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Antimicrobial activity of the seeds of *Hyoscyamus niger* L. (Henbane) on microorganisms isolated from urinary tract infections

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Abstract

Objective: The methanol extracts obtained from the seeds of *Hyoscyamus niger* L. (Solanaceae) were investigated for their antimicrobial activities against the pathogens causing complicated urine tract infections.

Methods: The seeds of plant were extracted with aqueous 60% methanol. The extract was screened against urinary tract pathogens (*Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Candida albicans*) by disc diffusion method and microdilution method. Some antibacterial and antifungal antibiotics were used as a positive reference standard to determine the sensitivity of the strains.

Results: The extracts showed strong antimicrobial activity against *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Candida albicans* with inhibition zones of 26.0, 19.0 and 16.0 mm, with MIC's and MBC's or MFC's of 4.0(8.0), 32(64) and 64(128) µg/mL, respectively. Also, the extracts exhibited moderate activity against the other test microorganisms.

Conclusion: Our findings support the use of *Hyoscyamus niger* L. in traditional medicine for the treatment against the urine tract pathogens.

Keywords: Urinary tract infection (UTI), antimicrobial activity, *Hyoscyamus niger* L.

1. Introduction

Plants produce a good deal of secondary metabolites which have benefited mankind in various ways including treatment of diseases [1]. These metabolites serve different purposes in the plant, including growth regulation, allelopath, defense against predators and infections or they may be waste products. Outside their intrinsic uses in the plant, these secondary metabolites have variously been shown to exhibit interesting biological and pharmacological activities and are important as prophylactics, chemotherapeutics or have served as the starting points in the development of modern medicines [2].

Hyoscyamus niger L. (Solanaceae), commonly known as Henbane, is widely distributed in Europe and Asia. The plant is said to possess anti-spasmodic, sedative and analgesic properties [3]. The narcotic alkaloids hyoscyamine, scopolamine, and atropine are derived from this foul smelling weed. Its name is derived from the Anglo-Saxon Henn (chicken) and Bana (murderer) because when fowls eat the seeds of this plant, they become paralyzed [4-6]. In our field trip, it is determined that the aqueous extracts obtained from the seeds of *H. niger* has been applied for spilling over the larvae from eye, so the name of plant is locally "shed-helmint". So, the aim of this work was to evaluate the antimicrobial activity of *H. niger* seeds that have been shown earlier to have biological activity against the pathogens causing complicated urine tract infections as wild-growing in Turkey.

2. Materials and Methods

2.1. Plant material

The plant seeds were obtained from a seller of medicinal herbs in Duzce, Turkey in June, 2014. The seeds were deposited in Department of Medical Biology of Duzce University in the author's personal collection (voucher number GD81-5).

2.2. Preparation of the extracts

The seeds of plant were extracted with aqueous 60% methanol. 10 g amounts of the seed materials were extracted in flasks placed in an ultrasonic bath first with 50 mL solvent for 60

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Min, then with 30 mL solvent for 45 min, and finally with 20 more mL solvent for 15 min, the overall extraction taking 120 min. The three extracts were combined, brought to a final volume of 100 mL with aqueous 60% methanol. The methanol was removed vacuum rotary at 40 °C until dryness. The extract yield obtained was 10.8%. The resulting dried extract was stored in labeled sterile screw-capped bottles at -20 °C. The extract (in the form of sticky black substances) was dissolved in 0.1 mL of DMSO (5 mg/g) (dimethyl sulfoxide) before testing.

2.3. 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.

2.4. Disc diffusion method

The paper disc diffusion method was employed [7]. Sterile 6 mm disc filter paper disc (Schleicher & 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 Hilton 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 CFU mL⁻¹ and 10⁵-10⁶ CFU 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.

2.5. Microdilution method

Determination of the minimum inhibitory concentration (MIC) was carried out according to the method described by Zgoda and Porter, with some modifications [8]. A dilution series of the extract, ranging from 10 to 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 to 0.25 µg/mL concentrations in microtitre plates.

3. Results and Discussion

The antimicrobial activities of *H. niger* 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 Table 2).

The methanol extracts obtained from the seeds of *H. niger* had strong antimicrobial activities against the pathogens, with inhibition zones of 9.0-26.0 mm. *E. faecalis* is more susceptible to the extract of *H. niger* as compared to all standard antibacterial antibiotics such as Penicillin, Ampicillin and Tobramycin (inhibition zone is 26.0 mm). *E. coli* is more resistant to the extracts. The extracts showed higher antibacterial activity on *K. pneumoniae* than those of all standard antibacterial antibiotics. *P. aeruginosa* and *P. mirabilis* are more susceptible and equal to the standard antibacterial agents. The yeast culture *C. albicans* is equal to the standard antifungal agent clotrimazole. The methanol 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 *E. faecalis*, followed *K. pneumoniae* and *C. albicans* (MIC values are 32 (64) and 64 (128) µg/mL, respectively). The extracts have weak antimicrobial effect against the other pathogens, with MICs and MBCs ranged from 1000 (1000)-500 (1000) µg/mL. These values are far below than the standard antibiotics. Especially, on *E. coli* which the extracts had no antibacterial activity.

Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than the other solvents [9-11]. According to present results, methanol extract has stronger and broader spectrum of antimicrobial activity. This information confirmed that the methanol has higher effective solvent for extraction of antimicrobial substances on *H. niger*.

There have been few studies on the antimicrobial activity studies on *H. niger*. In a previous study, antifungal activity of seeds of *H. niger* was investigated against some clinically relevant fungal pathogens such as *Candida* species (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13808, *C. guilliermondii* ATCC 6260, *C. krusei* ATCC 20298, *C. glabrata*

ATCC 2001 and *C. parapsilosis* ATCC 22019) and two *Cryptococcus* species (*C. neoformans* ATCC 90112 and *C. laurentii* ATCC 34142) by microbroth dilution method. Greater activity was shown against both *Cryptococcus* species, with MIC values of 15 µg/mL. In addition, the extracts displayed antifungal activity against the other test fungal cultures [12]. In this study, the extracts have more strong antifungal activity against *C. albicans* than those of the standard antifungal antibiotics. From this aspect, the findings obtained from this study are parallel to the above study. In another study, the methanol extract obtained from the seeds of *H. niger* L. was investigated for its antibacterial activity against *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 7064, *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 10538, *Proteus vulgaris* ATCC 6899, *Salmonella typhimurium* CCM 5445 and *Pseudomonas aeruginosa* ATCC 27853 by disc diffusion and microdilution method. The extracts showed strong antibacterial activity against *S. aureus*, with inhibition zones of 25.0 mm and with minimum

inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of 16 (32) µg/mL, respectively. Also, the extracts exhibited moderate activity on the other test bacteria but there was weak activity against *E. coli* with inhibition zone at 9.0 mm and no activity had shown in microdilution method [13]. In this study, the extracts against *E. coli* have shown the same effects. So, it is communicable that the *H. niger* extracts is impotent against *E. coli*.

Phytochemical analyses of *H. niger* have confirmed the occurrence of alkaloids [14], tyramine derivative [15], withanolides [15], lignanamides [16] and flavonoids [17]. Flavonoids may be responsible for their antibacterial activity [18]. This activity may be indicative of the presence of metabolic toxins or the mentioned plant compounds.

4. Conclusion

H. niger has a strong antibacterial effect against especially *E.*

faecalis and *K. pneumoniae*. Isolation of enterococci resistant to multiple antibiotics has become increasingly common in the hospital setting [19]. According to National Nosocomial Infections Surveillance (NNIS) data from January 2003 through December 2003, more than 28% of enterococcal isolates in ICUs of the more than 300 participating hospitals were vancomycin-resistant. Clonal spread is the dominant factor in the dissemination of multidrug-resistant enterococci in North America and Europe [20]. *K. pneumoniae* is an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma. New antibiotic resistant strains of *K. pneumoniae* are appearing and it is increasingly found as a nosocomial infections [21]. The result indicated that *H. niger* possessed significant activity against both bacteria. So, this plant extracts should be analyzed further, as it might provide a new compound effective against pathogens.

Table 1: Summary of antimicrobial activity of *H. niger* 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>	26.2	14.0	16.0	18.0	Nt	Nt	Nt
<i>Escherichia coli</i>	9.6	16.0	14.0	10.0	Nt	Nt	Nt
<i>Klebsiella pneumoniae</i>	19.2	18.0	14.0	15.0	Nt	Nt	Nt
<i>Pseudomonas aeruginosa</i>	11.2	8.0	10.0	12.0	Nt	Nt	Nt
<i>Proteus mirabilis</i>	14.4	13.0	16.0	14.0	Nt	Nt	Nt
<i>Candida albicans</i>	16.2	Nt	Nt	Nt	18.0	22.0	16.0

A includes 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 *H. niger*

Microorganisms	Extract (µg/mL)	MIC (MBC or MFC)		
		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>	-	4.0 (4.0)	32 (64)	Nt
<i>Klebsiella pneumoniae</i>	32 (64)	8.0 (16.0)	8.0 (8.0)	Nt
<i>Pseudomonas aeruginosa</i>	1000 (1000)	1.0 (1.0)	16 (32)	Nt
<i>Proteus mirabilis</i>	500 (1000)	4.0 (8.0)	0.5 (1.0)	Nt
<i>Candida albicans</i>	64 (128)	Nt	Nt	8.0 (16)

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

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