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Evaluation of Antimicrobial Activity of Two Endemic *Scrophulariaceae* Members

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 endemic *Scrophulariaceae* members *Verbascum vacillans* Murb. and *Scrophularia depauperata* Boiss. have been investigated for their antimicrobial activity. Antimicrobial activity was determined with *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC 57, *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, *Cryptococcus neoformans* ATCC 32308 and *Kluyveromyces fragilis* ATCC 8608 by disk diffusion method. While the extract of *V. vacillans* has strong antimicrobial activity against the Gram-positive bacteria and yeast cultures, the other plant has weak activity against the test microorganisms used in this study.

Key Words: Antimicrobial activity, *Scrophulariaceae*, *Verbascum vacillans* Murb., *Scrophularia depauperata* Boiss.

INTRODUCTION

The *Scrophulariaceae*, also known as the figwort family, comprise approximately 5100 species belonging to 268 genera[1]. Phytochemically this family is a rich source of iridoid glycosides, especially from the genera *Buddleja, Scrophularia* and *Verbascum*[2-4].

Some species of *Verbascum* L. (*Scrophulariaceae*) have been used widely 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[5].

Many *Scrophularia* L. species have been used for some medicinal treatments including scrophula, scabies, tumours and inflammatory affections since ancient times as folk remedies[6,7].

Many species of *Verbascum* L. have been used for ethnopharmacological effects among the common people in Turkey. Especially their flowers have been used. The drug prepared from their flowers has diuretic and expectorant effects. Leaves of plants have also been used for their diuretic, expectorant and sedative effects. Seeds of *Verbascum* species are used as poisonous seeds for hunting fish. *Verbascum* species are called 'fish plant' in the Northern Anatolia because of that property[8].

Verbascum vacillans Murb. and *Scrophularia depauperata* Boiss. are endemic to Turkey[9,10]. Turkey has a rich flora and still unstudied for phytochemistry or bioactivity. However, the strong ethnopharmaceutical literature of Turkey provides us with valuable information on the widely utilized plant material in folk medicine. During routine excursions, it was determined that these plants are used as diuretic and expectorant. However, these plants have not been previously investigated for their antimicrobial activities. Therefore, the aim was to determine the antibacterial and antifungal effects of the methanol extract obtained from these endemic plants against the test microorganisms.

EXPERIMENTAL

Aerial parts of *Verbascum vacillans* Murb. and *Scrophularia depauperata* were collected from different localities in Canakkale, Turkey during the months of September-October 2007. Voucher specimens of the plants was deposited in the Biology Department at Canakkale Onsekiz Mart University, Canakkale Turkey and identified by Dr. Emin Ugurlu from Celal Bayar University, Manisa, Turkey.

Preparation of extracts: The plant parts were air-dried. Dry powdered plant material (20 g) was extracted with 150 mL of 80 % methanol (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment[11]. The extract was filtered using Whatmann filter paper no. 1 and the filtrate was then evaporated under reduced pressure and dried using a rotary evaporator at 55 ºC. Dried extract was stored in labelled sterile screw-capped bottles at -20 ºC.

Microorganisms: *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC 57, *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, *Cryptococcus neoformans* ATCC 32308 and *Kluyveromyces fragilis* ATCC 8608 were used as test microorganisms.

Screening for antimicrobial activities: The dried plant extracts were dissolved in 10 % aqueous dimethylsulfoxide to a final concentration of 200 mg/mL and sterilized by filtration through an 0.45 µm membrane filter.

Empty sterilized antibiotic disk shaving 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 Mueller Hinton Agar (Oxoid Ltd., Hampshire, UK) plates (1 mL inoculum/plate). The disks injected with extracts were placed on the inoculated agar by pressing slightly. Petri dishes 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 35 ± 0.1 ºC for 24 h[12]. 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 microorganisms for comparison.

RESULTS AND DISCUSSION

The inhibition zones formed by the plant extracts and some standard antibiotics are indicated in Table-1.

TABLE-1
SUMMARY OF ANTIMICROBIAL ACTIVITY OF THE PLANTS AND SOME STANDARD ANTIBITOTICS

Microorganisms	Inhibition zone (mm)								
	Plant species		Standard antibiotics						
	VV	SD	P10	SAM20	CTX30	VA30	OFX5	TE30	NY100
Bacteria									
Escherichia coli	-	-	18.2	12.2	10.4	22.0	30.8	28.2	-
Staphylococcus aureus	18.8	12.0	13.4	16.8	12.6	13.4	24.4	26.4	-
Klebsiella pneumonia	-	-	18.2	14.4	13.4	22.4	28.2	30.6	-
Pseudomonas aeruginosa	-	-	8.6	10.8	54.2	10.8	44.0	34.8	-
Proteus vulgaris	-	-	10.2	16.2	18.4	20.0	28.6	26.2	-
Bacillus cereus	20.2	16.2	14.4	12.4	14.6	18.6	30.2	25.4	-
Mycobacterium smegmatis	13.2	15.2	15.8	21.0	11.8	20.0	32.2	24.6	-
Listeria monocytogenes	11.4	10.8	10.6	12.4	16.6	26.4	30.2	28.2	-
Micrococcus luteus	21.8	11.2	36.2	32.0	32.2	34.2	28.8	22.4	-
Fungi									
Candia albicans	20.8	14.4	-	-	-	-	-	-	20.0
Cryptococcus neoformans	17.2	13.2	-	-	-	-	-	-	16.8
Kluveromyces fragilis	18.8	12.0	-	-	-	-	-	-	18.2
Rhodotorula rubra	18.0	11.4	-	-	-	-	-	-	18.0

VV = *V. vacillans*, SD = *S. depauperata*; [a] Includes diameter of disk (6 mm)
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, KETO20 = Ketaconazole 20 µg = CLT10 = Clotrimazole 10 µg

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 been anti-yeast activity against all tested yeast cultures in different levels.

It was found that extracts of *V. vacillans* have more antibacterial and antifungal effects than *S. depauperata*. However, the extract of *S. depauperata* against the acid-fast bacterium *Mycobacterium smegmatis* is more effective than *V. vacillans*. *Staphylococcus aureus* and *Bacillus cereus* are more susceptible to the extract of *V. vacillans*, as compared to all standard antibacterial antibiotics, except for OFX5 and TE30. Similarly, in comparison to P10 standard, it was seen that *Listeria monocytogenes* is more susceptible. In addition, the extract of *V. vacillans* has a strong antiyeast effect against all the yeast cultures. Especially, *Candida albicans, Cryptococcus neoformans* and *Kluyveromyces fragilis* are more susceptible to the extract of *V. vacillans* in comparison to the standard antifungal antibiotic Nystatin. Notably, the extract of *V. vacillans* is equal to the standard Nystatin against *Rhodotorula rubra*.

Fungi used in this study were chosen primarily on the basis of their importance as opportunistic pathogens of humans, *Candida albicans* while naturally occurring in the intestinal flora, can cause oral thrush and systematic infections. *Cryptococcus neoformans* causes cryptococcocis, an opportunistic infection of the lungs especially in AIDS patients. According to findings from the National Nosocomial Infection Surveillance System (NNIS)[13], 61 % of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp. and *Cryptococcus* spp. Therefore, in this study, different strains of medical yeast cultures were investigated. Notably, *V. vacillans* can be used as antifungal agent in new drugs for the therapy of infectious diseases especially against *Candida* and *Cryptococcus* infections.

Verbascum L. species contain a wide range of compounds, such as glycosides[14-17], alkaloids[18] and saponins[19]. Members of the family Scrophulariaceae have been reported to contain a group of unusual macrocyclic spermine alkoloids[20,21]. The antimicrobial activities of nine *Verbascum* L. species have previously been reported[5]. 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 previous studies[22-26], the extracts obtained from some *Verbascum* species showed similar results against specific bacteria. It is determined that *Verbascum* L. species showed antimicrobial activity

against Gram-positive bacteria and yeast and no antibacterial activity was found against Gram-positive bacteria. The results in this study are similar to those reported in the mentioned studies. In general, Gram-positive bacteria have been found to be more resistant to extracts than Gram-positive bacteria, possibly because of their cell wall lipopolysaccharide[27-29].

Many *Scrophularia* species have been investigated and found to contain many classes of secondary metabolites including iridoids, phenyl propanoids, 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[30]. 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). In addition, the plant species could be considered as potentially antiseptic agents on bacteriological infections, especially in processes where Gram-positive bacteria are involved[31]. The antibiotic activity of two endemic Turkish *Scrophularia* spp. may be attributable to phenylethanoid glycosides[32,33].

The present results seem to confirm the traditional use of the members of *Verbascum vacillans* and *Scrophularia depauperata* as a medicinal herb. Besides, the extracts of these plants may be useful as an alternative antimicrobial agent in natural medicine for the treatment of many infectious diseases especially against candidiasis and cryptococcocis. These results also reinforce the concept that the traditional approach to screening plants as potential sources of bioactive substances could be successful.

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