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ANTIBACTERIAL AND ANTIFUNGAL SCREENING OF LEAF EXTRACT OF *AZADIRACHTA INDICA*

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ABSTRACT

The present investigation focuses on the antibacterial and antifungal screening of ethanolic leaf extract of *Azadirachta indica* against the selected bacterial and fungal strains. Leaf extract of *Azadirachta indica* was more potent in inhibiting the growth of *Klebsiella pneumoniae* with different degree of inhibition. *Azadirachta indica* completely inhibited *Curvularia luenata* at 500-1000ppm. The results support that the plant extract containing compound that can form the basis for the development of novel broad spectrum antimicrobial formulations against *Klebsiella pneumoniae* and *Curvularia luenata*.

INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds [1]. Plants are the largest biochemical and pharmaceutical stores ever known on our planet. These living stores are able to generate endless biochemical compounds. In their living, human and animals are using only a small portion (1 to10%) of plants available on Earth [2, 3]. Medicinal plants are rich in a numerous variety of secondary metabolites of antimicrobial properties such as saponines, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters [4, 5]. *Azadirachta indica*, a large tree of India, has been used for centuries in Asia as insecticides, fungicides, anticonceptionals in popular medicine almost every part of this tree seeds, leaves, roots, bark, trunk and branches has multiple uses [6] and has been recommended to plant in African and Asia by many international organizations. The present study is aimed at evaluating the antibacterial and antifungal potential of leaf extracts of *Azadirachta indica* against some pathogenic microorganisms.

MATERIALS AND METHODS

Collection, preparation extraction of plant material

Fresh leaves of *Azadirachta indica* was collected in Trichy, during the month of December. Plant material was dried under shade at room temperature, pulverized by a mechanical grinder and sieved through 40 meshes, then stored in airtight closed bottles until required. *Azadirachta indica* power, 100g was taken in a container. To this 300ml of ethanol added. This was kept in shaking condition at 150 rpm for 3 days. The extract was collected after 3 days and the plant material was subsequently extracted twice in fresh solvent in 3 days interval. The solvent was evaporated by roto-evaporation. Lyophilization technique was used to remove the water from the extract. This yielded a residue of 51.154g. The extract working stock was prepared by dissolving in DMSO.

Test Microorganism and culture media

The bacterial and fungal strains used for the test were both pathogenic and non pathogenic such as, *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella*

pneumoniae, Escherichia coli, Pseudomonas aeruginosa, *Bacillus species, Aspergillus flavus, Aspergillus niger, Botrytis cinereae, Curvularia luenata, Scopulariopsis* Sp. *Epidermophyton floccosum, Trichophyton rubrum* and *Trichophyton tonsurans*. All stock cultures obtained from Department of Microbiology, from Cauvery College, Trichy. Muller Hinton Agar (Himedia, India) was used as the media for the culturing of bacterial strains.

Screening antibacterial activity

Initial screening for antibacterial activity of the plant extracts was tested by agar dilution method. The plant compound which has been extracted was filter sterilized and stored in clean sterile vials. A stock of 100 mg/ml concentrations was prepared. Appropriate dilutions were made from the stock solution. The compounds were tested at 1000, 500, 250 and 125ppm. Different concentration of compounds 1000, 500, 250 and 125ppm were prepared and added to Muller Hinton Agar, then mixed well and plated. Plates were dried, inoculated with each organism in triplicates and incubated aerobically at 37^0 C.

Screening antifungal activity

The standardized inoculums 5 ml was taken in a sterile pipette and placed on the plant extract amended Muller Hinton Agar plate. The plates were incubated at room temperature (27^0 C) for 4 days or until the measurable growth. A positive control and a solvent contour plated also included in each day. The diameter of the fungal mycelium was measured in test and positive control. The percentage of mycelial growth inhibition was calculated using the formula. The percentage of mycelial growth inhibition = (Control – Test)/Control X 100.

RESULTS

From Table 1, it is evident that the organisms *Klebsiella pneumoniae* was found to be inhibited at 125ppm. No other bacterial culture was found to be inhibited by the ethanol extract of *Azadirachta indica*. Complete inhibition was seen in the culture of *Curvularia luenata* at all the concentrations. A minimum inhibition was found in *Aspergillus flavus* at 125ppm. Inhibition, 55% was observed at 1000ppm in *Trichophyton rubrum*. The minimum inhibition was noted in *Scopulariopsis* Sp. at 125ppm (Table 2).

Table 1 Antibacterial activity of ethanolic extract of *Azadirachta indica* against pathogenic bacteria

Organisms	Control		Test concentration (ppm)			
	DMSO	POSITIVE	1000	500	250	125
Staphylococcus aureus	+	+	+	+	+	+
Staphylococcus epidermidis	+	+	+	+	+	+
Klebsiella pneumoniae	+	+	-	-	-	-
Escherichia coli	+	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+	+
Bacillus species	+	+	+	+	+	+

+ No inhibition - Growth inhibition

Table 2 Antifungal activity of ethanolic extract of *Azadirachta indica*

Organisms	Positive control growth (mm)	125ppm		250ppm		500ppm		1000ppm	
		Growth (mm)	Growth inhibition (%)	Growth (mm)	Growth inhibition (%)	Growth (mm)	Growth inhibition (%)	Growth (mm)	Growth inhibition (%)
Aspergillus flavus	3.5	3.0	14.2	2.8	20.0	2.6	25.7	2.5	28.5
Aspergillus niger	3.3	2.7	18.1	2.6	21.2	2.5	24.2	2.3	30.0
Botrytis cinereae	3.6	1.9	47.0	1.8	50.0	1.6	55.5	1.5	58.3
Curvularia luenata	2.7	0	100	0	100	0	100	0	100
Scopulariopsis sp.	3.9	3.6	07.6	3.3	15.3	3.1	20.5	3.0	23.0
Epidermophyton floccosum	1.5	1.1	26.0	1.0	33.0	0.9	40.0	0.7	53.0
Trichophyton rubrum	0.9	0.7	22.2	0.6	33.3	0.5	44.4	0.4	55.5
Trichophyton tonsurans	1.2	1.1	08.3	1.0	16.6	0.9	25.0	0.9	25.0

DISCUSSION

Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents [7]. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs [8]. In the present results of inhibition studies using ethanolic extract of *Azadirachta indica* in selected bacterial and fungal culture. This study showed that *Azadirachta indica* leaves were an effective antibacterial and antifungal agent against *Klebsiella pneumoniae* and *Curvularia luenata*. The organism was inhibited at the minimum concentration at 125ppm *Klebsiella pneumoniae. Azadirachta indica* completely inhibited *Curvularia*

luenata at 500-1000ppm. Leaves of *Azadirachta indica* (locally known as neem) are used by indigenous people in different parts of India for curing gastrointestinal disorder such as diarrhea and cholera is wide spread [9, 10, 11, 12, 13,]. *Azadirachta indica* has been reported to have antibacterial and antifungal effect. It has been shown to be active against pathogenic bacteria such as *Staphylococcus aureus* and *Salmonella typhi* [14, 15] and against various pathogenic fungi belonging to the genera *Trichophyton*, *Epidermophyton*, *Microsporum*, *Geotrichium* and *Candida* [16].

CONCLUSION

This could be suggested that the leaves of *Azadirachta indica* extract found in our work may be used as potential source to develop novel antimicrobial compound against *Klebsiella pneumoniae* and *Curvularia luenata*. Further studies need to be done to characterize the active components of the leaf and could be of considerable interest of the development of new drugs.

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