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IN VITRO ANTIBACTERIAL PROPERTIES OF ORGANIC EXTRACTS OF *Cestrum nocturnum* L.M.A. KHATUN¹, S.M. NUR ALAM^{*1} AND M.N. HASAN²

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ABSTRACT

Khatun MA, Nur Alam SM, Hasan MN (2013) *In vitro* antibacterial properties of organic extracts of *Cestrum nocturnum* L. J. Innov. Dev. Strategy. 7(3), 7-9.

The aim of this study was to test the efficacy of various organic extracts (hexane, chloroform, ethyl acetate and methanol) of *Cestrum nocturnum* L. as a potential antibacterial agent against some representative food borne pathogenic and spoilage bacteria. The extracts revealed remarkable antibacterial activity against *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19166, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* KCTC 2004, *Salmonella typhimurium* KCTC 2515 and *Escherichia coli* ATCC 8739. The zones of inhibition of different concentrations of essential oil against the tested bacteria were found in the range of 17.5 ± 1.3 to 9.2 ± 0.6 mm and the MIC values recorded between 62.5 and 1000 $\mu\text{g/ml}$. The results obtained in this study suggest that the *C. nocturnum* may have potential use in food, pharmaceuticals and agro industries for preservatives or antimicrobial agents.

Key words: *Cestrum nocturnum* L.; organic extracts; antibacterial activity; food borne pathogen

INTRODUCTION

A variety of different chemical and synthetic compounds have been used as antimicrobial agent to inhibit pathogenic bacteria. The demands for more natural antimicrobials have driven scientists to investigate the effectiveness of inhibitory compounds such as extracts from plants (Zeng *et al.* 2011). *Cestrum nocturnum* L. is an evergreen shrub from the family Solanaceae (Kishore *et al.* 2012). Common names include night blooming jasmine, lady of the night and queen of the night, due to its special sweet fragrance at night. It is widely naturalized in tropical and subtropical regions throughout the world, including Australia, Southern China and Southernmost United states. It is also cultivated in Bangladesh in home yards and gardens (Odenwald *et al.* 1996). Practitioners use the plant externally for skin disorders, but several scientific reports demonstrate that it exhibits a wide spectrum of pharmacological activity when administered systemically or in isolated organ preparations (Reza *et al.* 2010). Most of the species of *Cestrum* have found several applications in folk medicine. *Cestrum parqui* is used in Chilean folk medicine as antifebrile and for the treatment of fever and inflammation (Patil *et al.* 2011). Chinese people use leaves of *C. nocturnum* for their pharmacological significance in burns and swellings. It is also used for treating epilepsy and as stupefying charm medicine in West Indian Islands. The volatile oil of the species is known to be mosquito repellent and hence *C. nocturnum* and *C. diurnum* are used to prevent malaria in several African Nations (Reza *et al.* 2010). Leaves of *C. auriculatum* are used in Peru and Santa externally to reduce wound inflammations and kill domestic fleas. It is also used for its antimicrobial, anti-infective and anti-inflammatory properties. *Cestrum parvifolium* is also reported to be used traditionally for fever, ulcers and skin disorders (Moreno-Murillo *et al.* 2001).

In this study, we tested the antibacterial efficacy of various organic extracts (hexane, chloroform, ethyl acetate and methanol) of *C. nocturnum* with emphasis for the possible future use of the extracts as an alternative to chemical bactericides.

MATERIALS AND METHODS**Plant materials**

The flowers of *C. nocturnum* were collected from Islamic University Campus, Kushtia and Chowgacha, Jessore of Bangladesh. The flowers were cleaned and then dried under shade, when the plant was properly dried they were pulverized into a coarse powder by grinding mill.

Preparation of organic extracts

The powdered flower parts (50g) of *C. nocturnum* were extracted with hexane, chloroform, ethyl acetate and methanol separately at room temperature for 7 days and the solvents were evaporated by vacuum rotary evaporator at 40°C. The extraction process yielded in hexane (7.3g), chloroform (6.3g), ethyl acetate (5.2g) and methanol (6.1g) extracts. Solvents (analytical grade) for extraction were obtained from commercial sources.

Microorganisms

The following bacterial strains were used in the antimicrobial test: *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19166, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* KCTC 2004, *Salmonella typhimurium* KCTC 2515 and *Escherichia coli* ATCC 8739.

Determination of antibacterial activity of organic extracts

The dried extracts were dissolved in the same solvent for their extraction and sterilized by filtration by 0.45 µm Millipore filters. The antibacterial activities of various organic extracts were tested by employing standard agar disc diffusion method. In the disc diffusion method, the discs were impregnated with 10 µl of 30 mg/ml extracts (300 µg/disc) and placed aseptically over the bacterial culture on agar medium plates. Negative controls were prepared using the same solvents employed to dissolve the samples. Negative controls were prepared using the same solvents employed to dissolve the samples. Standard antibiotic, streptomycin (10 µg/disc) was used as positive control for the tested bacteria. The plates were incubated micro aerobically at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested bacteria. Each assay in this experiment was replicated three times.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of the samples was tested by two-fold serial dilution method. The test samples of various extracts were first dissolved in 10% dimethylsulfoxide (DMSO) and incorporated into Luria-Broth to obtain a concentration of 2000 µg/ml and serially diluted to achieve 1000, 500, 250 and 62.5 µg/ml. 10 µl standardized suspension of each tested organism (10^8 CFU/ml) was transferred to each tube and incubated at 37°C for 24 h. the lowest concentration of the test samples, which did not show any growth of tested organism after macroscopic evaluation, was determined as MIC.

RESULTS

Antibacterial activity of organic extracts

The antibacterial activity of various organic extracts of *C. nocturnum* against the employed bacteria was qualitatively assessed by the presence or absence of inhibition zones. Various organic extracts of *C. nocturnum* revealed a great potential of antibacterial activity against all bacteria, at the concentration of 300 µg/disc (Table 1). Methanol extract showed the strongest antibacterial effect against *S. aureus* ATCC 6538, *L. monocytogenes* ATCC 19166 and *B. subtilis* ATCC 6633 (inhibition zones: 17.5±1.3 to 15.5±0.5 mm). On the other hand, ethyl acetate extract showed moderate to high antibacterial effects against most of the bacteria that have being tested (inhibition zones: 10±1.2 to 15.5±1.1mm). Hexane and chloroform extracts displayed a moderate inhibitory effect against most of the bacteria. In this study, in some cases, organic extracts exhibited higher or similar types of antibacterial activity than that of streptomycin. The blind control did not inhibit the growth of the bacteria tested.

Table 1. Antibacterial activity of various extracts of *Cestrum nocturnum* L.

Microorganism	Zones of inhibition (mm)				
	Various extracts				SM
	MeOH	Hexane	CHCl ₃	EtOAc	
<i>Staphylococcus aureus</i> ATCC 6538	17.5±1.3	13.0±0.9	14.0±1.5	15.5±1.1	13.1±0.5
<i>Listeria monocytogenes</i> ATCC 19166	17.0±1.1	10.5±0.6	13.5±0.6	15.0±1.0	12.5±0.7
<i>Bacillus subtilis</i> ATCC 6633	15.5±0.5	13.1±0.7	11.2±1.3	13.2±0.6	14.1±0.8
<i>Pseudomonas aeruginosa</i> KCTC 2004	15.0±1.1	10.5±1.2	10.5±1.7	12.5±0.8	17.8±0.7
<i>Salmonella typhimurium</i> KCTC 2515	12.1±1.0	9.5±1.2	10.0±1.4	11.0±1.5	13.2±0.6
<i>Escherichia coli</i> ATCC 8739	11.2±1.6	9.2±0.6	9.5±1.1	10±1.2	14.0±0.5

Standard antibiotics: SM, streptomycin (10 µg/disc)

Values are given as mean ± S.D. (n=3)

Minimum inhibitory concentration (MIC)

As shown in Table 2, the MIC values of various solvent extracts against the tested bacteria were found in the range 62.5-1000 µg/ml (Table 2). In this study, the gram-positive bacteria were found to be more susceptible to the various solvent extracts than Gram-negative bacteria.

Table 2. MIC of various extracts of *Cestrum nocturnum* L.

Microorganism	MIC ^a			
	Various extracts			
	MeOH	Hexane	CHCl ₃	EtOAc
<i>Staphylococcus aureus</i> ATCC 6538	62.5	250	250	62.5
<i>Listeria monocytogenes</i> ATCC 19166	125	500	125	250
<i>Bacillus subtilis</i> ATCC 6633	250	500	250	250
<i>Pseudomonas aeruginosa</i> KCTC 2004	500	1000	500	125
<i>Salmonella typhimurium</i> KCTC 2515	500	1000	1000	500
<i>Escherichia coli</i> ATCC 8739	250	1000	1000	1000

^a Minimum inhibitory concentration (values in µg/ml)

DISCUSSION

In this study, various organic extracts of *C. nocturnum* exhibited potential activity against some of the bacterial strains such as *S. aureus* ATCC 6538, *L. monocytogenes* ATCC 19166, *B. subtilis* ATCC 6633, *P. aeruginosa* KCTC 2004, *S. typhimurium* KCTC 2515 and *E. coli* ATCC 8739. This activity could be attributed to the presence of oxygenated mono and sesquiterpene hydrocarbons and these findings are in agreement with the previous reports (Shunying *et al.* 2005; Guillen *et al.* 1998).

In pharmaceutical industries there is a continuing need to find new and improved antimicrobial agents, especially in view of the increasing incidence of antibiotic resistance. One of the areas which is subjected to considerable interest is plant extracts. Also, the increasing consumer demand for effective and safe natural products means that quantitative data on plant extracts are required. Therefore, screening of *C. nocturnum* growing in Bangladesh, for antimicrobial activity Phytochemicals is important for finding potential new compounds for medicinal or other uses.

CONCLUSION

The organic extracts of *Cestrum nocturnum* L. in our study showed a great potential activity against *S. aureus* ATCC 6538, *L. monocytogenes* ATCC 19166, *B. subtilis* ATCC 6633, *P. aeruginosa* KCTC 2004, *S. typhimurium* KCTC 2515 and *E. coli* ATCC 8739. Therefore, organic extracts are being considered as potential alternatives to synthetic bactericides or as leading compounds for new classes of natural bactericides. The results demonstrate that the possibility of using organic extracts in food or pharmaceutical industry as natural source. However, further research is needed in order to establish the real application of *C. nocturnum* extracts in food or pharmaceuticals.

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