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Investigations on Antibacterial Activity of Leaf Extracts of *Azadirachta indica* A. Juss (Meliaceae): A Traditional Medicinal Plant of India

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ABSTRACT

The present study was carried out to screen and evaluate antimicrobial activity of leaf extracts of *Azadirachta indica* A. Juss. Petroleum ether, dichloromethane, chloroform, ethanol and aqueous extract of leaves of *A. indica* were tested against selected Gram positive and Gram negative bacterial species. Phytochemical leaf extracts of *A. indica* exhibited significant anti-bacterial activity against all the test microorganisms. However, inhibitory activities of the leaf extracts were both organism and solvent dependent. The leaf extracts limited the growth of both Gram-positive and Gram-negative bacterial species tested. Among the different extracted used in the study, ethanolic and dichloromethane leaf extracts of *A. indica* were found to be more active towards the bacterial species used in the study. Further, the aqueous leaf extract was moderately active. However, petroleum ether and chloroform extracts were not effective against any of the organisms tested, but for *Bacillus cereus* where the chloroform extract was moderately active. Growth of *Lactobacillus bulgaris* was not inhibited by any of the tested leaf extracts of *A. indica* but for dichloromethane. The study shows that ethanolic and dichloromethane leaf extracts of *A. indica* can be used as a potential source of antimicrobial agents.

KEYWORDS: *Azadirachta indica*; Medicinal Plants; Antibacterial Agents; phytochemicals; Disc Diffusion Assay (DDA).

INTRODUCTION

Medicinal plants are part and parcel of humans since the dawn of civilization. In India they form the backbone of several indigenous traditional systems of medicine. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti *et al.*, 2008). Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design (Chakravarthy and Gode, 1985; Ebi and Ofoefule, 2000). Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube, 2008).

WHO (2005), pointed out that more than 80% of world's population rely on plants based products to meet their primary health care needs. Overexploitation of selected medicinal plant species has led to significant reduction

in number of plants in the wild. Ruthless hunting has resulted in inclusion of their name in the red data book (Ahmedullah and Nayar 1999). In recent years, multiple drug resistance in both human and plant pathogens has been developed due to indiscriminate use of synthetic drugs. This drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and have fewer side effects (Prusti *et al.*, 2008).

Neem (*Azadirachta indica* A. Juss) is perhaps the most commonly used traditional medicinal plant of India. Almost all parts of the plant are endowed with medicinal property. During the past few decades, apart from studies in the chemistry of Neem compounds, considerable progress has been made in evaluating biological activity of phytochemicals for medicinal applications. In the modern era, Neem is considered as a valuable source of unique natural products for development of medicines against various diseases (Puri, 1999; Biswas *et al.*, 2002).

Azadirachta indica A. Juss (syn. *Melia azadirachta*) is well known in India and its neighboring countries as one of the most versatile medicinal plants having a wide spectrum of biological activity. *A. indica* and *M. azedarach* are two closely related species of Meliaceae. The former is popularly known as Indian Neem (margosa tree) or Indian lilac, and the latter as the Persian lilac (Parrotta and Chaturvedi, 1994; Biswas *et al.*, 2002). Neem is an evergreen tree, cultivated in various parts of the Indian subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity. Several pharmacological activities and medicinal applications of various parts of Neem have been documented in the ancient literature. Recently, biological activities and medicinal properties of Neem have been extensively reviewed by (Biswas *et al.*, 2002). Biological activity of Neem is reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil. However, only crude extract of different parts of Neem has been used as traditional medicine for the treatment of various diseases. Neem has been extensively used in Ayurveda, Unani, Homoeopathic and Siddha medicine and has become a cynosure of modern medicine (Varma, 1976). In the present study we have evaluated the antimicrobial potential of *A. indica*.

MATERIALS AND METHODS

Collection of Plant Material

Mature leaves of *A. indica* were collected from Vellore, Tamilnadu, India during Apr 2008. The Flora of Presidency of Madras (Gamble, 1935) and The Flora of Tamil Nadu Carnatic (Matthew, 1983) were used for identification and authentication of the plants. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grounded into powder.

Preparation of Phytochemical Extracts

The powder was extracted by maceration in different solvents used in the study using the cold percolation method. The plant extracts were concentrated using rotary evaporator (Buchi, Switzerland) and stored at 4 °C until used in the assay.

Test Organisms

Eight strains of Gram-positive bacteria - *Micrococcus glutamicus*, *Lactobacillus bulgaris*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Staphylococcus pyogenes*, *Micrococcus luteus*, *Bacillus cereus* and two strains of Gram negative bacteria - *Escherichia coli* and *Pseudomonas aeruginosa* were used to evaluate the antibacterial activity (Table 1). All bacterial cultures were maintained in NA slants/ plates; stored at 4 °C and periodically sub-cultured.

Antimicrobial Activity Test

Antimicrobial activity was tested using a modified disc diffusion assay (DDA) method originally described by Bauer (1966) and Ncube *et al* (2008). Plant extracts were dissolved in 20% DMSO treated water. The inoculums for each microorganism were prepared from broth cultures (10^5 CFU/ml). A loop of culture from the NA slant stock was cultured in LB medium overnight and spread with a sterile swab into Petri-plates. Sterile disc (6 mm dia, Hi-media, Mumbai, India) impregnated with the plant extracts (5 mg/ml) were placed on the cultured plates and incubated for 24 h at 37 °C. The solvent loaded disc without extracts in it served as control in the study. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate.

RESULTS AND DISCUSSION

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. Though bioactive products of Neem have been used in treatment of various ailments since time immemorial, role of phytochemical in inhibition of growth of microorganisms has gained less prominence (Sasidharan *et al.*, 1998; SaiRam *et al.*, 2000). In the present study, petroleum ether, dichloromethane, chloroform, ethanol and aqueous extract of leaves of *A. indica* were tested against selected Gram positive and Gram negative bacterial species (Table 1). The leaf extracts limited the growth of both Gram-positive and Gram-negative bacterial species tested. Different phytochemical leaf extracts of *A. indica* exhibited significant anti-bacterial activity against all test organisms. Further, inhibitory role of the leaf extracts was both organism and solvent dependent. Among the different extracted, ethanolic and dichloromethane leaf extracts of *A. indica* were found to be more active towards the bacterial species used in the study. Aqueous leaf extract of *A. indica* was moderately active against all the bacterial species tested except *M. glutamicus*, *L. bulgaris* and *S. pyogenes*.

Dichloromethane leaf extract of *A. indica* exhibited maximum inhibitory activity followed by ethanolic and aqueous extracts against various organisms tested (Table 2). Chloroform extract showed inhibition against only *B. cereus*. Petroleum ether extract was not effective against any of the organism tested. Among the different microorganisms tested maximum inhibition was found in *M. glutamicus* followed by *S. aureus*, *B. stearothermophilus*, *B. cereus* and *S. faecalis*.

Even though much work has been done on ethnomedicinal plants in India, interest in a large number of traditional natural products has increased of late. Several medicinal plants have been reported to possess antimicrobial, antifungal and other activity has been elucidated by various workers (Sasidharan, 1998; Sudharameshwari, 2007). Phytochemical extracts from Neem plant are potential sources of antiviral, antitumor and antimicrobial agents (Biswas *et al.*, 2002). Several workers have evaluated antibacterial, antisecretory, antihemorrhagic, insecticidal activity of *A. indica* based drugs to meet the health care needs (SaiRam *et al.*, 2000; Thakurta *et al.*, 2007).

CONCLUSION

Neem, the versatile traditional medicinal plant of India, is the rich source of bioactive compounds with diverse chemical structure. As of now, little work has been done on the biological activity and plausible medicinal applications of the phytochemical compounds and hence extensive investigation is needed to exploit the bioactive principles of Neem for therapeutic utility. In the present study antibacterial activity of *A. indica* extracts towards drug resistant/ clinically significant microbes has been investigated. Phytochemical studies on active constituents of Neem plant is expected to serve as lead in the development of novel bioactive antimicrobial compounds.

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Table 1. Bacterial strains used in the present study.

S. No	Bacterial strain	Gram (+/-)
1	<i>Escherichia coli</i>	-
2	<i>Pseudomonas denitrificans</i>	-
3	<i>Micrococcus glutamicus</i>	+
4	<i>Lactobacillus bulgaris</i>	+
5	<i>Streptococcus faecalis</i>	+
6	<i>Staphylococcus aureus</i>	+
7	<i>Bacillus stearothermophilus</i>	+
8	<i>Staphylococcus pyogenes</i>	+
9	<i>Micrococcus luteus</i>	+
10	<i>Bacillus cereus</i>	+

Table 2. Anti-microbial activity of *Azadirachta indica* leaf extracts.

S. No	Strains used	Zone of inhibition (mm)									
		PC	PLE	DC	DLE	CC	CLE	EC	ELE	AC	ALE
1	<i>E. coli</i>	-	-	-	15	-	-	-	13	-	10
2	<i>M. glutamicus</i>	-	-	-	18	-	-	-	15	-	-
3	<i>L. bulgaris</i>	-	-	-	15	-	-	-	-	-	-
4	<i>S. faecalis</i>	-	-	-	15	-	-	-	13	-	15
5	<i>S. aureus</i>	-	-	-	16	-	-	-	12	-	12
6	<i>B.stearothermophilus</i>	-	-	-	16	-	-	-	14	-	10
7	<i>S. pyogenes</i>	-	-	-	15	-	-	-	12	-	-
8	<i>P. denitrificans</i>	-	-	-	15	-	-	-	14	-	11
9	<i>M. luteus</i>	-	-	-	15	-	-	-	13	-	9
10	<i>B. cereus</i>	-	-	-	16	-	11	-	15	-	10

EC = Ethanolic control
 AC = Aqueous control
 DC = Dichloromethane control
 ELE = Ethanolic leaf extract
 ALE = Aqueous leaf extract
 DLE = Dichloromethane leaf extract
 PC = Petroleum ether control
 CC = Chloroform control
 - = No inhibition.
 PLE = Petroleum ether leaf extract
 CLE = Chloroform leaf extract