



Phytochemical Analysis and Antibacterial Activity of *Azadirachta indica*

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ABSTRACT

The methanol leaf extracts of *Azadirachta indica* showed significant antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Streptococcus pyrogens*, *Staphylococcus aureus*. The antibacterial potential of methanol extract of *Azadirachta indica* was tested by using Agar well diffusion method. The (100mg/ml) leaf extract showed maximum inhibition against *Pseudomonas aeruginosa* (18mm). Phytochemical tests were performed and showed that the antibacterial activity of plant *Azadirachta indica* leaves was due to the presence of phytochemical compounds like reducing sugar, glycosides, tannins, triterpenes.

Keywords: Methanol extract of *Azadirachta indica*, Phytochemical Analysis, Antibacterial activity.

INTRODUCTION

A*zadirachta indica* (Meliaceae) commonly known as neem is native of India and naturalized in most of tropical and subtropical countries is of great medicinal value and distributed wide spread in the world. The Chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones. *Azadirachtin* is actually a mixture of seven isomeric compounds labeled as *azadirachtin* A-G and *azadirachtin* E is more effective¹. Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin². Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases³. The plant extracts have been developed and proposed for use as antimicrobial substances⁴. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine⁵. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential⁶⁻⁸. The present study was aimed to evaluate the antibacterial potential of methanol extract of *Azadirachta indica* against bacterial pathogens and phytochemical analysis was done.

MATERIALS AND METHODS

Collection and Drying of plant materials

Mature leaves of were collected *Azadirachta indica* from Coimbatore in Tamil Nadu. The leaves were washed thoroughly three times with water and once with distilled water. The plant materials were air dried and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

Preparation of plant extract

10 g of powdered leaves were extracted successively with 100 ml of methanol at 40-50°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use⁹.

Test microorganisms

Five pathogenic bacteria, viz., *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Klebsiella pneumonia*, *Vibrio cholera* and *Pseudomonas aeruginosa* were used during the present study and were obtained from MTCC, Chandigarh. The cultures were sub-cultured and maintained on nutrient agar slants and stored at 4°C.

Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards.

Determination of antibacterial activity (Agar well Diffusion)

Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculums on the surface of the media. Wells (8 mm in diameter) were punched in the agar. Methanol extracts with same concentrations of 100 mg/ml were used. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm).

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, glycosides, flavonoids, reducing sugars, triterpenes,

phenolic compounds and tannins by the following procedure.

Test for Alkaloids (Meyer's Test)

The extract of *Azadirachta indica* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent¹⁰. The samples were then observed for the presence of turbidity or yellow precipitation¹¹.

Test for Glycoside

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated Sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer⁹.

Test for Flavonoid

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavonoid⁹.

Test for reducing sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Test for Triterpenes

300 mg of extract was mixed with 5 ml of chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well and observed for red colour formation.

Test for Tannins

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins¹².

Test for Saponins

2g of the powdered sample was boiled in 20 ml of distilled water in a water bath. 10ml of the filterable was mixed with 5 ml of distilled water shaken vigorously for a stable persistent broth. The following was mixed with 3 drops of Olive oil and shaken vigorously and then observed for the formation of emulsion.

RESULTS AND DISCUSSION

The present study aimed at testing the antibacterial activity of *Azadirachta indica* leaves against five human pathogens and the findings were summarized. The leaves of *Azadirachta indica* were collected from Coimbatore district. The collected leaves were dried and powdered. Powdered leaves were extracted successively using polar solvent viz methanol. The extracts of *Azadirachta indica*

were tested against pathogenic bacteria like *klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus pyrogen* and *Staphylococcus aureus* by agar well diffusion method. The methanol extract of *Azadirachta indica* (100mg/ml) showed maximum zone of inhibition (18mm) against *Pseudomonas aeruginosa*. *klebsiella pneumonia* showed (4mm) less zone of inhibition (Table 1). Antibacterial activity of *Azadirachta indica* plant leaves is due to the presence of phytochemical compounds like phenolic compounds, tannins, reducing sugar, glycosides, triterpenes (Table 2).

Table 1: Antibacterial activity of *Azadirachta indica* methanol extract against bacterial pathogens

Organism	Concentration of extract and zone of inhibition (mm)		
	50 µl	75 µl	100 µl
<i>Escherichia coli</i>	6mm	8mm	10mm
<i>Pseudomonas aeruginosa</i>	12mm	16mm	18mm
<i>Klebsiella pneumonia</i>	4mm	6mm	7mm
<i>Streptococcus pyogens</i>	11mm	13mm	14mm
<i>Staphylococcus</i>	10mm	12mm	13mm

Table 2: Phytochemical analysis of *Azadirachta indica* extract:

Test	Result
Reducing sugar	+
Tripenoid	-
Triterpenes	+
Tannins	+
Saponins	-
Glycosides	+
Flavonoids	-

Discussion

There are about 45,000 plant species in India with capacity to produce a large number of organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity^{13,14}. The result of phytochemicals in the present investigation showed that the plant leaves contain components like tannins, glycosides, reducing sugar and triterpenes. This study reports the presence of different phytochemicals with biological activity that can be valuable therapeutic index^{15,16}. In the present study, we have found that the biologically active phytochemicals were present in the methanolic extracts of few medicinal plants. The antibacterial properties of these extracts may be due to the presence of above mentioned phytochemicals.

CONCLUSION

It may be concluded from this study that *Azadirachta indica* leaf extract has antibacterial activity against pathogens. Phytochemical and antibacterial activity of *Azadirachta indica* extract showed that it is mainly due to

the presence of phytochemical compounds such like tannins, reducing sugar, glycosides and triterpenes. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs.

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