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Antibacterial Study Of Neempatra Extract On Escherichia Coli, Pseudomonas Aeuroginosa, Corynebacteria, Staphylococcus Aureus And Staphylococcus Epidermidis- An In Vitro Study

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ABSTRACT

The present study was carried out to evaluate antibacterial activity of leaf extract of Azadirachta Indica A. Juss. against Escherichia coli, Pseudomonas aeuroginosa, Corynebacteria, Staphylococcus aureus and Staphylococcus epidermidis. Azadirachta Indica is a multipurpose tree with multiple health benefits . Different part of the tree were shown to exhibit antimicrobial effects against a wide variety of microorganism screening of this medicinal plants for bioactive compounds may lead to development of less expensive new antimicrobial agents with improved safety and efficacy.

INTRODUCTION

Azadirachta indica belongs to the botanic family meliacae commonly known as a neem. It is used in traditional medicine as a source of many therapeutic agents. Azadirachta indica (leaf, bark & seed) are known to contain antibacterial and antifungal activities against different pathogenic microorganisms.

Apart from its various health benefits, neem also has immense benefits for your skin as well as hair weather it is pimples, annoying blackheads, fine lines , dandruff, hair fall so in ayurvedic text neem has been regarded as the *" sarv roga nivarini"* as it keeps all the disease at bay.

Extract of neem leaves and seeds produce pain relieving, anti-inflammatory and fever reducing compounds that can helps in healing cuts.

Neem has been extensively used in Ayurveda, Unani, Homoeopathy and Siddha medicine. In the present study we have evaluated the antimicrobial potential of Azadirachta Indica.

MATERIALS AND METHODS:-

Plant extract preparation :

The plant material used in this study were collected from Herbal Garden (SSAM) Hirawadi Nashik. It was identified and authenticated by the Dept of Botany, Panchavati College, Nashik, Maharashtra. Fresh leaves and ripened fruits were collected and dried in shade. The dried leaves were ground to powder and suspended in petroleum ether and kept in refrigerator overnight for removing all the fatty substance .After overnight incubation the supernatant was discarded and the residue was suspended in ethanol and ethyl acetate respectively in 250 ml conical flask and kept in 40o C overnight. Each 100 gms of powder leaf material were soaked in 250 ml of ethanol, ethyl acetate.

After overnight incubation the supernatants was filtered through whatmann filter paper No. 1 and the filtrate was dried to evaporate the organic solvent at room temperature. The sediment extract was weighed and dissolved in 5% dimethyl Sulphoxide (DMSo)

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 rotary evaporator and subsequently cure under reduced pressure (in Vaccume at 40 oC) and evaporate to dryness and stored at 4oC in air tight bottle

Methanol Extract:

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

Ethanol Extract:

50 gm of dried leaf powder of Azadirachta indica were taken in a separate container, to this 250ml of ethanol was added and kept for 24 hrs with periodic shaking filtered and filtrate was collected, the procedure was repeated three times. The collected filtrates were polled.

Micro-organism:

The pathogenic strains of E. Coli, P. aeuraginsa, Corynrbacteria ,S.aureus, staphylococcus epidermidis were used. These strains were obtained from HiMedia Laboratories Pvt. Ltd Mumbai 400086.

ANTIMICROBIAL SCREENING:

Agar disc diffusion method

This method (Kirby Bauer et al 1966) is suitable for organism that grows rapidly overnight at 35-37oC. The antibiotic (specific`111….) 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 conc. Zone of inhibition of bacterial growth around each disc is measured and the susceptibility is determined.

Medium:

3.8 gm of Muller Hinton Agar is added to 100 ml distilled water and autoclaved at 121 oC for 15 mins at 15 ibs and poured in sterile petri plates up to a uniform thickness of approximately 4 mm and the agar is allowed to set t ambient temperature and used.

Inoculums:

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

Method:

The sterile cotton 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 micro dilution 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	Escherichia coli	11 mm	-	-
2	Pseudomonas aeuroginosa	14mm	10mm	12mm
3	Corynebacteria	17 mm	15mm	10mm
4	Staphylococcus aureus	13 mm	9mm	12mm
5	Staphylococcus epidermidis	16mm	14mm	10mm

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

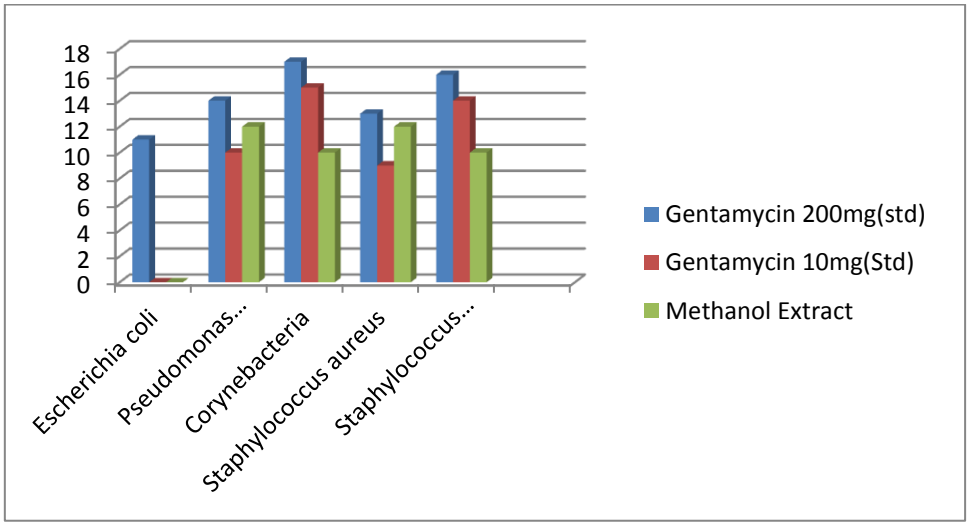
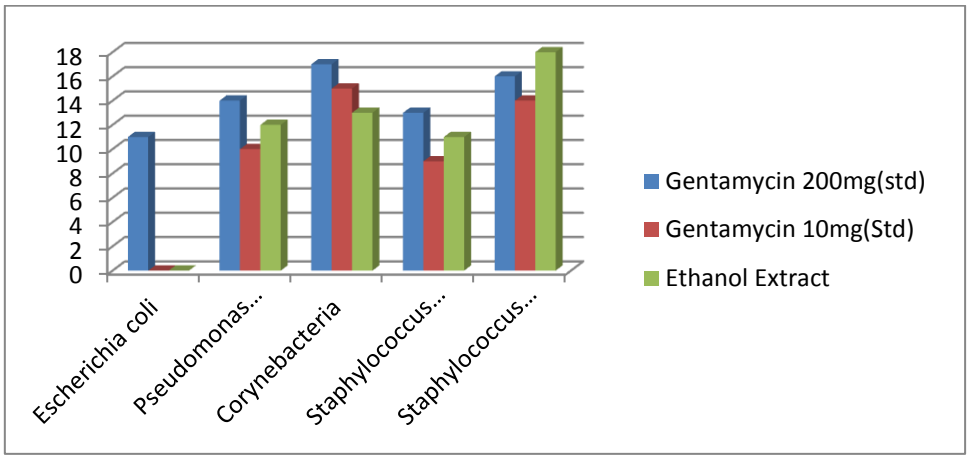


TABLE 2: Invitro activity of Neem leaves in Ethanol extract against opportunistic pathogens.

S.No	Name of the Organism	Gentamycin 200mg(std)	Gentamycin 10mg(Std)	Ethanol Extract
1	Escherichia coli	11 mm	-	-
2	Pseudomonas aeuroginosa	14mm	10mm	12mm
3	Corynebacteria	17 mm	15mm	13mm
4	Staphylococcus aureus	13 mm	9mm	11mm
5	Staphylococcus epidermidis	16mm	14mm	18mm

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 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).

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REFERENCES

1. 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.
2. Sonia Bajaj, Srinivasan B.P. (1999) Investigation into the Anti diabetic activity of *Azadirachta indica*. *Indian journal of pharmacology* 31:138-141.
3. Saseed A. Khan and Junaid Aslam (2008) Study on the effect of Neem (*Azhadirachta indica*) leaves smoke in controlling airborne Bacteria in Residential premises. *Current research in Bacteriology*1 (2): 64-66.
4. 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.
5. Srivastava A Shukla Kumar YN (2000) Recent development in plant derived antimicrobial constituents A Review. *J Med Arom Pl. Sci*.20: 717-72.
6. Faiza Aslam, Khalil.Ur. Rehman, Mohammad Asghar and Muhammed Sarwar (2009) Antibacterial activity of various Phytoconstituents of Neem. *Pak. J.Agri. Sci.*, Vol. 46(3), 456-463.
7. 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.
8. Saradha jyothi Koona, 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.