

Antimicrobial and Antimalarial Activities of Some Endemic Turkish *Verbascum* Species

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Summary : The antimicrobial activities of ethyl acetate and methanol extracts of five *Verbascum* species were studied. The dried extracts of the aerial parts of *Verbascum chionophyllum* Hub.-Mor., *V. cilicicum* Boiss., *V. pterocalycinum* var. *mutense* Hub.-Mor., *V. pycnostachyum* Boiss. & Helder. and *V. splendidum* Boiss. were tested *in vitro* against *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 90113), *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *S. aureus* (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853), *Aspergillus fumigatus* (ATCC 90906) and *Mycobacterium intracellulare* (ATCC 23068) using a 96-well microplate assay. Amphotericin B, ciprofloxacin and rifampin were used as positive controls. The antimalarial activities of the same extracts were also tested to *Plasmodium falciparum* clone [Sierra Leone D6 (chloroquine-sensitive)]. The antimalarial agents chloroquine and artemisinin were used as positive controls. None of the tested extracts showed important antimicrobial or antimalarial activities.

Keywords: *Verbascum chionophyllum*, *V. cilicicum*, *V. pterocalycinum* var. *mutense*, *V. pycnostachyum*, *V. splendidum*, *Scrophulariaceae*, Antimicrobial Activity, Antimalarial Activity

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Bazı Endemik *Verbascum* Türlerinin Antimikrobiyal ve Antimalariyal Aktiviteleri

Özet : Beş *Verbascum* türünün etilasetat ve metanol ekstraktlarının antimikrobiyal aktiviteleri çalışılmıştır. *Verbascum chionophyllum* Hub.-Mor., *V. cilicicum* Boiss., *V. pterocalycinum* var. *mutense* Hub.-Mor., *V. pycnostachyum* Boiss. & Helder. ve *V. splendidum* Boiss. 'in topraküstü kısımlarının kuru ekstraktları, 96 kuyulu mikrotitrasyon plak yöntemi ile, *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 90113), *Staphylococcus aureus* (ATCC 29213), meticillin-resistant *S. aureus* (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853), *Aspergillus fumigatus* (ATCC 90906) ve *Mycobacterium intracellulare* (ATCC 23068)'e karşı *in vitro* test edilmiştir. Amphotericin B, ciprofloxacin ve rifampin pozitif kontrol olarak kullanılmıştır. Aynı ekstraktların antimalariyal aktiviteleri ise *Plasmodium falciparum* klonu [Sierra Leone D6 (Chloroquine-duyarlı)]'na karşı çalışılmıştır. Antimalariyal ajanlar, chloroquine ve artemisinin pozitif kontrol olarak kullanılmıştır. Denenen ekstraktların hiçbiri önemli bir antimikrobiyal ve antimalariyal aktivite göstermemiştir.

Anahtar kelimeler: *Verbascum chionophyllum*, *V. cilicicum*, *V. pterocalycinum* var. *mutense*, *V. pycnostachyum*, *V. splendidum*, *Scrophulariaceae*, Antimikrobiyal Aktivite, Antimalariyal Aktivite

INTRODUCTION

The genus *Verbascum*, commonly known as "mullein", is a widespread genus of the family Scrophulariaceae, which comprises more than 2500 species worldwide. This taxon is represented by 228 species, 185 of which are endemic, in the flora of Turkey¹. Some *Verbascum* species have been used for their medicinal effects in Turkey. Their flowers have mu-

colytic and expectorant effects. The leaves have been used as diuretic, sudorific, expectorant, sedative and constipate in traditional Turkish medicine. Seeds of *Verbascum* species are used for fishing because of their saponin contents which are toxic to fish². *Verbascum* species are also used externally for desiccating wounds, anal fistula and pruritic conditions in uregenital organs³ and internally for hemorrhoids⁴. Additionally, several *Verbascum* species are known

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as piscicide, antiseptic, astringent, demulcent, emollient, expectorant, sedative, narcotic, diuretic and antimalarial, and as a treatment for tumors, inflammations, migraine, asthma and spasmodic coughs in Europe, Asia and Northern America⁵.

Antimicrobial effects of *Verbascum bombyciferum* Boiss. and *V. olympicum* Boiss. have been previously investigated^{6,7}. Moreover, *V. prusianum*⁷, *V. macrurum*⁸ and *V. qulebrium*⁹ have also been investigated for their antimicrobial activity. As was stated in a report by McCutcheon et al.¹⁰, extracts of *V. thapsus* revealed antiviral activity against herpes virus type 1. Furthermore, aqueous extracts of *V. fruticosum* L. demonstrated strong growth inhibition on the malaria parasite¹¹.

Malaria is caused by a protozoan of the genus *Plasmodium* that enters the circulatory system of humans from the salivary glands of infected female *Anopheles* mosquitoes. Malaria is still an endemic disease in more than 100 countries¹². The World Health Organization has estimated that more than 270 million people acquire malaria annually and one million may die¹³. The need for new antimalarial drugs is urgent, as antimalarials such as the most widely used chloroquine are failing due to the appearance of resistant *P. falciparum* strains^{14,15}.

Ethnobotanical information obtained from traditional healers may serve as a starting point for bioactive compounds. In addition, investigations confirm that higher plants used as anti-infective phytomedicines may serve as a valuable source for novel antibiotics¹⁶. *Verbascum chionophyllum* Hub.-Mor., *V. cilicicum* Boiss., *V. pterocalycinum* var. *mutense* Hub.-Mor., *V. pycnostachyum* Boiss. & Helder. and *V. splendidum* Boiss. are endemic to Turkey¹.

As a part of our ongoing research on the *Verbascum* species growing in Turkey, we investigated the antimicrobial and antimalarial properties of these *Verbascum* species against *C. albicans*, *C. neoformans*, *S. aureus*, methicillin-resistant *S. aureus*, *P. aeruginosa*, *A. fumigatus* and *M. intracellulare* and the

malarial parasite *P. falciparum* using a 96-well microplate assay protocol. This is the first report on antimicrobial and antimalarial properties of these species.

MATERIALS and METHODS

Plant material

Five species were collected from different regions of Turkey during flowering time. The voucher specimens were deposited in the herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Verbascum chionophyllum Hub.-Mor.: C4: Icel: 40 km from Mut to Ermenek, *Pinus brutia* fields, 550-600 m, July 2000 (HUEF 00180).

Verbascum cilicicum Boiss.: C5: Adana: Between Pozantı and Ulukışla, Alihoca village, July 2000 (HUEF 00183).

Verbascum pterocalycinum var. *mutense* Hub.-Mor.: C4: İçel: Between Mut and Karaman, *Pinus brutia* and *Pinus nigra* fields, 930-1100 m, July 2000 (HUEF 00184).

Verbascum pycnostachyum Boiss. & Heldr.: C4: Karaman: From Mut to Karaman, 1300 m, July 2000 (HUEF 00182).

Verbascum splendidum Boiss.: C5: Konya: Eregli, From Eregli to Karaman, 1150-1200 m, July 2000 (HUEF 00181).

Preparation of plant material

Ten samples of air dried parts (flower and leaf) of the plants (20 g) were extracted with methanol (150 ml) and ethyl acetate (150 ml) using evaporator without vacuum at room temperature. The extracts were evaporated to dryness at 40°C, and stored at -20°C for further analysis. The crude residues were used both in antimicrobial and antimalarial assays.

Antimicrobial assay

The antimicrobial activities of the extracts were determined by using a 96-well microplate assay protocol^{17,18}. The test organisms used were ATCC strains of *Candida albicans* (90028), *Cryptococcus neoformans* (90113), *Staphylococcus aureus* (29213), methicillin-resistant *S. aureus* (43300), *Pseudomonas aeruginosa* (27853), *Aspergillus fumigatus* (90906) and *Mycobacterium intracellulare* (23068). Amphotericin B, ciprofloxacin and rifampin were used as positive controls, with DMSO as a negative control.

Antimalarial/Parasite LDH assay

The *in vitro* antimalarial assay procedure¹⁹ utilized at the NCNPR, University of Mississippi, is an adaptation of the parasite lactate dehydrogenase (pLDH) assay developed by Makler et al.^{20,21}, using a 96-well microplate assay protocol with a *P. falciparum*

clone [Sierra Leone D6 (chloroquine-sensitive)]. The antimalarial agents chloroquine and artemisinin are used as positive controls, while DMSO is the negative (vehicle) control.

RESULTS and DISCUSSION

According to antimicrobial assay^{17,18}, the extracts were tested at 500 µg/ml concentration on a primary assay and then the active samples were screened at 500, 100, and 20 µg/ml concentrations in a secondary assay.

The antimicrobial activities of *Verbascum species* against Gram positive and Gram negative bacteria and one yeast-like fungi and one mold like fungi as % growth inhibitions and active concentrations (µg/ml) values are summarized. The primary assay results of the antimicrobial activity screening are given in Table I.

Table I. Antimicrobial activity of the methanol and ethyl acetate extracts of some *Verbascum species* (primary assay)

Plant	Plant Part	Solvent	Growth Inhibition (%)					*Active Concentration (µg/ml)	
			Ca	Cn	Sa	MRS	Pa	Af	Mi
<i>V. chionophyllum</i>	flower	MeOH	8	0	19	38	35	-	-
	leaf	MeOH	11	1	19	40	41	-	-
<i>V. cilicicum</i>	flower	MeOH	9	2	0	5	22	-	-
	leaf	MeOH	4	0	0	0	29	-	-
<i>V. pterocalycinum</i> var. <i>mutense</i>	flower	MeOH	17	0	9	0	51	-	-
	leaf	MeOH	13	0	0	3	40	-	-
<i>V. pycnostachyum</i>	flower	MeOH	9	30	1	39	23	-	-
	leaf	MeOH	7	0	18	22	41	-	-
<i>V. splendidum</i>	flower	MeOH	14	0	55	71	50	-	-
	leaf	MeOH	14	0	35	60	29	-	-
<i>V. chionophyllum</i>	flower	EtOAc	20	32	100	100	0	-	-
	leaf	EtOAc	36	41	100	100	66	-	-
<i>V. cilicicum</i>	flower	EtOAc	32	74	100	100	19	-	-
	leaf	EtOAc	41	72	100	100	44	-	-
<i>V. pterocalycinum</i> var. <i>mutense</i>	flower	EtOAc	-	-	-	-	-	-	-
	leaf	EtOAc	-	-	-	-	-	-	-
<i>V. pycnostachyum</i>	flower	EtOAc	18	18	72	83	0	-	-
	leaf	EtOAc	33	34	74	100	22	-	-
<i>V. splendidum</i>	flower	EtOAc	-	-	-	-	-	-	-
	leaf	EtOAc	-	-	-	-	-	-	-
Amphotericin B			0.10	0.35				1.25	
Ciprofloxacin					0.20	0.20	0.04		
Rifampin									0.78

Ca: *Candida albicans* (ATCC 90028), Cn: *Cryptococcus neoformans* (ATCC 90113), Sa: *Staphylococcus aureus* (ATCC 29213), MRS: methicillin-resistant *S.aureus* (ATCC 43300), Pa: *Pseudomonas aeruginosa* (ATCC 27853), Af: *Aspergillus fumigatus* (ATCC 90906), Mi: *Mycobacterium intracellulare* (ATCC 23068), "-" Not active, MeOH: Methanol, EtOAc: Ethyl acetate.*The active concentration is the lowest concentration (µg/ml) that significantly inhibits growth^{17, 18}.

The extracts showing % growth inhibition < 80 were considered inactive in primary assay. However, the samples showing % growth inhibition > 80 were considered active and these samples were screened in a secondary assay^{17,18}. Based on the representative results, the ethyl acetate extracts of *Verbascum chionophyllum* Hub.-Mor., *V. cilicicum* Boiss., and *V. pycnostachyum* Boiss. & Helder. were evaluated against *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *S. aureus* (ATCC 43300), *Pseudomonas aeruginosa* (ATCC 27853) in a secondary assay. IC₅₀ ≤ 150 µg/ml of the extracts was considered to be active in the secondary assay of the antimicrobial screening^{17,18}. None of the extracts was found to be active in the secondary assay.

All the data indicated that the investigated extracts of *Verbascum* species have no important antibacterial or antifungal activities.

The methanol extracts of these *Verbascum* species were also tested for antimalarial activity¹⁹⁻²¹. The primary screening involved determination of pLDH inhibition (%) of each sample tested at 15.9 µg/ml for extract. The tested species did not exhibit any activity in the antimalarial assay at the tested concentration.

CONCLUSION

The antimicrobial activity of nine *Verbascum* L. species was reported by Meurer-Grimes et al.⁶. They tested the 80% aqueous methanol extracts of the flowers, seeds, leaves and roots of *Verbascum* species and detected a strong growth inhibition. As a result of this study, antimicrobial activity against the Gram (+) bacterium *Staphylococcus aureus* ATCC 6538P and yeast *Candida albicans* ATCC 10231 was found.

We found that the five *Verbascum* L. species revealed no antimicrobial activity versus Gram (+) and Gram (-) bacteria and yeasts used. Moreover, the tested extracts did not show any antimalarial activity on *P. Falciparum*, while *V. fruticosum* has shown

strong inhibitory activity (growth inhibition 26.9%) against the same parasite¹¹.

Further screening of *Verbascum* species may lead to identification of additional species with their biological activities. Research in our laboratory is now underway to collect *Verbascum* species in different seasons and to isolate the antimicrobial agents from those species.

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