ORIGINAL ARTICLE

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Antibacterial Activity of Verbascum antinori ABSTRACT

Objective: In current study, biological screening of the methanol extract obtained from *Verbascum antinori* Boiss. et Heldr. (Scrophulariaceae) was carry out on antibacterial effects.

Methods: Antibacterial activity of the extract was evaluated for *Staphylococcus aureus* ATCC 6538P, *Proteus vulgaris* ATCC 8427, *Klebsiella pneumoniae* UC57, *Micrococcus luteus* CCM 169, *Escherichia coli* ATCC 11230, *Listeria monocytogenes* ATCC 15313, *Bacillus cereus* ATCC 7064 and *Pseudomonas aeruginosa* ATCC 27853 by disk diffusion method.

Results: The extract has potential effect against the Gram-positive bacteria, but no activity was observed against the Gram-negative bacteria used in the present study.

Conclusions: On the basis of the mentioned results, *V. antinori* may be a potential source of antibacterial agent.

Keywords: Antibacterial Activity, Verbascum antinori

Verbascum antinori Bitkisinin Antibakteriyal Aktivitesi ÖZET

Amaç: Bu çalışmada, *Verbascum antinori* Boiss. et Heldr. (Scrophulariaceae) bitkisinden elde edilen metanol ekstraktının antibakteriyal aktivitesi araştırılmıştır.

Gereç ve Yöntem: Ekstraktın antibakteriyal aktivitesi, *Staphylococcus aureus* ATCC 6538P, *Proteus vulgaris* ATCC 8427, *Klebsiella pneumoniae* UC57, *Micrococcus luteus* CCM 169, *Escherichia coli* ATCC 11230, *Listeria monocytogenes* ATCC 15313, *Bacillus cereus* ATCC 7064 ve *Pseudomonas aeruginosa* ATCC 27853 bakterilerine karşı disk difüzyon metodu kullanılarak belirlenmiştir.

Bulgular: Elde edilen ekstraktın, çalışmada kullanılan Gram-pozitif bakterilere karşı oldukça etkin olduğu, ancak Gram-negatif bakterilere karşı ise etkisiz olduğu saptanmıştır.

Sonuç: *V. antinori* bitkisinden elde edilen ekstraktın antibakteriyal ajan olarak kullanılabilirliği saptanmış ve ilerki çalışmalarda bu bitkinin antibakteriyal bileşiklerinin belirlenmesine yönelik çalışmaların gerekliliği öngörülmüştür. **Anahtar Kelimeler:** Antibakteriyal Aktivite, *Verbascum antinori*

INTRODUCTION

Verbascum L. (Scrophulariaceae) is composed of about 323 species and distributed worldwide. (1). In Turkey, the plant comprises about 245 species, 129 hybrids and 6 imperfectly known or doubtful records. Endemism ratio of this plant is very high with 193 (79%) species restricted to Turkey (2).

Verbascum L. has been known for centuries as a powerful medicinal plant according to "folk remedies" for cultures around the world. The leaves and flowers of the plant are known to have expectorant, mucolytic and demulcent properties which are used to treat respiratory disorders in Turkey. In addition, various species of the plant are used for a long time to treat hemorrhoids, rheumatic pain, diarrhea, wounds and superficial fungal infections, and have preventive and inhibitory activities against the murine lymphocytic leukemia and influenza viruses A2 and B (1). In Turkey, especially their flowers have been used. The drug which is prepared from their flowers has diuretic, expectorant and sedative effects (3).

During routine excursions, it was determined that *Verbascum antinori* Boiss. et Heldr. (Scrophulariaceae) is used as diuretic and expectorant. However, the plant has not been previously investigated for their antibacterial activity. Therefore, the aim was to determine the antibacterial effect of the methanol extract prepared from against the test bacteria.

MATERIAL AND METHODS

Plant materials: Aerial parts of *Verbascum antinori* Boiss. et Heldr. was collected from Canakkale, Turkey during the months of September - October 2009. Voucher specimen of the plant was deposited in Department of Biology of Duzce University in the author's personal collection.

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 24h by using Soxhlet equipment (4). The extract was filtered using Whatman filter paper no.1, and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55 °C (yield: 12.4% for methanol).

Microorganisms: Staphylococcus aureus ATCC 6538P, Proteus vulgaris ATCC 8427, Klebsiella pneumoniae UC57, Micrococcus luteus CCM 169, Escherichia coli ATCC 11230, Listeria monocytogenes ATCC 15313, Bacillus cereus ATCC 7064 and Pseudomonas aeruginosa ATCC 27853 were used as test microorganisms.

Screening for Antibacterial Activities: Determination of the antibacterial activity was carried out according to the method described by CLSI (2012) and Dulger (2006) by disk diffusion method (5-6). Studies were performed in triplicate. On each plate, an appropriate reference antibiotic disk was applied, depending on the test microorganisms for comparison.

RESULTS

In the present study, the inhibition zones formed by the extract and some standard antibacterial antibiotics are shown in Table 1.

Table 1. Antibacterial activity of the plant and some standard antibacterial agents

Microorganisms	Diameter of inhibition zone (mm)*					
		Standard antibacterial agents				
	Extract	AM10	CTX30	VA30	OFX5	TE30
Escherichia coli ATCC 10538	-	15.2	22.4	NT	30.8	28.2
Staphylococcus aureus ATCC 6538P	22.6	16.8	12.6	15.2	24.4	26.4
Klebsiella pneumonia UC57	-	11.0	13.4	NT	28.2	23.0
Pseudomonas aeruginosa ATCC 27853	-	19.6	21.0	NT	22.3	NT
Proteus vulgaris ATCC 8427	-	16.2	18.4	20.0	16.7	18.3
Bacillus cereus ATCC 7064	20.4	12.4	NT	18.6	30.2	25.4
Listeria monocytogenes ATCC 15313	14.2	12.4	NT	26.4	19.6	28.2
Micrococcus luteus CCM 169	17.4	32.0	32.2	34.2	28.8	22.4

* Includes diameter of disk (6mm)

AM10: Ampicillin 10 μg, CTX30: Cefotaxime 30 μg, V30: Vancomycin 30 μg, OFX 5: Oflaxacin 5 μg, TE30: Tetracycline 30 μg, NT: Not tested

No significant effect was observed against Gram-negative bacteria such as *E. coli*, *P. vulgaris*, *K. pneumoniae*, and *P. aeruginosa*. Antibacterial effect against the Gram-positive bacteria such as *S*. aureus, B. cereus, L. monocytogenes and M. luteus were determined with inhibition zone ranged from 14.2 to 22.6 mm. The extracts of V. antinori has shown the best antibacterial effect against Staphylococcus aureus, having inhibition zone of 22.6 mm, as compared to all standard antibacterial agents except for TE30 and OFX5. However, the lowest effect was determined against *Listeria monocytogenes* (inhibition zone is 14.2 mm). Bacillus cereus was susceptible to the extract, having inhibition zone of 20.4 mm, as compared to AM10 and VA30. The effect of the extract *Micrococcus luteus* (inhibition zone is 17.4 mm) has weaker than those of the standard all antibacterial antibiotics used in this study.

DISCUSSION

Although a large number of studies on the biological screening of *Verbascum* species have been done, the current study is the first report showing the antibacterial effect of *V. antinori* extracts according to literature scanning. Species of *Verbascum* L. exhibited a wide variation in antimicrobial activity studies. For instance, in our previously study, *V. cilicium* was declared to exhibit more antimicrobial activity than other *Verbascum* species and *Staphylococcus aureus* found to be more susceptible bacterium against the extracts of all *Verbascum* species (6). Similarly, *S. aureus* was found to be more sensitive to the extract of *V. antinori*, having the larger inhibition zones (22.6 mm) in present study (Table 1).

In previous studies, it is determined that *Verbascum* L. species contain, iridoid, flavonoids, saponins, monoterpene glycosides, phenylethanoid glycosides, neolignan glycosides, steroids and spermine alkaloids (7-10). It is known that iridoid and phenylethanoid glycosides are very common in *Verbascum* species (11).

The findings given in present study can be considered as the first report on the antibacterial activity of *V. antinori*. Thus, the activity was more consistently detected and a potential antibacterial effect on the *S. aureus* had been determined. *S. aureus* is one of the commoner causes of opportunistic nosocomial and community infections. These are pneumonia, osteomyelitis, septic arthritis, bacteraemia, endocarditis,

abscesses/boils and other skin infections. S. aureus has gained notoriety because of the increased incidence of Methicillin-resistant Staphylococcus aureus (MRSA) Infections (12). According to data from the German nosocomial infection surveillance system (KISS), 16.3 % of all nosocomial ICU infections are due to S. aureus. This percentage has been relatively constant over the years, however, during the last 6 years the percentage of methicillinresistant S. aureus (MRSA) infections has increased dramatically - from 8% in 1997 to 30% in the first 6 months of 2003 (13). In our previous studies, antimicrobial activity studies on some Verbascum species determined the similar findings against the tested bacteria (6, 14-17). Verbascum L. species used in these studies showed antimicrobial activity against Gram-positive bacteria and no significant effect was observed against Gram-negative bacteria. In previous study, it is declared that verbascoside isolated from Verbascum L. species has shown to have antagonistic effect in Grampositive bacteria (18). As major components, saponins and iridoids could be responsible for the antagonistic effects as explained before (19-20). The results in present study are parallel to those reported in the mentioned investigations. In general, the distinctive feature of gram-negative bacteria is the presence of a double membrane surrounding each bacterial cell. Although all bacteria have an inner cell membrane, gram-negative bacteria have a unique outer membrane. This outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than are gram-positive bacteria.

CONCLUSION

The extract of *V. antinori* had a good potential for therapeutic uses against some Grampositive pathogens especially *S. aureus*. Hence, suggesting possible exploitation of these plants for their antimicrobial active principles for the development of novel herbal-based antimicrobials.

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