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Antibacterial Activity of the Seeds of *Hyoscyamus niger* L. (Henbane)

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The methanolic extract obtained from the seeds of *Hyoscyamus niger* L. (Solanaceae) 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 *Staphylococcus 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 the other test bacteria. The results demonstrate that the methanolic extract of the seeds of *H. niger* has significant activity and suggest that it may be useful in the treatment of infections.

Key Words: *Hyoscyamus niger*, Henbane, Antibacterial activity.

INTRODUCTION

Medicinal plants have been used for a wide variety of purposes for many thousand of years in Turkey. In particular, extracts and oils of these plants have formed the basis of many applications, including raw and processed food preservation, pharmaceutical, alternative medicine and natural therapies. Although medicinal plant potential in Turkey is quite large, ethnobotanical and pharmaceutical studies on these plants are inadequate.

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[1]. 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[2-4].

A bibliographical survey showed that there were no reports on antimicrobial activity of this plant. So, the aim of this work is to evaluate the antibacterial activity of *H. niger* as wild-growing in Turkey.

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EXPERIMENTAL

The plant materials were collected from Kayseri, Turkey in September, 2009. Voucher specimens of the plant were deposited in the Biology Department at Canakkale Onsekiz Mart University.

Preparation of the extracts: The seeds of plant were extracted with aqueous 60 % methanol. 10 g of the seed materials were extracted in flasks placed in an ultrasonic bath first with 50 mL solvent for 1 h, then with 30 mL solvent for 45 min and finally with 20 more mL solvent for 15 min, the overall extraction taking 2 h. All the three extracts were combined, brought to a final volume of 100 mL with 60 % aqueous methanol. The methanol was removed vacuum rotary at 40 ºC until dryness. 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) before testing.

Test microorganisms: *in vitro* Antibacterial studies were carried out against the bacterial strains such as *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 obtained from the Microbiology Research Laboratory in Canakkale Onsekiz Mart University, Department of Biology, Turkey.

Disc diffusion method: The paper disc diffusion method was employed[5]. Sterile 6 mm filter paper discs (Schleicher & Schul, No. 2667, Dassel, Germany) were impregnated with 50 µL of the plant extracts, separately. The bacterial cultures were inoculated on nutrient broth (Oxoid) and incubated for 24 h at 37 ± 0.1 °C. Adequate amounts of Mueller Hinton Agar (Oxoid) were dispensed into sterile plates and allowed to solidity under aseptic conditions. The counts of bacterial cultures were adjusted to yield *ca.* $1.0 \times 10^7 - 1.0 \times 10^8$ mL^{-1} using the standard McFarland counting method. The test microorganisms (0.1 mL) were incubated with a sterile swab on the surface of appropriate solid medium in plates.

The agar plates inoculated with the test bacteria were incubated for 1 h before placing the extract impregnated paper discs on the plates. The bacterial plates were incubated at 37 ± 0.1 °C for 24 h. After incubation, all plates were observed for zones of growth inhibition and the parameters of these zones were measured in millimeters. All tests were performed under sterile conditions in duplicate and repeated three times. Penicillin (10 µg/disc), tobramycin (10 µg/disc) and ampicillin (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[6]. Dilution series of the extracts were prepared from 10 to 0.5 mg/mL in the test tubes then transferred to the broth in 96 well microtiter plates. Final concentrations were 1000 to 50 µg/mL in the medium. Before inoculation of the test microorganisms, the bacterial strains were adjusted to 0.5 McFarland and diluted 1:1000 in mueller hinton agar (Oxoid). Plates were incubated at 37 ± 0.1 °C

for 24 h. All the tests were performed in broth and repeated twice. Whereas the MIC values of the extracts were defined as the lowest concentration that showed no growth, minimum bactericidal concentration (MBC) was determined by plotting samples from clear wells onto Mueller Hinton Agar. Minimum bactericidal concentration (MBC) was defined as the lowest concentration yielding negative subcultures. Ampicillin and streptomycin were used as a standard antifungal agent. Their dilutions were prepared from 128 to 0.25 µg/mL concentration in microtiter plates.

RESULTS AND DISCUSSION

The antibacterial activity of *H. niger* extracts against the test bacteria examined in this study were qualitatively and quantitatively assessed by the presence of inhibition zones, MIC and MBC (Tables 1 and 2). The methanol extracts of *H. niger* were strong antibacterial effects against the tested bacterial strains, with inhibition zones at 9.0 to 25.0 mm. Notably, *Staphylococcus aureus* is more susceptible to the extract of *H. niger* as compared to standard antibacterial antibiotics ampicillin and tobramycin (inhibition zone is 25.0 mm).

TABLE-1
ANTIBACTERIAL ACTIVITY OF *H. niger* AND SOME STANDARD ANTIBIOTICS

Microorganisms	Inhibition zones (mm)*			
	Plant extract (µg/mL)	Standard antibiotics		
		Penicillin (10 µg/disc)	Ampicillin (20 µg/disc)	Tobramycin discs (10 µg/disc)
Bacillus cereus	15.0	14.0	12.0	24.0
Bacillus subtilis	16.0	13.0	16.0	18.0
Escherichia coli	9.0	16.0	14.0	10.0
Staphylococcus aureus	25.0	23.0	16.0	8.0
Pseudomonas aeruginosa	11.0	8.0	10.0	12.0
Proteus vulgaris	12.0	10.0	16.0	13.0
Salmonella typhimurium	10.0	13.0	13.0	10.0

*Includes diameter of disk (6 mm); mean value of three independent experiments.

TABLE-2
MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE EXTRACTS OF *H. niger*

Microorganisms	MIC (MBC)		
	Extract (µg/mL)	Standards	
		Streptomycin	Ampicillin
Bacillus cereus	250 (500)	0.5 (0.5)	0.5 (2.0)
Bacillus subtilis	64 (> 128)	4.0 (4.0)	8.0 (8.0)
Escherichia coli	-	4.0 (4.0)	64 (128)
Staphylococcus aureus	16 (32)	2.0 (4.0)	< 0.25 (0.35)
Pseudomonas aeruginosa	1000 (1000)	1.0 (1.0)	16 (32)
Proteus vulgaris	500 (1000)	8.0 (32)	0.5 (0.5)
Salmonella typhimurium	1000 (1000)	16 (32)	1.0 (4.0)

Similarly, the extracts showed higher antibacterial activity on both *Bacillus* species and *Pseudomonas aeruginosa* than those of penicillin and tobramycin antibiotics. However, *Escherichia coli, Proteus vulgaris* and *Salmonella typhimurium* are resistant to the extract, as compared to standard antibacterial antibiotics.

The methanolic extracts were further tested by microdilution to determine the MICs and MBCs. No antibacterial activity was seen against *E. coli*. The lowest MICs and MBCs of the extracts were 16 (32) µg/mL against *Staphylococcus aureus*, followed by *Bacillus subtilis* 64 (> 128) µg/mL. The extracts have a weak antibacterial activity against the other bacterial strains with MICs and MBCs ranged from 250 (500) to 1000 (1000) µg/mL. These values are far below than the standard antibacterial antibiotics.

Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than the other solvents[7]. 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 in *H. niger*.

There are no reports on the antimicrobial activity studies on *H. niger*. However, phytochemical analyses of *H. niger* have confirmed the occurrence of alkaloids[8], tyramine derivative[9], withanolides[9], lignanamides[10] and flavonoids[11]. Flavonoids may be responsible for their antibacterial activity[12]. The result indicated that *H. niger* possessed significant activity against bacteria. This activity may be indicative of the presence of metabolic toxins or the mentioned plant compounds. So, this plant extracts should be analyzed further, as it might provide a new compound effective against pathogens.

In conclusion, present results clearly show that *H. niger* has a strong antibacterial effect against *Staphylococcus aureus. Staphylococcus aureus* is one of the major causes of the both community and hospital-acquired infections[13]. It provides numerous toxins, including super-antigens and staphylococcal scarlet fever[14]. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most serious issues in public health in developed countries. It does not only a high prevalence (< 1-80 %), but it has also became resistant to almost all the currently available antibiotics except teicoplanin and vancomycin[15]. The rapid development of resistance to vancomycin, the last resort antibiotics against MRSA, recently has been reported in several countries[16,17]. *H. niger* may be useful for therapy of staphylococcal diseases. The extracts of *H. niger* may provide a candidate for a potential antibacterial agent against especially methicillin-resistant *Staphylococcus aureus*.

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