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Anti-Staphylococcal Activity of *Verbascum thapsus* L. against Methicillin-Resistant *Staphylococcus aureus*

ABSTRACT

Objective: In this study, it was aimed to investigate the anti-Staphylococcal activity of ethanol extract obtained from leaves of *Verbascum thapsus* L. (Scrophulariaceae) plant.

Methods: The anti-Staphylococcal activity of the extract of ethanol extracted from the leaves of *Verbascum thapsus* L. (Scrophulariaceae) was investigated against Methicillin-Resistant *Staphylococcus aureus* (MRSA) bacteria by Agar-well diffusion method and microdilution method.

Results: The ethanol extract showed antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) strains (MIC <1024 µg/mL). When compared with Methicillin and Gentamicin, the extract was more effective against MRSA strains.

Conclusion: The use of *Verbascum thapsus* L. (Scrophulariaceae) as an anti-Staphylococcal agent has been found to be appropriate in vitro and should be performed in vivo.

Keywords: *Verbascum thapsus* L., Agar-well diffusion method, microdilution method, Methicillin-resistant *Staphylococcus aureus*

Methicillin-Dirençli *Staphylococcus Aureus* Bakterilerine Karşı *Verbascum thapsus* L. Bitkisinin Anti-Staphylococcal Aktivitesi

ÖZET

Amaç: Bu çalışmada, *Verbascum thapsus* L. (Scrophulariaceae) bitkisinin yapraklarından elde edilen etanol ekstraktının anti-Staphylococcal aktivitesinin araştırılması amaçlanmıştır.

Yöntem: *Verbascum thapsus* L. (Scrophulariaceae) bitkisinin yapraklarından elde edilen etanol ekstraktının anti-Staphylococcal aktivitesi Methicillin-Dirençli *Staphylococcus aureus* (MRSA) bakterilerine karşı Agar-kuyu difüzyon metodu ve mikrodilüsyon metodu ile araştırılmıştır. **Bulgular:** Etanol ekstraktı Methicillin-Dirençli *Staphylococcus aureus* (MRSA) bakterilerine karşı (MIC <1024 µg/mL) antibakteriyel bir aktivite göstermiştir. Methicillin ve Gentamicin antibiyotikleri ile karşılaştırıldığında, ekstraktın MRSA suşlarına karşı oldukça etkili olduğu saptanmıştır.

Sonuç: *Verbascum thapsus* L. (Scrophulariaceae)'nin anti-Staphylococcal ajan olarak kullanılabilirliği in vitro olarak uygun bulunmuş ve in vivo çalışmaların yapılması gerektiği düşünülmüştür.

Anahtar Kelimeler: *Verbascum thapsus* L., Agar-kuyu difüzyon metodu, Mikrodilüsyon metodu, Methicillin-Dirençli *Staphylococcus aureus*

INTRODUCTION

Common mullein, also known as Woolly Mullein (*Verbascum thapsus* L., Scrophulariaceae) has been used as a medicinal herb since ancient times. The leaves and flowers are reported to have expectorant and demulcent features which are used to treat respiratory problems such as bronchitis, dry coughs, whooping cough, tuberculosis, asthma and hoarseness. The plant is reported to be mildly diuretic and to have a soothing and anti-inflammatory effect on the urinary tract, and to act as a mild sedative. It has also been used as a domestic remedy for pneumonia, fever, congestion, allergies, migraine, catarrhs and colic (1-3). During our routine field excursions, it was found that this plant is used to treat respiratory tract infections and externally boils and abscesses. Also, it has also been as traditional remedy to treat various ailments such as spasmodic, digestive disorders and menstrual problems. Therefore, the aim was to determine *V. thapsus* extracts that have been shown earlier to have biological activity against the urinary tract pathogens.

MATERIALS AND METHODS

Plant material: Leaves of the plant were collected from İkizdere, Rize, Turkey in September, 2011 and identified by Dr. Tulay Tutenocakli. A voucher specimen of the plant (voucher number GD70-4) was deposited in Department of Medical Biology of Duzce University in the author's personal collection.

Preparation of extracts: The leaves of the plant were dried in an oven at 40°C (12h) and powdered. Each dry powdered plant material (20 g) was extracted with 150 mL of 95% ethanol (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment (4). The extract was filtered using Whatman filter no.1, and the filtrate solvent was evaporated under vacuum using a rotary evaporator at 55°C (yield: 11.8% for ethanol). The resulting dried extract (in the sticky black substances) amounting to around 2 g was dissolved in 0.1 mL of DMSO (5 mg/mL) (dimethyl sulfoxide) before testing.

Strains: *Escherichia coli* (ATCC 11230 and ATCC 10536), *Pseudomonas aeruginosa* (ATCC 25619 and ATCC 9027), *Staphylococcus aureus* (ATCC 6538P and ATCC 25923) were used as positive controls. The clinical strains and methicillin-resistant *Staphylococcus aureus* (MRSA) were obtained from the research hospital at Canakkale Onsekiz Mart University, Canakkale, Turkey. All strains were stocked at room temperature on Brain Heart Infusion Agar slants (Oxoid), and prior to assay, the cells were grown overnight at 35-37°C in Brain Heart Infusion Broth (Oxoid).

Antimicrobial assay: The solid medium diffusion technique using agar wells was used for screening the extracts for antibacterial activity. 100 µL

of the bacterial suspension (approximately 10^5 cfu/mL) was uniformly spread on sterile Brain Heart Infusion Agar petri dishes, and 50 µL of extract (10 mg/mL) were added inside agar wells of 6 mm in diameter. The plates were incubated at 35-37°C for 24h. The data of antibacterial activity were used only when the growth inhibition zone had a diameter ≥ 10 mm. MICs were determined by microdilution method by adding 100 µL of each strain suspended in Brain Heart Infusion Broth (final concentration 10^5 cfu/mL) to a 96 well micro titer plate with wells containing 100 µL of two fold serial dilutions of extracts (5). The final concentrations of extract were 512 to 8 µg/mL. MIC was defined as the lowest concentration required for growth inhibition. The minimal bactericidal concentration (MBC) was determined by inoculating Brain Heart Infusion Agar (Oxoid) plates with sample from non-growth wells (6-7).

The MRSA strains 009-016 and 036 were assayed with methicillin and gentamicin (Sigma Co.) at final concentrations of 1024 to 1 µg/mL. All plates were incubated aerobically for 24h at 35-37°C. MBC was defined as the lowest concentration showing no growth. All antimicrobial assays were performed twice and the results were expressed as the average of two repetitions. The solutions of the antibiotics were prepared using the recommendations of Clinical and Laboratory Standard Institute – CLSI (8).

RESULTS AND DISCUSSION

Table 1 shows the inhibition zones generated by the extracts obtained from *V. thapsus* assayed against clinical isolates of *S. aureus* and bacteria used as positive controls. The extracts obtained from *V. thapsus* have shown a strong antibacterial activity against the ones used as positive controls with inhibition zones of 11.6-19.0 mm. Especially, *E. coli* is the most sensitive bacterium to the extracts (19.0 mm). The lowest effect has been shown against *P. aeruginosa* ATCC 9027 (11.6 mm). Notably, the extracts inhibited the growth of all MRSA strains with zones of 12.4-18.8 mm. Three strains showed inhibition zones with diameter ≥ 16.0 mm such as 009, 016 and 036. The smallest inhibition zones was found with the MRSA strain 001 (12.4 mm), while the largest one was found with the MRSA strain 036 (18.8 mm).

Table 2 shows the anti-Staphylococcal effect of the extract compared to the aminoglycoside Gentamicin and the β -lactam Methicillin. MIC and MBC values for the extracts were 512/1024 µg/mL, 1024/ ≥ 1024 µg/mL and $\geq 1024/\geq 1024$ µg/mL for the *S. aureus* strains 036, 016 and 099, respectively. The extracts were 1-2 times more effective in inhibiting *S. aureus* growth than these drugs against strain 036, but not against strain 009. The extracts against strain 016 were a little more effective than those of drugs.

Table 1. Origin, resistance profile of *Staphylococcus aureus* strains and inhibitory activity of *Verbascum thapsus* L.

Strain	Origin	PRP ^a	Inhibition (mm)
			<i>V. thapsus</i> extract
<i>E.coli</i> ATCC 11230	-	-	19.0
<i>E.coli</i> ATCC 10536	-	-	17.6
<i>P. aeruginosa</i> ATCC 25619	-	-	12.4
<i>P. aeruginosa</i> ATCC 9027	-	-	11.6
<i>S. aureus</i> ATCC 6538P	-	-	15.2
<i>S. aureus</i> ATCC 25923	-	-	14.4
MRSA 001	Surgical wound	1	12.4
MRSA 002	Surgical wound	2	14.0
MRSA 004	Abscess	3	14.4
MRSA 005	Abscess	3	12.8
MRSA 009	Surgical wound	2	17.6
MRSA 011	Surgical wound	4	15.2
MRSA 014	Abscess	2	14.6
MRSA 015	Windpipe secretion	3	14.4
MRSA 016	Surgical wound	3	18.0
MRSA 032	Abscess	1	14.2
MRSA 033	Abscess	1	13.4
MRSA 036	Surgical wound	2	18.8
MRSA 038	Surgical wound	3	13.8
MRSA 039	Surgical wound	4	15.4

^aPhenotypic Resistance Profile: PRP; 1: Oxacillin, Gentamicin, Tobramycin, Amikacin, Chloramphenicol, Rifamycin, Novobiocin; 2: Oxacillin, Gentamicin, Tobramycin, Amikacin, Kanamycin, Neomycin, Paromomycin, Butirocin, Sisomicin, Netilmicin; 3: Oxacillin, Penicillin, inductive Erythromycin, Kanamycin, Streptomycin, Gentamicin, Amikacin, Tobramycin, Minocycline, 4: Oxacillin, Penicillin, Constitutive Erythromycin, Kanamycin, Streptomycin, Gentamicin, Amikacin, Tobramycin, Minocycline.

Table 2. Comparative MICs and MBCs of ethanol extracts of *V. thapsus* and antibiotics against MRSA strains isolated from clinics ($\mu\text{g/mL}$)

Strain	Methicillin MIC/MBC	Gentamycin MIC/MBC	<i>V. thapsus</i> MIC/MBC
MRSA 009	$\geq 1024 / \geq 1024$	$\geq 1024 / \geq 1024$	$\geq 1024 / \geq 1024$
MRSA 016	$\geq 1024 / \geq 1024$	$\geq 1024 / \geq 1024$	$1024 / \geq 1024$
MRSA 036	$\geq 1024 / \geq 1024$	$\geq 1024 / \geq 1024$	512/1024

Some studies concerning the effectiveness of extraction methods highlight that ethanol extraction yields higher antimicrobial activity than the other solvents (9). According to present results, ethanol extract has stronger and broader spectrum of antibacterial activity. This information confirmed that the ethanol is a more effective solvent for extraction of antimicrobial substances in *V. thapsus*.

Verbascum L. species contain a wide range of compounds, such as glycosides, alkaloids, and saponins (10-14). Members of the family Scrophulariaceae have been reported to contain a group of unusual macrocyclic spermine alkaloids (15-16). Constituents of *V. thapsus* include polysaccharides; iridoid glycosides including harpagoside, harpagide and aucubin (especially in the leaf); flavonoids, including 3'-methylquercetin,

hesperidin and verbascoside; saponins and volatile oils (17-19). Flavonoids and saponins may be responsible for their antimicrobial activity. Activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Lipophilic flavonoids may also disrupt microbial membranes (20). It is reported that *V. thapsus* has antiviral activity against influenza in chicken embryos (21). In a previous study, leaf extracts of this plant have been shown to be active against bovine herpes virus type 1, and showed slight antibacterial and antifungal activity (22-24). Methanol extract has been shown to be effective against mosquito larvae (25). Khan et al assessed the biological activity of common mullein extracts and commercial mullein products using selected bench top bioassays, including

antibacterial, antitumor and two toxicity assays (brine shrimp and radish seed) and tested antibacterial activity with *Klebsiella pneumoniae*, *Staphylococcus aureus*, *S. epidermidis* and *Escherichia coli* and they found that aqueous extracts were the most effective ones (3). Only *K. pneumoniae* and *S. aureus* showed sensitivity to the mullein samples tested. The results indicated that *V. thapsus* possessed significant activity against bacteria. Our findings clearly show that *V. thapsus* has strong antibacterial effects against MRSA strains. The emergence of MRSA is one of the most serious issues in public health in developed countries. It does not only have a high prevalence (<1-80 %), but it

has also become resistant to almost the currently available antibiotics except teicoplanin and vancomycin (26). The rapid development of resistance to vancomycin, the last resort antibiotics against MRSA recently has been reported in several countries (27).

CONCLUSION

The extracts demonstrating especially antibacterial activity against Methicillin-resistant *Staphylococcus aureus* could result in the discovery of novel antibacterial agents, showing broad spectrum activities, this may help to discover new antibiotics that could serve as selective agents for infectious diseases.

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