



## ANTIMICROBIAL ACTIVITY IN LEAF EXTRACT OF NEEM (*AZADIRACHTA INDICA* Linn.)

<sup>1</sup>Maragathavalli, S., <sup>2</sup>Brindha, S., <sup>3</sup>Kaviyarasi, N.S., <sup>4</sup>B. Annadurai, B. & <sup>5</sup>Gangwar, S.K.

<sup>1</sup>Department of Biochemistry, King Nandi Varman College of Arts and Science, Thellar,

<sup>2</sup>Department of Bioinformatics, New Prince Arts and Science College, Chennai,

<sup>3</sup>Department of Biochemistry, Bangalore City College, Bangalore

<sup>4&5</sup>Department of Crop and Horticultural Sciences, Biotechnology Team, College of Dryland Agriculture and Natural Resources, Mekelle University, Mekelle,

### ABSTRACT

Antimicrobial activity in leaf extract of neem (*Azadirachta indica*) against human pathogenic bacteria. *E.coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Bacillus pumilus*. Antimicrobial activities of alcoholic extracts of neem leaves were used. Varying concentration of each extracts 200mg/ml, 150 mg/ml, 100mg/ml, 50mg/ml, 25mg/ml prepared by using disc diffusion method. When compared with gentamycin 200mg and gentamycin 10mg, the methanol and ethanol extract shows maximum inhibition on *Bacillus pumillus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an ascending order.

**KEY WORDS:** *Azadirachta indica*, *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Bacillus pumilus*.

### INTRODUCTION

Neem is used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries. Its twigs provide a chewing stick and are widely used in the Indian sub continent (Almaskand Al. lafi. T.R. *et al.*, 1995) earlier studies on Neem have showed that it contains active substances with multiple medicinal properties. (Md mohashine Bhuiyan *et al.*, 1997). *Azadirachta indica* in folklore medicine for the treatment of Diabetes and show the potential role of anti diabetic activity ( Shraavan kumar Dholi *et al.*, 2011). Aqueous extract of Neem leaf extract has a good therapeutic potential as anti hyperglycemic agent in IDDM and NIDDM (Sonia Bajaj, and Srinivasan B.P 1999). Abu syed Md *et al.*, 2008 suggests that anti inflammatory effect of Neem extract is less than that produced by dexamethasone. Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise (Saseed A. *et al.*, 2008, A.M. E.I. Mahmood *et al*, 2010) Supports the use of the Neem seeds in traditional medicine to treat infections conditions especially those involving the eye and ear. Administration of alcoholic extract of Neem flower disrupts the estrous cycle in Sprague Dawley rats and causes a partial block in ovulation and has the potential of an ideal antifertility agent ( Gbotolorun S. C. *et al.*, 2008). The great potential Neem aqueous extract as powerful chemotherapeutic and viral agent (Hassan Amer *et al.*, 2010).

The purpose of the present study was to investigate the antimicrobial activity of Neem leaves against human pathogenic bacteria, including *Escherichia coli*,

*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus pumilus*.

### MATERIALS AND METHODS

#### Selection of plant

The plant neem (*Azadirachta indica*) was selected for study. Its leaves were collected from DKM college Garden in Vellore District, Tamil nadu. The collected leaves were identified and authenticated by Dr. B. Annadurai.

#### Leaf extract

The completely shade dried material was coarsely powdered and allowed soxhlet for successive extraction with methanol and ethanol. The obtained liquid extracts were subjected to subjected to Rotary evaporator and subsequently concentrated under reduced pressure (in vacuum at 40°C) and evaporated to dryness and stored at 4°C in air tight bottle.

#### Methanol Extract

50g of dried leaf powder were taken in a separate container. To this 250ml of methanol was added and kept for 24 h with periodic shaking then filtered and the filtrate was collected. The procedure was repeated three times with fresh volume of methanol. The filtrates were pooled.

#### Ethanol Extract

50g of dried leaf powder of *Azadirachta indica* were taken in a separate container. To this 250 ml of ethanol was added and kept for 24 h with periodic shaking. Filtered and the filtrate was collected. The procedure was repeated three times. The collected filtrates were pooled.

#### Microorganism

The Pathogenic strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus pumilus* were used. These strains were

obtained from Sargam Laboratory Pvt. Ltd., Manappakkam, Chennai.

**Antimicrobial screening**

**Agar disc diffusion method**

This method (Kirby Bauer *et al*, 1966) is suitable for organism that grows rapidly over night at 35-37°C. The antibiotic (specific concentration) impregnated disc absorbs moisture from the agar and antibiotic diffuses in to the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases. There is a logarithmic reduction in the antibiotic concentration. Zone of inhibition of bacterial growth around each disc is measured and the susceptibility is determined.

**Medium**

3.8g of Muller Hinton Agar is added to 100 ml distilled water and autoclaved at 121° C for 15 minutes at 15 lbs and poured in sterile Petri plates up to a uniform thickness of approximately 4mm and the agar is allowed to set at ambient temperature and used.

**Inoculums**

The microorganisms were inoculated in peptone medium and incubated at 37° C for 3-4 hours and this was used as inoculums.

**Method**

A sterile cotton swab was inserted into the bacterial suspension and then rotated and compressed against the wall of the test tube so as to express the excess fluid. The surface of Muller Hinton Agar plate was inoculated with

the swab. To ensure that the growth is uniform and confluent (or semi confluent) the swab is passed three times over the entire surface, by repeating the procedure, taking care the second and third time to turn the plate through 60° leaf extract and which were prepared using Dimethylsulfoxide: Methanol (1:1) solvent to dissolve the plant extract and then placed on the inoculated agar surface using sterile forceps.

Standard disc of Streptomycin (10µg/disc) and Tetracycline (30µg/disc) (Himedia), 6 mm in diameter were used as positive control and the solvent used for preparing extract was used as negative control. The plates were incubated overnight at 37° C for 18-24 hours. Antimicrobial activity was evaluated by measuring zone of inhibition by using Hi Media zone scale.

**Determination of Minimum inhibitory concentration**

**Microdilution assay**

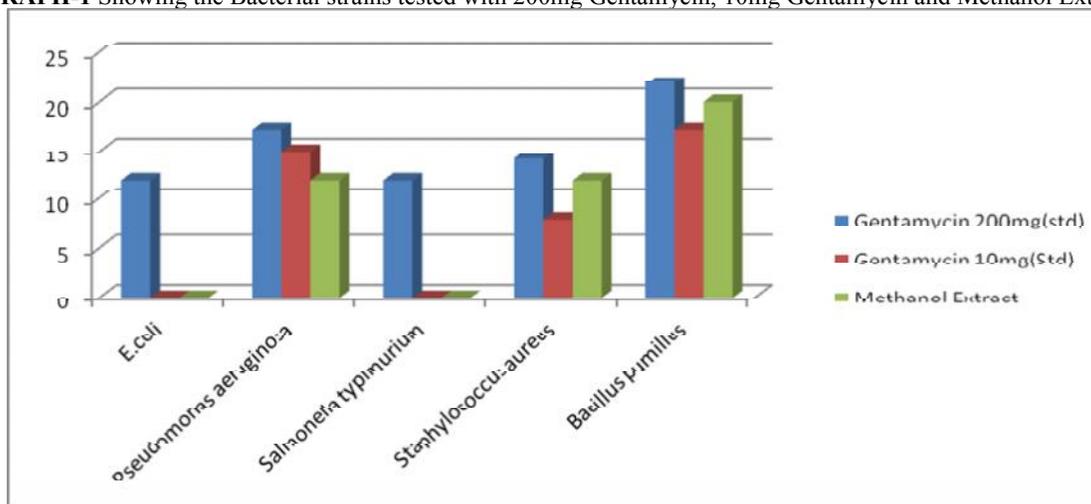
The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganisms (Kumar, G.S. *et al.*, 2007) The minimum inhibitory concentration values were determined by broth dilution assay of microdilution assay. Varying concentrations of the extracts (200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml) were prepared. 0.1ml of standardized test organism of Controls was equally set up by using solvents and test organisms without extract. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration.

**RESULTS**

**TABLE 1.** Invitro activity of Neem leaves in Methanol extract against opportunistic pathogens.

S.No	Name of the Organism	Gentamycin 200mg(std)	Gentamycin 10mg(Std)	Methanol Extract
1.	<i>E.coli</i>	12mm	-	-
2.	<i>Pseudomonas aeruginosa</i>	17mm	15mm	12mm
3.	<i>Salmonella typhimurium</i>	12mm	-	-
4.	<i>Staphylococcus aureus</i>	14mm	08mm	12mm
5.	<i>Bacillus pumillus</i>	22mm	17mm	20mm

**GRAPH-1** Showing the Bacterial strains tested with 200mg Gentamycin, 10mg Gentamycin and Methanol Extract



In Graph-1 *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus pumillus* were the Bacterial strains tested with 200mg Gentamycin, 10mg Gentamycin and Methanol Extract.

When compared with Gentamycin 200 mg and Gentamycin 10mg, the Methanol Extract shows maximum inhibition on *Bacillus pumillus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an ascending order.

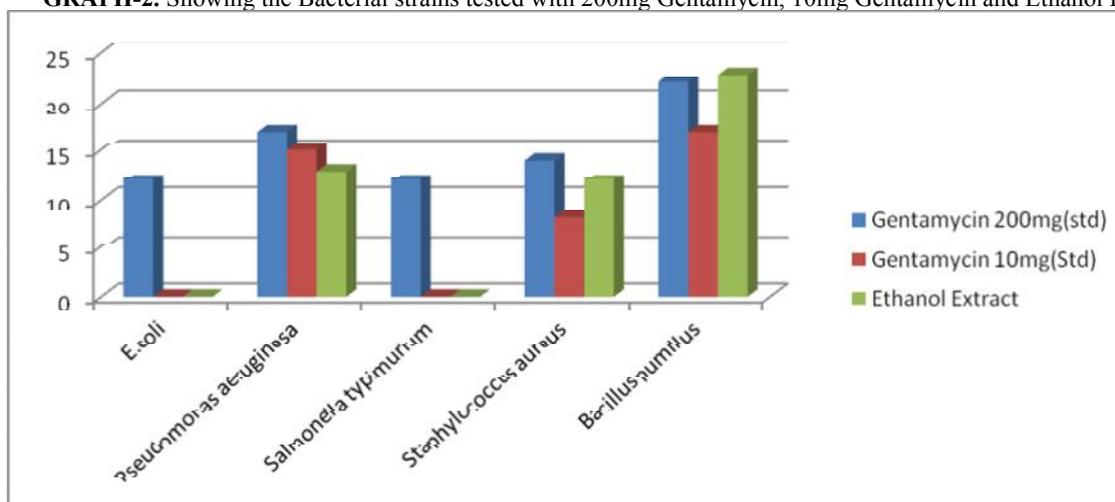
In Graph-2 *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus pumillus* were the Bacterial strains tested with 200mg Gentamycin, 10mg Gentamycin and Ethanol Extract.

When compared with Gentamycin 200mg and Gentamycin 10mg, the Ethanol Extract shows maximum inhibition on *Bacillus pumillus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an ascending order.

**TABLE 2:** Invitro activity of Neem leaves in Ethanol extract against opportunistic pathogens.

S.No	Name of the Organism	Gentamycin 200mg(std)	Gentamycin 10mg(Std)	Ethanol Extract
1.	<i>E.coli</i>	12mm	-	-
2.	<i>Pseudomonas aeruginosa</i>	17mm	15mm	13mm
3.	<i>Salmonella typhimurium</i>	12mm	-	-
4.	<i>Staphylococcus aureus</i>	14mm	08mm	12mm
5.	<i>Bacillus pumillus</i>	22mm	17mm	23mm

**GRAPH-2.** Showing the Bacterial strains tested with 200mg Gentamycin, 10mg Gentamycin and Ethanol Extract



## DISCUSSION

Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects ( Srivastava *et al.*, 2000 ). *Azadirachta indica* leaves possessed good anti bacterial activity, confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care (Saradha jyothi, Subbarao 2011). The extracts of Neem when used as medicinal plant, could be useful for the growth inhibition of the carcinogenic bacterium, *S. sobrinus*. (Md Mohashine Bhuiyan *et al.*, 1997). The phytoconstituents alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens ( Hafiza, 2000). The result was also supported by ( Faiza aslam *et al.*, 2009).

## ACKNOWLEDGEMENTS

I would like to thank my indebtedness to my academic guide Prof. Dr. B. Annadurai, Research coordinator, Department of Plant biology and Biotechnology, CAH college, Melvisharam, and Dr.R.Puvanakrishnan, Emeritus

scientist, Department of Biotechnology, Central leather Research institute, Adyar, Chennai, India, for his counsel and guidance during the research period.

## REFERENCES

- Abu. Syed Md. Mosaddek and Md. Mamun Ur Rashid (2008 ) A comparative study of Anti-inflammatory effect of aqueous extract of Neem leaf and dexamethasone. *Bangladesh J Pharmacol* 3: 44-47.
- Almas, K. Ansal lafi, T.R. (1995) The natural toothbrush. *World health Forum* 16: 206-210.
- Shravan Kumar Mankala, Kannappan Nagappan (2011) *invivo* Antidiabetic evaluation of Neem leaf extract in alloxan induced rats. *Journal of applied Pharmaceutical science*, 7, 100-105.
- Sonia Bajaj, Srinivasan B.P. (1999) Investigation into the Anti diabetic activity of *Azadirachta indica*. *Indian journal of pharmacology* 31:138-141.
- Saseed A. Khan and Junaid Aslam (2008) Study on the effect of Neem (*Azadirachta indica*) leaves smoke in

Antimicrobial activity in leaf extract *Azadirachta indica* Linn.

controlling airborne Bacteria in Residential premises. *Current research in Bacteriology* 1 (2): 64-66.

A.M. EI- Mahmood, O.B Ogbonna and M.Raji (2010) The antibacterial activity of *Azadirachta indica* (Neem) associated with eye and ear infections. *Journal of medicinal plant Research*, 4(14),1414-1421.

Gbotolorun S.C. Osinubi A.A., Noronha C.C., Okanlawon A.O (2008) Antifertility potential of Neem flower extract on adult female Sprague Dawley rats. *African Health Science* 8(3): 168-173.

Hassan Amer, Wafaa A. Helmy, Hanan A.A Taie (2010) *invitro* Antitumour activities of seeds and leaves Neem (*Azadirachta indica*) extracts. *International journal of Academic research*. 2(2), 165-171.

Md Mohashine Bhuiyan, Michiko Nishimura seishi matsumura and Tsutomu shimono (1997) Antibacterial

effects of the crude *Azadirachta indica* Neem bark extract on *Streptococcus sobrinus*, *Pediatric dental journal* 7(1): 61-64.

Srivastava A Shukla Kumar YN (2000) Recent development in plant derived antimicrobial constituents A Review. *J Med Arom Pl. Sci.*20: 717-72.

Saradhajothi Koon, Subbarao Budida (2011) Antimicrobial potential of the extracts of the leave of *Azadirachta indica* , Linn. *Nat Sci Biol*, 3(1) 65-69.

Hafiza, R.E. (2000) Peptides antibiotics, *Lancet* 349: 418-422.

Faiza Aslam, Khalil.Ur. Rehman, Mohammad Asghar and Muhammed Sarwar (2009) Antibacterial activity of various Phytoconstituents of Neem. *Pak. J.Agr. Sci.*, Vol. 46(3), 456-463.