ATCC 27294) and *M. avium* (ATCC 15769) were maintained on Lowenstein-Jensen medium and subcultured on Middlebrook 7H11 agar resuspended in 7H9-

Table 2. Folkloric usages and extraction yields of the plants.

Plant name	Utilization	Extract type	Extraction yield (%)*	Reference
AB	Cold, bronchitis	Decoction	24.83	17
AP	Cold	Infusion	11.91	13
AT	Cold	Infusion	27.73	13
AA	Cold	Decoction	20.74	12
CF	Cold, cough, catarrh	Infusion	4.90	111
CO	Cold	Infusion	22.83	13, 16
HH	Cold, catarrh	Decoction	24.26	14
LC.of	Cold, catarrh	Infusion	24.79	14
LC.opf	Cold	Infusion	25.28	14
Nİ	Cold	Infusion	32.73	7
OS	Bronchitis	Infusion	13.82	14
PS	Tonsillitis	Decoction	30.65	4
PL	Cold, bronchitis	Decoction	36.63	14
PM (flower)	Bronchitis	Decoction	17.97	10
PM (leaf)	Bronchitis	Decoction	31.79	8
PV (leaf)	Bronchitis	Infusion	30.00	4
RC (leaf)	Bronchitis	Decoction	24.81	5
RC (shoot)	Bronchitis	Decoction	22.99	5
RC (flower)	Bronchitis	Decoction	39.00	5
RC (root)	Bronchitis	Decoction	14.82	5
RS (shoot)	Cold	Decoction	21.93	5
RS (flower)	Cold	Decoction	32.83	5
RS (leaf)	Cold	Decoction	23.42	9
SF	Catarrh, cold	Decoction	20.85	29
SV	Catarrh, cold	Decoction	35.39	29
TP	Bronchitis	Decoction	21.50	6
UD	Cold	Decoction	23.76	5

*Values are mean ± SEM of 3 replications.

S broth medium supplemented with 10% OADC (0.1% casitone, 0.5% glycerol, supplemented oleic acid, albumin, dextrose, and catalase), 0.2% glycerol, and 0.1% Bacto casitone (Difco). Suspensions were prepared in 0.04% (v/v) Tween 80 and 0.2% bovine serum albumin adjusted to McFarland tube number 1. This was diluted to 1:20, and 100 µL of aliquot was used as inoculum. For the screening of the extracts as minimum inhibitory concentrations (MICs) against *M. tuberculosis* and *M. avium*, the resazurin microplate assay procedure (REMA) was carried out. First 100 µL of Middlebrook 7H9 broth was dispensed into each well of a sterile flat-bottom 96-well plate; then serial 2-fold dilutions (from 256 to 0.06 µg/mL) of each extract were prepared directly in the plate and 100 µL of inoculum was added to each well. A growth control and a sterile control were also included for each isolate. The plate was covered and incubated at 37 °C under normal atmosphere. After 7 days of incubation, 10 µg/mL of resazurin solution was added to each well, and the plate was reincubated overnight. A change in color from blue (oxidized state) to pink (reduced) indicated the growth of bacteria, and MICs were defined as the lowest concentration of drug that prevented this change in color. Isoniazid, ethambutol, and streptomycin were used as the standard antimycobacterial drugs (20). *Trichophyton rubrum* (RSKK 486), *Epidermophyton floccosum* (RSKK 3027), and *Microsporum gypseum* (NCPF 580) were used as the standard and isolated dermatophytic fungi species. Dermatophytes were subcultured onto potato dextrose agar plates at 28 °C for 7-14 days. The turbidity of supernatants was measured spectrophotometrically at a wavelength of 530 nm, and transmission was adjusted to 65%-75%. These stock suspensions were diluted 1:50 in RPMI medium to obtain final inoculum sizes ranging from 0.4×10^4 to 5×10^4 cfu/mL. The turbidity of the mixed suspension was measured at 530 nm to obtain 75%-77% transmission and adjusted to 1×10^6 cfu/mL by a spectrophotometric method (20,21). The MIC value of each extract was determined by using broth microdilution techniques as described by the CLSI for filamentous fungi. DMSO, H_2O, pure microorganisms, and pure media were used as control wells. The lowest concentration of the extracts that completely inhibited macroscopic growth was determined as the MIC (20). Bacteriostatic/bactericidal and fungistatic/fungicidal effects were noted when growth was not observed in liquid medium macroscopically, but growth was seen on agar plates and the viable cells were determined as cfu/mL (20-22).

DPPH radical scavenging assay

Radical scavenging activity of extracts against stable 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) was determined spectrophotometrically (23). The extracts were prepared by dissolving in methanol. The solution of DPPH in methanol (6×10^{-5} M) was prepared daily before the UV measurements, and 3 mL of the solution was mixed with 77 µL of extract solution. The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured at 515 nm on a spectrophotometer. BHT (50 µg/mL) was used as a reference compound. The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

% inhibition = $[(A_C - A_S) / A_C] \times 100$, where A_C is the absorption of blank sample (t = 0 min) and A_S is the absorption of tested extract solution (t = 15 min).

Determination of total phenolic content

Total phenolic content of extracts was determined with Folin-Ciocalteu reagent. The samples (0.25 mL, 10 mg/mL) or gallic acid were put into test tubes; 2.5 mL of Folin-Ciocalteu reagent and 2 mL of sodium carbonate solution were added. The tubes were vortexed and incubated at room temperature for 15 min. Afterwards, absorption was measured at 765 nm. The total phenol values are expressed in terms of gallic acid equivalent (GAE) (24).

Statistical analysis

All values are expressed as the mean ± the standard error of the mean (SEM); linear regression analyses and correlation coefficients to determine the relationship between 2 variables were calculated using MS-DOS software (GraphPad InStat statistical program).

Results and discussion

Antioxidant activity

In this study, the antioxidant activities of extracts were evaluated using DPPH free radical scavenging assay. Except for *C. foetida*, *H. helix*, *L. camara* (orange flowers), *N. italica*, *O. spinosa*, *P. vulgaris* (flower, leaf), and *U. dioica* extracts, all extracts showed DPPH radical scavenging activity. The most active plants against the DPPH radical were *A. biebersteinii*, *A. pseudocotula*, *A. tinctoria* var. *tinctoria*, *A. austriaca*, *C. oblonga*, *P. spina-christi*, *P. major* (leaf), *R. canina* (leaf, shoot, flower, root), *R. sanctus* (shoot, flower, leaf), *S. fruticosa*, *S. verticillata*, and *T. polium* with scavenging activities ranging between 81.4 ± 0.2% and 86.8 ± 1.4%. The DPPH radical scavenging abilities of these extracts were found to be the same as or close to that of the BHT (86.5 ± 0.5%, 50 μg/mL) used as reference (Table 3).

Table 3. Antioxidant activity and total phenolic content of plant extracts.

Plant name	DPPH inhibition (%)*	Total phenolic content (mg GAE/g extract)*
AB	83.0 ± 3.0	6.21 ± 0.01
AP	83.7 ± 1.0	6.47 ± 0.01
AT	86.3 ± 1.2	9.15 ± 0.02
AA	86.8 ± 1.4	8.35 ± 0.02
CF	41.8 ± 0.9	2.43 ± 0.02
CO	85.9 ± 0.5	11.85 ± 0.04
HH	32.0 ± 0.1	2.40 ± 0.01
LC.of	15.9 ± 0.6	3.97 ± 0.02
LC.opf	76.8 ± 3.5	9.47 ± 0.01
Nİ	49.8 ± 1.0	4.81 ± 0.01
OS	20.5 ± 0.8	3.09 ± 0.01
PS	88.5 ± 0.5	17.19 ± 0.01
PL	74.3 ± 0.7	5.26 ± 0.01
PM (flower)	87.5 ± 2.0	7.24 ± 0.02
PM (leaf)	79.4 ± 1.9	5.41 ± 0.01
PV (leaf)	46.0 ± 0.5	7.55 ± 0.01
RC (leaf)	85.7 ± 0.3	25.55 ± 0.03
RC (shoot)	85.5 ± 1.1	25.42 ± 0.04
RC (flower)	85.5 ± 1.3	27.06 ± 0.04
RC (root)	84.2 ± 0.5	34.40 ± 0.03
RS (shoot)	87.2 ± 0.7	25.54 ± 0.01
RS (flower)	81.4 ± 0.2	31.01 ± 0.02
RS (leaf)	85.6 ± 0.3	26.27 ± 0.03
SF	87.6 ± 0.7	23.70 ± 0.04
SV	86.8 ± 1.0	23.21 ± 0.01
TP	80.9 ± 0.6	5.54 ± 0.01
UD	21.5 ± 0.2	5.31 ± 0.01
BHT	86.5 ± 0.5	

*Values are mean ± SEM of 3 replications.

Total phenolic content

The total phenolic contents of 13 extracts are presented in Table 3. The total phenolic content of all extracts was found to be in the range of 2.40-34.40 mg GAE/g extract. *R. canina* root extract (34.40 ± 0.03 mg GAE/g extract) contained the highest level of total phenols, while the lowest level was observed in *H. helix* extract (2.43 ± 0.02 mg GAE/g extract). This study showed no correlation between antioxidant activity and phenolic content for extracts.

Antimicrobial activity

The results obtained for antibacterial activity against gram-negative bacteria, gram-positive bacteria, and 2 *Mycobacterium* strains and for antifungal activity against yeast-like fungi and dermatophytes are presented in Tables 4-7.

The results indicated that all extracts' activities were more pronounced against gram-positive bacteria than against gram-negative bacteria. All extracts exhibited inhibitory activities against gram-positive bacteria (*Streptococcus pneumoniae*, *S. pyogenes*, *S. aureus*, *S. epidermidis*), with MICs ranging from 16 to 64 µg/mL (Table 4). The *S. verticillata* extract displayed the best activity (MIC: 16 µg/mL) against all gram-positive microorganisms used in the study. *L. camara* (orange flowers, orange and pink flowers) extracts also showed remarkable activity against both *S. aureus* and *S. epidermides* at a concentration of 16 µg/mL. Additionally, *A. pseudocotula* and *A. austriaca* extracts exhibited antibacterial activity against *S. pneumoniae* and *S. pyogenes* at the same concentration, while *O. spinosa* extract was active against only *S. pyogenes* at 16 µg/mL. As seen in Table 5, based on MICs, all extracts displayed the best antibacterial activity for gram-negative bacteria against *Haemophilus influenzae* at 16-32 µg/mL. In particular, the extracts of plants belonging to families Lamiaceae and Asteraceae (MIC: 16 µg/mL) displayed promising antibacterial activity against *H. influenzae*. Lower than those of other extracts, antibacterial effects were seen against *A. baumannii* at MIC values of 32-64 µg/mL, which represents bactericidal and bacteriostatic effects at 128 and ≥32 µg/mL, respectively. Additionally, all extracts showed similar antibacterial activities against *K. pneumoniae* and *P. aeruginosa* at MIC values of 64-128 µg/mL.

Except for *T. polium*, *U. dioica*, *S. fruticosa*, *S. verticillata*, *R. canina* (flower, shoot, root), *R. sanctus* (leaf, flower, shoot), and *C. foetida*, the extracts also showed antimycobacterial activity against both *Mycobacterium tuberculosis* and *M. avium* with MICs ranging between 8 and 64 µg/mL, shown to be similar to the effect of the control (ethambutol MIC: 2 µg/mL) (Table 4).

The extracts were tested against *Candida albicans*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* in comparison to ketoconazole and fluconazole (Table 6). According to the results obtained, all extracts showed better antifungal activity against *C. parapsilosis* at 8 µg/mL, whereas they were less active against *C. albicans* and *C. tropicalis*, with MIC values of 16 µg/mL. Interestingly, all extracts (MIC: 32 µg/mL) were 2 times more active than fluconazole (MIC: 64 µg/mL) against *C. krusei*. Against yeast-like fungi, the minimum fungicidal and fungistatic values were seen in the range of 8 to ≥64 µg/mL.

The antidermatophytic activity results showed that *Tricophyton* and *Microsporum* species were more susceptible to all extracts. According to the MIC data, *R. sanctus* flower, *S. fruticosa*, and *A. tinctoria* var. *tinctoria* extracts were found to be the most active against *T. rubrum* with MIC values of 8 µg/mL, while *A. tinctoria* var. *tinctoria*, *P. major* (flower, leaf), *P. vulgaris*, and *S. fruticosa* extracts were also active against *Epidermophyton floccosum* with MIC values of 8 µg/mL. On the other hand, all extracts showed activity against *M. gypseum* with MIC values of 16 µg/mL. Antidermatophytic activities against isolated strains were shown at 8-64 µg/mL for the fungistatic effects of all assayed extracts (Table 7).

Plants used as folk remedies are one of the candidates known to be effective for the treatment of inflammation, arteriosclerosis, rheumatism, diabetes, liver diseases, gynecological diseases, and osteoporosis and bone resorption. Much scientific research has been conducted on plants used as folk remedies for new antimicrobial drug discoveries. In this report, we investigated the antibacterial, antifungal, antidermatophytic, and antioxidant activities of some plants used in the treatment of cold, cough, catarrh, tonsillitis, and bronchitis in Turkish traditional medicine and presented the results of the antioxidant and antimicrobial activities of 27 different extracts from 21 plants collected for this purpose.

Table 4. Antimycobacterial and antibacterial activities of the extracts and the control drugs as minimum inhibition concentration (MIC: µg/mL), as well as minimum bactericidal (MBC) and bacteriostatic (MBS) effects.

Extracts	Gram-positive microorganisms									
	S. pneumonia		*S. pyogenes*		*S. aureus*		*S. epidermidis*		*M.tb*	*M.a*
	MIC	MBC/MBS	MIC	MBC/MBS	MIC	MBC/MBS	MIC	MBC/MBS	MIC	MIC
AB	32	64/32	32	64/32	64	64/-	64	64/-	32	16
AP	16	-/≥16	16	64/ ≥16	32	64/32	32	64/32	64	16
AT	64	64/-	64	64/-	32	64/32	32	64/32	32	16
AA	16	-/≥16	16	64/ ≥16	32	64/32	32	64/32	16	8
CF	32	64/32	32	64/32	32	64/32	32	64/32	≥128	64
CO	64	64/-	64	64/-	32	64/32	32	64/32	16	8
HH	64	64/-	64	64/-	32	64/32	32	64/32	32	16
LC.of	32	64/32	32	64/32	16	64/≥16	16	64/≥16	16	8
LC.opf	32	64/32	32	64/32	16	64/≥16	16	64/≥16	32	16
Nİ	32	64/32	64	64/-	64	64/-	64	64/-	16	8
OS	32	64/32	16	64/≥16	32	64/32	32	64/32	16	8
PS	64	64/-	64	64/-	64	64/-	64	64/-	16	8
PL	64	64/-	64	64/-	64	64/-	64	64/-	16	8
PM (flower)	64	64/-	64	64/-	32	64/32	32	64/32	16	8
PM (leaf)	64	64/-	64	64/-	32	64/32	32	64/32	32	8
PV (leaf)	64	64/-	64	64/-	64	64/-	64	64/-	32	32
RC (leaf)	64	64/-	64	64/-	64	64/-	64	64/-	16	16
RC (shoot)	32	64/32	32	64/32	64	64/-	64	64/-	32	≥128
RC (flower)	32	64/32	32	64/32	64	64/-	64	64/-	≥128	≥128
RC (root)	32	64/32	32	64/32	64	64/-	64	64/-	≥128	≥128
RS (shoot)	32	64/32	32	64/32	32	64/32	32	64/32	≥128	≥128
RS (flower)	32	64/32	32	64/32	32	64/32	32	64/32	≥128	≥128
RS (leaf)	32	64/32	32	64/32	32	64/32	32	64/32	≥128	≥128
SF	64	64/-	64	64/-	32	64/32	32	64/32	≥128	≥128
SV	16	-/≥16	16	-/≥16	16	-/≥16	16	-/≥16	16	≥128
TP	64	64/-	64	64/-	64	64/-	64	64/-	32	≥128
UD	64	64/-	64	64/-	32	64/32	32	64/32	32	≥128
AC	<0.12	-	<0.12	-	<0.12	-	<0.12	-	-	-
LFX	-		0.12		0.25		0.25			
INH	-	-	-	-	-	-	-	-	0.125	0.125
ETB	-	-	-	-	-	-	-	-	2	2
STR	-	-	-	-	-	-	-	-	1	2

M.tb: M. tuberculosis, M.a: M. avium, LFX: levofloxacin, INH: isoniazid, ETB: ethambutol, STR: streptomycin.

Table 5. Antibacterial activity of the extracts and the control drugs as minimum inhibition concentration (MIC: µg/mL), as well as well as minimum bactericidal (MBC) and bacteriostatic (MBS) effects.

Extracts	Gram-negative microorganisms							
	K. pneumoniae		*H. influenzae*		*P. aeruginosa*		*A. baumannii*	
	MIC	MBC/MBS	MIC	MBC/MBS	MIC	MBC/MBS	MIC	MBC/MBS
AB	128	128/-	16	64/≥16	128	128/-	64	128/64
AP	64	128/64	16	64/≥16	64	128/64	32	128/≥32
AT	128	128/-	32	64/32	64	128/64	32	128/≥32
AA	64	128/64	16	64/≥16	64	128/64	32	128/≥32
CF	128	128/-	16	64/≥16	128	128/-	64	128/64
CO	64	64/-	32	64/32	64	64/-	32	128/≥32
HH	128	128/-	16	64/≥16	128	128/-	64	128/64
LC.of	64	128/64	32	64/32	64	128/64	32	128/≥32
LC.opf	64	128/64	32	64/32	64	128/64	32	128/≥32
Nİ	128	128/-	16	64/≥16	128	128/-	64	128/64
OS	64	128/64	32	64/32	64	128/64	32	128/≥32
PS	128	128/-	16	64/≥16	128	128/-	64	128/≥32
PL	64	128/64	16	64/≥16	64	128/64	32	128/≥32
PM (flower)	128	128/-	32	128/≥32	64	128/64	32	128/≥32
PM (leaf)	128	128/-	32	128/≥32	64	128/64	32	128/≥32
PV (leaf)	128	128/-	32	64/32	128	128/-	64	128/64
RC (leaf)	64	128/64	16	64/≥16	64	128/64	32	128/≥32
RC (shoot)	64	128/64	32	64/32	64	128/64	32	128/≥32
RC (flower)	64	128/64	32	64/32	64	128/64	32	128/≥32
RC (root)	64	128/64	32	64/32	64	128/64	32	128/≥32
RS (shoot)	64	128/64	32	64/32	64	128/64	32	128/≥32
RS (flower)	64	128/64	32	64/32	64	128/64	32	128/≥32
RS (leaf)	64	128/64	16	64/≥16	64	128/64	32	128/≥32
SF	128	128/-	16	64/≥16	64	128/64	32	128/≥32
SV	64	128/64	16	64/≥16	64	128/64	32	128/≥32
TP	64	128/64	16	64/≥16	64	128/64	32	128/≥32
UD	128	128/-	16	64/≥16	128	128/-	64	128/64
AMPC	<0.12		-		-		<0.12	
MEPM	-		0.12		-		-	
GM	-		-		0.5		-	

AMPC: ampicillin-clavulanate, MEPM: meropenem, GM: gentamicin

Table 6. Antifungal activity of the extracts and the control drugs as minimum inhibition concentration (MIC: µg/mL), as well as minimum fungicidal (MFC) and fungistatic (MFS) effects.

Extracts	*C. albicans*		*C. tropicalis*		*C. parapsilosis*		*C. krusei*	
	MIC	MFC/MFS	MIC	MFC/MFS	MIC	MFC/MFS	MIC	MFC/MFS
AB	16	64/≥16	16	64/≥16	8	16/8	32	64/32
AP	16	64/≥16	16	64/≥16	8	16/8	32	64/32
AT	16	64/≥16	16	64/≥16	8	16/8	32	64/32
AA	16	64/≥16	16	64/≥16	8	16/8	32	64/32
CF	16	64/≥16	16	64/≥16	8	16/8	32	64/32
CO	16	64/≥16	16	64/≥16	8	16/8	32	64/32
HH	16	64/≥16	16	64/≥16	8	16/8	32	64/32
LC.of	16	64/≥16	16	64/≥16	8	16/8	32	64/32
LC.opf	16	64/≥16	16	64/≥16	8	16/8	32	64/32
Nİ	16	64/≥16	16	64/≥16	8	16/8	32	64/32
OS	16	64/≥16	16	64/≥16	8	16/8	32	64/32
PS	16	64/≥16	16	64/≥16	8	16/8	32	64/32
PL	16	64/≥16	16	64/≥16	8	16/8	32	64/32
PM (flower)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
PM (leaf)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
PV (leaf)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
RC (leaf)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
RC (shoot)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
RC (flower)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
RC (root)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
RS (shoot)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
RS (flower)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
RS (leaf)	16	64/≥16	16	64/≥16	8	16/8	32	64/32
SF	16	64/≥16	16	64/≥16	8	16/8	32	64/32
SV	16	64/≥16	16	64/≥16	8	16/8	32	64/32
TP	16	64/≥16	16	64/≥16	8	16/8	32	64/32
UD	16	64/≥16	16	64/≥16	8	16/8	32	64/32
KTZ	1		1		1		4	
FLU	2		2		4		64	

KTZ: ketoconazole, FLU: fluconazole.

Table 7. Antifungal activity against dermatophytes of the extracts as minimum inhibition concentration (MIC: µg/mL), as well as minimum fungicidal (MFC) and fungistatic (MFS) effects.

Extracts	*T. rubrum*				*E. floccosum*				*M. gypseum*			
	RSKK 486		Isolated strain		RSKK 3027		Isolated strain		NCPF 580		Isolated strain	
	MIC	MFS/MFC	MIC	MFS/MFC	MIC	MFS/MFC	MIC	MFS/MFC	MIC	MFS/MFC	MIC	MFS/MFC
AB	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
AP	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
AT	8	≥8/-	16	≥16/-	8	≥8/-	16	≥16/-	16	≥16/-	32	≥32/-
AA	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
CF	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
CO	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
HH	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
LC.of	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
LC.opf	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
Nİ	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
OS	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
PSi	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
PL	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
PM (flower)	16	≥16/-	32	≥32/-	8	≥8/-	32	≥32/-	16	≥16/-	32	≥32/-
PM (leaf)	16	≥16/-	32	≥32/-	8	≥8/-	32	≥32/-	16	≥16/-	32	≥32/-
PV (leaf)	16	≥16/-	32	≥32/-	8	≥8/-	32	≥32/-	16	≥16/-	32	≥32/-
RC (leaf)	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
RC (shoot)	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
RC (flower)	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
RC (root)	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
RS (shoot)	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
RS (flower)	8	≥8/-	32	≥32/-	16	≥16/-	32	≥32/-	16	≥16/-	32	≥32/-
RS (leaf)	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
SF	8	≥8/-	32	≥32/-	8	≥8/-	32	≥32/-	16	≥16/-	32	≥32/-
SV	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
TP	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
UD	16	≥16/-	32	≥32/-	64	≥64/-	64	≥64/-	16	≥16/-	32	≥32/-
TBF	0.125		0.25		0.25		0.5		0.25		0.5	
GSF	0.5		1		0.5		1		0.5		1	
ITZ	0.25		0.5		0.125		0.25		0.125		0.25	

TBF: terbinafine, GSF: griseofulvin, ITZ: itraconazole.

Staphylococcus aureus can cause serious infections such as bloodstream infections, pneumonia, furuncles, cellulites, and bone and joint infections. *S. epidermidis* is one of the most often isolated bacterial pathogens in hospitals and is involved in nosocomial bloodstream infections, cardiovascular infections, and infections of the eye, ear, nose, and throat. *S. pneumoniae* is one of the most frequent causes of bacterial infection in children. Common infections caused by this pathogen include otitis media, sinusitis, occult bacteremia, pneumonia, and meningitis. On the other hand, acute *S. pyogenes* is the most common bacterial cause of acute pharyngitis and sore throat (25). In this work, *S. verticillata* extract displayed strong antibacterial activity against all gram-positive microorganisms. *A. pseudocotula*, *A. austriaca*, *L. camara* (orange flowers, orange and pink flowers), and *O. spinosa* extracts also showed promising antibacterial activity against gram-positive microorganisms.

Haemophilus influenzae is a common cause of epiglottitis, laryngotracheobronchitis, pneumonia, bronchiolitis, otitis media, and meningitis (26). Among all tested gram-negative microorganisms, *H. influenzae* was significantly inhibited by all of the extracts.

Based on the literature review, no previous work has been done on the antimycobacterial activity of these plants, excluding *A. tinctoria* var. *tinctoria*, *L. camara*, *P. vulgaris*, *P. major*, *R. canina*, *S. fruticosa*, and *U. dioica*. Ghaemi et al. showed that *R. canina* hydroalcoholic extract had limited effect on *Mycobacterium smegmatis* and *M. bovis* (27). Tosun et al. indicated that extracts prepared from *A. tinctoria* var. *tinctoria*, *P. vulgaris*, *P. major*, and *U. dioica* were inactive against *M. tuberculosis* H37Rv (28). Aşkun et al. found that *S. fruticosa* methanol extract showed moderate activity against *M. tuberculosis* (29). Kirimuhuzya et al. demonstrated that the methanol extract of *L. camara* exhibited strong antimycobacterial activity against 3 strains of *M. tuberculosis* (30). In our study, the extracts displayed strong activity against both *M. tuberculosis* and *M. avium*, except for *T. polium*, *U. dioica*, *S. fruticosa*, *S. verticillata*, *R. canina* (flower, shoot, root), *R. sanctus* (leaf, flower, shoot), and *C. foetida* extracts.

Various extracts (ethanol, methanol, chloroform, hexane, and dichloromethane extracts) prepared from some plants tested in this study were also evaluated previously against different and/or the same microorganisms and were found to exhibit different activities than those observed in this study (12,16,31-43). The difference between our results and those of other researchers may be due to several factors, including variations within each assay, extraction protocols, collection times, climate, and others.

Dermatophytes are categorized into 3 genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*, which include about 40 species. *T. rubrum* is a fungus that is the most common cause of tinea pedis (athlete's foot), jock itch, and ringworm. *E. floccosum* causes tinea pedis, while *M. gypseum* causes tinea capitis and tinea corporis. Many effective synthetic antifungal drugs (terbinafine, fluconazole, itraconazole, ketoconazole, and griseofulvin) have been used for treatment in dermatophytic infections (44). However, most of these drugs display different side effects. Therefore, plants are important medicinal sources for antifungal drug discovery research. Based on the literature review, no previous work has been done on the antidermatophytic activity of the plants presented here, excluding *S. fruticosa*. Ali-Shtayeh and Abu Ghdeib showed the antifungal activity of *S. fruticosa* infusion against *T. violaceum* (45). In this study, antidermatophytic activity results of *R. sanctus* (flower), *S. fruticosa*, *P. major* (flower, leaf), *P. vulgaris*, and *A. tinctoria* var. *tinctoria* extracts proved very hopeful for treatment of fungal infections caused by *T. rubrum* and *E. floccosum*.

Previous chemical profile reports were described for the following species: flavonoid, terpenoids, and volatile oil for *A. tinctoria* var. *tinctoria*, *A. biebersteinii*, *A. austriaca*, *A. pseudocotula*, and *Nepeta* spp. (35,41,46,47); flavonoids and phenolic acid derivatives for *R. sanctus* (48); flavonoids, terpenoids, anthocyanins, and carotenoids for *R. canina* (49); flavonoids and phenolic acids for *C. foetida* (50); organic acids and flavonol glycosides for *C. oblonga* (51); flavonoids, catechins, diterpenoids, triterpenoids, and volatile oil for *S. verticillata* and *S. fruticosa* (15); saponin, volatile oil, flavone glycoside, and sugar for *P. vulgaris* (52); terpenoids, flavonoids, and iridoids for *L. camara* (53); flavonoids, triterpenoids, and alkaloids for *P. spina-christi* (54); phenolic acids, flavonoids, and anthocyanins for *U.*

dioica (55); terpenoids, sterols, lectins, saponins, and flavonoids for *O. spinosa* (56); flavonoids, phenolic acids, and saponins for *H. helix* (57); phenolic acids, iridoids, flavonoids, and saponins for *Plantago* species (58); and diterpenoids, fatty acid esters, flavonoids, and steroids for *T. polium* (59). As noted above, these plants are rich in terpenoids and phenolic compounds known to possess antimicrobial activity (60). In our study, no relationship between total phenol content and the antimicrobial activities of extracts was observed. The antimicrobial activity of plants may vary depending on the types of terpenoids and flavonoids.

Antioxidants play an effective role in inhibiting and scavenging free radicals, thus providing support to the defense mechanism of the body against infection and chronic diseases. In this study, the findings did not show any relationship between the antioxidant and antimicrobial activities of the extracts.

The results of the present study may suggest that all extracts presumably possess compound(s) with antimicrobial properties against some bacterial and fungal pathogens. In conclusion, all plants used in this study could be potential sources of new antimicrobial agents. Additionally, antimicrobial activity results support the traditional uses of the plants. Further studies are being conducted to elucidate the components responsible for the antimicrobial activity as well as any pharmacological or toxicological properties that such extracts might have.

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Corresponding author:
Didem DELİORMAN ORHAN
Department of Pharmacognosy,
Faculty of Pharmacy, Gazi University,
06330 Ankara - TURKEY
E-mail: didemdeliorman@gmail.com

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