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

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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, 150mg/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 pumilus, Pseudomonas aeruginosa and Staphylococcus aureus in an ascending order.

KEY WORDS: Azadiricha 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. Iafi, 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 (Shravan kumar Dholi et al., 2011). Aqueous extract of Neem leaf extract has a good therapeutic potential as anti hyperglycemic agent in IIDDMA 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 aequous 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 and Bacillus pumilus were used. These strains were
Antimicrobial activity in leaf extract *Azadirachta indica* Linn.

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

**Antimicrobial screening**

**Agar disc diffusion method**

This method (Kirby Bauer *et al.*, 1966) is suitable for organisms that grow rapidly overnight at 35-37°C. The antibiotic (specific concentration) impregnated disc absorbs moisture from the agar and antibiotic diffuses into 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.

**Inoculum**

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.   | E.coli                     | 12mm                  | -                    | -                |
| 2.   | Pseudomonas aeruginosa     | 17mm                  | 15mm                 | 12mm             |
| 3.   | Salmonella typhimurium      | 12mm                  | -                    | -                |
| 4.   | Staphylococcus aureus       | 14mm                  | 08mm                 | 12mm             |
| 5.   | Bacillus pumilus            | 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: In vitro 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.   | *E.coli*                                | 12mm                  | -                    | -               |
| 2.   | *Pseudomonas aeruginosa*                | 17mm                  | 15mm                 | 13mm            |
| 3.   | *Salmonella typhimurium*                | 12mm                  | -                    | -               |
| 4.   | *Staphylococcus aureus*                 | 14mm                  | 08mm                 | 12mm            |
| 5.   | *Bacillus pumillus*                     | 22mm                  | 17mm                 | 23mm            |

### 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, flavonoids 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).

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