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In Vitro Antibacterial Prospective of Crude Leaf Extracts of *Melia azedarach* Linn. against Selected Bacterial Strains

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ABSTRACT

The present study was carried out to evaluate the antibacterial activity of the crude leaf extracts of *Melia azedarach* against selected Gram positive and Gram negative bacterial strains. Five plant extracts (Methanol, Ethanol, Dichloromethane, Ethyl acetate and Aqueous) under five different concentrations (1mg/ml and 5mg/ml) were tested by Disk diffusion method. Methanol, Ethyl acetate and Aqueous extracts of *M. azedarach* showed significant inhibition against bacteria tested.

KEY WORDS

Melia azedarach; Antibacterial activity; growth inhibition; leaf extracts; Disc Diffusion Assay (DDA).

INTRODUCTION

Plants have been exploited for treatment of human diseases by different ethnic groups in different parts of the world since the dawn of civilization. But the traditional cultures without proper scientific evidence are not able to ponder the importance of plant species for treatment of human diseases. Therefore, to ascertain the medicinal value of the phytochemicals pharmacological studies have been carried out by different groups world over (Prusti *et al*., 2008). Also because the phytochemicals from medicinal plants serve as lead compounds in drug discovery and design (Ebi and Ofoefule, 2000).

According to World Health Organization (WHO), infectious diseases are the number one cause of deaths world wide. Infectious diseases alone account for more than 50 % of the deaths in tropical countries. To combat these diseases in the last few decades, pharmacological industries have produced a number of antibiotics, but the resistance of microbes has also increased parallely. Further, it has been reported that bacterial strains have developed resistance to almost all the antibiotics that are available in the market. This has resulted multiple drug resistance in both human and plant pathogens due to indiscriminate use of synthetic drugs especially in the developing countries (Hart and Karriuri, 1998).

Further more, some antibiotics have serious undesirable side effects which limit their applications, hence, the ultimate goal of the leading drug companies and the academia is to hunt for novel therapeutic/ antimicrobial agents that are effective with minimal side effects.

According to WHO, about 80% of the population in the developing countries use traditional medicine in the treatment of various aliments. Nearly, 25 to 45% of modern prescriptions contain plant derived lead molecules as a basic source in drug formulations. Nevertheless, ruthless hunting has resulted in inclusion of their name in the red data book (Jain and Sastry, 1979; Ahmedullah and Nayar 1999). Therefore, plant species used by different ethnic groups should be investigated in order to tap the incredible bioresources for sustainable harvest of novel bioactive phyto pharmaceuticals (Cox, 1994). In-depth investigation is expected to provide better understanding of pharmacological properties, safety and efficacy (Chopra *et al*., 1997). In the last few years, a number of studies have been conducted worldwide to prove such efficacy (Khan et al., 2002). In view of enormous ethnomedicinal uses and medicinal applications, antimicrobial properties of *Melia azedarach* L. (Meliaceae), was investigated against selected bacterial strains.

Description of the plant:

Azadirachta indica and *Melia 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 (Biswas *et al*., 2002). The plant is a small-to medium-sized deciduous tree. It grows to a height of 5 to 15 m tall and 30 to 60 cm in diameter. The plant is characterized by the presence of a spreading, dense and dark green crown. Its bark is dark brown in color, relatively smooth, and fissured. The leaves are alternate, leaflets are short stalked and thin, hairless, dark green (ventral) and relatively pale (dorsal). Flowers are white with purple stripes and are charcteised by the presence of a typical fragrance (odor). Fruits or berries are yellow, round, smooth, and fleshy. Dried fruits are hard with 4 to 5 seeds. *M. azedarach* Linn. is native to tropical Asia. It is widespread and naturalized in most of the tropics and subtropical countries (Asolkar et al., 1992).

Medicinal uses and Pharmacology:

Leaves: leprosy, scrofula, anthelmintic, antilithic, diuretic, deobstruent, resolvent. Root: resolvent, deobstruent. Seeds: rheumatism. Leaves: Leaf extract has insecticidal property (azadirachtin) that repels insects in clothing. The leaves can also serve as feed for goats. Seed oil: The oil is the most active medicinal product of the plant. It is used as antiseptic for sores and ulcers that show no tendency to heal. It is also used for rheumatism and skin diseases such as ringworm and scabies. Internally, the oil is useful in malaria fever and leprosy. Powdered dust of fruit insecticidal, crude extract from wood and bark insecticidal, oil antibacterial. Alcoholic extract (50% EtOH) of leaf anthelmentic, oil with unspecified extract central nervous system depressant, mild analgesic, depression followed by stimulation in animals. Alcoholic extract (50% EtOH) of stem bark anticancerous, antispasmodic, antiviral (Rastogi and Mehrotra, 1991, 1993, Rastogi, 1998).

Table 1. Ethnomedicinal uses of *Melia azedarach*.

Disease	Plant part used and mode of application

Burns	Fresh leaf extract is applied externally.
Gingivitis	Fresh leaf extract is used as mouth wash
Gonorrhea	Stem bark infusion 30-50 ml is administered orally twice a day
Piles	Leaf extract 5 ml is administered orally thrice a day.
Pyrexia	Leaf extract 5-10 ml is administered orally twice a day for 7 days

Source (Khan et al., 2002).

MATERIALS AND METHODS

Collection of Plant Material

Mature leaves of *M. azedarach* were collected from the wild in Vellore District, Tamilnadu, India during Apr – Jun 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 ground to powder.

Preparation of Phytochemical Extracts

The powder was extracted by maceration in double distilled water. The plant extracts were concentrated using rotary evaporator (Buchi, Switzerland) and stored at 4℃ 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 2). All bacterial cultures were maintained in NA slants/ plates; stored at 4℃ 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, Hi-media, Mumbai, India) impregnated with the plant extracts (1.0 mg/disc and 5.0 mg/disc) were placed on the cultured plates and incubated for 24 h at 37℃. 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

Dichloromethane leaf extract of *M. azedarach* was found to be more effective against gram positive than gram negative bacterial strains used in the present study. While ethanol extract inhibited the growth of three gram positive and two gram negative bacterial strains, ethyl acetate, methanolic

fraction and aqueous extract of *M. azedarach* were found to be effective against all tested bacterial strains (Table 2).

Results of antibacterial activity of *M. azedarach* depict that all the extracts effectively inhibited the growth of *B. subtilis* with maximum zone of inhibition on the disc loaded with phytochemical extract (1 and 5 mg/ml/disk). Ethanol extract inhibited the growth of all the ten tested bacterial strains; however, maximum inhibition zone was recorded against the gram negative strains namely *E. coli* and *P. aeruginosa*. All the tested bacterial strains showed sensitivity against the ethyl acetate fraction *S. aureus* and *B. subtilis*. While methanolic fraction was also found to be effective against all the strains and maximum inhibition was recorded against *B. subtilis*. Aqueous leaf extract showed moderate degree of sensitivity against all tested bacterial strains.

From the results it is clear that leaves of *M. azedarach* are effective against both gram positive and gram negative strains. Ethyl acetate fractions exhibited maximum inhibition followed by Methanolic fractions. The *in vitro* screening studies confirm medicinal uses reported earlier (Kirtikar and Basu, 1935; Asolker et al., 1992; Khan et al., 2002; Jain 1991; Fransworth, 1988; Khan et al., 2002; Valsaraj et al., 1997).

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Table 2. Bacterial strains used in the present study.

S. No	Bacterial strain	Gram (+/-)
1	*Escherichia coli*	−
2	*Pseudomonas aeruginosa*	−
3	*Bacillus cereus*	+
4	*Bacillus subtilis*	+
5	*Lactobacillus bulgaris*	+
6	*Micrococcus glutamicus*	+
7	*Micrococcus luteus*	+
8	*Staphylococcus aureus*	+
9	*Staphylococcus pyogenes*	+

10	*Streptococcus faecalis*														

Table 3. *In vitro* antibacterial potential of *Melia azedarach* crude leaf extracts.

Organism	E			M			DCM			EA			A		
	C	1	5	C	1	5	C	1	5	C	1	5	C	1	5
Escherichia coli	−	++	+++	−	+	+	−	++	+	−	+	+	−	+	+
Pseudomonas aeruginosa	−	++	+++	−	+	+	−	++	+	−	+	+	−	+	+
Bacillus cereus	−	+	++	−	+	+	−	++	+	−	+	+	−	+	+
Bacillus subtilis	−	+	++	−	+	+	−	++	+	−	+	++	−	+	+
Lactobacillus bulgaris	−	+	++	−	+	+	−	++	+	−	+	+	−	+	+
Micrococcus glutamicus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Micrococcus luteus	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−
Staphylococcus aureus	−	−	+	−	−	−	−	+	−	−	+	++	−	−	−
Staphylococcus pyogenes	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−
Streptococcus faecalis	−	+	++	−	+	+	−	++	+	−	+	+	−	+	+

(Growth analysis: +++ = abundant; ++ = normal; + = less; − = no; C = control; 1 = 1 mg/ml; 5 = 5 mg/ml)

E = Ethanol M = Methanol DCM = Dichloromethane

EA = Ethyl acetate A = Aqueous