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# EFFECT OF ANTIBIOSIS ACTIVITY OF AZADIRACHTA INDICA AGAINST SOME WOUND MICROBES

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## ABSTRACT

In the present study antimicrobial activity of *Azadirachta indica* was evaluated against pathogenic bacteria (*Escherichia coli, Salmonella typhi, Klebisella pneumonia, Proteus* sp. and *Staphylococcus aureus*) and fungi are *Aspergillus flavus, A.niger, A.terreus, Penicillium* sp. and *Trichoderma* sp. performed and *Azadirachta indica* leaves were collected from Thanjavur District, Tamilnadu, India. All the test organisms were screened for their antibacterial activity against different concentration and different solvent extraction of *A. indica* leaves by agar well diffusion method. Leaf extract of *Azadirachta indica* showed maximum zone of inhibition in antibacterial activity against *E.coli* (14mm) and antifungal activity against *A.niger* (24mm) susceptible to neem extract. The highest zone of inhibition was measured in both antibacterial and antifungal activity from the higher concentration of 20% of ethanolic extracts.

**KEYWORDS:** *Azadirachta indica* leaves, bacteria and fungal culture, solvents.

## INTRODUCTION

Medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anemia for a long time period (Fola, 1993), but the potential of higher plants as source for new drugs is still largely unexplored (Gerhartz, 1985). Systematic screening of microbes may be result in the discovery of novel effective compounds (Tomoko *et al*., 2002). Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines which have made large contributions to human health and well-being. Generally many drugs have been replaced by more potent synthetic ones; trees remain a source for some drug ingredients (Thomson, 1978).

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 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 (Maragatharavlli *et al.*, 2012). Aqueous extract of neem leaf has a good therapeutic potential as antihyperglycemic agent in IDDM and NIDDM (Mossadek and Rashid, 2008; Patil *et al*., 2013). Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise (Saseed and Aslam, 2008; El-Mahmood *et al*., 2010).

Medicinal plant *Azadirachta indica* were studied by several workers. They were anti-pyretic (Okpanyi and Ezeukwk, 1981; Khattak *et al*., 1985), anti-malarial and anti-tumour effect (Fujiwara *et al.*, 1982), anti-ulcer effect (Pillai and Santhakumari, 1984), anti-diabetic effect (Patil *et al*., 2013), anti-fertility effect (Sinha *et al*., 1984), effect on central nervous system and antioxidant activity (Bandyopadhyay *et al*., 2002). Boiled neem leaf water makes an excellent antiseptic to clean wounds, soothes, swellings and eases skin problems (Bonjar and Holland, 2004).

## MATERIALS AND METHODS

### Collection of plant materials

Healthy and uninfected leaves of *Azadirachta indica* were collected from Thanjavur district, Tamil Nadu. The plant materials were cleaned free from soil or dirt particle and shade dried and to prepare in powder form. The identity of the plants was confirmed by refereing standard manual (Matthew, 1983).

### Preparation of leaf extracts

Aqueous, ethanol, ethyl acetate and methanol have been used as solvents for the extraction of leaf *Azadirachta indica*. Ten grams of the dried powdered leaf materials were soaked separately with 50 ml of solvents viz. aqueous, ethanol, ethyl acetate and methanol at soxhlet apparatus for 24 hours. At the end, each extract was filtered through muslin cloth and filtrates were

concentrated by evaporation at room temperature in order to reduce the volume.

**Test organisms**

Seven bacterial species were subjected to the antibacterial activity assay by using standard methods. They are *Klebsiella pneumoniae*, *E.coli*, *Salmonella* sp., *Proteus* sp., *S.aureus.* Five species of fungi viz., *Aspergillus niger*, *A. flavus*, *A. terreus*, *Penicillium* sp. and *Trichoderma* sp. were used for the antifungal activity.

**Antimicrobial Activity**

For antibacterial and antifungal activity by preparing nutrient agar and potato dextrose agar respectively. Then dispense the media into each of the petridish and allowed it to solidify. Transferred 1ml of 24 hrs old bacterial and fungal culture on to solidified plate and spread it with the help of sterile glass rod. After spreading, made the well at the centre of the media with the help of cork borer. Then transferred different concentration of leaf extracts in each of the well. Incubated the plates at 37°C for 24 hrs for antibacterial activity and at room temperature for 48 hrs for antifungal activity. After, the plates were observed to measure their diameter.

## RESULT AND DISCUSSION

In the present study, the leaf extracts of *Azadirachta indica* are more potential effects of antibiosis activity. Four different solvents (aqueous, ethanol, ethyl acetate and methanol) with four different concentrations (5, 10, 15 and 20 %) of leaf extracts were used, among the solvents ethanol extract with 20% of the leaf extract was excellent activity against bacteria as well as fungal pathogens.

**Table: 1 Antibacterial activity of *Azadirachta india* against various bacteria.**

| Name of the Bacteria | Zone of Inhibition (mm) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aqueous (%) | | | | Ethanol (%) | | | | Ethyl acetate (%) | | | | Methanol ( %) | | | |
| | 5 | 10 | 15 | 20 | 5 | 10 | 15 | 20 | 5 | 10 | 15 | 20 | 5 | 10 | 15 | 20 |
| *E.coli* | - | - | - | - | 13 | 15 | 16 | 24 | - | - | - | 24 | - | - | - | - |
| *K. pneumonia* | - | - | - | - | 9 | 10 | 11 | 14 | - | - | - | 23 | - | - | - | 7 |
| *Proteus sp.* | - | - | - | - | 7 | 8 | 6 | 6 | - | - | - | 10 | - | - | - | - |
| *Salmonella sp.* | - | - | - | - | 5 | 2 | 2 | 2 | - | - | - | 22 | - | - | - | - |
| *Staphylococcus aureus* | - | - | - | - | - | - | 14 | 15 | - | - | - | 22 | - | - | - | - |

**Table: 2 Antifungal activity of *Azadirachta indica* against various fungi.**

| Name of the Bacteria | Zone of Inhibition (mm) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aqueous (%) | | | | Ethanol (%) | | | | Ethyl acetate (%) | | | | Methanol (%) | | | |
| | 5 | 10 | 15 | 20 | 5 | 10 | 15 | 20 | 5 | 10 | 15 | 20 | 5 | 10 | 15 | 20 |
| *Aspergillus flavus* | - | - | - | - | 8 | 12 | 14 | 14 | 24 | 18 | 30 | 25 | 6 | 6 | 7 | 8 |
| *A.niger* | - | - | - | - | 24 | 24 | 30 | 30 | 34 | 34 | 34 | 34 | 5 | 8 | 9 | 10 |
| *A.terreus* | - | - | - | - | 18 | 24 | 24 | 30 | 34 | 34 | 34 | 34 | 6 | 6 | 6 | 6 |
| *Penicillium* sp | - | - | - | - | 14 | 14 | 14 | 16 | 32 | 32 | 32 | 32 | 6 | - | - | - |
| *Trichoderma* sp. | - | - | - | - | 14 | 16 | 18 | 16 | 14 | 16 | 14 | 16 | 8 | 8 | 8 | 8 |

The findings of this study coincide with the observations of several researchers. Oil from the leaves, seeds and bark possesses a wide spectrum of antibacterial activity action against gram-negative and gram-positive microorganisms. Koona and Budida (2011) reported the antimicrobial activity of the seed oil against a variety of pathogens. Antimicrobial effects of neem extract have been demonstrated against *Streptococcus* sp. (Mehrotra *et al*., 2010). The zones of inhibition (mm) for the different synthetic antibiotics were determined. Regarding the antibacterial activity was highest activity due to the action of ciprofloxacin against *Pseudomonas aeruginosa* (28mm) and lowest activity was erythromycin against *Proteus mirabilis* (4.4mm). In addition, the aqueous extract of neem leaves enhanced the growth of EcO157 although water extracts of neem chewing sticks were found inhibitory to supra-gingival plaque organisms including generic *E. coli* (Rao *et al*., 2014). However, in a different study, water extracts were not found to be inhibitory to multidrug resistant *E. coli* (Dahiya and Purkayastha, 2012). In our findings, *E.coli* was maximum zone of inhibition in ethanol extract with 20% of concentration followed by *S.aureus* (15mm), *K.pneumoniae* (14mm), *Salmonella* sp. (24mm). and *Proteus* sp. (6mm) when compared to other extract. The concentration was increased with the zone of inhibition also increased (Table 1).

Khan *et al.* (1987) reported that some leaf extracts including those from neem had a characteristic effect on dermatophytes especially for low polar extracts over the high polar ones. The authors suggested that one possible explanation for this is the flavonoid quercetin contained in the extracts. Shivpuri *et al*. (1997) noticed that the extracts in ethanol of *A. indica* had fungitoxic properties against five pathogenic fungi when tested under laboratory conditions at concentrations ranging between 500 and 1000 µg/ml. The results obtained during assay

with organic extracts were also in accordance with those recorded by Verma *et al*. (1998) who found that a purified fraction (ethyl acetate : chloroform, 3:1) of extracts in methanol from neem seed coat showed strong antifungal activity against *A. niger* and *Curvularia lunata* with MIC of 250 ppm. They found also that the extracts in petroleum ether from the neem leaves were highly active at a lower MIC (100 ppm) against the same pathogens.

Uma (2017) reported that the aqueous extract of pulp showed effective inhibitory action against *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*. The zone of inhibition of pulp extract of neem against *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* was measured about 23, 22 and 23mm respectively. The important antimicrobial activity of the *A.indica* and *P. guajava* extracts with clear zones of inhibition against the bacteria tested (Rasool *et al*., 2017).

The antifungal activity of *Azadirachta indica* observed the maximum zone of inhibition in *A.niger* (30mm) in ethanolic extracts when compared to other solvents (Table 2). Similarly Upasana *et al*. (2002) found that neem seed extract in methanol was effective against *Aspergillus niger, Fusarium oxysporum* and *Trichoderma resii* and that both dried and fresh organic extracts from leaves were effective only against *Trichoderma resii*. The comparative effect of aqueous, ethanolic and ethyl acetate extracts of neem leaves on growth of *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Candida albicans* and *Microsporum gypseum*. The study found that 20% ethyl acetate extract has the strongest inhibition compared with the activity obtained by the same concentration of aqueous and ethanolic extracts (Mahmoud *et al*., 2011).

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