

Azadirachta indica* (NEEM): antibacterial effects against *Escherichia coli* and *Salmonella

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Abstract

The main objective of the study is to evaluate antibacterial activity of Neem leaf extracts (methanolic) against *E. coli* and *salmonella* using Zone of Inhibition (ZOI) method. *Azadirachta indica* (neem) leaf extract was used to test antimicrobial activity against disease causing bacteria *E.coli* and *Salmonella* Methanol extracts of varying concentrations 0.5, 1.0, 1.5, and 2.0% was prepared and tested against test organisms using agar diffusion method. Gentamicin of same varying concentrations was used to compare the effect of antimicrobial activity of methanol leaf extract. Data revealed that methanol extract of Neem has shown highest antimicrobial activity as compared to other extracts.

Keywords: *Azadirachta indica*, *Escherichia coli*, *Salmonella*, Zone of Inhibition.

Introduction

Neem is used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries. Its twigs provide a chewing stick and are widely used in the Indian sub-continent earlier studies on Neem have showed that it contains active substances with multiple medicinal properties.¹ Knowledge of herbs has been handed down from generation to generation for thousands of years. Herbal drugs constitute a major part in all traditional systems of medicines.² Plants have been used for thousands of years to conserve food and treat health diseases. Recently, natural products have been evaluated as sources of antimicrobial agents with efficacy against a variety of microorganisms.³ *Azadirachta indica* is a tree in the mahogany family *Maliaceae*. Neem is the most useful traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical and semi-tropical countries. Its twigs are used as tooth brush and are widely used in the Indian sub-continent.⁴ Earlier studies on neem have showed that it contains active substances in almost every part of the seeds, leaves, roots, bark, trunk and

branches with multiple medicinal properties.⁵ It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.⁶ *A.indica* is used for the treatment of diabetes like neem biscuits and shows the potential role of anti-diabetic activity. Aqueous extract of neem leaf extract has a good therapeutic potential as anti-hyperglycemic agent.⁷ Anti-inflammatory effect of neem extract is less than that produced by dexamethasone. Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise.⁸ Neem seeds are used in traditional medicine to treat infections conditions especially those involving the eye and ear.⁹ Administration of alcoholic extract of neem flower disrupts the estrous cycle in Sprague Dawley rats and causes a partial block in ovulation and has the potential of an ideal antifertility agent.¹⁰ Neem aqueous extract has powerful chemotherapeutic and viral agent. The purpose of the present study was to investigate the antimicrobial activity of Neem leaves against disease causing bacteria, such as *Escherichia coli*, *Salmonella*.

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Material and Method

Selection of plant

Leaf extract

The completely shade dried material was coarsely powdered and allowed soxhlet for successive extraction with methanol. The obtained liquid extracts were subjected to Rotary evaporator and subsequently concentrated under reduced pressure (in vacuum at 40°C) and evaporated to dryness and stored at 4°C in air tight bottle.

Methanol Extract

50g of dried leaf powder were taken in a separate container. To this 250 ml 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.

Test microorganisms

The disease causing strains of *E.coli* and *Salmonella* were obtained from Indian Herbs Research and Supply Co. Ltd, Saharanpur.

Antimicrobial screening

Agar diffusion method-The method is suitable for organisms that grows rapidly overnight at 35-37°C. The well is made in medium after inoculation with microorganisms. When well is loaded with antibiotics, it diffuses in the medium and inhibits the growth of organism. There is logarithmic reduction in antibiotic concentration. The zone of inhibition of bacterial growth around each well is measured and the susceptibility is determined. Medium-Muller Hinton Agar (3.8 gm/100 ml of distilled water) was prepared, autoclaved at 121°C for 15 minutes at 15 lbs and poured in sterile petri plates up to a uniform thickness of approximately 5-6mm and the agar was allowed to set at ambient temperature and used. Inoculums-The microorganisms were inoculated in

The plant neem (*Azadirachta indica*) was selected for study. Its leaves were collected from Indian Herbs Research and Supply Co. Ltd, Saharanpur.

Nutrient broth and incubated at 37°C and were used as inoculums.

Method

25 µl of inoculum was spread over the MHA medium, using sterile spreader. After few minute, four wells were made in each Petri plate and loaded with 0.5, 1.0, 1.5 and 2.0% methanol extract. Similarly 0.5, 1.0, 1.5 and 2.0% gentamicin solution was added in another plate. Plates were incubated at 37°C for 24hrs. Antimicrobial activity was evaluated by measuring zone of inhibition by using Hi-media zone scale.

Result

The results of ZOI of the plant extract and its comparison with standard antibiotic Gentamycin is recorded in Table 3,4. The following 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. ZOI activity of the methanolic extract of the Neem found to be significant activity against *E. coli* and *salmonella* respectively which has been shown in Table-1,2. Methanolic extract of neem showed an average inhibitory zone diameter (2.0cm, 2.2cm, 2.4cm and 2.1cm) which indicates that methanolic extract gave best result having ZOI greater than that of standard antibiotic Gentamycin (2.4, 2.7, 2.5 and 2.6 cm) against *E.coli* while methanolic extract of Neem showed an average inhibitory zone diameter of (1.8, 2.2, 2.0, 2.1 cm) respectively which indicates that methanolic extract showed best result having ZOI greater than of the standard antibiotic Gentamycin (3.4, 3.3, 2.8, 3.1cm) against *salmonella*.

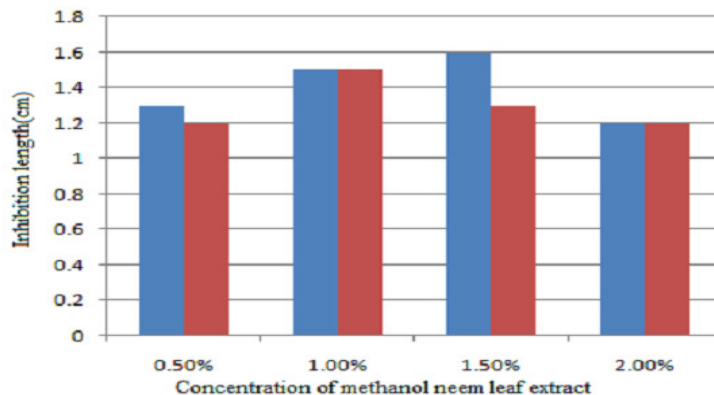
The methanol extract of *A. indica* against *E. coli* and *Salmonella bacteria* showed varieties zone of inhibition.

Table.1. Antibacterial activity of methanol neem leaf extracts against *Salmonella*.

Concentration	Well Diameter(cm)	Zone of inhibition(cm)	Inhibition length(cm)
0.5%	0.8	1.8	1.2
1.0%	0.8	2.2	1.5
1.5%	0.8	2.0	1.3
2.0%	0.8	2.1	1.2

Table.2. Antibacterial activity of methanol neem leaf extracts against *E.coli*

Concentration	Well Diameter(cm)	Zone of inhibition(cm)	Inhibition length(cm)
0.5%	0.8	2.0	1.3
1.0%	0.8	2.2	1.5
1.5%	0.8	2.4	1.6
2.0%	0.8	2.1	1.2



Blue-*E.coli*, Red-*Salmonella*

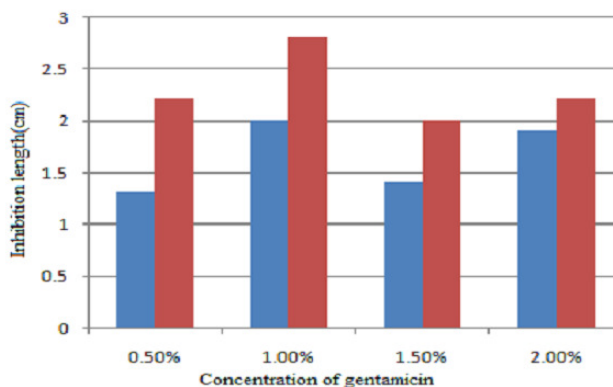
Graph.1.Antibacterial activity of methanol neem leaf extract against *E.coli* and *Salmonella*

Table.3 Antibacterial activity of Gentamicin against *Salmonella*

Concentration	Well Diameter(cm)	Zone of inhibition(cm)	Inhibition length(cm)
0.5%	0.8	3.4	2.1
1.0%	0.8	3.3	2.6
1.5%	0.8	2.8	2.3
2.0%	0.8	3.1	2.1

Table.4 Antibacterial activity of Gentamicin against *E.coli*

Concentration	Well Diameter(cm)	Zone of inhibition(cm)	Inhibition length(cm)
0.5%	0.8	2.4	1.2
1.0%	0.8	2.7	2.0
1.5%	0.8	2.5	1.4
2.0%	0.8	2.6	1.9



Blue- *E. coli*, Red- *Salmonella*

Graph 2: Antibacterial activity of Gentamicin against *E.coli* and *Salmonella*

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. *A. indica* leaves possessed good antibacterial activity confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care. The extract of *A.indica* when used as medicinal plant, could be useful for the growth inhibition of the carcinogenic bacterium. The alkaloids, glycosides, flavanoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanisms of the plants against pathogens.

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