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Antimicrobial Activity of Some Endemic *Verbascum* and *Scrophularia* Species from Turkey

BASARAN DULGER* and NURCIHAN HACIOGLU
Department of Biology, Faculty of Science & Arts
Canakkale Onsekiz Mart University, 17100, Canakkale, Turkey
Fax: (90)(286)2180533; E-mail: basarandulger@yahoo.com

The methanol extracts obtained from three endemic *Verbascum* L. species (*V. cariense* Hub.-Mor., *V. adenophorum* Boiss. and *V. inulifolium* Hub.-Mor.) and two endemic *Scrophularia* L. species (*S. depauperata* Boiss. and *S. mersinensis* Lall) have been investigated for their antimicrobial activity. Antimicrobial activity was determined with *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538-P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27583, *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, *Kluyveromyces fragilis* ATCC 8608, *Debaryomyces hansenii* DSM 70238 and *Hanseniaspora guilliermondii* DSM 3432 by the disk diffusion method. The extracts of all plant species had strong antimicrobial activity against the Gram-positive bacteria and yeasts, but no activity was observed against the Gram-negative bacteria used in this study.

Key Words: Antimicrobial activity, *Verbascum cariense* Hub.-Mor., *Verbascum adenophorum* Boiss., *Verbascum inulifolium* Hub.-Mor., *Scrophularia depauperata* Boiss., *Scrophularia mersinensis* Lall.

INTRODUCTION

The demand for herbal medicines is increasing rapidly due to their lack of side effects. Further as health care costs continue to escalate, the attraction for low-cost remedies has stimulated consumers to re-evaluate the potential of alternatives[1]. Approximately 60-80 % of the world's population still depends on traditional medicines for the treatment of common ailments and diseases[2]. Inadequate medicinal facilities that are often too far from communities living in isolated villages and insufficient access to the most current pharmaceuticals on the market or to most current diseases therapies, make it difficult to successfully treat certain health conditions in

Turkey. Bacterial and viral infections and a high infant mortality rate are relatively common throughout the country. Traditional remedies have a long-standing history in many Turkish communities and continue to provide useful tools for treating diseases.

Some species of *Verbascum* L. (Scrophulariaceae) have widely been used throughout centuries to treat internal and external infections. Many internal and external uses of the leaves and flowers of several *Verbascum* L. species have been documented in many societies in Europe, Asia, Africa and North America[3].

Many *Scrophularia* L. species have been used for some medicinal treatments including Scrophula, scabies, tumors and inflammatory affections since ancient times as folk remedies[4,5].

Verbascum L. species used in this study have been used for ethnopharmacological effects among common people in Turkey. Especially their flowers have been used. The drug, prepared from their flowers, has diuretic and expectorant effects. Leafs of plants have also been used for their diuretic, expectorant and sedative effects. Seeds of *Verbascum* species poisonous seeds for hunting fish. *Verbascum* L. species are called 'fish plant' in the northern Anatolia because of that property[6].

Verbascum cariense Hub.-Mor., *V. adenophorum* Boiss. and *V. inulifolium* Hub.-Mor., *Verbascum cariense* Hub.-Mor., *Verbascum adenophorum* Boiss., *Verbascum inulifolium* Hub.-Mor., *Scrophularia depauperata* Boiss. and *Scrophularia mersinensis* Lall were endemic to Turkey[7,8]. Although there are many investigations on this species, these plants have not been previously investigated. Therefore, our aim was to determine the antimicrobial effects of plant extracts obtained from these endemic species against microorganisms.

EXPERIMENTAL

Aerial parts of plants were collected from different localities in Turkey during the months of September-October 2006. Voucher specimens of the plants were deposited in the Biology Department at Çanakkale Onsekiz Mart University, Çanakkale, Turkey and identified by Emin Ugurlu from Celal Bayar University, Manisa, Turkey.

Preparation of extracts: The plant parts were air-dried. Each dry powdered plant material (20 g) was extracted with 150 mL of 80 % methanol (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment[9]. The extract was filtered using Whatmann filter paper no. 1 and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55 ºC. Dried extracts were stored in labeled sterile screw-capped bottles at -20 ºC.

Microorganisms: *Escherichia coli* ATCC 11230, *Staphylococcus aureus* 6538-P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27583, *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, *Kluyveromyces fragilis* ATCC 8608, *Debaryomyces hansenii* DSM 70238 and *Hanseniaspora guilliermondii* DSM 3432 were used as test microorganisms.

Screening for antimicrobial activities: The dried plant extracts were dissolved in 10 % aqueous dimethyl sulfoxide to a final concentration of 200 mg/mL and sterilized by filtration through an 0.45 μm membrane filter. Empty sterilized antibiotic disks having a diameter of 6 mm (Schleicher & Schull No: 2668, Dassel, Germany) were each impregnated with 50 μL of extract (10 mg/disk) at a concentration of 200 mg/mL. All the bacteria mentioned above were incubated at 35 ± 0.1 ºC for 24 h by inoculation into nutrient broth (Difco Laboratories, MI, USA) and the yeast cultures studied were incubated in malt extract broth (Difco Laboratories, MI, USA) at 25 ± 0.1 ºC for 48 h an inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Muller-Hinton Agar (Oxoid Ltd., Hampshire, UK) plates (1 mL inoculum/plate). The disks injected with extracts were placed at 4 ºC for 2 h, plaques injected with the yeast cultures were incubated at 25 ± 0.1 ºC and bacteria were incubated at 35 ± 0.1 ºC for 24 h[10,11]. At the end of the period, inhibition zones formed on the medium were evaluated in millimeters. Studies were performed in triplicate. On each plate, an appropriate reference antibiotic disk was applied, depending on the test microorganism for comparison.

RESULTS AND DISCUSSION

Table-1 shows antimicrobial activity of the plant extracts and the inhibition zones formed by standard antibiotic disks are indicated in Table-2. As can clearly be seen from Table-1, no significant activity was found against Gram-negative bacteria such as *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Notably, activity against the Gram-positive bacteria such as *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Micrococcus luteus* and acid-fast bacterium *Mycobacterium smegmatis* were found. Besides, the plant extracts have an anti-yeast activity against all tested the yeast cultures in different levels.

It was found that extracts of *V. inulifolium* have more antibacterial and antifungal activity than the other *Verbascum* L. species. *Staphylococcus aureus* is more susceptible to the extracts of all *Verbascum* L. species, as compared to standard antibiotics, except for TE 30 and OFX5. Similarly, in

TABLE-1
SUMMARY OF ANTIMICROBIAL ACTIVITY OF STUDIED PLANTS

Plant species	Microorganisms/inhibition zone (mm)*													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
V. cariense	-	12.8	-	-	-	12.2	14.0	11.2	12.6	13.8	12.8	11.4	12.6	15.6
V. adenophorum	-	16.8	-	-	-	14.4	19.2	11.8	16.2	10.8	11.2	18.4	10.6	15.2
V. inulifolium	-	22.2	-	-	-	24.6	16.8	13.8	20.8	14.2	16.4	22.2	11.8	14.6
S. depauperata	-	11.4	-	-	-	14.4	12.8	15.2	15.4	10.2	10.4	11.6	9.8	10.8
S. mersinensis	-	16.8	-	-	-	15.2	16.4	13.8	15.2	12.6	14.8	15.8	15.2	12.6
Methanol (control)	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1 = *Escherichia coli*; 2 = *Micrococcus luteus*; 3 = *Klebsiella pneumoniae*; 4 = *Pseudomonas aeruginosa*; 5 = *Proteus vulgaris*; 6 = *Bacillus cereus;* 7 = *Mycobacterium smegmatis*; 8 = *Listeria monocytogenes;* 9 = *Staphylococcus aureus;* 10 = *Candida albicans*; 11 = *Kluyveromyces fragilis*; 12 = *Rhodotorula rubra;* 13 = *Hanseniaspora guilliermondii*; 14 = *Debaryomyces hansenii*; *Includes diameter of disk (6 mm).

TABLE-2
ANTIMICROBIAL ACTIVITIES OF SOME STANDARD ANTIBIOTICS

Microorganisms/Antibiotics	Inhibition zone (mm)								
	P10	SAM20	CTX30	VA30	OFX5	TE30	NY100	KETO20	CLT10
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	21	15
Kluyveromyces fragilis	-	-	-	-	-	-	18	16	18
Rhodotorula rubra	-	-	-	-	-	-	18	22	16
H. guilliermondii	-	-	-	-	-	-	21	24	22
Debaryomyces hansenii	-	-	-	-	-	-	16	14	18

P10 = Penicillin G (10 Units), SAM20 = Ampicillin 10 µg, CTX30 = Cefotaxime 30 µg, V30 = Vancomycin 30 µg, OFX5 = Oflaxacin 5 µg, TE30 = Tetracyclin 30 µg, NY100 = Nystatin 100 µg, KETO20 = Ketaconazole 20 µg, CLT10 = Clotrimazole 10 µg.

comparison to P10, SAM20, CTX30 and VA30 standard, it was seen that *Bacillus cereus* is more susceptible. In addition, the extracts of *V. adeophorum* and *V. inulifolium* have a greater anti-yeast effect than the standard antifungal antibiotic Nystatin against *Rhodotorula rubra* and *Kluyveromyces fragilis*. Notably, the extract of all *Verbascum* L. species has more antiyeast effect than those of the Standard KETO20.

Verbascum L. species contain a wide range of compounds, such as glycosides[12-15], alkaloids[16] and saponins[17]. Members of the family Scrophulariaceae have been reported to contain a group of unusual macrocyclic spermine alkaloids[18,19]. The antimicrobial activities of nine *Verbascum* L. species have previously been reported[3]. They used extracts from flowers, seeds, leaves and roots and detected a strong growth inhibition. As a result of that study, antimicrobial activity was more consistently detected and activity against the Gram-positive bacterium *Staphylococcus aureus* and the yeast cultures had been found. In our previous studies, the extracts obtained from some *Verbascum* species showed similar results against specific bacteria[20-23]. It is determined that *Verbascum* L. species showed antimicrobial activity against Gram-positive bacteria and yeasts and no antibacterial activity had found against Gram-negative bacteria. The results in this study are similar to those reported in the mentioned studies. In general, Gram-negative bacteria have been found to be more resistant to extracts than Gram-positive bacteria, possibly because of their cell wall lipopolysaccharide[24-26]. The extracts obtained from *Scrophularia* L. were found to be effective against Gram-positive bacteria, but they were weakly active against the yeast cultures used in this study. When the results obtained with both *Scrophularia* species were compared to those of some standard antibiotics; it was determined that *Staphylococcus aureus* and *Listeria monocytogenes* are susceptible to the extract. The acid-fast bacterium *Mycobacterium smegmatis* is more resistant to the extracts in comparison to the standard antibacterial antibiotics, except for CTX30.

Many *Scrophularia* species have been investigated and found to contain many classes of secondary metabolites including iridoids, phenylpropanoids, phenolic acids, flavanoids and saponins. Some of these compounds were shown to have antiinflammatory, antibacterial, fungicidal, protozoocidal, molluscicidal, cytotoxis, hepatoprotective, immunomodulator, cardivascular, diuretic and antitumor activities. According to the literature, antibacterial activity of *Scrophularia* L. species can be attributed to the presence of phenolic acids (ferutic, isovanillic, *p*-hydroxy benzoic, syringic, caffeic, gentisic, protocetechuric, *p*-coumaric and vanillic acids)[27]. The plant species could be considered as potentially antiseptic agents on bacteriological infections, especially in processes where Gram-positive bacteria are involved[28].

The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new antiinfective agents[29-31]. Herbal medicines are a valuable and rapidly available resource for primary health care and complementary health care systems. Undoubtedly, the plant Kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered; large numbers of plants are constantly being screened for their antimicrobial effects. Finally, the results of the present study provide evidence that *Verbascum* L. and *Scrophularia* L. species continue to represent an important asset to the health care in communities in Turkey. More pharmacological investigations are necessary.

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