Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: Basaran Dülger, Serap Kirmizi, Hülya Arslan & Gürcan Güleryüz (2002) Antimicrobial Activity of Three Endemic Verbascum Species, Pharmaceutical Biology, 40:8, 587-589, DOI: 10.1076/phbi.40.8.587.14657

To link to this article: https://doi.org/10.1076/phbi.40.8.587.14657

Pharmaceutical Biology
2002, Vol. 40, No. 8, pp. 587–589

1388-0209/02/4008-587$16.00

Antimicrobial Activity of Three Endemic *Verbascum* Species

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Abstract

The extracts obtained from three *Verbascum* L. species (*Verbascum olympicum* Boiss., *Verbascum prusianum* Boiss., and *Verbascum bombyciferum* Boiss.) have been investigated for their antimicrobial activity. Growth inhibition, using the agar disc diffusion assay, was determined against *Escherichia coli* ATCC 11230, *Micrococcus luteus La 2971*, *Staphylococcus aureus* ATCC 6538P, *Salmonella thyphi* ATCC 19430, *Klebsiella pneumoniae* UC57, *Pseudomonas aeroginosa* ATCC 27893, *Corynebacterium xerosis* CCM 2824, *Bacillus cereus* ATCC 7064, *Bacillus megaterium* DSM 32, *Mycobacterium smegmatis* CCM 2067, *Proteus vulgaris* ATCC 8427, *Candida albicans* ATCC 10231, *Rhodotorula rubra*, and *Saccharomyces cerevisiae* ATCC 9763. We found that *Verbascum* L. species showed antimicrobial activity against the Gr(+) bacteria and yeasts, but no activity was seen against the Gr(−) bacteria used in this study.

Keywords: Antimicrobial activity, *Verbascum* L. spp., *V. olympicum* Boiss., *V. prusianum* Boiss., *V. bombyciferum* Boiss.

Introduction

Some species of *Verbascum* L. (*Scrophulariaceae*) have widely been used throughout centuries to treat internal and external infections. According to Meurer-Grimes et al. (1996), Hildegard von Bingen has mentioned in her first book *Physica* "De wullena", with which she probably refers to *Verbascum thapsus* L. She mentions its use against hoarseness in a concoction of wine, fennel, and mullein leaves. Since then, many other 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 northern America (Meurer-Grimes et al., 1996).

Verbascum L. species contain a wide range of compounds, such as glycosides (Klimek, 1996; Skaltsounis et al., 1996; Elgindi & Mabry, 2000; Kalpoutzakis et al., 1999), alkoloids (Youhnovski et al., 1999), and saponins (Hartleb & Seifert, 1994). In addition, species of the family *Scrophulariaceae* have been reported to contain a group of unusual macrocyclic spermine alkoloids (Koblikova et al., 1983; Seifert et al., 1982). This group of compounds has not been previously investigated for antimicrobial activity.

As it was stated in a report by McCutcheon et al. (1995), extracts of *V. thapsus* revealed antiviral activity against Herpes virus type 1. Furthermore, aqueous extracts of *V. fruticulosum* L. demonstrated strong growth inhibition on the malaria parasite (Sathiyamoorthy et al., 1999).

Verbascum phlomoides L., *Verbascum densiflorum* Bertol. and *Verbascum thapsus* L. species have been used for their 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 are poisonous because of the saponins contained. People use these poisonous seeds for hunting fish. *Verbascum* species are called 'fishplant' in the nothern Anatolia because of that property (Zeybek, 1985; Baytop, 1999).

Ethnobotanical information obtained from traditional healers may serve as an intial lead for bioactive compounds (Cox & Balick, 1994). In addition, investigations confirm that higher plants used as antiinfective phytomedicines may serve as a valuable source for novel antibiotics. *Verbascum olympicum* Boiss., *Verbascum prusianum* Boiss., and *Verbascum bombyciferum* Boiss. are endemic to Uludağ Mount-Bursa, Turkey and Euro-Siberian elements. Antimicrobial effects of *Verbascum bombyciferum* Boiss. and *Verbascum olympicum* Boiss. have been previously investigated by Meurer-Grimes et al. (1996). In this study, our aim was to determine the antimicrobial effects of plant extracts obtained from three endemic *Verbascum* L. species against microorganisms. Further investigation on *Verbascum* L. species are necessary to provide additional knowledge about this plant.

Accepted: May 8, 2002

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Materials and methods

Materials

Three species of *Verbascum* L. (*Verbascum olympicum* Boiss., *Verbascum prusianum* Boiss., and *Verbascum bombyciferum* Boiss.) were collected from Uludağ Mount-Bursa. These species are distributed on different altitudes and they are only known in this area (Davis, 1978). *Verbascum bombyciferum* Boiss. is distributed on the lower region of the mountain, for example on the roadsides, public gardens and archeological sites in the city of Bursa, and it flowers around May and June. *Verbascum bombyciferu*m Boiss. is widespread around the winter sports center, roadsides and picnic areas at higher altitudes, it flowers in June and August. *Verbascum prusianum* Boiss. can be seen around the Bursa-Inegol roads in May (Güleryüz & Malyer, 1998).

Extraction

Plant material was ground to fine powder. Fifteen grams of the powder were extracted twice with 80% aq. methanol. The extract was evaporated to dryness at 40 °C, and stored at −20 °C for further analysis.

Bioassay

In vitro antimicrobial studies were carried out by the agar-disk diffusion method against test microorganisms (Collins & Lyne, 1987; NCCLS, 1993; Board & Lovelock, 1975; Favel et al., 1994). Five hundred micrograms of crude plant extracts were dissolved in 20 µl of 80% aq. methanol and applied to a 6 mm diameter paper disk for every test. Penicillin for the bacteria, and sulconazole for the yeasts (10 µg/disc; both obtained from Sigma), and 80% aq. methanol were used as controls. Mueller Hinton Agar plates (Oxoid) in the exponential growing phase (approximately 5 CPU). *Escherichia coli* ATCC 11230, *Micrococcus luteus La2971*, *Staphylococcus aureus* ATCC 6538P, *Salmonella thyphi* ATCC 19430, *Klebsiella pneumoniae* UC57, *Pseudomonas aeroginosa* ATCC 27893, *Corynebacterium xerosis* CCM 2824, *Bacillus megaterium* DSM 32, *Mycobacterium smegmatis* CCM 2067, *Proteus vulgaris* ATCC 8427, *Candida albicans* ATCC 10231, *Rhodotorula rubra*, *Saccharomyces cerevisiae* ATCC 9763 were used for testing the antimicrobial activity. Inhibition zone diameters were measured after 24–48 h. The degree of growth inhibition was quantitatively evaluated after 16 h by comparison with the growth inhibition resulting from the positive control.

Results and discussion

Table 1 gives a summary of the investigated *Verbascum* L. species and the result of the antimicrobial screening. No significant activity was found against Gram (−) bacteria such as *Escherichia coli* ATCC 11230, *Proteus vulgaris* ATCC 8427,

Table 1. Survey of antimicrobial activity of three *Verbascum* species.

	Collector/Collection number		
Microorganisms	1 11772GG	2 11771GG	3 11770GG
Escherichia coli ATCC 11230	−	−	(+)
Micrococcus luteus La 2971	+	++	+
Proteus vulgaris ATCC 8427	(+)	−	−
Staphylococcus aureus ATCC 5538P	+	+++	+
Mycobacterium smegmatis CCM 2067	+	+	+
Corynebacterium xerosis CCM 2824	+	+	+
Klebsiella pneumoniae UC 57	−	−	−
Pseudomonas aeruginosa ATCC 27853	−	(+)	(+)
Bacillus megaterium DSM 32	+	++	+
Salmonella thyphi ATCC 19430	−	−	−
Candida albicans ATCC 10231	+	++	+
Saccharomyces cerevisiae ATCC 9730	+	+	(+)
Rhodotorula rubra	(+)	+	+

1: *Verbascum olympicum* Boiss.; 2: *Verbascum prusianum* Boiss.; 3: *Verbascum bombyciferum* Boiss. Collector (GG): Gürcan Güleryüz

(+): Inhibition zone less than 1 mm surrounding the 6 mm paper disk

+: Inhibition less than positive control

++: Inhibition comparable to positive control

+++: Inhibition more than 10 µg penicillin or sulconazole/disk; inhibition zones of references = 12–14 mm diam.

Klebsiella pneumoniae UC57, *Pseudomonas aeroginosa* ATCC 27893, and *Salmonella thyphi* ATCC 19430. Activity against the Gram (+) bacteria such as *Micrococcus luteus* LA 2971, *Corynebacterium xerosis* CCM 2824, *Bacillus megaterium* DSM 32, *Staphylococcus aureus* ATCC 5538P, and acid fast bacterium *Mycobacterium smegmatis* CCM 2067 were found from extracts of all *Verbascum* L. species. Antimicrobial activity was most consistently detected in the species *Verbascum prusianum* Boiss., especially against *Staphylococcus aureus* ATCC 6538P, *Micrococcus luteus* La 2971, *Bacillus megaterium* DSM 32 and *Candida albicans* ATCC 10231. Notably, *Verbascum prusianum* Boiss. exhibits more activity against yeasts and bacteria, while the others exhibit less effect against the microorganisms used in this study. This indicates that antifungal and antibacterial activities in *Verbascum* species may be associated with different compounds.

The antimicrobial activity of nine *Verbascum* L. species have previously been reported by Meurer-Grimes et al. (1996). 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 (+) bacterium *Staphylococcus aureus* ATCC 6538P, and the fungus *Candida albicans* ATCC 10231 had been found.

Our findings are similar to those reported in the mentioned study. We have found that the three *Verbascum* L. species revealed antimicrobial activity to some Gram (+) bacteria and yeasts. Despite this fact the extracts have not shown any antagonistic effect against the Gram (−) bacteria used. *Staphylococcus aureus* ATCC 6538P, *Micrococcus luteus* La 2971, and *Candida albicans* ATCC 10231 were found to be the most sensitive microorganisms. *Verbascum* species are under further investigation for the isolation and characterization of the antimicrobial compounds.

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