Pharmacophore 2015, Vol. 6 (2), 81-87 USA CODEN: PHARM7 ISSN 2229-5402

Pharmacophore

(An International Research Journal)

Available online at http://www.pharmacophorejournal.com/

Original Research Paper

THE INVESTIGATION OF ANTIBACTERIAL ACTIVITY OF *CESTRUM NOCTURNUM*

Yash S. Punjabi*, Veena L. Khilnani and Padmaja N. Damle

K.J. Somaiya College of Science and Commerce, 97/5, Yash Kutir, Agra Road, Mulund (W), Mumbai-400082, Maharashtra, India

ABSTRACT

Plants are proven to be reliable sources of antimicrobial compounds in traditional medicinal systems. The leaf and stem extracts of *Cestrum nocturnum* were screened for antimicrobial activity. The extracts prepared in various solvents were examined against *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella paratyphi A, Salmonella paratyphi B, Shigella flexineri, Staphylococcus aureus.* The ethyl acetate stem and leaf extracts were found to be most active. The results were compared with Penicillin and Streptomycin.

Keywords: *Cestrum nocturnum*, Antimicrobial activity, Triphenyltetrazolium chloride, Well-diffusion method

INTRODUCTION

Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules which exhibit antimicrobial properties. Thus, it is important to characterize different types of medicinal plants for their antimicrobial potential (Memnune, S *et al.*; 2009). The spread of drug resistant pathogens is one of the most serious threats in treatment of microbial diseases. Therefore, there is considerable interest to investigate antimicrobial effects of different extracts against a range of bacteria to develop other classes of natural antimicrobials (Bakri, IM *et al.*; 2005). Down the ages, plants and plant extracts have aroused interest in treatment of microbial diseases as source of alleo chemicals. They have been screened for their potential uses as alternate remedies for the treatment of various infectious diseases (Tepe, B *et al.*; 2009). Herbal medicines are employed in a variety of health related applications ranging from treatment of common cold to cancer (Kumar, GS *et al.*; 2004). Various chemical compounds synthesised in plants give them their medicinal properties. Thus, plants have been screened extensively for antimicrobial compounds. Extreme interest in plants with microbial activity has revived as a result of current problems such as resistance associated with use of antibiotics obtained from microorganisms. Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of (plant) species extinction (Lewis, WH *et al.*; 1995). There is a feeling among natural-product chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of

being lost irretrievably (Borris, RP; 1996). There is a continuous and urgent need to determine new antibacterial compounds with diverse chemical structures and novel mechanisms of action against new and reemerging infectious diseases. *Staphylococcus aureus* is etiological agent responsible for postoperative wound infection, toxic shock syndrome, endocarditis and food poisoning (Mylotte, JM *et al.*; 1987). *Escherichia coli* is present in human intestine and cause urinary tract infection, coleocystitis or septicemia (Singh, R *et al.*; 2000). *Klebsiella* is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics (Carpenter, JL; 1990). *Pseudomonas aeruginosa* is a prevalent opportunistic pathogen in humans, causing chronic lung infections in cystic fibrosis patients, burn victims, and other immunocompromised people (Singh, PK *et al.*; 2002 & Singh, PK *et al.*; 2000). Secreted factors such as elastase and protease cause the degradation of host proteins such as elastin, collagen, and transferrins, destroying the integrity of the host tissues (Aumercier, M; *et al.* 1990 & Kawaharajo, K *et al.*; 1975). Practitioners use *C.nocturnum* externally for skin disorders but several scientific reports demonstrate that it exhibits a wide spectrum of pharmacological activity when administered systematically or in an isolate organ preparation. For example, it is used as abortive analgesic, diuretic, antispasmodic, dyspeptic, antiviral and smooth muscle relaxant and to treat arterial hypotension. It also has negative inotropic and chronotrotic actions (Pe'rez-Saad, *et al*., 2008). 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 a mosquito repellant and hence is used to prevent malaria in several African countries (Ntonifor, N. N. *et al.*, 2006). The genus *Cestrum* which belongs to the Solanaceae contains more than 300 species most of them native to sub-tropical and tropical areas of America .*Cestrum nocturnum* commonly known as queen of the night is a native to West Indies. It is an evergreen shrub growing to about 4 metres, the leaves are simple, narrow, lanceolate, broad, smooth & glossy. Flowers are greenish white in colour produced in cymose inflorescences & they release a sweet powerful perfume during the night. The fruit is a berry of aubergine colour. The antimicrobial activity of the whole plant of *Cestrum nocturnum L*. against pathogenic organisms was performed by Khan et al. 2011. In the present study, the solvent extracts of leaf and stem of *Cestrum nocturnum L*. were investigated for antibacterial activity. Therefore, the aim of the present study is to evaluate antibacterial activity of the extracts prepared in various polar and non polar solvents against eight different human pathogens to identify the potentiality of the antibacterial activity with emphasis for the possible future use of essential oil and plant extract as an alternative to chemical bacteriocides.

MATERIALS AND METHODS

Plant Material

The fresh leaves and stem of *Cestrum nocturnum* free from disease was collected from Mumbai, Maharashtra in the month of November-December 2011. The leaves and stem were separately washed with tap water and then deionised water and shade dried. The plant material was regularly checked for fungal growth or rotting. After the plant material was dried it was powdered with the help of an electric blender and sieved through size 80 sieves to obtain a uniform fine particle size. This plant material was stored in airtight containers at 4ºC.

Extraction

10 grams of the above powdered material was put in a thimble and extracted with 200 mL of various polar as well as non polar solvents in a Soxhlet's extractor for 72 hrs. The solvents used were hexane, pet ether, toluene, chloroform, ethyl acetate, ethanol, and methanol. Leaves and stem extracts were prepared separately. The extracts so obtained were dried under vacuum and reconstituted with the respective solvents by dissolving 10,000 µg of solid extract in 1 mL of

dimethyl sulphoxide. These extracts were kept in amber coloured sample bottles in a refrigerator at 4° C until further use (Gupta, V.P. *et al.*,1996; Pinto, C.MF. *et al.*,1998).

Bacterial Strains

The following strains were used which were maintained by the Microbiology department of K. J.Somaiya College of Science and Commerce.

- *Escherichia coli*
- *Klebsiella pneumonia*
- *Proteus vulgaris*
- *Pseudomonas aeruginosa*
- *Salmonella paratyphi A*
- *Salmonella paratyphi B*
- *Shigella flexineri*
- *Staphylococcus aureus*

The above bacterial strains were sub cultured a day before they were used.

Preparation of Inoculums

The bacteria were subcultured on nutrient agar slants for 24 hours. Loopful of these cultures were suspended in sterile saline (0.85% NaCl w/v) to obtain a transmittance of 25% at 560 nm using an UV-Vis spectrophotometer. This concentration corresponded to 10^6 colony forming units (CFU) per mL. McFarland standards were used to adjust the turbidity of bacterial suspension. The McFarland standard was made by adding 0.05 mL of $BaCl_2$ (1.17% w/v of $BaCl_2.2H_20$) to 9.95 mL of 0.18 M H_2SO_4 with constant mixing. This standard was kept in a test tube and sealed to prevent loss by evaporation (Rashmi Pa *et. al.*, 2012).

Preliminary Analysis

All the extracts were screened for their antibacterial activity by agar streak method. In this method, 15 mL of molten sterile agar butts were taken and 1 mL of extract (10,000 µg/mL) was added to it. This mixture was poured into a sterile petri dish and allowed to solidify. Now, the bacterial strains were added to sterile saline suspension and optical density was adjusted to 0.1. The suspensions were streak inoculated onto the above agar plates using a sterile nichrome loop. These plates were incubated at 37° C for 24 hrs. The results were recorded as growth or no growth as shown in Table 1aand 1b.

Minimum Inhibitory Concentration

The extracts found to be active were analyzed for their MIC. The minimum inhibitory concentration was determined by micro-well dilution method in nutrient broth (Himedia, Mumbai). 0.01%w/v 2,3,5-triphenyltetrazolium chloride (TTC Sigma Aldrich, India) was used as a visual indicator of growth. In each well of the 96 well-plate 95 µL nutrient broth and 5 µL inoculums was added. Then to this 100 µL extract of concentrations 500 µg/mL, 250 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 10 µg/mL &5 µg/mL and 100 µL of TTC were added. Negative control was solvent in place of extract and as positive control penicillin and streptomycin were used. The plate was incubated at 37 ºC for 24 hrs. In presence of bacteria the TTC gets converted to red formazan. The red formazan indicates the viability or activity of the cells (Ellof, 1998).The results were noted as growth or no growth based on the colour of TTC and are represented in table 2a and 2b.

Well Diffusion Method

The extracts which were found to be active by the previous method were examined for their zone of inhibition by agar well diffusion method. The bacterial cultures were sub cultured a day before on agar slants and the inoculums were prepared as stated earlier. The inoculums were added to 20 mL of sterile molten nutrient agar. This was homogenized well and poured in petri dishes. The agar was allowed to solidify and harden. Later the required four wells were made using sterile metallic cork borer. The wells were filed with the extracts (0.1mL). Streptomycin and Penicillin were used as reference standards and the solvent (DMSO) was used as negative control. The plates were incubated at 37 °C for 24 hours. The zone of inhibition was measured using vernier calipers and reported in table 3a and 3b.

RESULTS AND DISCUSSION

The present study was designed to obtain preliminary information on the antibacterial activity of *Cestrum nocturnum*. Among the extractions assayed, the non polar extracts

showed least activity whereas polar extracts were strongly antibacterial. The stem extracts were showed higher inhibition of the bacterial growth than the leaf extracts. The ethyl acetate stem extract showed the highest activity and inhibited all the organisms used in the study. This was followed by the ethanol stem extract. Among the leaf extracts only polar extracts showed activity against the tested strains and ethyl acetate leaf extract showing the best activity. Deans, *et al.* 1995; investigated the susceptibility of Gram positive and Gram negative bacteria to plant volatile oils and found no evidence for a difference in sensitivity between Gram negative and Gram positive organisms. However, a few oils appeared to be more specific inhibiting Gram positive bacteria to a greater extent. Similarly, in the present study, the leaf and stem extracts appear to be more active against Gram positive bacteria than gram negative. Unlike the Gram positive bacteria the lipopolysaccharide along with proteins and phospholipids are major components in the outer surface of the Gram negative bacteria. This outer lipopolysaccharide layer hinders the entry of most compounds to the peptidoglycan layer of the cell wall. This explains the resistance of Gram negative organisms to many antibiotics.

The activity against both types of bacteria is indicative of a broad spectrum of antibacterial compounds or just general metabolic toxins.

CONCLUSION

The leaf and stem extracts of *Cestrum nocturnum* show considerable antibacterial activity. The plant extracts can be further studied to isolate the phytochemical responsible for the antibacterial activity.

Table 1a: Preliminary testing by agar streak method of the leaf extract of *C. nocturnum*

Bacteria	EtOAc	MeOH	Ethanol	Chloroform	Hexane	Pet Ether
Klebsiella pneumonia	+	+	+	-	-	-
Salmonella paratyphi A	+	+	+	-	-	-
Shigella flexineri	+	+	+	-	-	-
Staphylococcus aureus	+	+	+	-	-	-
Escherichia coli	+	+	+	-	-	-
Proteus vulgaris	+	+	+	-	-	-
Salmonella paratyphi B	+	+	-	-	-	-
Pseudomonas aeruginosa	+	+	-	-	-	-

Table 1b: Preliminary testing by agar streak method of the stem extract of *C. nocturnum*

Bacteria	EtOAc	MeOH	Ethanol	Chloroform	Hexane	Pet Ether
Klebsiella pneumonia	+	+	+	+	-	-
Salmonella paratyphi A	+	+	+	-	-	-
Shigella flexineri	+	+	-	+	-	-
Staphylococcus aureus	+	+	+	+	-	-
Escherichia coli	+	+	+	-	-	-
Proteus vulgaris	+	+	+	-	-	-
Salmonella paratyphi B	+	+	-	-	-	-
Pseudomonas aeruginosa	-	+	+	-	-	-

Table 2a: Minimum inhibitory concentration of leaf extracts of *C. nocturnum* in µg/mL

Bacteria	Ethyl acetate	Methanol	Ethanol	Penicillin	Streptomycin
Klebsiella pneumonia	100	50	150	250	100
Salmonella paratyphi A	50	25	100	25	100
Shigella flexineri	200	150	250	No activity	00
Staphylococcus aureus	100	150	200	10	150
Escherichia coli	50	25	200	250	200
Proteus vulgaris	250	200	250	No activity	00
Salmonella paratyphi B	100	25	150	No activity	150
Pseudomonas aeruginosa	350	300	500	No activity	00

Table 2b: Minimum inhibitory concentration of Stem extracts of *C.nocturnum* in µg/mL

Bacteria	Ethyl acetate	Methanol	Ethanol	Chloroform
Klebsiella pneumonia	100	25	100	200
Salmonella paratyphi A	50	25	100	No activity
Shigella flexineri	150	100	No activity	175
Staphylococcus aureus	75	200	150	200
Escherichia coli	50	25	100	No activity
Proteus vulgaris	150	150	175	No activity
Salmonella paratyphi B	100	25	No activity	No activity
Pseudomonas aeruginosa	No activity	250	400	No activity

Table 3a: Determination of zone of inhibition of leaf extracts of *C. nocturnum* in mm

Bacteria	Ethyl acetate	Methanol	Ethanol	Penicillin	Streptomycin
Klebsiella pneumonia	16	18	17.8	No activity	13
Salmonella paratyphi A	14	09	15	26	20
Shigella flexineri	24	08	18	No activity	No activity
Staphylococcus aureus	13	08	17	20	14
Escherichia coli	13	12	14	No activity	16
Proteus vulgaris	14	06	19	No activity	18
Salmonella paratyphi B	28	04	21	No activity	16
Pseudomonas aeruginosa	17	10	18	No activity	No activity

Table 3b: Determination of zone of inhibition of stem extracts of *C.nocturnum* in mm.

Bacteria	Ethyl acetate	Methanol	Ethanol	Chloroform
Klebsiella pneumonia	20	20	16	03
Salmonella paratyphi A	14.9	12	14	01
Shigella flexineri	19	5	No activity	03
Staphylococcus aureus	29	10	09	02
Escherichia coli	40	16	18	No activity
Proteus vulgaris	26	5	03	No activity
Salmonella paratyphi B	34	4	No activity	No activity
Pseudomonas aeruginosa	No activity	17	11	No activity

REFERENCES

1. Aumercier, M., DM, Murray and JL, Rosner (1990), "Potentiation of susceptibility to aminoglycosides by salicylates in *Escherichia coli*. Antimicrob. Agents", ***Chemother.***, 23,835-845.
2. Bakri, IM and Douglas, CWI (2005), "Inhibitory effect of garlic extracts on oral bacteria", ***Archives of Oral Biology***, 50, 645-651.
3. Borris, RP (1996), "Natural products research: perspectives from a major pharmaceutical company", ***J Ethnopharmacol.***", 51,29-38.
4. Carpenter, JL (1990), "*Klebsiella* pulmonary infections: occurrence at one medical center and review", ***Rev Infect Dis.***, 12,672-682.
5. Gupta, VP; Govindaiah and Datta, RK (1996), "Plant extracts: A non-chemical approach to control Fusarium diseases of mulberry", ***Current Science***, Vol. 71, 406-40.
6. JN, Eloff (1998), "A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria", Planta Medica, Vol. 64, No. 8, 711-713.
7. Kawaharajo, K; JY, Homma; Y, Aoyama; K, Okada and K, Morihara (1975), "Effects of protease and elastase from *Pseudomonas aeruginosa* on skin", ***Jpn. J. Exp. Med***., 45,79-88.
8. Kumar, GS; Jayaveera, KN; Sanjay, UP; Swamy, BMV and Kumar, DVK (2007), "Antimicrobial effects of Indian medicinal plants against acne inducing bacteria", ***Tropical Journal of Pharmaceutical Research***, 6(2),717-723.
9. Lewis, WH and Elvin-Lewis, MP (1995), "Medicinal plants as sources of new therapeutics", ***Ann. Mo. Bot. Gard***., 82,16-24.
10. Memnune, S; Hilal, Y; Neva, G; Bulent, C; Zeynet, E and Sezai, E (2009), "Total phenoic content, antioxidant and antimicrobial activities of some medicinal plants", ***Pak. J. Pharm. Sci.***,Vol.22, No.1, 102-106.
11. Mylotte, JM; Mcdermott, C and Spooner, JA (1987), "Prospective study of 114 consecutive episodes of Staphylococcus aureus bacteria", ***Review of Infectious Diseases***, 9, 891-907.
12. Ntonifor, NN; Ngufor, CA; Kimbi, HK and Oben, BO (2006), "Traditional use of mosquito repellant to protect human against mosquito and other insect bites in rural community of Cameroon", ***East African Medical Journal***, 83, 553-558.
13. Pe'rez-Saad, H and Buznego, MT (2008), "Behavorial and antiepileptic effects of acute administration of the extract of the plant *Cestrum nocturnum* L.", ***Epilepsy and behavior***, 12, 366-372.
14. Pinto, CMF; Casali, VWD & Cardoso, AA (1998), "In vitro effect of plant leaf extracts on mycelial growth and sclerotial germination

of Sclerotinum cepivorum", ***J. Phytopathology.***, Vol. 146, 421-425.

15. Rashmi, Pa and Linu, Mathew (2012), "Antimicrobial activity of leaf extracts of Justicia adhatoda L. incomparison with vasicine", ***Asian Pacific Journal of Tropical Biomedicine***, S1556-S1560.
16. Singh, R; Chandra, R; Bose, M and Luthra, PM (2000), "Antibacterial activity of Curcuma longa rhizome extract on pathogenic bacteria", ***Current Science***, 83, 737-740.
17. Singh, PK; AL, Schaefer; MR, Parsek; TO, Moninger; MJ, Welsh and EP, Greenberg (2000), "Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms", ***Nature***.
18. Singh, PK; MR, Parsek; EP, Greenberg and MJ, Welsh **(**2002), "A component of innate immunity prevents bacterial biofilm development", ***Nature***, 417,552-555.
19. Tepe, B; Daferera, D; Sokmen, M; Polissiou, M and Sokmen, A (2009)**, "**In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of Thymus eigii", ***Journal of Medicinal Plants Research***, Vol. 3(3), 174-178.

Correspondence Author:
Yash S. Punjabi
K.J. Somaiya College of Science and Commerce, 97/5,Yash Kutir, Agra Road, Mulund (W), Mumbai-400082, Maharashtra, India

Cite This Article: Punjabi, Yash S; Khilnanim, Veena L and Padmaja, N Damle (2015), "the investigation of antibacterial activity of *Cestrum nocturnum*", ***Pharmacophore***, Vol. 6 (2), 81-87.