International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 9 | Special Issue 1- NCBTIL | 2019 | 200-205
National Conference on Biochemistry –Transcending and Integrating Life Sciences (NCBTIL) -2019
Held @ Kristu Jayanti College, K Narayanapura, Kothanur PO, Bengaluru-560077, January 17th & 18th
| Conference Proceedings | Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

Comparative Study on Antibacterial Activity of Neem (*Azadirachta Indica*) Ethanol and Methanol Extracts Against Uropathogens

***[1]R Katherin Sylvia, [1]E Sheeba, [1]Pooja Meena, [1]K Vinod Kumar, [1]P Rajarajan**

[1]Department of Microbiology, Centre for Research & PG Studies, Indian Academy Degree College Autonomous, Hennur Cross, Bangalore, Karnataka, India-560043

Received: 10 Dec 2018 / Accepted: 30 Dec 2018 / Published online: 10 Jan 2019
Corresponding Author Email: sonukathy@gmail.com, sheeba.elavalli@gmail.com

Abstract

Tribal people of India have been using several types of plants as medicine since the ancient time which has not been studied extensively. Plants have been an important source of medicine for thousands of years. The rich resource of plants is decreasing at an alarming rate as a result of over-exploitation. Neem is used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries. **Aim and Methods**: In the present study, different concentrations of ethanol and methanol extracts (100 µg – 1000 µg) were checked against uropathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* by well diffusion method. **Result**: Ethanol extracts of leaves were more effective against all pathogens than methanol extract. Out of five bacterial pathogens, ethanolic extract of neem showed best response against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. MIC was detected for both extracts by broth dilution method. **Conclusion**: However, further studies are needed, including toxicity evaluation and purification of active antibacterial constituents from *Azadirachta indica* extracts looking toward a pharmaceutical use.

Keywords
Antibacterial activity, *Azadirachta indica*, MIC, Uropathogens

*****

INTRODUCTION

Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by

antibiotics will be active against drug resistant microbial pathogens [1]. However, a very little information is available on such activity of medicinal plants and small number of plants are analysed for their antimicrobial activity. *Azadirachta indica* (Neem) is perhaps the most useful traditional medicinal plant. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine [13]. Every part of the tree has been used as traditional medicine for household remedy against various human ailments. The tree is still regarded as "Village dispensary" in India. Most of the parts of the plant such as fruits, seeds, leaves, bark and roots contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer and antifungal properties [5]. It is evergreen, but in serious drought it may lose most or nearly all of its leaves. The branches are spread far apart. In the present study, the comparative study of antibacterial activity of ethanol and methanol extracts of neem was carried out to detect the effectiveness of the different extracts against uropathogens.

MATERIALS AND METHODS

The leaves of *Azadirachta indica* were collected from field-grown plants and washed with distilled water to remove the adhering dust particles. Then they were dried in the shaded place. The dried leaves were fine powdered and stored in airtight bottles. Ethanol and methanol were used as a solvent to extract the bioactive compounds and used as positive control. Susceptibility tests were performed using five strains of uropathogens obtained from Department of Microbiology, Indian Academy Degree College Autonomous, Bangalore. The cultures were Gram positive (one strain) and Gram-negative (four strains) bacteria. Selected pathogenic bacteria were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa.* All the test bacterial species were maintained on nutrient agar medium. The bacterial cultures were inoculated in nutrient broth and incubated at 37˚C on a rotary shaker at 100 rpm. After 48 hours incubation, the bacterial suspension was centrifuged at 10000 rpm for 15 minutes. The pellet was resuspended in sterile distilled water and the concentration was adjusted to 1×10^8 cfu/ml using UV-visible spectrophotometer by reading the OD of the solution to 0.45 (A_{610nm}) and used for further studies. The extract was obtained by Soxhlet extraction method and flash evaporator used for evaporation of the solvent. 30 g of powdered leaf powder of *Azadirachta indica* and 500 ml of ethanol or methanol used for extraction. Antimicrobial assay was performed based on well diffusion method. The Mueller Hinton agar (Hi Media, Mumbai) plates were prepared and the test bacterial strains were swabbed on the MHA plates using sterile cotton swabs. Five wells were made on the surface of the MHA plates. Different concentrations of leaf extract (100,250,500,750, 1000 µg/ml) poured in the wells. Then the plates were incubated at 37˚C for 24 hours. After 24 hours, zones of inhibition were observed and recorded. The tests were performed in triplicates for each bacterium evaluated and statistical analysis was performed. Zone of inhibition were measured in millimetres (mm). The results obtained from leaf extract with ethanol and leaf extract with methanol were compared to know the effectiveness of extracts. The statistical analysis was performed in SPSS 20.

MIC

The lowest concentration of the crude extract that inhibited the growth of microorganisms was considered as MIC. The medium for MIC of bacterial cultures was Mueller Hinton Broth Different concentrations of leaf extracts (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 µg/ml) were added to the sterilized medium with broth cultures of bacteria. Incubation period was at 37˚C for 24 hours on a rotary shaker at 100 rpm. After the incubation, OD was taken at 600 nm and MIC determined based on the readings.

RESULT

Statistical analysis was carried out to study the effectiveness of different concentration of ethanol and methanol extract of *Azadirachta indica.* Based upon the result obtained ethanol extract was more effective against *E. coli* and minimum effectiveness was showed against *Staphylococcus aureus* (Table -I). Methanol extract was effective against *Escherichia coli* and minimum activity was showed against *Klebsiella pneumoniae* (Table - II). All the five bacterial species tested were showed zone of inhibition with ethanol as a solvent. In methanol extract, 100µg/ml showed resistance against *Staphylococcus aureus* and *Proteus*

mirabilis. Ethanol extract was more effective than methanol extract.

In ethanol extract of *Azadirachta indica*, *Proteus mirabilis* was not showed significant value in between 500 µg/ml and 750 µg/ml. In methanol extract, *Klebsiella pneumoniae* not showed significant value in 100 µg/ml and 250 µg/ml and in between 750 µg/ml and 1000 µg/ml. Minimum inhibitory concentration was calculated and tabulated (Table III and IV).

Table – I: Statistical analysis of antibacterial activity of ethanol extract of *Azadirachta indica*

Sl.No.	Name of the Bacterium	Concentration of the ethanolic extract (µg/ml)	Mean ± SD (mm)	Maximum (mm)	Minimum(mm)	F value
1	*Staphylococcus aureus*	100	3.33 ± .57^{a}	4.00	3.00	79.667
		250	8.00 ± 1.73^{b}	9.00	6.00	
		500	11.00 ± .00^{c}	11.00	11.00	
		750	12.66 ± .57^{d}	13.00	12.00	
		1000	15.33 ± .57^{e}	16.00	15.00	
2	*Escherichia coli*	100	5.33 ± .57^{a}	6.00	5.00	450.000
		250	7.33 ± .57^{b}	8.00	7.00	
		500	11.33 ± .57^{c}	12.00	11.00	
		750	16.66 ± .57^{d}	17.00	16.00	
		1000	22.66 ± .57^{e}	23.00	22.00	
3	*Klebsiella pneumoniae*	100	3.00 ± .00^{a}	3.00	3.00	137.056
		250	6.33 ± .57^{b}	7.00	6.00	
		500	12.00 ± 1.00^{c}	13.00	11.00	
		750	13.33 ±1.15^{d}	14.00	12.00	
		1000	15.66 ± .57^{e}	16.00	15.00	
4	*Proteus mirabilis*	100	2.66 ± .57^{a}	3.00	2.00	203.500
		250	4.00 ± .00^{b}	4.00	4.00	
		500	7.33 ± .57^{c}	8.00	7.00	
		750	10.66 ± .57^{c}	11.00	10.00	
		1000	12.66 ± .57^{d}	13.00	12.00	
5	*Pseudomonas aeruginosa*	100	4.66 ± .57^{a}	5.00	4.00	103.125
		250	6.66 ± .57^{b}	7.00	6.00	
		500	9.33 ± .57^{c}	10.00	9.00	
		750	12.33 ± .57^{d}	13.00	12.00	
		1000	15.33 ±1.15^{e}	16.00	14.00	

All values are mean ± SD.
Values in the column superscripted by different letters are significantly (P< 0.05) different from each other (Duncan's multiple range test).
Separate analysis was done for each column.

Table – II: Statistical analysis of antibacterial activity of methanol extract of *Azadirachta indica*

Sl.No.	Name of the Bacterium	Concentration of the ethanolic extract (µg/ml)	Mean ± SD (mm)	Maximum (mm)	Minimum(mm)	F value
1	*Staphylococcus aureus*	100	.00 ± .00^{a}	0.00	0.00	109.143
		250	2.66 ± .57^{b}	3.00	2.00	
		500	5.33 ± .57^{c}	6.00	5.00	
		750	7.33 ± .57^{d}	8.00	7.00	
		1000	10.66± 1.15^{e}	12.00	10.00	
2	*Escherichia coli*	100	1.33 ± .57^{a}	2.00	1.00	557.000
		250	4.00 ± .00^{b}	4.00	4.00	
		500	6.66 ± .57^{c}	7.00	6.00	

Sl.No.	Name of the Bacterium	Concentration of the ethanolic extract (µg/ml)	Mean ± SD (mm)	Maximum (mm)	Minimum(mm)	F value
3	*Klebsiella pneumoniae*	750	10.00 ± .00^{d}	10.00	10.00	36.818
		1000	14.00 ± .00^{e}	14.00	14.00	
		100	2.66 ± .57^{a}	3.00	2.00	
		250	4.66 ± .57^{a}	5.00	4.00	
		500	10.00± 2.64^{b}	12.00	7.00	
		750	13.00 ± 1.73^{c}	14.00	11.00	
		1000	14.66± .57^{c}	15.00	14.00	
4	*Proteus mirabilis*	100	.00 ± .00^{a}	0.00	0.00	227.875
		250	3.66 ± .57^{b}	4.00	3.00	
		500	5.33± .57^{c}	6.00	5.00	
		750	8.66 ± .57^{d}	9.00	8.00	
		1000	11.66 ± .57^{e}	12.00	11.00	
5	*Pseudomonas aeruginosa*	100	2.00± .00^{a}	2.00	2.00	236.500
		250	6.66 ± .57^{b}	7.00	6.00	
		500	9.33 ± .57^{c}	10.00	9.00	
		750	12.33 ± .57^{d}	13.00	12.00	
		1000	13.33 ± .57^{e}	14.00	13.00	

All values are mean ± SD.
Values in the column superscripted by different letters are significantly (P< 0.05) different from each other (Duncan's multiple range test).
Separate analysis was done for each column.

Table –III: Minimum Inhibitory Concentration of Neem in ethanol

Sl.No.	Name of the bacterium	MIC(µg/ml)
1	*Staphylococcus aureus*,	1000
2	*Escherichia coli*	400
3	*Klebsiella pneumoniae*	900
4	*Proteus mirabilis*	600
5	*Pseudomonas aeruginosa*	700

Table IV: Minimum Inhibitory Concentration of Neem in Methanol

Sl.No.	Name of the bacterium	MIC(µg/ml)
1	*Staphylococcus aureus*,	1000
2	*Escherichia coli*	300
3	*Klebsiella pneumoniae*	900
4	*Proteus mirabilis*	800
5	*Pseudomonas aeruginosa*	700

Table – II: Statistical analysis of antibacterial activity of methanol extract of *Azadirachta indica*

Sl.No.	Name of the Bacterium	Concentration of the ethanolic extract (µg/ml)	Mean ± SD (mm)	Maximum(mm)	Minimum(mm)	F value
1	*Staphylococcus aureus*	100	.00 ± .00^{a}	0.00	0.00	109.143
		250	2.66 ± .57^{b}	3.00	2.00	
		500	5.33 ± .57^{c}	6.00	5.00	
		750	7.33 ± .57^{d}	8.00	7.00	
		1000	10.66± 1.15^{e}	12.00	10.00	
		100	1.33 ± .57^{a}	2.00	1.00	557.000

Sl.No.	Name of the Bacterium	Concentration of the ethanolic extract (µg/ml)	Mean ± SD (mm)	Maximum(mm)	Minimum(mm)	F value
2	*Escherichia coli*	250	4.00 ± .00^{b}	4.00	4.00	
		500	6.66 ± .57^{c}	7.00	6.00	
		750	10.00 ± .00^{d}	10.00	10.00	
		1000	14.00 ± .00^{e}	14.00	14.00	
3	*Klebsiella pneumoniae*	100	2.66 ± .57^{a}	3.00	2.00	36.818
		250	4.66 ± .57^{a}	5.00	4.00	
		500	10.00± 2.64^{b}	12.00	7.00	
		750	13.00 ± 1.73^{c}	14.00	11.00	
		1000	14.66± .57^{c}	15.00	14.00	
4	*Proteus mirabilis*	100	.00 ± .00^{a}	0.00	0.00	227.875
		250	3.66 ± .57^{b}	4.00	3.00	
		500	5.33± .57^{c}	6.00	5.00	
		750	8.66 ± .57^{d}	9.00	8.00	
		1000	11.66 ± .57^{e}	12.00	11.00	
5	*Pseudomonas aeruginosa*	100	2.00± .00^{a}	2.00	2.00	236.500
		250	6.66 ± .57^{b}	7.00	6.00	
		500	9.33 ± .57^{c}	10.00	9.00	
		750	12.33 ± .57^{d}	13.00	12.00	
		1000	13.33 ± .57^{e}	14.00	13.00	

All values are mean ± SD.
Values in the column superscripted by different letters are significantly (P< 0.05) different from each other (Duncan's multiple range test).
Separate analysis was done for each column.

Table –III: Minimum Inhibitory Concentration of Neem in ethanol

Sl.No.	Name of the bacterium	MIC(µg/ml)
1	*Staphylococcus aureus*,	1000
2	*Escherichia coli*	400
3	*Klebsiella pneumoniae*	900
4	*Proteus mirabilis*	600
5	*Pseudomonas aeruginosa*	700

Table IV: Minimum Inhibitory Concentration of Neem in Methanol

Sl.No.	Name of the bacterium	MIC(µg/ml)
1	*Staphylococcus aureus*,	1000
2	*Escherichia coli*	300
3	*Klebsiella pneumoniae*	900
4	*Proteus mirabilis*	800
5	*Pseudomonas aeruginosa*	700

DISCUSSIONS

The purpose of the present research work is to evaluate the medicinal compounds present in *Azadirachta indica* against different pathogenic bacteria. Uwimbabazi *et al* mentioned in the research that *Azadirachta indica* was effective against *Staphylococcus aureus* and resistant against *E. coli*. In our present research, *A. indica* was effective against *E. coli* and less effective against *Staphylococcus aureus*. Gauri *et al* and Mahmood *et al* findings were supported our studies. Synthetic drugs have many side effects. Compounds extracted from medicinal plants have extensive power against many pathogens. Purification of these compounds in industrial scale has importance in the near future as the antibiotic resistant bacteria are present in the environment.

According to Wendy *et al* neem leaves are effective against MRSA strains. MIC detected for each bacterium. Further analysis is required to identify the chemical compound responsible for antibacterial activity.

REFERENCES

[1] Anjana Sharma, Rani Verma and Padmini Ramteke., Antibacterial Activity of some Medicinal Plants used by tribals against UTI causing pathogens.World Applied Sciences Journal, 7(3): 332 -339,(2009)

[2] E. Sheeba., Antibacterial activity of Solanum surattense Burm. F.Kathmandu University Journal of Science, Engineering and Technology, 6(1): 1-4,(2010).

[3] Gauri Parashar, Ninad Sutar and Sachin Sanap., Antibacterial activity of mixture of leaf extracts of Neem (*Azadirachta indica* Linn.) and Tantani (Lantana camara). International Journal of Pharmaceutical Science and Research, 9(6): 2545 -2549, (2018).

[4]Jayakumar Jerobin, Pooja Makwana, RS Suresh Kumar, Rajiv Sundaramoorthy, Amitava Mukherjee, and Natarajan Chandrasekaran., Antibacterial activity of neem nanoemulsion and its toxicity assessment on human lymphocytes in vitro. International Journal of nanomedicine, 10(1): 77-86, (2015).

[5]Kunjal S. Mistry, Zarna Sanghvi, Girish Parmar, and Samir Shah., The antimicrobial activity of Azadirachta indica, Mimusops elengi, Tinospora cardifolia, Ocimum sanctum and 2% chlorhexidine gluconate on common endodontic pathogens: An in vitro study. European Journal of Dentistry, 8(2):172 -177, (2014).

[6] Leila Maleki, Tahereh Sadeghian-Rizi, Mostafa Ghannadian, Mohammad Hossein Sanati, Shahin Shafizadegan, and Hojjat Sadeghi-Aliabadi., Antibacterial Activity of Azadirachta indica Leaf Extracts against Some Pathogenic Standards and Clinical Bacterial Isolates. Avicenna Journal of Clinical Microbiology and Infection, (2017).

[7] Maragathavalli, S., Brindha, S., Kaviyarasi, N.S., B. Annadurai, B. and Gangwar, S.K., Antimicrobial Activity in leaf extract of neem (Azadirachta Indica Linn.). International Journal of Science and Nature, 3(1): 110 -113, (2012).

[8] Mahmood Rasool, Arif Malik, Mahwish Arooj, Mohammad Zubair Alam, Qamre Alam, Marium Awan, Muhammad Asif, Mahmood Husain Qazi, Ahmad Zaheer, Sami Ullah Khan, Absarul Haque, Peter Natesan Pushparaj, Hani Choudhry, Mohammad Sarwar Jamal., Evaluation of antimicrobial activity of ethanolic extracts of Azadirachta indica and Psidium guajava against clinically important bacteria at varying pH and temperature. Biomedical Research, 28(1): 134 -139, (2017).

[9] M.Priyadharsini, Sakshi Bhardwaj and E.Sheeba., Isolation, identification of microbial isolates from urinary tract infection patients and evaluation of antimicrobial activity using plant extracts. International Journal of Current Microbiology and Applied Sciences, 3(4): 154 -160, (2014).

[10] Mukul Chauhan, Paras Mani And Tanuj Kumar Sharma., The Antibacterial Activities of Neem [Azadirachta Indicia] Seed Oil, A Review. IOSR Journal of Applied Chemistry, 11(5): 58 -63, (2018).

[11] Raja Ratna Reddy Y, Krishna Kumari C, Lokanatha O , Mamatha S, Damodar Reddy C., Antimicrobial activity of Azadirachta indica(neem) leaf, bark and seed extracts. International Journal of Research in Phytochemistry and Pharmacology, 3(1):1-4, (2013).

[12] Ranjit R. Raut, Ajit R. Sawant and Bhagyashree B.Jamge., Antimicrobial activity of *Azadirachta indica* (Neem)against Pathogenic Microorganisms. Journal of Academia and Industrial Research, 3(7): 327 -329, (2014).

[13] Supriya R and Nagini S., Medicinal properties of neem leaves: a review. Current Medicinal Chemistry - Anti-Cancer Agents, 5(2): 149 -156, (2005).

[14] Quazi Rubyath Banna, Feroza Parveen and Md. Jalaluddin Iqbal., Growth inhibitory effect of ethanolic neem leaves extract on Klebsiella, Salmonella and Staphylococcus aureus. Bangladesh Journal of Pharmacology, 9:347-350, (2018).

[15]Sanjoy Banerjee, Lee Mei Kim, Mohamed Shariff, Helena Khatoon and Fatima Md. Yusoff., Antibacterial Activity of Neem (Azadirachta indica) Leaves on Vibrio spp. Isolated from Cultured Shrimp. Asian Journal of Animal and Veterinary Advances, 8(2): 355 -361, (2013)

[16] Uwimbabazi Francine, Uwimana Jeannette, Rutanga Jean Pierre., Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on Staphylococcus aureus and Escherichia coli. Journal of medicinal plants studies, 3(4):85 -91, (2015).

[17] Wendy C. Sarmiento, Cecilia C. Maramba, Ma. Liza M. Gonzales., An in-vitro study on the antibacterial effect of neem (*Azadirachta indica*) Leaf Extract on Methicillin-sensitive and Methicillin-resistant Staphylococcus aureus. PIDSP Journal, 12(1): 40 -45, (2011).