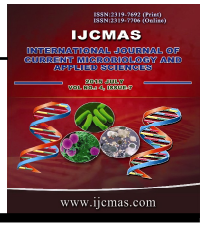
Int.J.Curr.Microbiol.App.Sci (2015) 4(7): 211-214

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 211-214**
http://www.ijcmas.com



Original Research Article

Antimicrobial Potential of Aqueous, Methanolic and Ethanolic Extracts of *Azadirachta indica* against Bacterial Pathogens Isolated from Urinary Tract Infection Patients

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Keywords

Neem, Agar well diffusion method, Urinary tract infection

A B S T R A C T

Medicinal plants offer a number of bioactive compounds which can lead to development of new antimicrobial agents. These agents will be cheaper with improved safety and efficacy than the synthetic drugs. In the present study we compared the aqueous, methanolic and ethanolic extracts of *Azadirachta indica* (Neem) leaves against human pathogenic bacteria causing urinary tract infections. Soxhlet method was used for extraction of the various extracts while agar well diffusion method was used to determine the antimicrobial activity against bacteria. Our results suggest that ethanolic extracts exhibit highest antimicrobial activity with more activity against Gram negative than Gram positive.

Introduction

Selman Waksman, the discoverer of Streptomycin, for the first time defined Antibiotic as "any class of organic molecule that inhibits or kills microbes by specific interactions with bacterial targets, without any consideration of the source of that particular compound or class". Antibiotics are the mainstay of therapy for bacterial infections and have always been considered one of the wonder discoveries of the 20th century (Demain and Elander, 1999).

However, the successful use of any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed. This has created immense problems in the treatment of diseases. Further these antibiotics have several side effects. Therefore, there is a need to develop antimicrobial agents which are safer, cheaper and more efficacious.

Medicinal plants offer a number of bioactive compounds which can lead to development of new antimicrobial agents (Sumathi and Parvathi, 2010). About 10% of all flowering plants on earth are used to treat various infections, although only 1% have gained recognition by modern scientists (Lewis and

Ausubel, 2006). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flaconoids, which have been found in vitro to have antimicrobial properties (Subapriya and Nagini, 2005).

Amongst these medicinal plants *Azadirachta indica* (Neem) is a source of potential therapeutic agents. *Azadirachta indica* belongs to family Meliaceae. It exhibits antibacterial, antifungal and antiviral activities (Biswas, 2002). Also exhibit wide pharmacological activities including: antioxidant, antimalarial, anticarcinogenic, anti-inflammatory, antiulcer, antimutagenic, antihyperglycaemic, and anti-diabetic properties (Talwar *et al.*, 1997; Subapriya and Nagini, 2005).

The present study was designed to compare the three extracts of neem leaves: aqueous, methanol and ethanol against human pathogens causing UTI.

Materials and Methods

Collection of raw materials: The neem leaves were collected from Forest Research Institute (F.R.I.), Dehradun and authenticated by a botanist.

Preparation of different leaf extracts: For this purpose, shade dried powder of leaves was used for extraction with different solvents. The preparation of the three extracts was carried out according to the Soxhlet method (Gabriela *et al.*, 2010) (Fig. 1).

Study population: Non repetitive 100 patients with significant bacteuria attending Muzaffarnagar Medical College, Muzaffarnagar were included in the study. For isolation of bacterial strains, loop full of urine samples were streaked on Cystiene Lactose Electrolyte Deficient (CLED) medium (Hi Media, India). After overnight incubation at 37ºC organism was identified on the basis of standard biochemical tests (Koneman *et al.*, 1997).

Antibacterial Susceptibility assay: The antibacterial activity of the three extracts was carried out using the Agar well diffusion method (Perez *et al.*, 1990). Lawn culture of the test organism adjusted to 0.5 McFarland turbidity Standard was made on the Mueller Hinton Agar (HiMedia) plates using sterile cotton swabs (11, 12). A sterile cork borer was then used to make wells (6mm diameter) for the three extracts. 100µl of the extracts were introduced into the wells with the help of micropipettes.

The culture plates were allowed to stand on the working bench for 30 min for pre-diffusion and were then incubated in upright position at 37°C for 24 h. After 24 hrs, antibacterial activity was determined by measurement of diameter of zones of inhibition (mm). Standard antibiotic discs of Ampicillin (10mcg/disc) and Nitrofurantoin (300mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

Results and Discussion

Amongst 100 urine samples which were included in the study *E. coli* was the commonest organism isolated 55 times (55%) alone and 4 times (4%) with *E. faecalis*. Other organisms isolated were *K. pneumonia* (19%), *P. aeruginosa* (7%), *P. mirabilis* (3%), *E. faecalis* (6%) and *S. aureus* (6%) each (Table 1).

The three extracts aqueous, methanolic and ethanolic were examined for their antimicrobial activity against the above mentioned bacteria, showed good antimicrobial potential (Table 2). Ethanolic

extracts exhibited higher antimicrobial effect than aqueous and methanolic extracts. Similar results were also obtained in studies by Tabassum *et al.* (2003).

Table.1 Bacteria isolated from Urine culture

S. No.	Bacteria isolated	Number	Percentage (%)
1.	*Escherichia coli*	55	55
2.	*Klebsiella pneumoniae*	19	19
3.	*Pseudomonas aeruginosa*	7	7
4.	*Proteus mirabilis*	3	3
5.	*Enterococcus faecalis*	6	6
6.	*Staphylococcus aureus*	6	6
7.	*Escherichia coli+ Enterococcus faecalis*	4	4

Table.2 Antimicrobial activity of various extracts of A. indica leaf

Extract/Drug	Diameter of inhibition zone (average) in mm.					
	E. coli	*K. pneumoniae*	*P. aeruginosa*	*P. mirabilis*	*E. faecalis*	*S. aureus*
Aqueous	16	9	11	10	12	12
Methanol	19	12	16	11	16	15
Ethanol	23	14	20	14	19	17
Ampicillin	15	13	12	13	15	14
Nitrofurantoin	25	24	20	18	18	21

Fig.1 Soxhlet Apparatus

Further maximum activity of ethanol extracts was seen on *E. coli* and least on *K. pneumoniae*. These results are in compliance with the results obtained by Sohail *et al.*

(2014), Raja Ratna *et al.* (2013) and Priyadharsini *et al.* (2014).

Based on the results obtained in this study leaf extracts of Neem expressed broad spectrum antibacterial activity on bacterial urinary tract infection isolates with highest activity recorded for ethanolic extracts.

Prevalence of drug resistance to various antibiotics among pathogenic bacteria has paved way for the search of new compounds that could replace the synthetic antimicrobial agents. Medicinal plants offer immense antimicrobial potential; all that is needed is to evaluate their antimicrobial activity. It is clear from the results obtained in our study that the leaf extracts of Neem exhibits good antimicrobial activity against urinary tract pathogens.

References

Biswas, K., Ishita, C., Ranajit, K.B., Uday, B. 2002. Biological activities and medicinal properties of Neem (*Azadiracta indica*). *Curr. Sci.,* 82: 1336–45.

Demain, A.L., Elander, R.P. 1999. The beta-lactam antibiotics: past, present, and future. *Antonie Van Leeuwenhoek*, 75(1-2): 5–19.

Gabriela, E.D., Villaneuva-Jimenez, J.A., Lopez-Collado, J., Rodriguez-Lagunes, D.A. 2010. Azadirachtin extraction using cold process and soxhlet method. *Boipestic Int.,* 6(1): 45–51.

Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., Winn, W.C. 1997. Color atlas and textbook of diagnostic microbiology, 5th edn. Lippincott-Raven Publishers, Philadelphia. Pp. 893–952.

Lewis, K., Ausubel, F.M. 2006. Prospects of plant derived antibacterials. *Nat. Biotechnol.,* 24: 1504–07.

Perez, C., Pauli, M., Barzerque, P. 1990. An antibiotic assay by agar-well diffusion method. *Acta Biol. Med. Exp.,* 15: 113–5.

Priyadharsini, M., Bhardwaj, S., Sheeba, E. 2014. Isolation,identification of microbial isolates from urinary tract infection patients and evaluation of antimicrobial activity using plant extracts. *Int. J. Curr. Microbial. Appl. Sci.,* 3(4): 153–160.

Raja Ratna, R.Y., Krishna, K.C., Lokanatha, O., Mamatha, S., Damodar, R.C. 2013. Antimicrobial activity of *Azadiracta indica* (neem) leaf, bark and seed extracts. *Int. J. Res. Phytochem. Pharmacol.,* 3(1): 1–4.

Sohail, M., Sarwar, S., Rasool, K., Iqbal, M.S. 2014. Antimicrobial activity of various plant extracts against bacterial pathogens isolated from urinary tract infection patients. *Th. Pharma. Chem. J.,* 1(2): 26–31.

Subapriya, R., Nagini, S. 2005. Medicinal properties of neem leaves: A review. *Curr. Med. Chem. Anticancer Agents*, 5: 149–156.

Subapriya, R., Nagini, S. 2005. Medicinal properties of neem leaves: A review. *Curr. Med. Chem. Anticancer Agents,* 5(2): 149–160.

Sumathi, P., Parvathi, A. 2010. Antimicrobial activity of some traditional medicinal plants. *J. Med. plants Res.,* 4: 316–21.

Tabassum, H., Ali, M.N., Al-Jameil, N., Khan, F.A. 2003. Evaluation of antibacterial potential of selected plant extracrs on bacterial pathogens isolated from urinary tract infections. *Int. J. Curr. Microbial. Appl. Sci.,* 2(10): 353–368.

Talwar, G.P., Raghuvanshi, P., Mishra, R., Mukherjee, S., Shah, S. 1997. Plant immunomodulators for termination of unwanted pregnancy and for conception and reproductive health. *Immunol. Cell Biol.,* 75: 190–2.