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Antimicrobial Activity of Some Turkish Medicinal Plants

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Abstract: In this study, antimicrobial activity of *Rhododendron ponticum* L., *Prunus laurocerasus* L., *Agrimonia eupatoria* L., *Cornus mas* L., *Vitis vinifera* L., *Punica granatum* L., *Anthemis cotula* L., *Cichorium intybus* L., *Viscum album* L., *Papaver hybridum* L., *Malva rotundifolia* L. and *Rhus coriaria* L. were investigated. The ethanolic extracts of these plants were tested against *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403 and *Kluyveromyces fragilis* ATCC 8608 by disc diffusion method. Of the 12 plants tested, nine showed antimicrobial activity. Each plant species has unique against different microorganisms. The fruit extract of *Rhus coriaria* had the highest antimicrobial effect with an inhibition zone of 12-52 mm against all the bacteria, but not shown antiyeast effect. Except for the extracts of *Rhus coriaria*, *Agrimonia eupatoria* and *Anthemis cotula*, all additional extracts of generated inhibition zones smaller than those generated by several reference antibiotics.

Key words: Antimicrobial activity, Turkish plants, disc diffusion

INTRODUCTION

Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments. In recent years there has been focus on plants with antimicrobial activity. Although the antimicrobial activity of various plant extracts has been studied on the growth of many microorganisms in Turkey[1-7], ethnobotanical and pharmaceutical studies on these plants are inadequate. Hence this *in vitro* study was aimed at screening selected Turkish medicinal plants for their antimicrobial activity, evaluating their potential use and determining whether their use in traditional medicine is justified.

MATERIALS AND METHODS

Plant materials: Twelve plant species commonly used in folk medicine in Turkey were selected (Table 1). Mature plants and their parts were collected from different cities in Turkey during the months of April-May of 2002. Voucher specimen of the plants was deposited in the herbarium of the Biology Department at Uludag University, Bursa-Turkey.

Preparation of extracts: The plant parts were air-dried. Each dry powdered plant material (20 g) was extracted with 150 mL 80% ethanol (Merck, Darmstadt) for 24 h by using Soxhlet equipment[8]. The extract was filtered using Whatman filter paper no.1 and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extract were stored in labeled sterile screw capped bottles at –20°C.

Microorganisms: *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403 and *Kluyveromyces fragilis* ATCC 8608 were used as test microorganisms.

Screening for antimicrobial activities: The dried plant extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 200 mg mL^{-1} and sterilized by filtration through a 0.45 μm membrane filter. Empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher and Schull No. 2668, Germany) were each impregnated with 50 μL of extract (10 mg $disc^{-1}$) at a concentration of 200 mg mL^{-1}. All the bacteria mentioned above were incubates at 35±0.1°C for 24 h by inoculation

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Table 1: Ethnobotanic data of studied plants

Botanical name (family, genus, species and voucher specimen)	Plant part(s) used	Traditional medicinal use(s)
Compositae		
Anthemis cotula L. (BD101)	Flower and leaves	Used to increases stool excretion and urine outflow
Cichorium intybus L. (BD105)	Leaves	Used to increases stool excretion and urine outflow Used for urinary tract infections
Rosaceae		
Agrimonia eupatoria L. (BD112)	Leaves	Treatment of whooping cough
Treats asthma		
Prunus laurocerasus L. (BD83)	Leaves	Used for cough
Treats asthma		
Ericaceae		
Rhododendron ponticum L. (BD45)	Leaves	Acts as antirheumatic
Cornaceae		
Cornus mas L. (BD92)	Bark	Decreases fever Increases stool excretion
Vitaceae		
Vitis vinifera L. (BD77)	Leaves	Acts as antiseptic Treats cutaneous abscesses
Punicaceae		
Punica granatum L. (BD81)	Bark	Treats cutaneous abscesses
Papaveraceae		
Papaver hibridum L. (BD116)	Flower and leaves	Used for cough
Malvaceae		
Malva rotundifolia L. (BD113)	Leaves	Acts as antiseptic
Treats abscesses		
Loranthaceae		
Viscum album L. (BD117)	Leaves	Used for diuretic
Anacardiaceae		
Rhus coriaria L. (BD107)	Fruit Decreases fever	Acts as antiseptic

into Nutrient Broth (Difco) and the yeast cultures studied were incubated in Malt Extract Broth (Difco) at 25±0.1°C for 48 h. An inoculum containing 10^6 bacterial cells or 10^8 yeast cells mL^{-1} was spread on Mueller-Hinton Agar (Oxoid) plates (1 mL inoculum/plate). The discs injected with extracts were placed on the inoculated agar by pressing slightly. Petri dishes were placed at 4°C for 2 h, placks injected with the yeast cultures were incubated at 25±0.1°C and bacteria were incubated at 35±0.1°C for 24 h[9,10]. At the end of the period, inhibition zones formed on the medium were evaluated in mm. Studies were performed in triplicate. On each plate an appropriate reference antibiotic disc was applied depending on the test microorganisms for comparing.

RESULT AND DISCUSSION

Table 2 shows antimicrobial activities of the plant extracts. Besides, the inhibition zones formed by standard antibiotic discs are indicated in Table 3.

As can clearly be seen from Table 2, all plants in this study show antimicrobial activity against the test microorganisms with the exception of *Prunus laurocerasus*, *Malva rotundifolia* and *Papaver hybridum* which have no antimicrobial effect. The extracts provided from the fruits of *Rhus coriaria* were found to be effective against all tested microorganisms except for the yeast cultures, having inhibition zones of 12-52 mm. When the results obtained were compared to those of standard antibiotics, it was determined that the extracts of *R. coriaria* have higher effect against bacteria. Digrak *et al.*[11] reported that the fruit extracts of *R. coriaria* showed antibacterial activity, showing inhibition zones of 36-51 mm. However, they were not effective against *Escherichia coli* and *Candida albicans*. In another study, the leaf extracts of *R. coriaria* have strong antimicrobial activity, but showing no antifungal effect[12]. In similar study, the same extract of *R. coriaria* inhibited gram-positive and gram-negative bacteria (including *Escherichia coli*), but they have no antifungal activity[13]. The findings obtained from this study are similar to those stated above. It is thought that observed differences may result from the doses used in this study. Besides, It was reported that the leaves of *R. coriaria* acted as an antibiotic medicine due to chemical compounds such as tannins and flavone derivates (fisetine) and were not harmful in the treatment of constipation[12,14].

It is also clear from Table 2 that extracts of *Agrimonia eupatoria* have antimicrobial effect against all tested microorganisms, having 8-16 mm. *Staphylococcus aureus* is more susceptible to the extracts

Table 2: Antimicrobial activity of plant extracts

	Microorganisms / inhibition zone (mm)*											
Plant species	1	2	3	4	5	6	7	8	9	10	11	12
Rhododendron ponticum	-	12	-	8	11	10	12	8	12	-	-	-
Anthemis cotula	-	14	-	-	-	12	14	10	12	-	-	-
Vitis vinifera	8	10	10	8	11	12	-	-	10	-	8	9
Rhus coriaria	12	52	40	44	25	38	23	44	40	-	-	-
Cornus mas	-	9	-	9	10	-	-	-	9	-	-	-
Punica granatum	-	12	-	-	10	12	-	8	10	12	10	8
Cichorium intybus	10	12	8	-	8	12	8	9	10	10	12	12
Viscum album	10	8	-	-	8	8	9	-	-	-	8	9
Agrimonia eupatoria	8	16	8	9	9	12	13	15	15	10	12	14
Prunus laurocerasus	-	-	-	-	-	-	-	-	-	-	-	-
Papaver hybridum	-	-	-	-	-	-	-	-	-	-	-	-
Malva rotundifolia	-	-	-	-	-	-	-	-	-	-	-	-
Ethanol (control)	-	-	-	-	-	-	-	-	-	-	-	-

1: *Escherichia coli*, 2: *Staphylococcus aureus*, 3: *Klebsiella pneumoniae*, 4: *Pseudomonas aeruginosa*, 5: *Proteus vulgaris*, 6: *Bacillus cereus*, 7: *Mycobacterium smegmatis*, 8: *Listeria monocytogenes*, 9: *Micrococcus luteus*, 10: *Candida albicans*, 11: *Kluyveromyces fragilis*, 12: *Rhodotorula rubra*
Inactive (-); moderately active (8-13); higher active (>14)
* includes diameter of disc (6 mm)

Table 3: Antimicrobial activities of some standard antibiotics

	Inhibition zone (mm)						
Microorganisms	P10	SAM20	CTX30	VA30	OFX5	TE30	NY100
Escherichia coli	18	12	10	22	30	28	-
Staphylococcus aureus	13	16	12	13	24	26	-
Klebsiella pneumoniae	18	14	13	22	28	30	-
Pseudomonas aeruginosa	8	10	54	10	44	34	-
Proteus vulgaris	10	16	18	20	28	26	-
Bacillus cereus	14	12	14	18	30	25	-
Mycobacterium smegmatis	15	21	11	20	32	24	-
Listeria monocytogenes	10	12	16	26	30	28	-
Micrococcus luteus	36	32	32	34	28	22	-
Candida albicans	-	-	-	-	-	-	20
Kluyveromyces fragilis	-	-	-	-	-	-	18
Rhodotorula rubra	-	-	-	-	-	-	18

P10: Penicillin G (10 Units), SAM20: Ampicillin 10 μg, CTX30: Cefotaxime 30 μg, V30: Vancomycin 30 μg, OFX 5 : Oflaxacin 5 μg, TE30 : Tetracyclin 30 μg, NY100 : Nystatin 100 μg

of *Agrimonia eupatoria*, as compared to standard antibiotics except for OFX5 and TE30. Similarly, in comparison to SAM20 standard, it was seen that *Listeria monocytogenes* is more susceptible. Coumarins, flavonoids, tannins and terpenoids from aerial parts of this plant are isolated[15,16]. Copland *et al.*[17] reported that n-Hexane, dichloromethane and methanol extracts of the seeds of *Agrimonia eupatoria* have strong antibacterial activity. These findings are similar to those stated above.

The extracts obtained from *Anthemis cotula* have an antibacterial effect, having an inhibition zone of 12-14 mm. When the results were compared to those of standard antibiotics, it was determined that they have strong effect against *Staphylococcus aureus* and *Mycobacterium smegmatis*. Quarenghi *et al.*[18] reported that the flavonoid containing total extracts of *Anthemis cotula* flowers, tested at the concentration of 200 μg mL^{-1}, showed antimicrobial activity against gram-negative and gram-positive bacteria (especially against *Staphylococcus* species). The findings obtained from this study are similar to those stated above. It is thought that observed differences may result from the doses used in this study. In addition, microorganisms variable sensivity to chemical substances relates to different resistance levels between the strains[19].

The extracts obtained from *Rhododendron ponticum*, *Vitis vinifera*, *Cornus mas*, *Punica granatum*, *Cichorum intybus* and *Viscum album* have moderately active against the test microorganisms in this study. Extracts obtained from *Cornus mas* species were found to be have inhibition zones against *Staphylococcus aureus*, *Pseudomomas aeruginosa*, *Proteus vulgaris* and *Micrococcus luteus*, having inhibition zones of 8-10 mm. However, *Cornus mas* was not effective against the other bacteria and the yeast cultures. The extracts of *Punica granatum* and *Cichorum intybus* have antiyeast effects against all tested the yeast cultures, but *Rhododendron ponticum* was not effective against the yeast cultures. The extract of *Vitis vinifera* and *Viscum album* were found to be effective against all the yeast cultures studied with the exception of *Candida albicans*.

It may be concluded from this study that the extracts of examined plants are active against the tested

microorganisms. Further phytochemical studies are required to determine the types of compounds responsible for the antimicrobial effects of these medicinal plants. In addition, the results confirm the use of these plants in traditional medicine for the treatment of infections.

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