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The Antibacterial and Antifungal Effects of *Rhododendron* Derived Mad Honey and Extracts of Four *Rhododendron* Species

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Abstract: The antibacterial and antifungal activities of mad honey and the crude extracts (leaves and flowers) of *Rhododendron ponticum* L. subsp. *ponticum*, *Rhododendron luteum* L., *Rhododendron smirnovii* L., and *Rhododendron caucasicum* L. (Ericaceae) were investigated. A total of 17 microbial organisms belonging to 16 species of bacteria, namely *Staphylococcus aureus*, *Streptococcus salivarius*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella enteritis*, *Streptococcus pneumoniae*, *Bacillus cereus*, *Listeria monocytogenes*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Bacillus licheniformis*, *Micrococcus luteus*, *Bacillus subtilis*, *Proteus vulgaris*, and *Candida albicans* were studied using a disk-diffusion and agar dilution (minimal inhibition concentration) method. The antimicrobial activity of the antibacterial and antifungal activity of the mad honey and the crude extracts obtained from the 4 *Rhododendron* spp. turned out to be more effective in the case of bacteria than against fungus. The antimicrobial activity against gram-positive bacteria was more pronounced than against gram-negative ones. Each of the crude extracts of the *Rhododendron* spp. and mad honey exhibited more or less pronounced antibacterial and antifungal potencies in the case of both gram-positive and gram-negative bacteria and fungus. In particular, the crude samples of *Rhododendron caucasicum* flowers (RCF), *Rhododendron ponticum* leaves (RPL) and *Rhododendron ponticum* flowers (RPF), and *Rhododendron smirnovii* leaves (RSL) showed antibacterial and antifungal activity against the tested organisms. The crude RCF sample required an MIC of ≥ 0.5 mg/ml for *S. enteritis*, *B. cereus*, *L. monocytogenes*, and *S. mutans* and of ≥ 2 mg/ml for *C. albicans*.

Key Words: Antimicrobial activity, *Rhododendron* sp., mad honey, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*

Rhododendron'dan Üretilen Deli Bal ve Dört *Rhododendron* Türünün Ekstraktlarının Antibakteriyel ve Antifungal Etkileri

Özet: Bu çalışmada temel olarak orman gülü çiçeklerinden üretilen deli balın ve orman gülü türleri olan *Rhododendron ponticum* L. subsp. *ponticum*, *Rhododendron luteum* L., *Rhododendron smirnovii* L., ve *Rhododendron caucasicum* L. (Ericaceae) çiçek ve yapraklarının ham özütlerinin antibakteriyel ve antifungal aktiviteleri araştırılmıştır. Toplam olarak 16 bakteri (*Staphylococcus aureus*, *Streptococcus salivarius*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella enteritis*, *Streptococcus pneumoniae*, *Bacillus cereus*, *Listeria monocytogenes*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Bacillus licheniformis*, *Micrococcus luteus*, *Bacillus subtilis*, *Proteus vulgaris*) ve *Candida albicans* mantarı disk-diffüzyon ve agar dilüsyon (minimum inhibisyon konsantrasyonu tayini için) yöntemleri kullanılarak çalışıldı. Dört orman gülü türünden elde edilen ham özütlerin ve deli balın antimikrobiyal aktivitelerinin testte kullanılan mantara göre bakterilerde daha yüksek olduğu görülmüştür. gram-pozitiflerde görülen antimikrobiyal aktivitelerin gram-negatiflerde belirlenene göre daha belirgin olduğu tespit edilmiştir. Ham özüt ve deli bal örneklerinin her biri orta veya ileri seviyede antibakteriyal ve antifungal potansiyele sahiptir. RCF (*Rhododendron caucasicum* çiçek) özütü, RPL (*Rhododendron ponticum* yaprak) ve RPF (*Rhododendron ponticum* çiçek) özütleri ile RSL (*Rhododendron smirnovii yaprak*) özütü testlerde mikroorganizmalara karşı daha yüksek antibakteriyal ve antifungal aktivite göstermiştir. RCF (*Rhododendron caucasicum* flowers) özütü *S. enteritis*, *B. cereus*, *L. monocytogenes* ve *S. mutans*`a karşı ≥ 0,5 mg/ml, *C. albicans*`a karşı ≥ 2 mg/ml MIC değeri göstermiştir.

Anahtar Sözcükler: Antimicrobial aktivite, *Rhododendron* sp., deli bal, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*

Introduction

Honey has a long and interesting history. In addition to its use as a food, honey has been used in medicine as a dressing for wounds and inflammations, both internal and external. Recently, the medicinal use of honey has been rediscovered by the medical profession and is gaining acceptance as an antibacterial agent for treating ulcers, wounds, and other surface infections. Honey has been used in medicine throughout recorded history and is still widely used in folk medicine (1). There are many reports of the beneficial effects of honey used as a topical treatment for a wide range of wounds, ulcers, and abscesses (2,3-5). The beneficial effect of honey in the treatment of wounds and burns is not restricted to its antibacterial action. The antibacterial activity of honey was first reported in 1892 (6). Honey has been shown to be active against a diverse range of microorganisms, and reports of its inhibitory effect on specific microorganisms are numerous (7). Honey has been shown to be effective against gram-positive and gram-negative organisms, and aerobic and anaerobic bacteria, as well as inhibiting spore germination of *Bacillus cereus* (8). The major acid present in honey is gluconic acid (9), produced by glucose oxidase. Another compound produced by this reaction is hydrogen peroxide. Several researchers have proposed that the antibacterial activity exhibited by some honeys is due to a specific compound or group of compounds in the particular honey. In a large survey of New Zealand honeys, Molan and Russell (10) found a correlation between high levels of antibacterial activity and non-peroxide content. Allen et al. (11) suggested that the variation in activity of New Zealand honeys might be attributable to the floral source.

Rhododendron ponticum is native to countries in the Western and Eastern Mediterranean such as Spain, Portugal, and Turkey and also occurs eastwards of Asia. Cases of human poisoning are also known, which are caused by the consumption of honey produced from *Rhododendron* flowers. Potentially toxic chemicals, particularly 'free' phenols and diterpenes, occur in significant quantities in the tissues of plants of *Rhododendron* species. Diterpenes, known as grayanotoxins, occur in the leaves, flowers, and nectar of rhododendrons. Ingestion of honey derived from this plant may cause profound hypotension and bradycardia. This honey is known locally as mad honey (12,13-15).

In the present study, we aimed to investigate the antimicrobial activity of 8 ethanolic extracts from some *Rhododendron* species collected in the mountains of the Eastern Black Sea region of Turkey and mad honey on gram-positive and gram-negative bacteria and a fungus. Some *Rhododendron* species that were previously screened by other investigators by different methods (16,17), microorganisms, and strains were included in this study. Finally, this study investigated the correlation between *Rhododendron* species and the activity of particular *Rhododendron* species-derived honeys: mad honey.

Materials and Methods

Plant material and honey sample

Plant materials of *Rhododendron ponticum* L. subsp. *ponticum*, *Rhododendron luteum* L., *Rhododendron smirnovii* L., and *Rhododendron caucasicum* L. (Ericaceae) were collected during March to June 2006 and 2007 in the mountains of the Eastern Black Sea region of Turkey. The identification of these specimens was carried out using *Flora of Turkey* (13).

The honey sample, so-called mad honey, used in the antimicrobial tests was collected from 50 bee hives located in Çambaşı Plateau, Ordu province, Turkey, where, within bee flight distance, *Rhododendron ponticum* L. subsp. *ponticum* and *Rhododendron luteum* L. were the major flowers and, among some others, *Rhododendron caucasicum* L. and *Rhododendron smirnovii* L. were minor ones. The honeys from each hive were then combined to form the test honey sample.

Preparation of plant extracts

Fresh leaves and flowers of the plants were dried at 23-25 °C for 1-2 weeks. The extracts of the plants were prepared according to the methods described by Ertürk et al. (18) and Holopainen et al. (19), with slight modification. Dried leaves and twigs of the plants were extracted with 95% ethanol (50 g 1/5 ethanol) at room temperature. The extracts were kept at 4 °C for a day, they were filtered through a 45 µm membrane filter, and then the solution was dried with an evaporator. The crude extracts were stored at -20 °C until used. They are abbreviated as follows: *R. ponticum* leaves (RPL), *R. luteum* leaves (RLL), *R. smirnovii* leaves (RSL), *R. caucasicum* leaves (RCL), *R. ponticum* flowers (RPF), *R. luteum* flowers (RLF), *R. smirnovii* flowers (RSF), and *R. caucasicum* flowers (RCF) (20).

Preparation of honey samples

The honey sample, so-called mad honey, used in the antimicrobial tests was collected from 50 bee hives located on Çambaşı Plateau, Ordu. The honey sample was extracted with 95% ethanol (50 g 1/5 ethanol) at room temperature. The extracts were kept at 4 °C for a day, and they were filtered through a 45 µm membrane filter. The honey sample was stored at -20 °C until used. Each honey sample was diluted with 70% ethanol.

Microorganisms tested and culture media

Strains of bacteria and fungus were obtained from ATCC (American Type Culture Collection, Rockville, Maryland, USA). Antimicrobial activities of 8 crude extract samples obtained from the 4 *Rhododendron* species were assayed against *Staphylococcus aureus* ATCC 6538, *Streptococcus salivarius* RSHE 606, *Klebsiella pneumoniae* ATCC 5041, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 29988, *Salmonella enteritis* ATCC 13076, *Streptococcus pneumoniae* ATCC 10015, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* NCTC 5348, *Streptococcus mutans* RSHE 676, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus licheniformis* B1001, *Micrococcus luteus* B1018, *Bacillus subtilis* B209, *Pseudomonas aeruginosa* B2679, *Proteus vulgaris* B123, and *Candida albicans* ATCC 25922. The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Broth (Merck). *C. albicans* was grown in Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid). The concentrations of bacterial suspensions were adjusted to 10^8 cells/ml and those of fungal suspensions to 10^7 cells/ml.

Antifungal assay and Antibacterial assay

Antibacterial and antifungal activity was measured using diffusion disk plates on agar (21). In order to test antibacterial and antifungal activity, the fractions of 4 plant samples and 1 honey sample were dissolved in 70% ethanol. For bacteria Mueller Hinton Agar medium (Merck) (20 ml) and for fungus Sabouraud Dextrose Agar (Oxoid 20 ml) were poured into each 15 cm petri dish. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) for 24 h at 37 °C, and *C. albicans* was grown in Sabouraud Dextrose Broth (Difco) at 27 °C for 48 h. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Mueller Hinton Broth medium for the bacteria and Sabouraud Dextrose Broth for the fungus. Suspensions (100 µl) with approximately 10^8 bacteria and fungus per milliliter were placed in petri dishes, over agar and dispersed. Then sterile paper disks (6 mm diameter) were placed onto the agar to load 15 µl of each plant and honey sample (20 mg/ml). One hundred units of nystatin, and for the bacteria Ampicillin and Cephazolin obtained from a local pharmacy were used as a positive control with alcohol as a negative control. Inhibition zones were determined after incubation at 27 °C for 48 h. All tests were performed in triplicate.

Minimum inhibition concentration

The agar dilution method described by Vanden Berghe and Vlietinck (16) was used for the antibacterial screening with slight modifications. Instead of 96 well microtiter plates 24 well tissue culture (Corning) plates were used. The crude extracts and honey sample were dissolved in 70% ethanol and physiological Tris buffer (Amresco 0826-500G) 1:4 and mixed with an equal amount of 3% agar solution at 45 °C to a final concentration of 4, 2, 1, and 0.5 mg of extract/ml. From the solutions 400 µl was transferred into each well of the tissue culture (Corning) plate. After solidification each well was inoculated with 10 µl of freshly prepared bacterial suspension of 10^8 bacteria/ml and incubated at 37 °C for 24 h. Ampicillin and Cephazolin, obtained from a local pharmacy, were used at 4, 2, 1, and 0.5 mg/ml (1 g/ml stock) as a positive control for bacteria, nystatin was used as a positive control for the fungus, and 70% alcohol was used as a negative control. The bacterial growth was assessed using a stereomicroscope after the incubation period. All tests were performed in triplicate.

Statistical analysis

The data were analyzed using SPSS for Windows (v. 13.0). The differences between the means of the inhibition zones were tested with one-way variance analysis followed by Tukey's HSD test. The results are evaluated in the confidence limit of 0.05.

Results and Discussion

The antibacterial and antifungal activity of the mad honey and the crude extracts of *Rhododendron* spp. was initially evaluated by the disk diffusion method using 16 strains of (gram-positive, gram-negative) bacteria and 1 yeast strain (*C. albicans*). The 9 tested compounds exhibited relatively strong antibacterial and antifungal activity. The results obtained in the disk diffusion assay regarding the growth inhibition zones of the tested microbes are shown in Table 1A. As indicated, in some

Table 1. A Results of antimicrobial screening some *Rhododendron* species extracts determined by the agar diffusion method (inhibition zone in mm).

Plant species and family	Part used	Collection Time	Collection site	Microorganisms Inhibition zone (mm) (mean ± S.E.)																
				E.c. 88	*E.c.*22	*S.a.*	*S.s.*	*K.p.*	*S.e.*	*S.p.*	*B.c*	*L.m.*	*S.m*	*P.a.* 53	*B.l.*	*M.l*	*B.s*	*P.a.*79	*P.v.*	*C.a.*	Total mm
Rhododendron ponticum L. subsp. ponticum (Ericaceae)	Lf,	March-June 2006-2007	Ordu	14.33±0.33 A	12.33±0.33 A	15.33±0.33 A	8.33±0.33 A	15.33±0.33 A	16.33±0.33 A	6.67±0.33 A	15.00±0.00 A	7.00±0.00 A	15.33±0.33 A	16.33±0.33 A	17.33±0.33 A	16.33±0.33 A	20.33±0.33 A	17.33±0.33 A	15.33±0.33 A	7.00±0.00 A	232
	Fr,			15.33±0.33 A	14.33±0.33 A	8.33±0.33 B	9.00±0.00 A	12.67±0.33 A	12.00±0.00 C	0.00±0.00 B	15.33±0.33 A	21.33±0.33 D	0.00±0.00 E	20.33±0.33 A	15.33±0.33 E	22.33±0.33 E	20.33±0.33 A	13.00±0.00 B	13.33±0.33 A	17.00±0.00 B	227
Rhododendron luteum L. (Ericaceae)	Lf,	March-June 2006-2007	Ordu	11.33±0.33 B	11.33±0.33 A	14.33±0.33 A	9.00±0.58 A	0.00±0.00 B	7.00±0.00 B	0.00±0.00 B	0.00±0.00 B	8.00±0.00 A	14.33±0.33 A	15.33±0.33 A	20.33±0.33 A	8.00±0.58 B	15.33±0.33 B	0.00±0.00 C	16.67±0.33 A	13.33±0.33 C	162
	Fr,			10.00±0.00 A	11.67±0.33 A	13.33±0.33 A	12.33±0.58 C	12.33±0.58 A	14.00±0.00 D	0.00±0.00 C	0.00±0.00 B	14.33±0.33 B	0.00±0.00 E	0.00±0.00 C	7.00±0.00 B	7.00±0.58 B	16.67±0.33 B	7.00±0.00 D	20.33±0.33 B	0.00±0.00 D	145
Rhododendron smirnovii L. ***(Ericaceae)***	Lf	March-June 2006-2007	Ordu	16.00±0.58 C	14.33±0.33 B	14.33±0.33 A	15.33±0.33 B	14.00±0.00 A	11.00±0.11 C	18.00±0.00 C	15.00±0.00 A	12.00±0.00 B	13.00±0.00 A	10.00±0.00 B	16.33±0.33 A	15.33±0.33 A	9.67±0.33 C	15.00±0.00 A	16.33±0.33 A	15.00±0.00 E	239
	Fr			16.33±0.33 C	14.00±0.58 A	8.67±0.33 B	11.33±0.33 C	17.33±0.33 A	11.33±0.33 C	17.67±1.15 C	0.00±0.00 B	13.00±0.00 B	15.33±0.33 B	18.33±0.33 A	21.67±0.33 A	15.33±0.33 A	20.33±0.33 A	0.00±0.00 C	17.67±0.33 A	11.33±0.33 C	227
Rhododendron caucasicum L. (Ericaceae)	Lf	March-June 2006-2007	Ordu	15.33±0.33 A	12.33±0.33 A	9.67±0.33 B	12.00±0.00 C	12.33±0.33 A	0.00±0.00 F	17.33±0.33 A	15.33±0.33 A	16.33±0.33 C	11.33±0.33 A	0.00±0.00 C	16.33±0.33 A	15.33±0.33 A	16.33±0.33 B	9.00±0.58 D	15.33±0.33 A	15.33±0.33 E	206
	Fr			12.33±0.33 B	15.33±0.33 B	9.67±0.88 B	20.33±0.33 D	0.00±0.00 B	16.00±0.00 A	7.00±0.00 A	20.33±0.33 C	20.33±0.33 D	19.00±0.00 B	13.33±0.33 A	14.33±0.33 A	16.33±0.33 A	16.33±0.33 B	15.33±0.33 A	20.33±0.33 B	16.00±0.00 E	249
Mad Honey		2006-2007	Ordu	12.67±0.67 B	12.33±0.33 A	13.33±0.33 A	12.33±0.33 C	10.33±0.33 A	9.00±0.00 B	7.00±0.00 D	7.00±0.00 D	15.33±0.33 B	12.33±0.33 A	0.00±0.00 C	6.67±0.33 B	12.33±0.33 C	11.33±0.33 C	0.00±0.00 C	12.00±0.00 C	11.33±0.33 C	141
Ampicillin				15.33±0.33 A	15.33±0.33 B	10.00±0.58 B	9.67±0.33 A	13.33±0.33 A	34.67±0.33 E	27.67±0.33 D	27.33±0.33 E	25.00±0.00 E	24.67±0.33 C	10.00±0.00 B	29.67±0.33 C	0.00±0.00 D	35.67±0.33 D	28.00±0.58 E	27.67±0.33 D	NT	335
Cephazolin				10.33±0.33 B	15.33±0.33 B	0.00±0.00 C	0.00±0.00 E	10.00±0.00 A	35.67±0.33 E	22.33±0.33 E	22.67±0.33 F	32.00±0.00 F	29.67±0.33 D	27.33±0.33 D	24.67±0.33 D	34.67±0.33 F	37.67±0.33 D	24.33±0.33 F	0.00±0.00 E	NT	327
Nystatin				NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	15	15
70% ethanol				0.00±0.00 D	0.00±0.00 C	0.00±0.00 C	0.00±0.00 E	0.00±0.00 B	0.00±0.00 F	0.00±0.00 C	0.00±0.00 B	0.00±0.00 G	0.00±0.00 E	0.00±0.00 C	0.00±0.00 F	0.00±0.00 D	0.00±0.00 E	0.00±0.00 C	0.00±0.00 E	0.00±0.00 D	

The differences between the means in the same column followed by the same letter are not statistically significant, $P > 0.05$

NT: Not tested, Parts used: Fr, flower; Lf, leaf; Microorganisms: *S. salivarius* RSHE 606, *S. aureus* ATCC 6538, *K. pneumoniae* ATCC 5041, *E. coli* ATC 25922, *E. coli* ATC 29988, *S. enteritis* ATCC 13076, *S. pneumonia* 10015, *B. cereus* ATCC 11778, *L. monocytogenes* NCTC 5348, *S. mutans* RSHE 676, *P. aeruginosa* ATCC 27853, *B. licheniformis* B1001, *M. luteus* B1018, *B. subtillis* B209, *P. aeruginosa* B2679, *P. vulgaris* B123, and *C. albicans* ATCC 25922.

cases we extracted different parts of the plants: flowers and leaves, separately, meaning that a total of 8 extracts were tested. The 8 crude extracts of *Rhododendron* spp. showed antibacterial activity against gram-positive and gram-negative bacteria, and fungus. All 8 crude extracts of *Rhododendron* spp. contain phenethyl alcohol and benzyl alcohol (16). It has been demonstrated that the lethal effect of 2 aromatic alcohols (phenethyl alcohol and benzyl alcohol) stop when protein synthesis is inhibited, and so it is likely that both possess specific mechanisms of action (22). The crude RPF sample obtained from *R. ponticum* had its highest antibacterial activity against *B. subtilis*, *P. aeruginosa* (20 mm/15 µl inhibition zone), *M. luteus* (22 mm/15 µl inhibition zone), and *L. monocytogenes* (22 mm/15 µl inhibition zone). However, no significant differences were evident between RPL and RPF crude extracts in terms of the antimicrobial spectrum. The flower extracts of all the species showed better antimicrobial activity against the test organism than the leaf extracts of all the species. The crude RCF sample obtained from *R. caucasicum* showed antibacterial and antifungal activity (7-20 mm/15 µl inhibition zone) against the test organisms. The crude RPF sample obtained from *R. ponticum* showed the highest antifungal activity (17 mm/15 µl inhibition zone) against *C. albicans*. Tasdemir et al. (20) reported that the flower of *R. luteum* contains high linalool. The purified linalool fraction was only inhibitory for *C. albicans.* The purified linalool fraction is not toxic to mammalian cells, which suggests that the purified components may be useful to control the microbial population in patients (23). The test extracts showed better antimicrobial activity against *P. vulgaris, B. subtilis, B. licheniformis*, and *L. monocytogenes* than against the other test microorganism. On the other hand, all of the test extracts showed weak antimicrobial activity against *S. pneumoniae.* The extracts of rhododendron species derived honey, mad honey, showed antibacterial activity against *L. monocytogenes, E. coli* 22, *E. coli* 88, and *S. pneumoniae* (15, 12, 13, and 13 mm/15 µl inhibition zones, respectively). However, this honey extract did not show antibacterial activity against either *P. aeruginosa* strain. It did show weak anti-fungal activity against *C. albicans* (11 mm/15 µl inhibition zone), which is generally similar to the results reported by Aksoy and Dıgrak (24). The antibacterial and antifungal activity of the mad honey and the crude extracts of *Rhododendron* spp. against bacteria was more effective than against the fungus, which is consistent with the results published by Avato et al. (25) and Zavalas et al. (17). Küçük et al. reported the antimicrobial activities of 3 honeys from northeastern Anatolia (26). They tested the antimicrobial activities of 2 monofloral honeys of *R. ponticum* and chestnut origin. The 3 honeys tested have been reported to have moderate antibacterial activity against 4 out of 8 bacteria and no antifungal activity against *C. albicans* and *C. tropicalis*. They reported the rhododendron derived honey to be less active compared to chestnut and heterofloral honeys. Mad honey tested in the current study apparently showed better antimicrobial activity, in comparison to the literature. The difference in activity is probably due to the floral origin being monofloral in the literature and multifloral, 3 rhododendron species, in our case. As is widely known, the floral origin markedly affects the biological activities of honeys.

Evaluation of MIC of the crude extracts obtained from *Rhododendron* species and *Rhododendron* species-derived mad honey by means of agar dilution is reported in Table 1B. The crude RCF sample obtained from *R. caucasicum* required a MIC of ≥ 0.5 mg/ml for *S. enteritis*, *B. cereus*, *L. monocytogenes*, and *S. mutans*, and of ≥ 2 mg/ml for *C. albicans*. However, the concentration of this RCF was observed to inhibit the growth of the other test microorganism. The crude RPL sample obtained from *R. ponticum* required an MIC of ≥ 0.5 mg/ml for *E. coli* 22, *E. coli* 88, *S. aureus*, and *S. enteritis*, and of ≥ 4 mg/ml for *C. albicans*. The crude RPL sample obtained from *R. ponticum* required an MIC of ≥ 0.5 mg/ml for *E. coli* 22 and *L. monocytogenes*, ≥ 1 mg/ml for *E. coli* 88, and lower MIC values (≥1 mg/ml) were required against *C. albicans*. The crude RSL sample obtained from *R. simirnoii* required an MIC of ≥ 1 mg/ml for *E. coli* 88, of ≥ 0.5 mg/ml for *L. monocytogenes*, and of ≥ 2 mg/ml for *C. albicans*.

The extracts of *Rhododendron* species-derived mad honey required a MIC of ≥ 1 mg/ml for *E. coli* 22, *E. coli* 88, *S. aureus, S. enteritis*, and *S. mutans,* of ≥ 0.5 mg/ml only for *L. monocytogenes*, and of ≥ 2 mg/ml for *C. albicans*. Much research effort has been centered on establishing the properties of honey to which its antibacterial activity may be attributed. Such factors as osmolarity, acidity, hydrogen peroxide content, and chemical components of honey have all been considered to contribute to the inhibition of bacterial growth. Honey is a super saturated hyperosmotic solution of carbohydrates with a moisture content of 12%-14% (27). When microorganisms enter a hypertonic medium, the osmotic

Table 1.B Results of antimicrobial screening of *Rhododendron* species extracts determined by the agar-well diffusion method (minimum inhibitory concentration, MIC, in mg.

Plant species and family	Part used	Collection Time	Collection site	Microorganisms MIC (mg/ml)															
				E.c. 88	*E.c.*22	*S.a.*	*S.s*	*K.p.*	*S.e.*	*S.p.*	*B.c.*	*L.m.*	*S.m.*	*P.a.* 53	*B.l.*	*M.l*	*B.s.*	*P.a.*7 9	*P.v.*	*C.a.*
Rhododendron ponticum L. subsp. *ponticum* (Ericaceae)	Lf,	March-June 2006-2007	Ordu	≥0.5	≥0.5	≥0.5	≥4	≥4	≥0.5	≥4	≥1	≥4	≥2	≥2	≥2	≥2	≥1	≥2	≥4	≥4
	Fr,			≥1	≥0.5	≥2	≥4	≥4	≥4	≥4	≥1	≥0.5	≥4	≥2	≥2	≥1	≥1	≥4	≥4	≥1
Rhododendron luteum L. (Ericaceae)	Lf,	March-June 2006-2007	Ordu	≥0.5	≥2	≥1	≥4	≥4	≥4	≥4	≥4	≥4	≥2	≥4	≥1	≥4	≥2	≥4	≥2	≥4
	Fr,			≥1	≥2	≥1	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥4	≥2	≥4	≥2	≥4
Rhododendron smirnovii **L. *(Ericaceae)***	Lf	March-June 2006-2007	Ordu	≥1	≥4	≥1	≥1	≥4	≥4	≥4	≥1	≥4	≥4	≥4	≥2	≥4	≥4	≥4	≥2	≥4
	Fr			≥1	≥2	≥2	≥4	≥4	≥4	≥4	≥4	≥4	≥2	≥4	≥0.5	≥4	≥2	≥4	≥2	≥4
Rhododendron caucasicum L. (Ericaceae)	Lf	March-June 2006-2007	Ordu	≥1	≥2	≥2	≥4	≥1	≥4	≥2	≥1	≥0.5	≥4	≥4	≥2	≥4	≥2	≥4	≥4	≥2
	Fr			≥1	≥2	≥2	≥1	≥1	≥0.5	≥4	≥0.5	≥0.5	≥0.5	≥4	≥4	≥2	≥2	≥4	≥4	≥2
Mad Honey		2006-2007	Ordu	≥1	≥1	≥1	≥1	≥4	≥4	≥4	≥4 -	≥0.5	≥1	≥4	≥2	≥2	≥2	≥4	≥4	≥2
Ampicillin				≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	NT
Cephazolin				≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	NT
Nystatin				NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	≥2
70% ethanol				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

pressure differential is so great that organisms lose water (28). Characteristically honey is an acidic medium. Full strength honey pH ranges from 3.2 to 4.5 and is generally considered to be an average of 3.9 (29). Such an acidity level would be inhibitory to the growth of most bacterial species. The average MIC values of the other tested extracts (leaves and flowers) of *Rhododendron* spp. were also determined, and there were no significant differences in their values.

In recent years, although technology and medicine have developed extensively, due to a decrease in natural richness and the drawbacks, some countries have made it obligatory to use natural products for many goals. For these reasons, like in other countries in the world, in Turkey also medicinal plants and their crop known by local people are collected and used for treating various diseases. However, *Rhododendron* species and *Rhododendron* species-derived honeys, including the nectar, contain toxic diterpenes known as grayanotoxins. Ingestion of honey derived from this plant may cause profound hypotension and bradycardia (12,13-15). Mad honey decreases blood glucose and lipid levels. Most likely this effect is due to the stimulation of grayanotoxins on the parasympathetic nervous system (30). The results of this study suggest that mad honey in small doses can be used for treating various diseases.

In conclusion, the results indicated that each the crude extracts of *Rhododendron* spp. exhibited more or less pronounced antibacterial and antifungal potencies in the case of both gram-positive and gram-negative bacteria and fungus. In particular, the crude RCF samples from *R. caucasicum*, the crude RPL and RPF samples from *R. ponticum* and the crude RSL samples from *R. smirnovii* showed antibacterial and antifungal activity against the tested organisms.

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