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Full Length Research Paper

Antimicrobial activities of the whole plant of *Cestrum nocturnum* against pathogenic microorganisms

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The crude methanol extract of the whole plant of *Cestrum nocturnum* L. (Solanaceae) and its subsequent fractions were tested against various bacterial and fungal strains. With the exception of *Salmonella typhi,* the tested samples showed marked antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aurous, Bacillus subtilis, Escherichia coli* and *Shigella flexenari*. The minimum inhibitory concentrations (MICs) ranged from 19 to 280 µg/ml. The crude extract and fractions were also susceptible to *Candida* species and *Microsporium canis*. The minimum inhibitory concentrations (MICs) for various fungi ranged from 170 to 290 µg/ml. In phytochemical analysis, the crude form and fractions of plant showed the presence of various phytochemical chemical groups like glycosides, alkaloids, saponins, phenols, flavonoids, sterols and tannins. Therefore, the current findings can be attributed to these groups.

Key words: *Cestrum nocturnum* L., antibacterial, antifungal, phytochemicals.

INTRODUCTION

The genus *Cestrum* contains more than 300 species, and most of them are native to warm subtropical and tropical areas of America. *Cestrum nocturnum* L. (Solanaceae), commonly known as night-blooming Jessamine, is an evergreen shrub with glossy, smooth, simple leaves; vine-like stems; and greenish, creamy white tubular flowers. The flower's volatile compounds were identified as phenylacetylaldehyde and linalool (Li et al., 1988). The leaves of *C. nocturnum* have pharmacological significance in Chinese folk medicine and have been used for the treatment of burns and swellings (Xiao, 1989). The leaves of the plant have shown significant analgesic and bactericidal activity (Huang et al., 2006; Chatterjee and Bhattacharjee, 2007). Local anesthetic effect, inhibitory effect on central nerve system and cardiac arrhythmic effect of plant is also documented (Zeng et al., 2002; Zeng et al., 2003; Zeng et al., 2003).

Mature leaf holds a calcinogenic glycoside that escorts to vitamin D toxicity and is accountable for elevated serum calcium level (Mello, 2003). Some of glycosides such as (25*R*)-spirost-5-ene-2R,3,-diol pentaglycosides (nocturnoside A) (Ahmad et al., 1991), (25*R*)-spirost-5-en-3,-ol tetraglycoside (nocturnoside B) (Ahmad et al., 1995) and phenolic glucosides (cesternosides A and B) (Sahai et al., 1994), two new flavonol glycosides and seven steroidal saponins including four new ones (Mimaki et al., 2001), and eight new steroidal glycosides have been isolated from the leaves of *C. nocturnum* (Mimaki et al., 2002). In the present study, we investigated the antibacterial and antifungal activities of the whole plant of *C. nocturnum*.

MATERIALS AND METHODS

Plant material

C. nocturnum L. (Solanaceae), as a whole plant was collected from

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Swat, Khyber Pukhtoonkhawa (Pakistan) during the month of June 2007. Authentication of the plant material was done by the Botany department of the PCSIR Laborites Peshawar.

Extraction

The shade dried plant material was chopped into small pieces and finally pulverized into fine powder. The powdered plant material (8.5 Kg) was soaked in methanol with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) using rotary evaporator (Khan et al., 2009). A crude extract (877 g) was obtained.

Fractionation

The crude methanol extract (877 g) was suspended in distilled water (500 ml) and sequentially partitioned with *n*-hexane (3 x 500 ml), chloroform (3 x 500 ml), ethyl acetate (3 x 500 ml) and *n*-butanol (3 x 500 ml) to yield the *n*- hexane (83 g), chloroform (154 g), ethyl acetate (210g), *n*-butanol (203 g) and aqueous (110 g) fractions, respectively.

Determination of antimicrobial activity

Antibacterial assay

The crude extract and its various fractions in the concentration of 3 mg/ml were screened against various human pathogens by agar well diffusion method (Khan et al., 2008). In this method, 10 ml aliquots of nutrients broth (Sigma-Aldrich, Germany) was inoculated with the test organism and incubated at 37°C for 24 h. Using a sterile pipette, 0.6 ml of the broth culture of the test organism was added to 60 ml of molten agar, mixed well and poured into a sterile Petri dish (for the 9 cm Petri dish, 0.2 ml of the culture was added to 20 ml of agar). Duplicate plates of each organism were prepared. The agar was allowed to set and harden and the required number of wells was dug in the medium with the help of a sterile metallic cork borer. Agar plugs were removed. Stock solutions of the test samples in the concentration of 1 mg/ml were prepared in the sterile dimethyl sulfoxide (DMSO) and 100 and 200 µl of each dilution was added in their respective wells. Control well received only 100 and 200 µl of DMSO. 'Imipinem' was used as standard drug (10 µg/ml). The plates were left at room temperature for 2 h to allow diffusion of the samples and then incubated in an upward direction at 37°C for 24 h. The diameter of the zones of inhibition was measured to the nearest mm (the well size was also noted).

Antifungal assay

Antifungal activity of the crude extract and various fractions were evaluated by agar tube dilution method (Atta-ur-Rehman et al., 1991). The samples in the concentrations of 24 mg/ml were dissolved in the sterile (autoclaved) dimethyl sulfoxide (DMSO, Merck), which served as stock solution. Sabouraud dextrose agar (SDA, Sigma–Aldrich, Germany) was prepared by mixing 32.5 g sabouraud, 4% glucose agar and 4.0 g of agar-agar in 500 ml distilled water. It was then stirred with a magnetic stirrer to dissolve it. Then 4 ml amount was dispensed into screw cap tubes, which were autoclaved at 120°C for 15 min and then cooled to 15°C. The non-solidified SDA media was poisoned with stock solution (66.6 µl) giving the final concentration of 400 µg of the extract per mL of SDA. Tubes were then allowed to solidify in the slanted position at room temperature. Each tube was inoculated with a piece (4 mm diameter) of inoculums removed from a seven days old culture of fungi for non-mycelial growth; an agar surface streak was employed. Other media supplemented with dimethyl sulfoxide (DMSO) and reference antifungal drugs served as negative and positive control respectively. Inhibition of fungal growth was observed after 7-days of incubation at 28 ± 1°C. Humidity (40 to 50%) was controlled by placing an open pan of water in the incubator. After incubating for 7-days, the test tubes were analyzed for the visible growth of the microorganisms.

Minimum inhibitory concentration determination (macrodilution method)

To determine the MIC, extracts (10 mg/ml) were dissolved in DMSO and serially diluted with sterile water in micro-plates in a laminar flow cabinet. The same volume of an actively growing culture of the tested pathogen was added to the different wells and cultures were grown overnight in 100% relative humidity at 37°C. The following morning, all the wells were supplemented with tetrazolium violet. Growth was indicated by a violet color of the culture. MIC was rated by the lowest concentration of the test solution that inhibited growth. The negative control acetone had no influence on the growth at the highest concentration used (Atta-ur-Rehman et al., 1991). Imipenem, Amphotericin-B and Miconazole were used as controls for comparison

Microorganisms

The bacterial strains used in the test were *E. coli* ATCC 25922*, Bacillus subtilis* ATCC 6633*, Shigella flexeneri (clinical isolate), Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 *and Salmonella typhi* ATCC 19430. The reference fungal strains were *Trichophyton longifusus (clinical isolate), C. albicans* ATCC 2091*, Aspergillus flavus* ATCC 32611*, Microsporium canis* ATCC 11622*, Fusarium solani* 11712 *and Candida glaberata* ATCC 90030.

Phytochemical tests

Preliminary phytochemical tests were performed for the detection of various pharmacologically active chemical groups like glycosides, alkaloids, saponins, phenols, flavonoids, sterols and tannins (Nisar et al., 2009).

RESULTS

Effect of antibacterial activity

The methanol extract of the whole plant of *C. nocturnum* and its solvent fractions were subjected to antibacterial in agar diffusion method. With the exception of *S. typhi,* the tested samples showed marked antibacterial activity against *P. aeruginosa, S. aureus, B. subtilis, E. coli* and *S. flexeneri* as shown in Tables 1 and 2. The minimum inhibitory concentrations (MICs) were in the range of 19 to 280 µg/ml. Imepenem was used as standard antibacterial drug.

Effect of antifungal activity

The antifungal activity of the methanol extract and

Table 1. Antibacterial activity of crude extract and fractions of *Cestrum nocturnum* L.

Name of bacteria	Zones of inhibition of bacterial growth (mm)						
	Std	CN-1	CN-2	CN-3	CN-4	CN-5	CN-6
E. coli	24	13	10	-	14	13	9
B. subtilis	23	12	-	13	13	10	12
S. flexeneri	28	8	12	-	-	14	-
S. aureus	27	15	-	16	13	14	15
P. aeruginosa	20	17	9	13	11	11	12
S. typhi	26	-	-	-	-	-	-

Standard drug: Imepenem, control= DMSO, CN-1 =Crude extract; CN-2= *n*-hexane fraction; CN-3 = Chloroform fraction; CN-4 = Ethyl acetate fraction; CN-5 =*n*-Butanol fraction and CN-6 = Aqueous fraction. Activity is represented in zones of inhibition of bacterial growth (in mm).

Table 2. Minimum inhibitory concentration (µg/ml) in the antibacterial activity of crude extract and the fractions of *Cestrum nocturnum* L.

Name of Bacteria	MIC (µg/ml)						
	Std	CN-1	CN-2	CN-3	CN-4	CN-5	CN-6
E. coli	0.19	84	195	-	95	175	280
B. subtilis	0.22	75	-	75	54	67	65
S. flexeneri	0.13	177	220	-	-	180	-
S. aureus	0.17	36	-	22	24	24	55
P. aeruginosa	0.31	31	200	19	21	75	62
S. typhi	0.17	-	-	-	-	-	-

Standard drug: Imepenem, CN-1 =Crude extract; CN-2= *n*-hexane fraction; CN-3 = Chloroform fraction; CN-4 = Ethyl acetate fraction; CN-5 =*n*-Butanol fraction and CN-6 = Aqueous fraction.

Table 3 Antifungal activity of the crude extract and fractions of *Cestrum nocturnum* L. represented in % inhibition of fungal growth.

Name of Fungi	% Inhibition of fungal growth by various samples						
	Std	CN-1	CN-2	CN-3	CN-4	CN-5	CN-6
T. longifusus	100[1]	-	-	-	-	-	-
C. albicans	100[1]	40	-	60	-	65	65
A. flavus	100[2]	-	-	-	-	-	-
M. canis	100[1]	50	-	-	40	30	50
F. solani	100[1]	-	-	-	-	-	-
C. glaberata	100[1]	30	-	-	60	60	-

[1]Standard drug = Miconazole, [2]Standandard Drug = Amphotericin B, Control= DMSO, CN-1 =Crude extract; CN-2= *n*-hexane fraction; CN-3 = Chloroform fraction; CN-4 = Ethyl acetate fraction; CN-5 =*n*-Butanol fraction and CN-6 = Aqueous fraction.

subsequent fractions of the plant are presented in Tables 3 and 4. The crude extract and fractions demonstrated some activity only against the *Candida* species and *M. canis*. The minimum inhibitory concentrations (MICs) for various fungi ranged from 170 to 290 µg/ml. Miconazole and Amphotericin-B were used as standard antifungal drugs.

DISCUSSION

Ethnobotanicals to treat diseases stand for century's old healing modality, practiced in almost all cultures of the world (Khan et al., 2010; Saeed et al., 2010). Numerous plants and secondary metabolites isolated from them have been reported with antimicrobial properties (Ali and Qasir, 2009; Qadrie et al., 2009; Nisar et al., 2010). In the present work we have investigated the antimicrobial spectrum of whole plant of *C. nocturnum* against various pathogenic bacteria. Our results clearly indicate the great potential of the whole plant against a variety of bacteria used in the study. The extensive use of antibiotic in the therapeutic management of infectious diseases causes

Table 4. Antifungal activity of crude extract and the fractions of *Cestrum nocturnum* L represented in minimum inhibitory concentration (µg/ml).

Name of fungus	Minimum inhibitory concentration (µg/ml)						
	Std	CN-1	CN-2	CN-3	CN-4	CN-5	CN-6
T. longifusus	1.4[1]	-	-	-	-	-	-
C. albicans	1.8[1]	210	-	190	-	175	170
A. flavus	2.3[2]	-	-	-	-	-	-
M. canis	1.6[1]	230	-	-	290	250	250
F. solani	1.1[1]	-	-	-	-	-	-
C. glaberata	0.5[1]	290	-	-	190	190	-

[1]Standandard Drug = Miconazole, [2]Standandard Drug = Amphotericin-B CN-1 = Crude extract; CN-2 = *n*-hexane fraction; CN-3 = Chloroform fraction; CN-4 = Ethyl acetate fraction; CN-5 = *n*-Butanol fraction and CN-6 = Aqueous fraction.

Table 5. Preliminary phytochemical tests of the crude extract and subsequent solvent fractions of the whole plant of *Cestrum nocturnum* L.

Test materials	Different chemical groups						
	Glycoside	Alkaloids	Saponins	Phenols	Sterols	flavonoids	Tannins
Crude	+	+	+	+	+	+	+
n-Hexane	+	–	–	–	+	–	–
Chloroform	+	+	+	–	–	+	–
EtOAc	+	+	+	+	–	+	+
n-Butanol	+	–	+	+	+	–	–
Aqueous	+	–	–	+	–	+	+

bacterial resistance especially to *S. aureus* (Timothy and Whitman, 2008). Different fractions of the plant expressed significant antibacterial activity against Gram-positive bacteria and the MICs for *B. subtilis* and *S. aureus* were measured from 54 to 75 and 22 to 55 µg/ml, respectively. Multidrug resistant bacteria have limited the efficacy of current clinical oral antibiotic against infections caused by the Gram-negative bacteria (Abbanat et al., 2008). Promising antibacterial activity was shown by the plant fractions tested against various Gran-negative bacteria including *P. aeruginosa, S. flexeneri* and *E. coli*. Therefore, it covers a wide range of infections and offers an alternative oral therapeutic option for the managements of infections caused by these bacteria.

Similarly, the crude extract and fraction of the plant showed sensitivity against tested fungus. *Candida* species are the major player in nosocomial bloodstream infections (Klotz et al., 2007). The plant showed notable sensitivity against *Candida* species. The crude methanol extract and subsequent fractions of the plant indicated the accumulation of glycosides, alkaloids, saponins, phenols, flavonoids, sterols and tannins. Thus our finding in the study can be attributed to the presence of these chemical groups. Further purification and characterization of the active principle(s) from the plant will provide better understanding of these activities.

The results of our study revealed outstanding antimicrobial activity against various pathogens responsible for wide variety of infections. Dramatic global increased in the morbidity and mortality of the infectious diseases is predominantly due to the prevalence of multidrug resistant pathogens. New safe and effective therapies are urgently required to address the current issue of antibiotic efficacy altered by resistance. As a striking feature of this study, the crude extract of the whole plant of *C. nocturnum* and its subsequent solvent fractions offered significant antimicrobial activities. As a natural character, it can be considered for low risk of resistance development. Moreover, this study can be used as a tool for bioactivity guided isolation of pure antimicrobial from the plant.

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