

Original article

Effect of AZADIRACHTA INDICA (Neem) leave extract on Some Selected Bacteria

DOI: <https://doi.org/10.47648/zhswwmcj.2021.v0302.01>

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Abstract

Azadirachta indica (Neem) is an herbal plant widely distributed in our subcontinent during all seasons. This study was carried out to detect the effect of *Azadirachta indica* (Neem) leave extract on some selected bacteria. The prospective study was carried out during the period of July 2005 to June 2006. Micro-organisms which are commonly responsible for infection in our country such as *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* were considered. Extract of Neem leave that is Aqueous Neem leave extract (ANLE) was prepared by filtration and evaporation. Effect against bacteria was examined by detection of minimum inhibitory concentration (MIC) by using 'Broth dilution method' and by detection of bacterial susceptibility by 'Agar disc diffusion method' and compare with cefepime. For ANLE the MICs against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia Coli* and *Pseudomonas aeruginosa* was 800 µg/ml, 1200 µg/ml, 1400 µg/ml and 1400 µg/ml respectively. With those MICs the average diameter of zone of inhibition against *staphylococcus aureus* with ANLE and cefepime was 20mm and 30mm respectively. *Salmonella typhi* exhibit sensitivity with zone of inhibition of 19mm, 26.5mm against ANLE and cefepime. *Escherichia coli* were sensitive against ANLE and cefepime with 17.5mm, 23.5mm zone of inhibition respectively. For *Pseudomonas aeruginosa* zone of inhibition was 16mm, and 21mm by ANLE, and cefepime respectively. Aqueous Leave extract of *Azadirachta indica* (Neem) have antibacterial activity against '*staphylococcus aureus*, *salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*' compared with Cefepime. Among all test bacteria *staphylococcus aureus* has lowest MICs.

Key words: *Azadirachta indica* (Neem), Cefepime, Aqueous Neem leave extract (ANLE), Antibacterial activity.

Introduction

Bangladesh is a developing country. Health status of its majority population is poor. Maximum people of this country cannot afford their expenditure for modern medical treatment. Bangladesh has friendly environment for cultivation and growth of many kinds of medicinal plants. The herbal medicines are used along with modern medicine and gives significant effects. So, we cannot avoid the usefulness and beneficial effect of herbal medicine.

Herbs are used for thousands of centuries by many cultures for their medicinal values. Herbal treatment is very popular because it is easily available, cheap and less toxic. *Azadirachta indica* (Neem) is a herbal plant widely distributed in our subcontinent during all seasons. Each part of Neem tree has some medicinal property. Neem leave, bark extracts and Neem oil are commonly used for therapeutic purpose¹.

So, Neem has been extensively used in Ayurvedic, Unani and Homeopathic medicine. But all of its constituents are not scientifically evaluated. The Sanskrit name of

Neem tree is "Arishtha" means reliever of sickness and hence it is considered as "sarbaroganibarini"^{2,3}. The tree is still regarded as Village dispensary. Food and drug Administration (FDA) believes that anything from neem has to be good. According to R.O. Larson Neem a tree for global problems because Neem is a versatile multipurpose tree².

Neem leave, bark extracts and neem oil have been therapeutically used to control leprosy, intestinal helmenthiasis, Respiratory disorders, Constipation, antifertility, antiulcer, antitumour, hypoglycemic effect, antimalarial, antifungal, antibacterial, antiviral, dental care and also in Rheumatic heart disease and specially for some important skin disease³.

Oil from leaves, seeds & bark of Neem possesses a wide spectrum of anti bacterial action against Gram-negative and Gram-positive microorganisms including *M. tuberculosis*. In vitro it inhibits *Vibrio cholerae*, *Klebsiella pneumoniae*, *M. tuberculosis*, *M. pyogenes*, *Streptococcus mutans*, *S. faecalis*, *Staphylococcus*

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aureus and *salmonella typhora*^{4,5}.

This antimicrobial potential of *Azadirachta indica* leaves extract may be due to its constituents. Indeed, the phyto-constituents alkaloids, glycosides, flavanoids and saponins which are important components of *Azadirachta indica* contain antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens⁶.

The study was designed to find out the effect of *Azadirachta indica* (Neem) Leave Extract on Bacteria.

Materials & Methods

Aqueous Neem leave extracts (ANLE) was obtained by crushed leaves in mortar under pestle then mixed with distilled water. Kept for 24 hours then filtrated by fine cloth followed by vacuum pump. Then concentrated by Rotary vacuum pump after that make powdered by using freeze dryer. After extraction the ANLE was diluted with different amount of distilled water to obtain different concentration of extracts. *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*. Individual bacterial strains in pure state (as single colony isolate) were obtained from the Microbiology department of Dhaka Medical College. The growth of test organism in each concentration of ANLE was examined and compared with controls by matching their turbidity. The clear preparation was considered as no growth. The lowest concentration at which bacteria were inhibited as judged by lack of turbidity considered as MICs.

The disc containing plant extracts at different MICs was poured to the disc and cefepime (30µg/disc) were placed on the plate with a sterile forceps and each of them was slightly pressed against agar surface. The diameter of zone of inhibition denotes the relative susceptibility to a particular antimicrobial agent which was detected by the formation of a clear zone around the disc. The diameter of zone of inhibition measured in mm on the under surface of the petridish using a transparent scale.

Results

Effects of leave extract have been studied against some Gram-positive and Gram-negative organisms. A perusal of the data in Table I reveals that the extracts of Neem leave possess antibacterial activity depending upon concentration.

By ANLE the zone of inhibition against *Staphylococcus aureus* was 20 mm with disc potency of 800 µg/ml. For *Salmonella typhi* the disc strength was 1200 µg/ml and zone of inhibition was 19 mm. against *Escherichia coli* and *Pseudomonas aeruginosa* disc strength was 1400 µg/ml but inhibitory zone was 17.5 mm and 16 mm respectively.

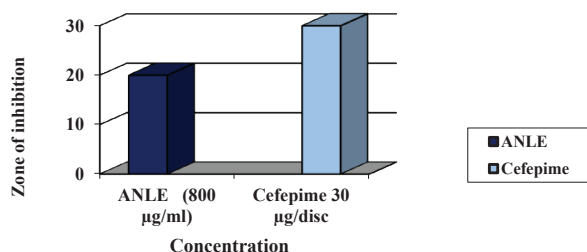
In case of *Staphylococcus aureus*, *salmonella typhi*, *Escherichia coli* & *pseudomonas aeruginosa* was sensitive against cefepime at 30µg/disc potency which compare with zone interpretative chart. That is diameter of zone of inhibition was more than 18mm. So, all bacteria were sensitive to Aqueous Neem leave extracts (ANLE).

Table I: Diameter of zone of inhibition by different MICs of ANLE and cefepime against test bacteria:

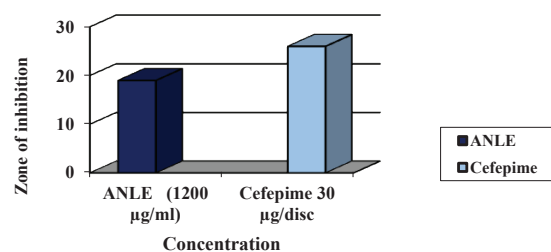
Neem Leave extract & Antimicrobial discs	Name of Bacteria with Average diameter of Zone of Inhibition							
	Staphylococcus aureus		Salmonella typhi		Escherichia coli		Pseudomonas aeruginosa	
	Disc Strength	Zone of inhibition	Disc Strength	Zone of inhibition	Disc Strength	Zone of inhibition	Disc Strength	Zone of inhibition
ANLE	800µg/ml	20 mm	1200µg/ml	19 mm	1400µg/ml	17.5 mm	1400µg/ml	16 mm
Cefepime	30µg/disc	30 mm	30µg/disc	26 mm	30µg/disc	23.5 mm	30µg/disc	21 mm

***ANLE – Aqueous Neem leave extract**

A. Diameter of zone of inhibition by Neem leave extract and Cefepime against *Staphylococcus aureus*



B. Diameter of zone of inhibition by Neem leave extract and Cefepime against *Salmonella typhi*



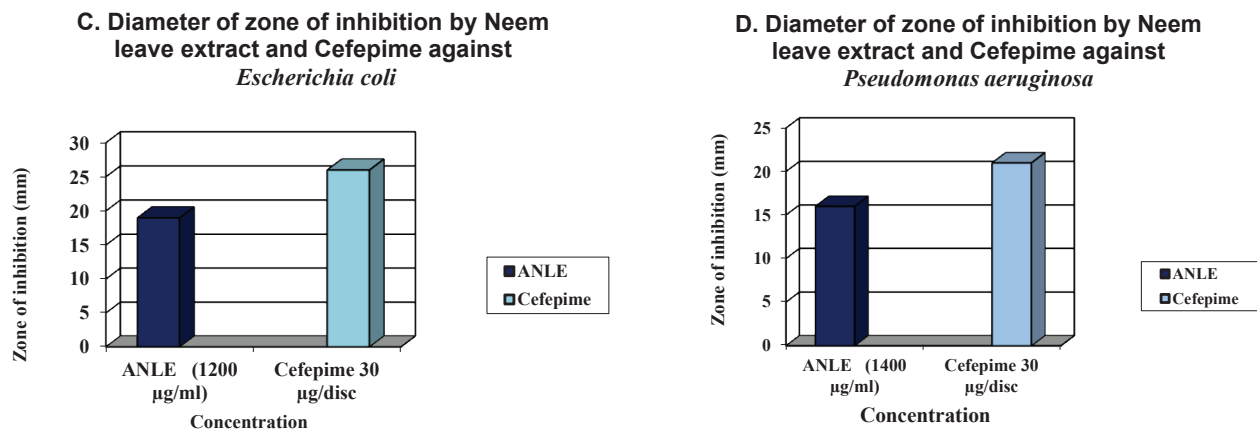


Fig.1. Bar diagrams shows minimum inhibitory concentration of ANLE along with their diameter of zone of inhibition of A.

Discussion

Nowadays, the up growing resistance of microorganisms to the convectional antimicrobial agents is a source of great concern to clinical microbiologists. Bacteria evolve some changes in their genome with time, as a result, a large number of bacterial species particularly *Salmonella* and *E. coli* ⁷ have become resistant to the antibacterial drugs due to extensive use and often create a problem in treatment of infectious diseases.

Medicinal plants having antimicrobial compounds in comparison with antibiotics, usually with fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature⁸.

The results of the present work revealed that the leaves extract of neem showed an interesting inhibitory action on a wider spectrum of microorganism using agar well diffusion technique. The antibacterial property of *Azadirachta indica* leaves reported in this study is in line with the report by Faiza aslam *et al.* ⁹

Azadirachta indica leaves has been reported to possessed good anti bacterial activity and this lead Saradhajyothi and Subbarao ¹⁰ to conclude it confirmation as a great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care.

Zone of inhibition produced by Aqueous neem leave extract show a linear relationship with the concentration of the juice. Higher concentration produced bigger zone of inhibition. This is sufficient to prove that fresh Aqueous neem leave extract contains compound

with antibacterial activities. By comparing the size of inhibition zone, *Staphylococcus aureus* showed a higher sensitivity to Aqueous neem leave extract.

Compared to the inhibitory zone produced by commercial antibiotics, the inhibitory zone of Aqueous neem leave extract was smaller. This may be because the active compound in Aqueous neem leave extract is not high or concentrated enough to produce the same antibacterial effect as compared to commercial antibiotics where their bacteriostatic and bactericidal effect has been determined by a lot of research.

Convincingly, *Azadirachta indica* leaves extracts demonstrated bactericidal potential against both gram positive and gram-negative bacteria and as such an indication that the plant can be a source antibacterial drug. Hence, extracts of *Azadirachta indica* used as medicine, could be useful in the inhibition of human biotic bacterium. Further studies are recommended to resorting the herbal compounds having antimicrobial characteristics instead of synthetic antibiotic drugs.

Conclusion

In this study it is observed that, leave extract of *Azadirachta indica* (Neem) that is Aqueous Neem Leave extract have antibacterial activity against '*staphylococcus aureus*, *salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa*' compared with Cefepime. Among all test bacteria *staphylococcus aureus* has lowest MICs and Gram-positive organisms are more sensitive/ susceptible than Gram-negative organisms.

Staphylococcus aureus , *B. Salmonella typhi* , *C. Escherichia coli* , *D. Pseudomonas aeruginosa*.

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