

***In Vitro* Antibacterial Potential of *Melia azedarach* Crude Leaf Extracts Against Some Human Pathogenic Bacterial Strains**

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Abstract

The emphasis of this paper is upon the sensitivity of the crude leaf extracts of *Melia azedarach* against some of the hospital isolated human pathogenic bacterial strains. Five plant extracts (Petrol, Benzene, Methanol, Ethyl acetate and Aqueous) under five different concentrations (1mg/ml, 2mg/ml, 5mg/ml, 10mg/ml and 15mg/ml) were tested by the *Disk diffusion method*. Methanol, Ethyl acetate and Aqueous extracts of the plant showed significant inhibition against bacteria tested. A comparison was made among the sensitivity demonstrated by the leaf extracts and known medicinal and ethnobotanical uses.

Key Words: *Melia azedarach*, Pathogenic bacteria, inhibition.

Introduction

Plant species have been exploited for the treatment of disease by different ethnic societies residing in different parts of the world. But traditional cultures without the knowledge of modern research are not able to trace the importance of plant species for human beings and science has identified the unique techniques to test the beneficiary effects of plant compounds.

According to the World Health Organization (WHO), infectious diseases are the number one cause of deaths world wide and account for 50 % of the deaths in tropical countries. The number of Multiple Drug Resistant microbial strains or those with a reduced susceptibility to antibiotics are increasing yearly, and this is attributed to indiscriminate use of broad spectrum antibiotics, surgery, epidermidis of HIV infections etc. In the last few decades, pharmacological industries have produced a number of antibiotics, but the resistance of microbes has increased. It has been reported that bacterial strains have developed resistance to almost all the antibiotics. Further more, some antibiotics have serious undesirable side effects which limit their applications, hence, our ultimate goal is to develop antimicrobial agents which are very effective with minimal unwanted side effects.

Reports say that higher plants are the potential source of novel antibiotics. According to WHO, about 80% of individuals from developing countries use traditional medicine, therefore such plant species should be investigated in order to better understand their pharmacological properties, safety and efficacy. Use of plants extracts and their constituents, both with known antimicrobial properties, can be of great significance in therapeutic treatment. In the last few years, a number of studies have been conducted worldwide to prove such efficacy. Investigations in this field revealed that many plants possess anti microbial properties that are due to compounds produced by the plants, as, for example, phenolic compounds, tannins and nitrogenous compounds (Cox,1994, Khan & Khan,2003, Khan et al.,2002).

In view of the ethnobotanical uses and medicinal properties of *Melia azedarach* L. [Meliaceae], it was conjectured

that this plant might possess antibacterial properties; hence, crude leaf extracts were employed in tests designed to measure the sensitivity of this species against some hospital isolated pathogenic bacterial strains.

Common Names

China berry, Persian lilac, Pride of India, China tree, Pride of China, Umbrella tree, Umbrella China berry, Indian lilac, Bead tree (English); Bakain, Drek, Dek, Pejri, Padrai (India); Bakainu (Nepal); Thamaga (Burma); Mindi (Java); Inia (Hawaii); Alelaila (Puerto Rico); Jacinto (Panama); Aleli (Venezuela); West Indian lilac, Lilac (West Indies); Lilas (Haiti, French); Cinnamumo (Brazil) and Ois rouge (New Caledonia).

Scientific name: *Melia azedarach* Linn.

Family: Meliaceae

Description: The plant is a small-to medium-sized deciduous tree, 5 to 15 m tall and 30 to 60 cm in diameter. It has a spreading, dense and dark green crown. Its bark is dark or reddish brown, smooth, and becoming fissured. The leaves are alternate. Leaflets have short stalks and are thin, hairless, dark green on the upper surface and paler underneath. They emit a pungent smell when crushed. Flowers are purple and fragrant. Fruits or berries are yellow, nearly round, smooth, and fleshy. They are as hard as stone, containing 4 to 5 black seeds.

Distribution: *Melia azedarach* Linn. is native to tropical Asia. It is widespread and naturalized in most of the tropics and subtropical countries. It was introduced and naturalized in the Philippines and now cultivated even in Manila. (Kirtikar & Basu, 1935, Anonymous, 1976, Asolkar et al., 1992).

Known medicinal uses:

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.

Ethnomedicinal uses in Northern India

***Burns:** Fresh leaf extract is applied externally. ***Gingivitis (Inflamed bleeding gums):** Fresh leaf extract is used as mouth wash. ***Gonorrhea:** Stem bark infusion 30-50 ml is administered orally twice a day. ***Spicy food** is not allowed during the course of treatment. **Piles (Bleeding):** 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. (** less known uses*) (Khan, 2002).

Chemical constituents: Bakayanin, quercitrin, rutin, backalactone 6 b-hydroxy-4-stigmastem-3-one and 6 b-hydroxy-4-campesten-3-one, 4, 5-dihydroxy-7-0-a-L-rhamnopyranosyl-(1→4)-b-D-glucopyranoside, cystine, serine, arginine, glycine, glutamic acid, threonine, methionine, leucine, lycine, and proline.

Pharmacology: Powdered dust of fruit insecticidal, crude extract from wood and bark insecticidal, oil antibacterial. Alcoholic extract (50% EtOH) of leaf anthelmintic, 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 & Mehrotra, 1991, 1993, Rastogi, 1998, Satyavati, etal, 1987).

Materials and Methods

Plant material

Aerial plant parts of *Melia azedarach* L. [Meliaceae], were collected from different localities of *Aligarh* district, India. Voucher specimen number [AV014, AV206] of the plant were deposited in the Department of Botany, Faculty of Life Sciences, Aligarh Muslim University, Aligarh , 202002, India.



***Melia azedarach* L.**

Preparation of extracts

Crude plant extracts; were prepared according to the protocol described below (Harbone,1973):

- 1) Freshly dried and healthy plant material (leaf) is ground into fine powder in an electric grinder. Powder so obtained is stored in a dessicator.
- 2) Five hundred g plant powder is refluxed with 95% methyl alcohol (MeOH) in a round bottom flask in a water bath for 10 hours. Mother liquor (Crude MeOH extract) is filtered out and residual plant material is again refluxed with 95% methyl alcohol for 10 hours. The process is repeated four times to obtain maximum yield of MeOH extract. The extract is evaporated to dryness at 35°C under reduced pressure.
- 3) The dried methanolic extract is refluxed with light petrol (60-80°C) for five hours. After filtration, the residual methanolic extract is again refluxed with petrol for five hours and filtered. This process is repeated five times. Petrol is evaporated under reduced pressure to obtain petrol soluble extract.
- 4) Petrol insoluble fraction of methanolic extract obtained in step 3 is refluxed with benzene for five hours. Thereafter, it was filtered and refluxed again with benzene for five hours and filtered. The process was repeated five times. Benzene is evaporated under reduced pressure to obtain benzene soluble extract.
- 5) Benzene insoluble fraction obtained in step 4 is refluxed with ethyl acetate for five hours. Thereafter, it is filtered and refluxed again with ethyl acetate for five hours and filtered. The process is repeated five times. Ethyl acetate is evaporated

under reduced pressure to obtain ethyl acetate soluble extract.

6) Ethyl acetate insoluble fraction obtained in step 5 is refluxed with methyl alcohol (95%) for five hours, filtered and is repeatedly refluxed for five times with methyl alcohol (Methanol). The methanolic soluble fraction is evaporated under reduced pressure to obtain methanolic extract, while methanol insoluble residue is discarded.

Preparation of aqueous extract

Shade dried plant material (500 g) is ground to a fine powder, poured with distilled water, and left for 72 hours at room temperature.

The flask is then refluxed over a hot water bath for 10 hours and the mother liquor is filtered. The solute is again added with solvent (distilled water) that is again refluxed and filtered; this process is repeated for 4 times. The filtrate, thus obtained, is evaporated to complete dryness under reduced pressure on a water bath. The residue thus obtained is the aqueous plant extract.

Yields per 1000 g dry material: petrol $\simeq$ 11 g, Benzene $\simeq$ 12.5 g, EtOAc $\simeq$ 9.0 g and MeOH $\simeq$ 14.0 g. Aqueous extract material (500 g) (yield $\simeq$ 48.0 g). Dried plant extract were stored in labeled sterilized screw capped bottles at -20°C

Microorganisms

The leaf extracts were tested for possible antibacterial activity in the disk assay using eighteen (18) human pathogenic bacteria listed in Table No. 1. The bacteria were obtained from the bacterial stock, Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh, India. The bacterial cultures were maintained at 4°C on nutrient agar.

Anti microbial assay

Agar plates (Mueller & Hinton, 1941) are inoculated using a sterile swab dipped into culture inoculums adjusted to 1.5×10^8 bacterial/ml using 0.5 Farland turbidity standard, the agar is streaked in three directions turning the plates by 60° by each streak. All the extracts are sterilized by filtration through 0.45 μm membrane filter. The paper disk (Whatman filter paper no 1) with, 1mg/ml, 2mg/ml, 5mg/ml, 10mg/ml and 15mg/ml plant extracts were dried and placed on the agar surface with the help of a sterile forceps. Finally press the sensitivity disc with forceps to make complete contact with the surface of the medium. Plates are kept at room temperature for 45 minutes (Pre diffusion time). Inoculated Petri dishes are incubated at 37°C over night and at the end of the period, inhibition zones formed on the medium are evaluated in mm (Bauer et al., 1966, Cruickshank, 1968; Colle & Marr, 1989). Experiments were repeated thrice and the mean of the triplicate of the results is summarized in Table No. 1.

Studied activity

Antibacterial activity by disc diffusion method (Bauer et al., 1966, Cruickshank, 1968). Diameters of Petri dish and disc 9.0 cm and 0.6 cm respectively.

Results

Petrol leaf extract of the plant was found to be effective against six of the gram positive and nine of the gram negative pathogenic bacteria. While benzene extract inhibited the growth of three gram positive and nine gram negative bacteria. Ethyl acetate, Methanolic fraction and Aqueous extract of the plant were found to be effective against all the tested bacterial strains (Table No.1).

Discussion

Results of the in vitro antibacterial activity brought to light Interesting facts. Petrol fraction shows maximum inhibition against *Bacillus subtilis*, *Proteus mirabilis* and *Shigella flexneri*. (zone of inhibition 6mm/1mg/ml/disk). Benzene extract inhibited the growth of eleven tested bacteria and the maximum

inhibition zone was recorded against *Proteus mirabilis* and *Shigella flexneri* (zone of inhibition 6mm/1mg/ml/disk). All the tested pathogens showed sensitivity against the ethyl acetate fraction and the most affected bacteria were *Staphylococcus aureus* and *Bacillus subtilis* (zone of inhibition 9mm/1mg/ml/disk). While methanolic fraction was also found to be effective against all the strains and maximum inhibition was recorded for *Shigella dysenteriae* and *Plesiomonas shigelloides*. (zone of inhibition 7mm/1mg/ml/disk/each),. Aqueous leaf extract showed moderate degree of sensitivity against all tested pathogenic bacteria.

From the results it is clear that leaves of *Melia azedarach* L. are effective in controlling bacterial infections caused by both gram positive and gram negative strains. During these investigations it became clear that the most effective crude extract was ethyl acetate, which demonstrated maximum inhibition followed by Methanolic fraction that inhibited the growth of all the tested human pathogens. The petrol and benzene extracts, as compared to the other three extracts, showed anti microbial action against fifteen and twelve pathogens, respectively. The phytochemical screening of this plant gave positive test for Phenolic compounds (Harbone, 1973). It was also noticed that Methanolic, Ethyl acetate and Aqueous extracts showed antibacterial activity against all of the pathogens (Table No.1). The above results show that plant extracts can be effective antibiotics, both in controlling gram positive and gram negative human pathogens. The in vitro screening also confirms medicinal uses reported earlier. (Kirtikar & Basu, 1935, Anonymous, 1976, Asolker et al., 1992, Khan, 2002, Jain 1991, Fransworth, 1988, Khan et al., 2002).

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Table 1. In vitro antibacterial potential of *Melia azedarach* crude leaf extracts against some human pathogenic bacterial strains

Inhibition zone (mm)																			
Gram Positive Bacteria										Gram Negative Bacteria									
1	2	3	4	5	6	7	1	2	3	4	5	6	7	8	9	10	11		
PETROL																			
1	5	-	-	5	5	-	6	-	4	-	6	3	3	4	4	-	6	2	
2	8	4	4	10	8	-	10	4	6	-	10	5	6	7	7	-	10	5	
5	10	7	7	16	11	-	13	7	12	-	14	9	11	11	11	-	14	11	
10	16	11	12	17	16	-	19	9	17	-	17	11	12	15	15	-	19	14	
15	21	18	17	22	18	-	23	13	22	-	21	13	17	19	20	-	22	17	
BENZENE																			
1	5	-	-	-	5	-	4	4	4	-	6	3	3	4	4	-	6	5	
2	8	-	-	-	8	-	7	6	6	-	10	5	6	7	7	-	10	11	
5	10	-	-	-	11	-	10	11	11	-	14	9	11	11	11	-	14	14	
10	16	-	-	-	16	-	15	14	16	-	17	11	12	15	15	-	19	18	
15	21	-	-	-	18	-	19	19	21	-	21	13	17	19	20	-	22	21	
ETHYL ACETATE																			
1	9	5	6	6	6	7	9	6	7	6	7	7	9	6	6	7	9	7	
2	12	7	11	10	9	10	12	10	11	11	12	12	12	12	9	10	13	11	
5	19	11	17	16	16	15	17	13	17	16	17	17	18	17	16	17	18	19	
10	22	16	19	19	20	18	20	17	19	18	22	22	23	19	20	22	22	23	
15	26	21	23	23	26	23	26	20	23	23	25	27	26	24	24	27	26	27	
METHANOL																			
1	5	-	4	6	5	5	5	4	3	2	5	6	5	4	5	7	4	7	
2	7	4	6	10	9	9	7	7	5	5	9	10	7	7	8	10	7	11	
5	12	6	11	16	16	15	12	9	8	9	14	15	10	12	12	16	12	16	

10	16	10	15	19	20	19	17	13	13	14	18	19	13	16	18	20	18	19
15	21	16	20	23	26	21	22	17	17	18	22	23	19	21	21	23	22	21
AQUEOUS																		
1	4	-	4	6	5	2	4	3	5	2	2	3	2	2	4	2	4	4
2	7	2	7	10	9	6	7	5	9	5	5	6	5	4	6	5	7	7
5	10	6	11	12	13	9	10	9	11	8	9	11	8	7	11	10	12	11
10	15	8	15	16	18	11	15	11	14	12	14	15	11	12	14	12	15	16
15	18	11	19	19	20	16	19	13	16	13	18	19	15	17	19	16	20	19
CHLORAMPHENICOL																		
	18	18	16	-	-	-	16	18	16	-	16	18	-	16	17	19	18	20

Gram Positive Bacteria

1. *Staphylococcus aureus** 2. *Staphylococcus aureus* ATCC 25923 3. *Staphylococcus epidermidis**, 4. Group-A *Streptococcus** 5. Group-B *Streptococcus** 6. *Enterococcus faecalis** 7. *Bacillus subtilis**,

Gram Negative Bacteria

1. *Escherichia coli* ATCC 25922. *Edwardsiella tarda** 3. *Klebsiella pneumoniae** 4. *Proteus mirabilis* * 5. *Proteus vulgaris** 6. *Pseudomonas aeruginosa** 7. *Salmonella typhi* *8. *Shigella boydii** 9. *Shigella dysenteriae** 10. *Shigella flexneri** 11. *Plesiomonas shigelloides**.

ATCC American type culture collection,* Hospital isolated pathogenic strains

CHLORAMPHENICOL 10mg/disk, ^aValues are the mean of replication of three; -, no inhibition.