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Evaluation of antimicrobial activity of *Azadirachta indica* bark extract

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Abstract
Antimicrobial activity in bark extract of neem (*Azadirachta indica*) against human pathogenic bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Antimicrobial activities of chloroform and alcoholic extracts of neem bark were used. Varying concentration of each extracts 1250 mg/ml, 625 mg/ml, 312mg/ml, 156mg/ml were prepared by using disc diffusion method. When compared with cefpodoxime 500mg the chloroform and ethanol extract shows maximum inhibition on *the microorganisms in* ascending order.

Keywords: Neem, chloroform extract, zone of inhibition, antimicrobial activity, bacteria

Introduction
The neem *A. indica* is a common medicinal plant in India having high and wide spectrum of biological activity and well known for its insecticidal properties and one of the most promising natural compounds [1]. Neem is called "Arista" in Sanskrit - a word that means "perfect, complete and imperishable". This eco-friendly native tree of India is perhaps most researched tree in the world. It is a fast growing broadleaved tree, native to the arid regions of the Indian subcontinent to be found in most tropical countries [2]. It has been in use since ancient times, to treat a number of human ailments and also as household pesticide [3]. It is renowned for its relative paucity of natural pests and pathogens, with over 300 compounds from the tree have been isolated and characterized [4]. The neem extract from the bark, leaves, fruits and roots have been used to control leprosy, intestinal helminthiasis and respiratory disorders [5]. Every part of the neem tree has been used as traditional medicine for house-hold remedy against various human ailments from antiquity. The tree is still regarded as Village dispensary'. It is a plant known over 2000 years as one of the most versatile medicinal plants having a wide spectrum of activity in both developing countries [6].
Several pharmacological activities and medicinal applications of various parts of neem are well known [7]. Biological activity of neem is reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil [8]. However, crude extract of different parts of neem have been used as traditional medicine for the treatment of various diseases. Various parts of the neem tree have been used as traditional ayurvedic medicine in India from time immemorial. The medicinal utilities have been described, especially for leaf, fruit and bark. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident. Neem oil finds use to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis. However, apart from these uses, there are several reports on the biological activities and pharmacological actions of neem based on modern scientific investigations [9].

Material and Methods
Collection of plant bark material
The barks of *Azadirachta indica* were collected and were shade-dried, cut into small pieces and coarsely powdered. The coarse powder was used for extraction with various solvents.

Solvent
Chloroform and Methanol were used as solvent for extraction as they are purchased from Merck Indian Pvt. Ltd.

Preparation of plant extracts
Chloroform extract
Thousand grams of dry powdered barks were taken in conical flask; 2.5 liters of solvents (water and chloroform) were used and the mixtures were shaken approximately for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled. The extracts were filtered before drying using Whatman filter paper no.1 on a Buchner funnel and the solvent was removed by vacuum at 40 ˚C; the extracts were placed in pre-weighed flasks before drying. Finally the chloroform extracts of *A. indica* barks were used for the antibacterial activity.

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. Again the chloroform extracts of *A. indica* barks were used for the antibacterial activity.

Antibacterial Activity
Chloroform extracts of *A. indica* were subjected for antimicrobial activity by disc diffusion method against Gram negative organism known as *Pseudomonas aeruginosa* and *Staphylococcus aureus* where zone of inhibition area was measured.

Agar disc diffusion method
This method 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 [10].

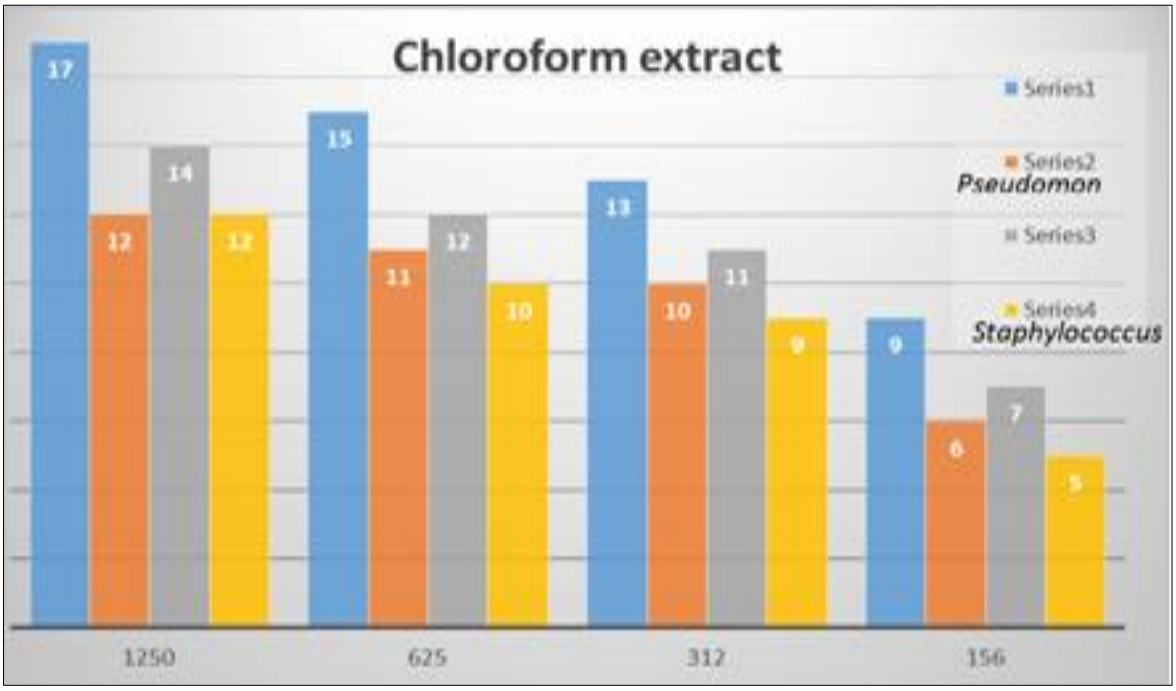
Preparation of cultures
Bacteria was cultured in sterile nutrient broth medium which had been autoclaved at 121 °C under a pressure of 15 atmospheres for 15 min. and left to grow for 48 h at 37 ^{0}C in an incubator. The bacterial cultures obtained were diluted with autoclaved Nutrient. This culture served as the inoculums for the antimicrobial experiments.

Preparation of agar plates for Antimicrobial activity
After checking the solubility of neem extract required Nutrient agar plates were prepared by mixing Nutrient agar (20 g) with sufficient peptone, beef extract and NaCl in 1000 ml distilled water boiled to dissolve the medium completely. Nutrient agar solution was sterilized by autoclaving at 121 ^{0}C for 15 min at 15 lb pressure. After cooling (45 ^{0}C), agar solution (25 ml) were poured into sterilized Petri dishes and left to solidify. Agar plates were inoculated with an overnight bacterial culture, using spread plate method after appropriate serial dilutions. Nutrient agar plates were used for *Pseudomonas aeruginosa*. The extract was aseptically put into the wells in different concentration made in agar plates making lawns of *Pseudomonas aeruginosa*. The Nutrient agar plates were then incubated at 37 ^{0}C for 24 h. The diameter of inhibitory zone surrounding disc was then measured after 24 hours and compared with standard. Two cross sectional points and the average was taken as the inhibition zone and the size of the zone diameter was measured. The plates were then photographed individually. Antibiotic solution was prepared as standard solution in the experiment as cefpodoxime was used. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The minimum inhibitory concentration values were determined by broth dilution assay of microdilution assay. Varying concentrations of the extracts (1250mg/ml, 625mg/ml, 312mg/ml and 156mg/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 and Discussion

Table 1: *In vitro* activity of Neem leaves in Chloroform extract against opportunistic pathogens

Serial No.	Concentration(ug/ml)	Name of the Organism	Standard Drug (Zone of inhibition in mm)	Extract (Zone of inhibition in mm)
1	1250	*Pseudomonas aeruginosa*	17	12
2	625		15	11
3	312		13	10
4	156		09	06
5	1250	*Staphylococcus aureus*	14	12
6	625		12	10
7	312		11	09
8	156		07	05

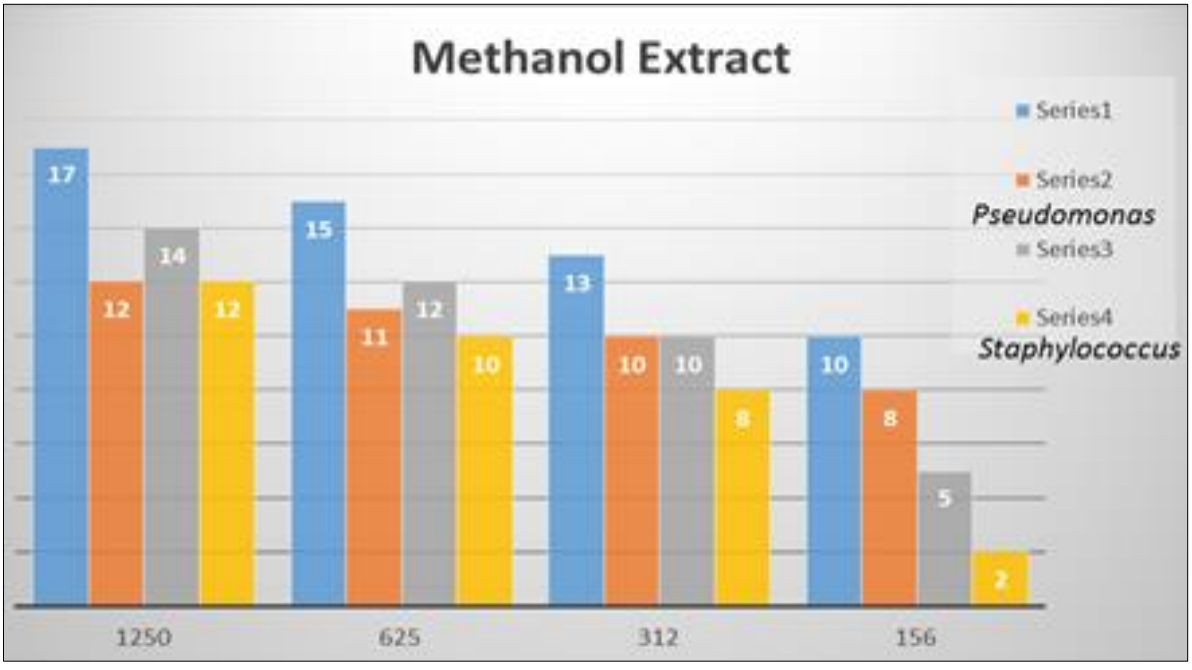


From the above result it is evident that the extract shows its antimicrobial activity in ascending order.

Fig 1: Zone of inhibition of Chloroform extract

Table 2: *In vitro* activity of Neem leaves in Methanol extract against opportunistic pathogens.

Serial No.	Concentration(ug/ml)	Name of the Organism	Standard Drug (Zone of inhibition in mm)	Extract (Zone of inhibition in mm)
1	1250	*Pseudomonas aeruginosa*	17	12
2	625		15	11
3	312		13	10
4	156		10	08
5	1250	*Staphylococcus aureus*	14	12
6	625		12	10
7	312		10	08
8	156		05	02



Again from the above table it is evident that the extract shows its antimicrobial activity in ascending order.

Fig 2: Zone of inhibition of Methanol extract

Conclusion

It is evident from the above experiment that Azadirachta indica bark extracts has potent antimicrobial effect and in future this plant can be used further against other pathogenic microbes for antibacterial effect.

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