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Effects of Plain, Hill and Coastal Neem (*Azadirachta indica*) Extracts Against Human Pathogenic Bacteria

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Abstract: The present study was finding out the *in-vitro* inhibitory (Antibacterial) effect of the aqueous extracts of *Azadirachta indica* from three different ecological habitats (The plains, hills and costal region) on selected bacterial species. Plant leaves extract were used for *in-vitro* inhibitory (Antibacterial) effects on selected bacterial species. The bacterial strains *Bacillus subtilis*, *Escharichia coli*, *Salmonella typhi*, Serratia sp, Hexomonas sp microorganisms by perez agar well diffusion method. The present study result showed the neem leaves (*A. indica*) extract exhibited maximum zone of inhibition in plain against *Salmonella typhi*. It was recorded that the neem (*A.indica*) extracts exhibited moderate levels of inhibition in coast area against *bacillus subtilis*. The present work to find that antibacterial activity of *A.indica* plant leaves extracts showed a good result in plains hills and coastal area for both gram positive and gram negative bacteria. The present finding suggesting that the plant *A. indica* may offer various biocontrol agents towards crop production strategies for antibacterial activity against important bacterial, various health benefits and eco-friendly in environment.

Key words: Azadirachta Indica · *In-vitro inhibitory* · *Salmonella typhi* · Ecological habitats

INTRODUCTION

The neem tree serve as a valuable source of unique natural products for medicine, industry, integrated pest management and other purposes. It has become important in the global context today because it offers answer to the major concerns facing mankind [1]. Two species of *Azadirachta indica* have been reported, *A. indica,* native to Indian subcontinent and *Azadirachta excelsa* Kack. Confined to Philippines and Indonesia. Presently neem trees can be found growing successfully in about 72 countries worldwide, in Asia, Africa, Australia, North, Central and South America [2].

The neem tree is perhaps the most useful traditional medicinal plant in India. Studies on various parts of neem tree have found more than 135 chemical compounds so far. These compounds have been divided into two major classes: isoprenoids (Like diterpenoids and triterpenoids containing protomeliacins, limonloids, azadirone and its deravatives, gadunin and its deravatives, vilasinin type of compounds and C- secomiliancins such as nimbin, salanin and azadirachtin) and non-isoprenoids, which are proteins, amino acids and carbohydrates (Polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochateone, coumarins, tannins and aliphatic compounds. Among these, many bioactive compounds such as azadirachtin, nimbin, salanin, meliantriol, etc., have been identified and the most active ingredient is reported as azadirachtin [3]. To date over 195 species of insects are affected by neem extracts at concentrations ranging from 0.12 to 1000 ppm and more important insects that have become resistant to synthetic pesticides are also controllable with these extracts[4].

Ecology: The neem tree has adaptability to a wide range of climatic, topographic and edaphic factors. It thrives well in dry, stony shallow soils and even on soils having hard calcareous or clay pan, at a shallow depth.

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The chemical ecology of *Azadirachta indica* is little known. There is a controversy over variations in azadirachtin content in *A. indica* seeds among various provenances in India was undertaken by Sidhu *et al*. [5] to evaluate qualitative and quantitative variability in azadirachtins A and B among various neem provenances or individual neem trees. The study also revealed that there are individual genetic differences among neem trees. The study was done in 43 provenances of India covering the five agro climatic regions of the country. They found wide variations in oil percentage and azadirachtin A and B contents quantified using reverse phase analytical HPLC among different provenances. Azadirachtin A ranged from 556.9 to 3030.8 mg kg^{-1} of kernels, whereas azadirachtin B was in the range 43.1-590.6 mg kg^{-1} of kernels among the provenances investigated. Variations among individual trees of a particular provenance indicated that climatic factors such as rainfall, humidity, or temperature did not influence azadirachtin content in the neem trees.

The present study was carried out with the objective of finding out the *in-vitro* inhibitory (Antibacterial) effect of the aqueous extracts of *Azadirachta indica* from three different ecological habitats (The plains, hills and costal region) on selected bacterial species.

MATERIALS AND METHODS

Collections of Plant Materials: Healthy, fresh and mature leaves *Azadirachta indica* were collected from different ecological habitats. The plains sample was collected from Tiruchengode, Namkkal District, the hills sample was collected from Yercaud (Servarayan hills near Nagalur), Salem District and the coastal region sample was collected from Thondi (Latitude: 9° 44' N and Longitude: 79° 00E), (Ramnadu district).

Preparation of Neem Leaf Extract: Mature fresh neem leaves collected from the three ecological habitats (Plains, hills and costal) were washed thoroughly with sterile distilled water and 25gm of the leaves were weighed in sterile disposable cup and were added to 50ml absolute ethanol. The mixture was macerated for 1-2 mins and care was taken to prevent temperature rise beyond 45-50°C. The extract was than filtered through muslin cloth for coarse residue. Extraction process was repeated again using course residue and 25 ml ethanol. Both the extract pooled together and filtered through a fast filter paper. The alcohol part of extract was removed by keeping the extract was water bath at 75°C, for about 30 mins. Resultant solution (25ml) was collected and transferred to airtight amber bottle and stored at 4°C in refrigerator. The antimicrobial activities of the neem extract was tested in 50, 75, 100 µl concentration.

Test Microorganisms: The following bacterial cultures were used for the screening of anti-bactrial activity of the ethanolic extract of neem. The bacterial strains Bacillus spp., *Escherichia coli*, Salmonella spp., Serratia spp. and Hexomonas spp. were sub cultured from culture collection centre of SASC. One loop full of pure culture was taken from each bacterial strain and inoculated in to a 10ml nutrient broth, the cultures then incubated at 37°C for 24-48 hrs. After the incubation period the culture were labelled stored at 4°C in refrigerator.

Determination of Anti- Bacterial Activity: The Antibacterial activity was tested using agar well diffusion method perez *et al*. [6] Briefly, the nutrient agar plates were prepared and bacterial broth cultures were spread onto each plate. Using sterile cork borer, four wells (6mm diameter) were made in each plate in four directions and filled with different concentration of plant extracts were added into the well. Plates were incubated at 37°C for 24 to 48 hours. Proper controls of the respective solvent of plant extracts were maintained. After incubation, the zones of incubation were expressed on mm.

RESULTS

In the present study, an attempt was made to determine the antibacterial activity of the ecologically diverse (Hill, plain and coastal) population of neem (*Azadirachta indica*) against five bacterial strains such as *Bacillus subtilis, Escherichia coli, Salmonella typhi,* Hexomonas spp and Serratia spp. The zone of inhibition was measured with various concentrations of extracts such as 100mg/ml, 75mg/ml and 50mg/ml. Antibacterial activity of neem leaves (*Azadirachta indica*) from plains, hills and coastal area showed good antimicrobial activity against human pathogens (Table 1). In plains the maximum zone of inhibition at 100mg/ml concentration was observed as 24mm against *S. typhi*. The minimum zone of inhibition 50mg/ml concentration was observed as 18mm against *S. typhi*. In contrast hill shown ethanol extract, the maximum zone of inhibition at 100mg/ml concentration was observed as 22 mm against *S. typhi*.

Table 1: Antimicrobial activity of different ecology environment against human pathogens

	Inhibition in different concentrations and zone of diameter in mg/ml								
	100mg/m			l75mg/ml			50mg/ml		
Bacterial Pathogens	P	H	C	P	H	C	P	H	C
Bacillus subtilis	21	20	23	19	17	18	17	14	15
Escherichia coli	22	21	18	18	18	17	16	15	12
Salmonella typhi	24	22	19	22	16	17	18	14	16
Hexomonas sp	23	21	22	19	15	19	18	13	17
Serratia sp	23	21	20	21	19	19	17	16	13

P- Plains; H- Hilly; C- Coastal

The minimum zone of inhibition 50mg/ml concentration was observed as 14mm against *S. typhi*. Likewise coastal neem extract showed the highest activity at 100mg/ml concentration as 23mm against *Bacillus subtilis*. The minimum activity 50mg/ml concentration was recorded 11mm *Bacillus subtilis*.

DISCUSSION

The methonol of extract plain of the leaves of *Azadirachta indica* exhibited pronounced activity 28mm against *Bacillus subtitles*, followed by 18mm to *staphylococcus auras* and 1.1cm against *Escherichia coli*. Leaves of *Azadirchta indica* showed high activity (2.8-1.8cm) against *Bacillus subtitles* and *Escherichia coli* respectively. The ethanol neem stick extract had higher *antibacterial* properties tham the leaves extract. Neem leaves had the active component of Azadirachtin (1-3%). Whereas the active components present neem sticks is tannin (6%) [7].

The difference in the antibacterial effects was probably because of the different percentage of the active compounds of phenol group found in both neem leaves and sticks. Neem leaves and roots are good for blood circulation and blood purifiers. Neem has been became extensively used in Ayurvedla, Unani and Homeopollic medicine. Neem leaf and its constituents have been demonestrated to exhibit immunodulatory, anti-inflamatory, antilyparlycaemic, antiulcer, antimalarial, antifungal antibacterial and anti carcinogenic properties.

Tested neem seed oil showed bactericidal activity against 14 strains of pathogenic bacteria. Crude aqueous and solvent extracts of neem were tried against 20 strains of pathogenic bacteria wherein crude extract produced better results Baswa *et al*. [8]. But in the present study, the neem leave extracts obtained from the hill, plains and coastal region showed different inhibiting activity against human pathogenic bacteria.

The antibacterial activity of neem leaves from in the plains. Ethanol extract residues were obtained by crude extract method to prepare various concentrations such as 50, 75 and 100mg/ml respectively. *Salmonella typhi* in the ethanol extract, the maximum zone of inhibition 100mg/ml concentration was observed as 24mm.The minimum zone of inhibition 50mg/ml concentration was observed as 18mm. No inhibition found in control.

The antibacterial activity of neem leaves (*Azadirachta indica*) from the hills. Likewise, the maximum zone of inhibition 22 mm observed against *Salmonella typhi* at 100mg/100ml concentration. The minimum zone of 14mm inhibition found at 50mg/ml concentration. The control was observed as no inhibition. In contrast coastal neem plant extracts show the maximum zone of inhibition at100 mg/ml concentration as 24mm against *Bacillus subtilis*. The moderate level 12mm of inhibition were observed at 50mg/ml concentration against *E. coli*. Similarly, Noor *et al*. [9] has been reported that 15 mm, 16.5mm and 20mm zone of inhibition was observed neem extracts against human pathogenic bacteria *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureure*. In contrast, Dhayanithi *et al*. [10] reported that Entrobacter and *E. coli* showed 14 and 15 mm zone in inhibition at methanolic neem extracts. Antibacterial activity of neem leaves (*Azadirachta indica*) different ecological variation of plains hills as well as coastal area. More antibacterial activity seen in ethanol extract obtained from neem leaves in plains in the hilly region. The present studies was concluded that hilly and plains neem extracts exhibiting strong inhibition activity than the coastal neem extracts.

CONCLUSION

The finding of the present study indicates that *Azadirachta indica.,* may be used as a biocontrol agent

towards crop production strategies for antibacterial activity against important bacterial activity against important bacterial pathogens and forms an important steps in developing plant based bacterial which are eco-friendly for the management of the bacterial pathogens and development commercial formulation based on field trail and toxicological experiment.

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