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To study the antibacterial activity of leaf extract of *Azadirachta indica* on *Staphylococcus aureus* and *Escherichia coli*

Arpana Ashokrao Durge

Department of Biochemistry, Guru Nanak College of Science, Ballarpur (M. S.), India

ABSTRACT

***Azadirachta indica* also known as neem in India is a widely used plant for its great medicinal potential. The ethanolic extracts of the *A. indica* showed highest antimicrobial potential against the gram-positive bacteria *S. aureus* with 2.7±0.2 mm zone of inhibition. The aqueous extracts of *A. indica* also inhibited the *S. aureus* with 2.2±0.2 mm zone of inhibition. *E. coli* was not inhibited by either aqueous or ethanolic extracts of *A. indica*.**

Keywords: *Azadirachta indica*, antimicrobial properties, *Staphylococcus aureus*, *Escherichia coli*

INTRODUCTION

Since ancient times plants have been used for their medicinal properties to treat diseases. Especially in non industrialised areas of the world, people have knowledge of variety of herbs and their potential to cure many ailments. Medicinal plants are traditionally used against several human ailments and are rich source of numerous antibacterial agents [1]. *Azadirachta indica* known as neem in local language is one such traditional medicinal plant wide spread around the world. The phytoconstituents of neem contain pharmacologically active components and the derived medicines have made large contributions to human health and well-being. Traditional medicinal plants being cheaper and safer source of drugs are immensely important for the treatment of human ailments like anemia, diabetes, hypertension, malaria etc since long time [2]. Neem oil is frequently used as insect repellent and also effective on dermal infections. other plant parts like stem, flower, fruit, leaf as well as root, found to cure burning sensations, itching and skin ulcers [3]. The *in vitro* phytochemical analysis performed on neem indicates the presence of biologically active components e.g., saponins, tannins, glycosides, alkaloids, terpenoids, and flavonoids. This study on neem plant is aimed primarily at screening the effectiveness of leaf extracts against the infectious pathogens and its potency against some disease-causing microbes e.g. *Escherichia coli* and *Staphylococcus aureus* that are causative agents for intestinal infections [4,5] Considering all the above-mentioned positive results obtained in past about the medicinal uses of neem plant, this study is being focused on the antibacterial activity of neem plant against *E. coli* and *S. aureus*. This will help people in finding a great alternative for antibiotics which could be hazardous to health if overused [6]. If proved active for its antibacterial potential, neem could be cultivated as medicinal herb on large scale and developing countries would be benefited with the less expensive drugs.

MATERIALS AND METHODS

Collection of the plant sample (leaf) and its identification

The fresh leaves of the neem plant were collected from local area of Bamani, District Chandrapur, Maharashtra (19°50’60” North, 79°22’33” East). The leaves were washed properly with distilled water to remove any dirt or dust. These are then shed dried for 4 to 5 days at room temperature (37^0C).

Preparation of aqueous and ethanol extract of neem leaves

The dried neem leaves are weighed on crude balance, then the leaves were crushed into fine powder using mixer grinder, pestle and mortar can also be used. For extract preparation 20 gm of leaf powder was taken and soaked in 250 ml distilled water and then incubated for 24 hrs. After 24 hrs, it was filtered through sterile muslin cloth and the filtrate was collected as extract and refrigerated at 5^0C for further use. The ethanolic extract was processed using rotary vacuum evaporator to concentrate and refrigerated it at 5^0C further use.

Preparation of pure bacterial cultures of *S. aureus* and *E. coli*

The two bacterial cultures important for the study i.e, *E. coli* and *S. aureus* were obtained from college laboratory. Bothe the organisms were isolated in the pure form. Both the cultures were first inoculated in the freshly prepared nutrient broth and then incubated for 24 hrs at 37^0C temperature.

Antimicrobial activity of neem leaves

Preparation of range of concentrations for antibacterial assay: The dried extract was dissolved in dimethyl sulfoxide (DMSO) and various concentrations were made (1, 0.5, 0.3 mg/ml). The disc was cut from whatmann No. 1 filter paper and dipped in the extracts. The nutrient agar was made in petri plates. The sterile cotton swab dipped in bacterial cultures was used to swap the petri plates. After swabbing the disc dipped in different concentration of the extracts were placed in the plates. The disc dipped in DMSO served as the control. After incubation at 37 °C for 24 h the plates were taken out of incubator to note down the zone of inhibition. The zone of inhibition was measured with the help of the ruler [5,7].

Statistical analysis

All the data were repeated thrice and the values mentioned are expressed as mean ± standard deviation. Microsoft excel was used for the calculation of standard deviation.

RESULT AND DISCUSSION

Both aqueous and ethanolic extracts showed the antibacterial activities against *S. aureus*. Highest zone of inhibition was obtained for the ethanolic extracts and are positively correlated with the increase in the concentration of the crude extracts.

Table 1 Antibacterial activity of neem extracts on *S. aureus*

Concentration of neem extract (mg/ml)	Zone of inhibition (mm)	
	Aqueous extract	Ethanol extract
1 mg	2.2±0.2	2.7±0.2
0.5 mg	1.8±0.1	2.2±0.1
0.3 mg	1.2±0.3	1.5±0.4

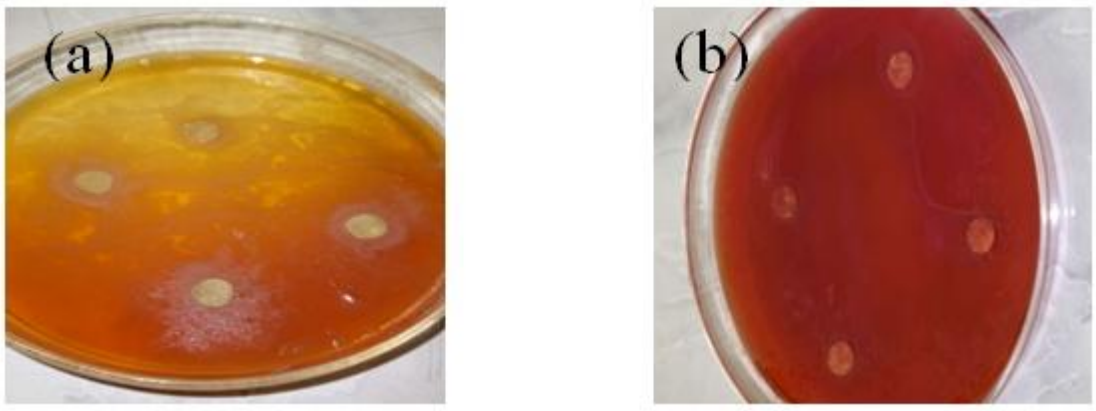

Figure 1 Nutrient agar plates showing the antibacterial activities of neem extracts on *S. aureus* (a) and *E. coli* (b)

Neem plant is known to possess the antibacterial activities and is being used from old times in the ayurveda. Our results showed that only gram-positive bacteria were inhibited by the aqueous extracts and no effect was seen on the gram-negative bacteria. Gram-negative bacteria are capable of genetic recombination and mutation. This alters the cell

membrane structure and can also alter the pockets where antibacterial compounds can bind and inhibit the growth of the microbe. Our results are in contradiction to the results obtained by Gajendra Singh et al., which showed *E. coli* as the most inhibiting bacterium to aqueous and ethanol extracts [7,8]. Dosage dependent increase in the zone of inhibition indicates that the secondary metabolites are present in the proportional quantities which are responsible for the killing of a greater number of microorganisms. Absence of the zone of inhibition don't indicate that the extract is not capable of inhibiting the microbes, it can be due to less concentration of metabolites in the extracts.

This research indicates that both aqueous and ethanolic extracts of *Azadirachta indica*, will inhibit the gram-positive bacteria and make it easier for dosage determination and chemotherapeutic index of the extract if they were to be processed into drugs [9].

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