Turkish Journal of Biology

Volume 29 | Number 2 Article 4

1-1-2005

Antimicrobial Effects of Verbascum georgicum Bentham Extract

MERYEM ŞENGÜL

HATİCE ÖĞÜTÇÜ

AHMET ADIGÜZEL

FİKRETTİN ŞAHİN

AYŞE AYDAN KARA

See next page for additional authors

Antimicrobial Effects of Verbascum georgicum Bentham Extract

Authors
MERYEM ŞENGÜL, HATİCE ÖĞÜTÇÜ, AHMET ADIGÜZEL, FİKRETTİN ŞAHİN, AYŞE AYDAN KARA, İSA KARAMAN, and MEDİNE GÜLLÜCE

Turk J Biol
29 (2005) 105-110

Antimicrobial Effects of *Verbascum georgicum* Bentham Extract

Meryem ŞENGÜL[2], Hatice ÖĞÜTCÜ[2], Ahmet ADIGÜZEL[1], Fikrettin ŞAHİN[1], Ayşe Aydan KARA[1], İsa KARAMAN[1], Medine GÜLLÜCE[2]

[1]Biotechnology Application and Research Center, Atatürk University, 25240, Erzurum - TURKEY

[2]Department of Biology, Faculty of Arts and Science, Atatürk University, 25240, Erzurum - TURKEY

Received: 16.03.2005

Abstract: The methanol extract of *Verbascum georgicum* Bentham (*Scrophulariaceae*) was investigated for its in-vitro antimicrobial properties. A total of 143 microorganisms belonging to 56 bacteria and 4 fungi and a yeast species were studied using the disk-diffusion method and microdilution assays. The results indicated that the methanol extract of V. geopgicum had an inhibitory effect on the growth of all *Candida albicans* isolates and 17 strains in 10 different species of bacteria, namely *Bacillus amyloliquefaciens, B. subtilis, B. cereus, B. pumilus, B. megaterium, B. lentimorbus, B. licheniformis, Pseudomonas putida, P. syringae* and *Escherichia coli*, at different concentrations ranging from 37.5 to 300 µg/ml. However, it did not show antifungal activity against the isolates of the 4 fungal species tested. Thus, the results suggest that *V. georgicum* extract possesses compounds with antimicrobial properties that might be utilized for developing new drugs.

Key Words: *Verbascum georgicum*, Antimicrobial activity, Medicinal plant

Verbascum georgicum Bentham Ekstraktının Antimikrobial Etkileri

Özet: *Verbascum georgicum* Bentham'ın metanol ekstraktının antimikrobiyal özelliği in-vitro ortamda araştırıldı. Bir maya 4 fungus 56 bakteri türüne ait 143 mikroorganizma disk-difüzyon ve mikrodilüsyon yöntemleri kullanılarak test edildi. *Verbascum georgicum* Bentham'ın metanol ekstraktının *Candida albicans* izolatlarının hepsine ve *Bacillus amyloliquefaciens, B. subtilis, B. cereus, B. pumilus, B. megaterium, B. lentimorbus, B. licheniformis, Pseudomonas putida, P. syringae, Escherichia coli* türlerini içeren 10 farklı bakteri türünün 17 suşunda 37.5-300 µg/mL' konsantrasyonlarında inhibisyon zonları oluşturduğu tesbit edildi. Bununla birlikte test edilen 4 fungus türünün izolatlarında antifungal aktivite göstermemiştir. Böylece, bu sonuçlara göre *Verbascum georgicum* Bentham'ın metanol ekstraktının antimikrobiyal özellikte bileşikler içerdiği ve bunun yeni ilaç geliştirilmesinde antimikrobiyal ajan olarak kullanılabileceği önerilebilir.

Anahtar Sözcükler: *Verbascum georgicum*, Antimicrobial aktivite, Tıbbi Bitki

Introduction

Many plant species among the flora of Turkey play an important role in traditional and conventional medicine. Based on the literature, there are approximately 9000 plant species in Turkey's flora, of which more than 500 are widely used in folkloric medicine due to their antimicrobial and anticarcinogenic properties (1). Furthermore, these species provide minerals, fiber, vitamins and essential fatty acids and enhance taste and color in food. *Verbascum* is the richest genus, represented by 233 species in Turkey (2,3). One of the well known *Verbascum* species is *V. thapsus* L., which has been used for the treatment of different diseases including asthma, spasmodic cough, migraine and earache (4-7). *V. thapsus*, *V. fruticulosum* and *V. undulatum* have been investigated for their antibacterial, antifungal, antiviral, and antimalarial activities through in-vitro and in-vivo tests (5,6,8-11). *V. georgicum* is an abundant biennial herb growning in the Eastern Anatolia region of Turkey, and used traditionally in the treatment of various medical conditions. However, *V. georgicum*, like many other *Verbascum* species, has not been investigated sufficiently in terms of its antimicrobial activities. In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs/chemicals commonly applied in the treatment of infectious diseases (12-14). This situation has prompted scientists to search for new antimicrobial substances from various sources, including medicinal

plants (15,16). The aim of this study was to evaluate the antimicrobial potential of *V. georgicum* tested on a wide range of microorganisms.

Materials and Methods

Plant material and extraction procedure

Aerial parts (leaves, flowers and stems) of *Verbascum georgicum* Bentham (*Scrophulariaceae*) plants were collected from Aşkale, Erzurum. The taxonomic identification of plant materials was confirmed by plant taxonomist Meryem Şengül at the Department of Biology, Atatürk University, Erzurum. Collected plant material was dried in the shade and ground to 2 mm. The dried and powdered plant materials (500 g) were extracted successively with 1 l of methanol by Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent (17). The aqueous extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40 °C using a rotary evaporator. The residues obtained were stored at -80 °C until the tests.

Microorganisms

A total 159 microbial cultures belonging to 56 bacteria, 1 yeast and 4 fungal species were used in this study. The microorganisms used are listed in Tables 1 and 2. They were provided by the Department of Clinical Microbiology, Faculty of Medicine, and the Plant Diagnostic Laboratory, Faculty of Agriculture, at Atatürk University, Erzurum. The identity of the microorganisms used was confirmed by the Microbial Identification System at the Biotechnology Application and Research Center at Atatürk University.

Table 1. Antimicrobial activity of *Verbascum georgicum* extracts (300 µg/disk) against the bacterial strains tested based on disk diffusion method.

	Inhibition zone in diameter (mm) around test disk			
Microorganisms	Number of Strains[a]	*V. georgicum* Extract	NCa	Standard Antibiotic Disks[b]
Bacteria				
Acinetobacter baumanii	3	-	-	NT
Acinetobacter calcoaceticus	2	-	-	16 mm (OFX)
Acinetobacter lwoffii	1	-	-	27 mm (OFX)
Acinetobacter johnsonii	2	-	-	25 mm (OFX)
Alcaligenes pacificus	1	-	-	32 mm (OFX)
Alcaligenes xylosoxydans	2	-	-	12 mm (SCF)
Agrobacterium radiobacter	1	-	-	28 mm (SCF)
Bacillus amyloliquefaciens	1	12 mm/1	-	27 mm (SCF)
Bacillus atrophaeus	2	-	-	21 mm (SCF)
Bacillus cereus	2	7-21 mm/2	-	30 mm (OFX)
Bacillus licheniformis	1	27 mm/1	-	- (SCF)
Bacillus macerans	1	-	-	26 mm (SCF)
Bacillus megaterium	4	18-26 mm/2	-	9 mm (SCF)
Bacillus pumilus	3	20-24 mm/3	-	23 mm (OFX)
Bacillus sphaericus	1	-	-	18 mm (OFX)
Bacillus substilis	4	9-17 mm/4	-	28 mm (OFX)
Brevundimonas diminuta	1	-	-	34 mm (SCF)
Brucella abortus	15	-	-	12 mm (SCF)
Brucella melitensis	11	-	-	18 mm (OFX)
Burkholdria cepacia	1	-	-	-mm (SCF)
Burkholdria gladioli	1	-	-	22 mm (NET)
Citrobacter freundii	1	-	-	23 mm (SCF)
Clavibacter michiganense	1	-	-	25 mm (SCF)
Curtobacterium flaccumfaciens	2	-	-	37 mm (OFX)
Enterobacter aerogenes	1	-	-	12 mm (OFX)
Enterobacter cloacae	2	-	-	10 mm(OFX)
Enterobacter intermedius	2	-	-	27 mm (SCF)
Enterobacter pyrinus	1	-	-	NT
Erwinia amylovora	1	-	-	12 mm (OFX)
Erwinia carotovora	1	-	-	30 mm (OFX)

Table 1. Continued.

Microorganisms	Inhibition zone in diameter (mm) around test disk			
	Number of Strains[a]	*V. georgicum* Extract	NCa	Standard Antibiotic Disks[b]
Erwinia chrysanthemi	1	-	-	(SCF)
Escherichia coli	1	18 mm/1	-	- (OFX)
Flavobacterium blastinum	1	-	-	32 mm (OFX)
Klebsiella pneumoniae	2	-	-	12 mm (OFX)
Klebsiella trevisanii	1	-	-	23 mm (OFX)
Kocuria varians	1	-	-	18 mm (OFX)
Leclercia adecarboxylata	1	-	-	NT
Micrococcus luteus	1	-	-	- (OFX)
Neisseria spp	1	-	-	- (OFX)
Pantoea agglomerans	3	-	-	24 mm(NET)
Plesiomonas shigelloides	1	-	-	- (SCF)
Proteus vulgaris	1	-	-	12 mm (OFX)
Pseudomonas aeruginosa	20	-	-	22 mm (NET)
Pseudomonas fluorescens	5	-	-	30 mm (OFX)
Pseudomona huttiensis	2	-	-	10 mm (OFX)
Pseudomonas putida	1	23 mm/1	-	24 mm (SCF)
Pseudomonas syringae pvs.	5	24 mm/1	-	24 mm (OFX)
Ralstonia pickettii	1	-	-	25 mm (OFX)
Salmonella typhimurium	1	-	-	27 mm (SCF)
Staphylococcus aureus	5	-	-	22 mm (SCF)
Staphylococcus epidermis	5	-	-	NT
Stenotrophomonas maltophilia	2	-	-	NT
Streptococcus pneumoniae	2	-	-	- (OFX)
Streptococcus pyogenes	2	-	-	10 mm (OFX)
Xanthomonas campestris pvs.	4	-	-	20 mm (SCF)
Total 55 bacterial species	142	7-27 mm/16 strains	-	

[a]NC: Negative Control (MeOH).
[c]OFX = Ofloxacin (10 µg/disk); SCF = Sulbactam (30 µg) + Cefoperazona (75 µg) (105 µg/disk); NET = Netilmicin (30 µg/disk) were used as positive reference standards antibiotic disks (Oxoid); NT=Not tested.

Table 2 Antimicrobial activity of *Verbascum georgicum* extracts (300 µg/disk) against yeast and fungus isolates tested based on the disk diffusion method.

Microorganisms	Inhibition zone in diameter (mm) around test disk			
	Number of Strains[a]	*V. georgicum* Extract	NC[a]	Standard Antibiotic Disks[b]
Yeast				
Candida albicans	8	9-23 mm/8	-	- (SCF)
Fungi				
Alternaria alternata	2	-	-	NT
Aspergillus flavus	2	-	-	NT
Fusarium oxysporum	2	-	-	NT
Penicillium spp.	2	-	-	NT
16 isolates		9-23 mm/8 isolates	-	

[a]NC: Negative Control (MeOH).
[c]OFX = Ofloxacin (10 µg/disk); SCF = Sulbactam (30 µg) + Cefoperazona (75 µg) (105 µg/disk); NET = Netilmicin (30 µg/disk) were used as positive reference standards antibiotic disks (Oxoid); NT=Not tested.

Antimicrobial activity

Disk-diffusion assay

The dried plant extracts were dissolved in the same solvent (methanol) to a final concentration of 30 mg/ml and sterilized by filtration through 0.45 µm Millipore filters. Antimicrobial activity tests were then carried out by disk diffusion (18) using 100 µl of suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast and 10^4 spore/ml of fungi spread on nutrient agar (NA), Sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) media, respectively. The disks (6 mm in diameter), containing 10 µl of the extracts (300 µg/disk) at a concentration of 30 mg/ml, were impregnated in the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ofloxacin (10 µg/disk), sulbactam (30 µg) + cefoperazone (75 µg) (105 µg/disk) and/or netilmicin (30 µg/disk) were used as positive reference standards to determine the sensitivity of one strain/isolate for each microbial species tested. The inoculated plates were incubated at 37 ºC for 24 h in the case of clinical bacterial strains, for 48 h for yeast and for 72 h for the fungal isolates. Plant associated microorganisms were incubated at 27 ºC. Antimicrobial activity was evaluated by measuring the inhibition zones in reference to the test organisms. Each assay in this experiment was repeated twice.

Microdilution assays

The minimum inhibitory concentration (MIC) values were also studied for the microorganisms that were determined as sensitive to *V. georgicum* extract in the disk diffusion assay. The inocula of microorganisms were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The *V. georgicum* extract dissolved in 10% dimethylsulfoxide (DMSO) was first diluted to the highest concentration (600 µg/ml) to be tested, and then serial 2-fold dilutions were made in a concentration range from 9.37 to 600 µg/ml in 10 ml sterile test tubes containing nutrient broth. MIC values of *V. georgicum* extract against bacterial strains and *Candida albicans* isolates were determined based on a micro-well dilution method (19-21).

The 96-well microtiter plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inoculum into each well. Then 100 µl of *V. georgicum* extract initially prepared at a concentration of 600 µg/ml was added to the first wells, and 100 µl from their serial dilutions was transferred into 6 consecutive wells. The last well, containing 195 µl of nutrient broth without the compound and 5 µl of the inoculum on each strip, was used as a negative control. The final volume in each well was 200 µl. Maxipime (Bristol-Myers Squibb) at the concentration range of 7.8-500 µg/ml was prepared in nutrient broth and used as the standard drug for positive control. The plate was covered with a sterile plate sealer. The contents of each well were mixed on a microtiter plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by absorbance values at 600 nm using an ELx 800 universal microtiter plate reader (Biotek Instrument Inc, Highland Park, Vermont, USA) and confirmed by plating 5 µl samples from clear wells on nutrient agar media. The extract tested in this study was screened twice for each organism. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

Results and Discussion

In the present study, the antimicrobial compounds from the aboveground parts of *V. georgicum* plants collected from Aşkale in the Eastern Anatolia region of Turkey were extracted, and evaluated against a wide range of microorganisms on the basis of disk diffusion and microdilution assays. In previous studies, it was reported that methanol was a better solvent for the consistent extraction of antimicrobial substances from medicinal plants compared to other solvents such as water, ethanol and hexane (17,22,23). Therefore, methanol was used for plant extraction in this study and antimicrobial activities were quantitatively assessed by the presence or absence of and inhibition zone and zone diameters (Tables 1, 2), and MIC values (Table 3). Our results showed that the methanol extract of *V. georgicum* has inhibitory effect on the growth of all *Candida albicans* isolates and 17 strains of 10 bacterial species, namely *Bacillus amyloliquefaciens, B. subtilis, B. cereus, B. pumilus, B. megaterium, B. lentimorbus, B. licheniformis, Pseudomonas putida, P. syringae,* and *Escherichia coli* (Tables 1, 2). The maximal inhibition zones and MIC values for bacterial strains and *C. albicans* isolates sensitive to *V. georgicum* extract were 7-27 mm and 125-250 µg/ml and 9-23 mm and 37.5 µg/ml, respectively (Tables 1-3). Findings in this study confirmed

Table 3. The MIC values of Verbascum georgicum extracts against the the microorganisms tested in microdilution assay (MIC in µg/ml).

Microorganisms	*V. georgicum* Extract	Standard Drug (Maxipime)
Bacillus amyloliquefaciens	300	NT
Bacillus cereus	300	250
Bacillus licheniformis	150	-
Bacillus megaterium	300	-
Bacillus pumilus	300	31.25
Bacillus substilis	150	125
Escherichia coli	300	15.62
Pseudomonas syringae	150	31.25
Pseudomonas putida	300	125
Candida albicans	37.5	-

the observations of some other researchers, namely that some *Verbascum* species contain substances with antimicrobial properties (5,6,8-11). However, this is the first study to provide data that *V. georgicum* plants possess potential antibacterial and anticandidal activities.

On overall evaluation of the results showed that except for *Candida albicans* isolates there is no uniform response within or between the bacterial strains of the same species in terms of susceptibility to antimicrobial compounds in *V. georgicum* extract, even though 82% of bacterial strains sensitive to the *V. georgicum* extract were Gram-positive (24). These kinds of differences in susceptibility among the bacterial strains against antimicrobial substances in plant extracts may be explained by the differences in cell wall composition and/or inheritance of antimicrobial-resistance genes on plasmids that can easily be transferred among bacterial strains.

Conclusions

The results in the present study suggest that *V. georgicum* extract possesses compounds with antibacterial and anticandidal properties that can be used as antimicrobial agents in the development of new drugs for the treatment of infectious diseases.

Acknowledgments

This study was supported by a grant from the Research Funds appropriated to Atatürk University.

Corresponding author:
Meryem ŞENGÜL
Department of Biology, Faculty of Arts and Science,
Atatürk University, 25240, Erzurum - Turkey.
E-mail: sengulm@atauni.edu.tr

References

1. Özturk M, Özcelik H. Doğu Anadolu'nun şifalı Bitkileri. SİSKAV Vakfı, Semih Ofset Matbaacılık, 1991: pp. 27-28.
2. Davis PH. Flora of Turkey and the East Aegean Islands. Univ. Press, Edinburgh , 515-16, 1988.
3. Baytop T. Türkiye'de Bitkiler ile Tedavi Geçmişte ve Bugün, İst. Üniv. Yay. No: 3255, İstanbul, 235-36, 1984.
4. Baskin JM, Baskin CC. Seasonal changes in germination responses of buried seeds of *Verbascum thapsus* and *V. blattaria* and ecological implications. Canadian J Bot, 59: 1769-75, 1981.
5. Türker AU, Camper ND, Gurel E. In vitro culture of common mullein (*Verbascum thapsus* L.). In Vitro Cell Dev Biol-Plant, 37, 1: 40-43, 2001.
6. Magiatis P, Spanakis D, Mitaku S et al. Verbalactone a new macrocyclic dimer lactone from the roots of *Verbascum undulatum* with antibacterial activity. Journal of Natural Products, 64: 1093-94, 2001.
7. Sarrell EM, Mandelberg A, Cohen HA. Efficacy of naturapathic extracts in the management of ear pain associated with acute otitis media. Archives of Pediatrics and Adolescent Medicine 155: 796-99, 2001.

8. Meurer Grimes B, McBeth DL, Hallihan B et al. Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae. International Journal of Pharmacognosy, 34: 243-48, 1996.

9. Qureshi S, Rai MK, Agrawal SC. *In vitro* evoluation of inhibitory nature of extracts of 18 plant species of Chindwora against 3-keratinophilic fungi. Hindustan Antibiotics Bulletin, 39: 56-60, 1997.

10. Zanon SM, Ceriatti FS, Rovera M et al. Search for antiviral activity of certain medicinal plants from Cordoba, Argentina. Revista Latinoamericana de Microbiologia 41: 59-62, 1999.

11. Sathiyamoorthy P, Lugasi-Evgi H, Schlesinger P et al. Screening for cytotoxic and antimalarial activities in desert plants of the Negev and Bedouin market plant products. Pharmaceutical Biology, 37: 188-195, 1999.

12. Loper JE, Henkels MD, Roberts RG et al. Evaluation of streptomycin, oxytetracycline, and copper resistance of *Erwinia amylovora* isolated from pear orchards in Washington State. Plant Disease, 75: 287-90, 1991.

13. Davis J. Inactivation of antibiotics and the dissemination of resistance genes. Science, 264: 375-82, 1994.

14. Service RF. Antibiotics that resist resistance. Science, 270: 724-27, 1995.

15. Clark AM. Natural products as resource for new drugs. Pharmaceutical Research, 13: 1133-41, 1996.

16. Cordell GA. Biodiversity and drug discovery a symbiotic relationship. Phytochemistry, 55: 463-80, 2000.

17. Lin J, Opoku AR, Geheeb-Keller M et al. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. Journal of Ethnopharmacology, 68: 267-74, 1999.

18. Murray PR, Baron EJ, Pfaller MA et al. Manual of Clinical Microbiology. 6th ed; vol. 6, ASM, Washington. 214-15, 1995.

19. Şahin F, Karaman İ, Güllüce M et al. Evaluation of antimicrobial activities *Satureja hortensis* L., Journal of Ethnopharmacology 87: 61-65, 2002.

20. Karaman İ, Şahin F, Güllüce M et al. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L., Journal of Ethnopharmacology, 85: 231-35, 2003.

21. Güllüce M, Adıgüzel A, Ö?ütçü H et al. Antimicrobial effects of *Quercus ilex* L. extract., Phytotherapy Research, 18: 208-11, 2004.

22. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology, 62: 183-193, 1998.

23. Eloff JN. Which extract should be used for the screening and isolation of antimicrobial components from plants. Journal of Ethnopharmacology, 60: 1-8, 1998.

24. Dülger B, Kırmızı S, Arslan H et al. Antimicrobial activity of three endemic *Verbascum* species. Pharmaceutical Biol. 40: 587-589, 2002.