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ANTIMICROBIAL SCREENING OF LEAF EXTRACT OF *Azadirachta indica* A. *Juss.*

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Key words : Antibacterial potential, Azadirachta indica, methanolic extracts, Salmonella.

The present investigation revealed that most of the plant extracts are found to be effective against Gram-negative bacteria. Azadirachta indica crude extracts is found to have an effective antibacterial properties. *A indica* methanolic leaf extracts are found to be effective specially against *E.Coli* and *Salmonella typhimurium*.

INTRODUCTION

The human beings still dependent on nature for remedies is well apparent from the fact that all the major systems of medicines eg. Ayurveda Unani and Homeopathy are largely based on drugs of plant origin. In spite of tremendous development in the field of allopathy during 20th century, plants still remain one of the major sources of drugs of modern as well as traditional systems of medicine throughout the world. Chemically, depending on their active principles, plants may have alkaloids, glycosides, steroids or other groups of compounds which may have marked various pharmaceuticals actions. *Azadirachta indica* A. Juss. Syn. *Melia azadirachta* Linn was selected for screening for their antibacterial activities against *Salmonella typhi*, Bacillus. subtilis, Staphyllococceaus. aureus, *E. coli*, *Pseudomonas aeruginosa* under methanolic and ethanolic leaf extracts.

Azadirachta indica of family Meliaceae is a highly esteemed tree and has been closely related with the socio-cultural and religious aspect of Indian life since ancient times. Siddiqui *et al.* (1992) isolated a new limonoid which showed significant antibacterial activity against various Gram-positive and Gram-negative bacteria. Das et. al (1999) tested the emulsified product of neem kernel against fish pathogenic bacteria and found reduction in the bacterial cell population. The bark extract showed high bactericidal property as compared to the leaves (Fabry et.al, 1998). Vanka et.al., (2001) found some lactobacilli resistant to the *A. indica* extract.

MATERIALS AND METHODS

Azadirachta indica A. Juss was selected for the present investigation. Specific parts (fresh leaves) of these plant were utilized for the preparation of crude extracts. The completely shade dried material was coarsely powdered and allowed for successive extractions with methanol and ethanol. The obtained liquid extracts were subjected to Rotary evaporator and subsequently concentrated under reduced pressure (in Vaccum at 40°C) and evaporated to dryness and stored at 4°C in air tight bottle.

Methanol extract

50 gms of dried leaf powder of fresh leaves of concerned plant species were taken in a separate container, 250 ml of methanol was added and kept 24 hrs with periodic shaking then filtered and the filtrate was collected. The process was repeated three times with fresh volume of methanol.

Ethanol extracts

50 gms of dried leaf powder of fresh leaves of concerned plant species were taken in a separate container and then 250 ml of ethanol was added and kept for 24 hrs with periodic shaking, filtered and filtrate was collected. Procedure was repeated three times. The clear liquid thus obtained was filtered through Whatman filter paper (No. 1) and stored in Pre-sterilized screw capped containers.

Microorganisms

The pathogenic strains of *Escherichia coli, Salmonells typhimurium, Staphylococcus aureus, Bacillus subtilis* and *Proteus mirabilis* were used. All these bacterial strains were obtained from Sargam Laboratory Pvt. Ltd. Manappakkam, Chennai. Antimicrobial screening was done by Agar-diffusion method (Murray et.al , 1995) at 30-37°C. The well was prepared in medium after inoculation with microorganisms. When well is loaded with antibiotics, it diffuses in the medium and inhibits the growth of organisms. There is logarithmic reduction in antiobiotic concentration. The zone of inhibitions of bacterial growth around each

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well is measured and the susceptibility is determined. MMH Agar (3.8 gm/100 ml of distilled water) was prepared and autoclaved at 120°C for 15 minutes at 15 lbs and poured in sterile petriplates up to a uniform thickness of approximately 5-6 mm and the agar was allowed to set at ambient temperature and then used.

RESULTS AND DISCUSSION

The results showed that the extract possessed antimicrobial activity against the tested organisms, depending upon the nature of the active ingredients present in the extracts and their capacity for diffusion into agar medium. Zone of inhibition activity of the methanolic extract of the *A. indica* found to be significant against *E.coli* and *Salmonella* respectively which has been shown in Table 1,2,3 & 4. Methanolic extract of *A. indica* showed an average inhibitory zone diameter (2.0 cm, 2.2 cm, 2.4 cm and 2.1 cm) which indicates that methanolic extract gave best result having zone of inhibition greater than that of antibiotic Gentamycin (2.4, 2.7, 2.5 and 2.6 cm) against E.coli while methanolic extract of A.indica showed an average inhibitory zone diameter of 1.8, 2.2, 2.0 and 2.1 cm respectively which indicates that methanolic extract showed the best result having zone of inhibition greater than of the standard antibiotic Gentamycin (3.4, 3.3, 2.8 and 3.1 cm) against *Salmonella*. The methanol extract of *A.indica* against *E.coli and Salmonella* bacteria showed varieties of zone of inhibition.

Table - 1 : Antibacterial activity of methanol leaf extracts of *A. indica against Salmonella*.

Concentrations	Well Diameter (cm)	Zone of inhibition (cm)	Inhibition length (cm)
0.50%	0.8	1.8	1.2
1.00%	0.8	2.2	1.5
1.50%	0.8	2	1.3
2.00%	0.8	2.1	1.2

Table - 2 : Antibacterial activity of methanol leaf extracts of *A. indica against E.coli*.

Concentrations	Well Diameter (cm)	Zone of inhibition (cm)	Inhibition length (cm)
0.50%	0.8	2	1.3
1.00%	0.8	2.2	1.5
1.50%	0.8	2.4	1.6
2.00%	0.8	2.1	1.2

Table - 3 : Antibacterial activity of Gentamycin against *Salmonella.*

Concentrations	Well Diameter (cm)	Zone of inhibition (cm)	Inhibition length (cm)
0.50%	0.8	3.4	2.1
1.00%	0.8	3.3	2.6
1.50%	0.8	2.8	2.3
2.00%	0.8	3.1	2.1

Table - 4 : Antibacterial activity of Gentamycin against *E.coli*.

Concentrations	Well Diameter (cm)	Zone of inhibition (cm)	Inhibition length (cm)
0.50%	0.8	2.4	1.2
1.00%	0.8	2.7	2
1.50%	0.8	2.5	1.4
2.00%	0.8	2.6	1.9

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