

Full Length Research Paper

Antimicrobial activity of the leaves of *Verbascum sinuatum* L. on microorganisms isolated from urinary tract infection

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The ethanolic extracts obtained from the leaves of *Verbascum sinuatum* L. (Scrophulariaceae) were investigated for their antimicrobial activities against the pathogens causing complicated urine tract infection (*Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Candida albicans*) disk diffusion method and microdilution method. Some antibacterial and antifungal antibiotics were used as a positive reference standard to determine the sensitivity of the strains. The extracts showed strong antimicrobial activity against *Enterococcus faecalis*, *Proteus mirabilis* and *Candida albicans* with inhibition zones of 20.0, 18.0 and 20.0 mm, with MIC's and MBC's of 4.0 (8.0), 8.0 (16.0) and 8.0 (16.0) µg/mL, respectively. Also, the extracts exhibited moderate activity against the other test microorganisms. The results demonstrate that the ethanol extract of the aerial parts of *Verbascum sinuatum* L. has significant activity and suggest that it may be useful in the treatment of infections.

Key words: Urinary tract infection (UTI), antimicrobial activity, *Verbascum sinuatum*

INTRODUCTION

Urinary tract infections are a serious health problem affecting millions of people each year. A urinary tract infection (UTI) is very common infection that occurs when bacteria enter and multiply anywhere along the normally sterile urinary tract. UTI has a number of causes. Most are caused by bacteria normally present on the skin or in the intestinal tract that invade the urinary tract. Leading etiological agents of UTI's include *Escherichia coli*, *Candida albicans*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Svanborg and Godaly, 1997). Women are especially prone to UTIs for reasons that are not yet well understood. One woman in five develops a UTI during her lifetime. The incidence of acute uncomplicated UTI is estimated to exceed 0.5 episodes per annum among women between 18 - 20 years (Meyhoff et al., 1981; Farrell et al., 2003; Warren, 2005).

The leaves and flowers of *Verbascum* (Scrophulariaceae) reported to have expectorant, mucolytic and

demulcent properties, and are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis and asthma in traditional Turkish medicine. The species are also used to treat hemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea, and have inhibitory activities against the murine lymphocytic leukemia and influenza viruses A2 and B. The oil made from the flowers is used to help soothe earache and can be applied externally for eczema and other types of inflammatory skin conditions (Tatli and Akdemir, 2004). Therefore, the aim was to determine the antibacterial effects of the ethanolic extracts obtained from *Verbascum sinuatum* against the pathogen causing complicated urine tract infection.

MATERIALS AND METHODS

Plant material

Aerial parts of *V. sinuatum* L. were collected from Icel, Turkey during the months of September-October 2008. A voucher specimen (voucher specimen BD136) of the plant was deposited in the Biology Department at Canakkale Onsekiz Mart University,

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Preparation of extracts

The leaves of the plant were dried in an oven at 40°C for 12 h and powdered. Each dry powdered plant material (20 g) was extracted with 150 mL of 95% ethanol (Merck, Darmstadt, Germany) for 24 h using a Soxhlet extractor (Khan et al., 1988). The extract was filtered with Whatman filter paper no.1 and the filtrate was evaporated under vacuum in a rotary evaporator at 55°C. The extract yield obtained was 12.4%. The dry extract, which was sticky and black, was stored in labeled sterile screw-capped bottles at -20°C pending use. Prior to testing, 1 g was dissolved in 0.2 L of dimethyl sulfoxide (DMSO) (5 mg/mL).

Microorganisms

Urinary tract pathogens (*Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Candida albicans*) were isolated from the urine of patients diagnosed with urinary infections in Faculty of Medicine at Canakkale Onsekiz Mart University, Canakkale, Turkey and from Trakya University, Edirne, Turkey.

Disc diffusion method

The paper disc diffusion method was employed (Collins et al., 1989). Sterile 6 mm disc filter paper disc (Schleicher and Schul, No. 2668, Dassel, Germany) were impregnated with 50 µL of the plant extract. The bacterial cultures were inoculated on Nutrient Broth (Oxoid) and incubated for 24 h at 37 ± 0.1°C, while the yeast cultures were inoculated on Malt Extract Broth (Oxoid) and incubated for 48 h at 28.0 ± 0.1°C. Adequate amounts of Mueller Hinton Agar (Oxoid) were dispensed into sterile plates and allowed to solidify under aseptic conditions. The counts of bacterial and yeast cultures were adjusted to yield 10⁷ - 10⁸ and 10⁵ - 10⁶ mL⁻¹, respectively, using the standard McFarland counting method. The test microorganisms (0.1 mL) were inoculated with a sterile swab on the surface of appropriate solid medium in plates. The agar plates inoculated with the test microorganisms were incubated for 1 h before placing the extract impregnated paper disc on the plates. The bacterial plates were incubated at 37 ± 0.1°C for 24 h while yeast plates were incubated at 28 ± 0.1°C for 48 h. After incubation, all plates were observed for zones of growth inhibition and the diameter of these zones was measured in millimeters. All tests were performed under sterile conditions in duplicate and repeated three times. Penicillin (10 µg/disc), tobramycin discs (10 µg/disc), ampicillin (20 µg/disc), nystatin (30 µg/disc), clotrimazole (30 µg/disc) and ketoconazole (20 µg/disc) discs were used as positive controls.

Microdilution method

Determination of the minimum inhibitory concentration (MIC) was carried out according to the method described by Zgoda and Porter, with some modifications (Zgoda and Porter, 2001). A dilution series of the extract, ranging from 10 - 0.5 mg/mL, were prepared and then transferred to the broth in 96 well microtitre plates. The final concentrations were in the range 1000 to 50 µg/mL in the medium. Before inoculation of the test organisms, the bacterial and yeast strains were adjusted to 0.5 McFarland and diluted 1:1000 in Mueller Hinton Broth (Oxoid) and Malt Extract Broth (Oxoid), respectively. The plates were incubated at 35°C for 18 - 24 h for bacteria and 30°C for 48 h for the yeast cultures. All the tests were

performed in broth and repeated twice. While the MIC values of the extracts were defined as the lowest concentration that showed no growth, minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by plating samples from clear wells onto Mueller Hinton Agar and Malt Extract Agar, respectively. MBC and MFC were defined as the lowest concentration yielding negative subculture. Ampicillin and streptomycin were used as the standard antibacterial agents, while nystatin was used as the standard antifungal agent. Their dilutions ranged from 128.0 - 0.25 µg/mL concentrations in microtitre plates.

RESULTS AND DISCUSSION

The antimicrobial activities of *Verbascum sinuatum* L. extracts against the pathogens causing complicated urinary tract infections examined in this study were qualitatively and quantitatively assessed by the presence of inhibition zones, MIC, MBC and MFC (Table 1 and 2). The ethanolic extracts obtained from the leaves of *Verbascum sinuatum* L. were strong antimicrobial activities against the pathogens, with inhibition zones at 11.0 - 20.0 mm. *Enterococcus faecalis* and *Candida albicans* are more susceptible to the extract of *V. sinuatum* as compared to standard antibacterial antibiotics Penicillin, Ampicillin and Tobramycin, and antifungal antibiotic Nystatin (inhibition zone is 20.0 mm) respectively. Similarly, the extracts showed higher antibacterial activity on *Proteus mirabilis* than those of all standard antibacterial antibiotics. *Pseudomonas aeruginosa* is more susceptible and equal to the standard antibacterial agents.

The ethanol extracts were further tested by microdilution to determine the MICs and MBCs. The lowest MICs and MBCs or MFCs of the extract were 4.0 (8.0) µg/mL against *Enterococcus faecalis*, followed *Proteus mirabilis* and *Candida albicans* (MIC values is 8.0 (16.0) µg/mL). The extracts have weak antimicrobial effect against the other pathogens, with MICs and MBCs ranged from 500 (1000) - 250 (500) µg/mL. These values are far below than the standard antibiotics.

Based on the results, it is possible to conclude that ethanol extract has stronger and broader spectrum of antibacterial activity as compared to the others. This information confirmed the evidence in previous study reported that ethanol is a better solvent for extraction of antimicrobial substances from medicinal plants than water and methanol (Jonathan and Fasidi, 2003).

Verbascum L. species contain biologically active compounds such as flavanoids, phenyl ethanoid and neolignan glycosides saponins, and iridoid and monoterpene glycosides (Tatli and Akdemir, 2004). There are many investigations on antimicrobial activities of various *Verbascum* species. The methanol extracts obtained from endemic *V. gypsicola* Vural and Aydogdu, *V. pseudoholotrichum* Hub.-Mor., *V. cymigerum* Hub.-Mor., *V. cholorostegium* Bornm and Murb, *V. linguifolium* Hub.-Mor., *V. pellitum* Hub.-Mor., *V. dalamicum* Hub.-Mor., *V. chionophyllum* Hub.-Mor., *V. cilicium* Boiss, *V. trapifolium*

Table 1. Summary of antibacterial activity of *V. sinuatum* and some standard antibiotics.

Microorganisms	Plant Extracts (µg/mL)	Inhibition zones (mm) ^a					
		Standard antibiotics					
		P 10	AMP 20	TOB 10	NYS 30	KETO 20	CLT 30
<i>Enterococcus faecalis</i>	20.0	14.0	16.0	18.0	Nt	Nt	Nt
<i>Escherichia coli</i>	11.0	16.0	14.0	10.0	Nt	Nt	Nt
<i>Klebsiella pneumoniae</i>	12.0	18.0	14.0	15.0	Nt	Nt	Nt
<i>Pseudomonas aeruginosa</i>	12.0	8.0	10.0	12.0	Nt	Nt	Nt
<i>Proteus mirabilis</i>	18.0	13.0	16.0	14.0	Nt	Nt	Nt
<i>Candida albicans</i>	20.0	Nt	Nt	Nt	18.0	22.0	16.0

^aincludes diameter of disk (6 mm); mean value of three independent experiments; Nt = not tested; P = penicillin (10 µg/disc); TOB = tobramycin discs (10 µg/disc); AMP = ampicillin (20 µg/disc); NYS = nystatin discs (30 µg/disc); KETO = ketoconazole (20 µg/disc); CLT = clotrimazole (30 µg/disc).

Table 2. Minimum inhibitory concentration (MIC) of the extracts of *V. sinuatum*.

Microorganisms	MIC (MBC or MFC)			
	Extract (µg/mL)	Standards		
		ST	AMP	NYS
<i>Enterococcus faecalis</i>	4.0 (8.0)	2.0 (4.0)	1.0 (4.0)	Nt
<i>Escherichia coli</i>	500 (1000)	4.0 (4.0)	32 (64)	Nt
<i>Klebsiella pneumoniae</i>	250 (500)	8.0 (16.0)	8.0 (8.0)	Nt
<i>Pseudomonas aeruginosa</i>	250 (500)	1.0 (1.0)	16 (32)	Nt
<i>Proteus mirabilis</i>	8.0 (16.0)	4.0 (8.0)	0.5 (1.0)	Nt
<i>Candida albicans</i>	8.0 (16.0)	Nt	Nt	8.0 (16)

Nt: not tested; ST: Streptomycin, AMP: Ampicillin, NYS: Nystatin

(Stapf) Hub-Mor., *V. meinckeanum* Murb and *V. lyratifolium* Kochel were investigated for their antimicrobial activities. Antimicrobial activity was revealed against *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *B. cereus*, *M. smegmatis*, *L. monocytogenes*, *M. luteus*, *C. albicans*, *R. rubra* and *K. fragilis* by disk diffusion method. The *Verbascum* L. extracts had a strong antimicrobial activity against the Gram-positive bacteria and the yeast cultures used in these studies (Dulger and Gonuz, 2004; Dulger and Ugurlu, 2005; Dulger et al., 2005; Dulger, 2006). In another study, the methanolic extracts of the leaves, flowers, roots and seeds of *V. blattaria*, *V. bombyciferum*, *V. chaixii*, *V. dumulosum*, *V. nigrum*, *V. olympicum*, *V. phlomoides*, *V. phoeniceum* and *V. roripifolium* were studied for their antimicrobial activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans* (Meurer-Grimes et al., 1996). Senatore et al. (2007) was determined that antimicrobial activity of the methanolic extract of *V. sinuatum* showed inhibition against all the bacterial strains tested (MIC between 15.5 and 250 µg/mL). In that study, Gram-positive bacteria were most sensitive to the extract; among these, *S. epidermidis* showed the lowest MIC (15.5 µg/mL). The

Gram-negative bacteria were less sensitive; the extract showed an antibacterial activity (MIC 62 µg/mL) only against *P. vulgaris*, *P. mirabilis* and *C. diversus*. The results in this study are similar to those reported in the above studies. In general, Gram-negative bacteria have been found to be more resistant to extracts than Gram-positive bacteria, possibly because of their cell wall lipopolysaccharide (Farbood et al., 1976; Cetin and Gurler, 1989; Outtara et al., 1997).

In conclusion, the extracts demonstrating especially antibacterial activity against *Enterococcus faecalis* 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 against infectious diseases.

REFERENCES

- Cetin TE, Gurler N (1989). The experiment for antibiotic sensitivity of bacteria. J. Kukem. 12: 2-5.
 Collins CH, Lyne PM, Grange JM (1989). Microbiological Methods, 6th Ed. London, Butterworths, p. 410.
 Dulger B, Gonuz A (2004). Antimicrobial activity of some endemic

- Verbascum*, *Salvia* and *Stachys* species. Pharm. Biol. 42: 301-304.
- Dulger B, Ugurlu E (2005). Evaluation of antimicrobial activity of some endemic Scrophulariaceae members from Turkey. Pharm. Biol. 43: 275-279.
- Dulger B, Ugurlu E, Aki C, Suerdem TB, Camdeviren A, Tazeler G (2005). Evaluation of antimicrobial activity some endemic *Verbascum*, *Sideritis* and *Stachys* species from Turkey. Pharm. Biol. 43: 270-274.
- Dulger B (2006). Antimicrobial activity of some endemic Scrophulariaceae from Turkey. Pharm. Biol. 44: 672-676.
- Farbood MI, MacNeil JH, Ostovar K (1976). Effects of rosemary spice extractive on growth of microorganisms in meats. J. Milk Food Technol. 39: 675-679.
- Farrell DJ, Morrissey I, De Rubeis D, Robbins M, Felmingham D (2003). A UK multicentre study of the antimicrobial susceptibility of bacterial pathogens causing urinary tract infection. J. Infect. 46: 94-100.
- Jonathan SG, Fasidi IO (2003). Antimicrobial activities of two Nigerian edible macrofungi, *Lycoperdon pusillum* and *L. giganteum*. Afr. J. Biomed. Res. 6: 85-90.
- Khan NH, Nur-E Kamal MSA, Rahman M (1988). Antibacterial activity of *Euphorbia thymifolia* Linn. Indian J. Med. Res. 87: 395-397.
- Meurer-Grimes B, Macbeth DL, Hallihan B, Delph S (1996). Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae. Pharm. Biol. 34: 243-248.
- Meyhoff H, Nordling J, Gammelgaard P, Vejlsgaard R (1981). Does antibacterial ointment applied to urethral meatus in women prevent recurrent cystitis? Scand. J. Urol. Nephrol. 15(2): 81-83
- Outtara B, Simard RE, Holley RA, Pietta GJP, Begin A (1997). Antimicrobial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. 37: 155-162.
- Senatore F, Rigano D, Formisano C, Grassia A, Basile A, Sorbo S (2007). Phytogrowth-inhibitory and antibacterial activity of *Verbascum sinuatum*. Fitoterapia 78: 244-247.
- Svanborg C, Godaly G (1997). Bacterial virulence in urinary tract infection. Infect. Dis. Clin. N. Am. 11: 513-529.
- Tatli II, Akdemir ZS (2004). Chemical constituents of *Verbascum L.* species. FABAD J. Pharm. Sci. 29: 93-107.
- Warren JW, Mandell GL, Bennett JE, Dolin R (2005). Nosocomial urinary tract infections. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 6th ed. Philadelphia: Churchill Livingstone, pp. 3370-3381.
- Zgoda JR, Porter JR (2001). A convenient microdilution method for screening natural products against bacteria and fungi. Pharm. Biol. 39: 221-225.