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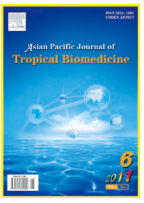

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Antibacterial efficacy of the seed extracts of *Melia azedarach* against some hospital isolated human pathogenic bacterial strains

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ABSTRACT

Objective: To investigate the antibacterial potential of the polar and non-polar extracts of the seeds of *Melia azedarach* (*M. azedarach*) L. (Meliaceae) against eighteen hospital isolated human pathogenic bacterial strains. **Methods:** Petrol, benzene, ethyl acetate, methanol, and aqueous extracts at five different concentrations (1, 2, 5, 10 and 15 mg/mL) were evaluated. Disk diffusion method was followed to evaluate the antibacterial efficacy. **Results:** All extracts of the seeds demonstrated significant antibacterial activity against tested pathogens. Among all extracts, ethyl acetate extract revealed the highest inhibition comparatively. The present study also favored the traditional uses reported earlier. **Conclusions:** Results of this study strongly confirm that the seed extracts of *M. azedarach* could be effective antibiotics, both in controlling gram-positive and gram-negative human pathogenic infections.

1. Introduction

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. In developing countries like India, different plant species are explored by ethnic societies, exploiting them for treatment of various diseases and disorders. Infectious diseases have continued to ravage most developing nations of the world. Approximately half of all deaths caused by infectious diseases each year can be attributed to just three diseases: tuberculosis, malaria, and AIDS. Altogether, these diseases cause over 300 million illnesses and more than 5 million deaths each year[1]. Coupled with the scourge of infectious diseases is the recent emergence of drug-resistant microorganisms that have reduced the effectiveness of antimicrobial agents[2,3]. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs. Bioactive compounds with antibacterial effects need to be explored from local natural resources. Being new, such compounds may not have the problem of microbial resistance, and with some structural modification their activity could be diversified. Seeking remedies for human ailments from the environment has formed the basis for therapeutics. Various plants are used traditionally by ethnic societies from remote past in the forms of extracts of different plant parts (*e.g.* fruits, leaves, stem-bark and roots) in hot water or alcohol. They can be used in the form of infusions, decoctions or concoctions[4,5].

Melia azedarach (*M. azedarach*) L. (Meliaceae) (local name: Bakain) is one of the most useful traditional medicinal plant like *Azadirachta indica* (*A. indica*) A. juss (local names: Neem, Margose) from the family Meliaceae in India. Each part of *M. azedarac*h has some medicinal properties like *A. indica* and thus is commercially exploitable. During the last twenty years, apart from the chemistry of this plant, considerable progress has been achieved regarding the biological activity and medicinal applications.

*M. azedarac*h L. is native to tropical Asia. It is wide spread and naturalized in most of the tropics and subtropical countries. It was introduced and naturalized in Philippines, United States of America, Brazil, Argentine, many African and Arab countries[6].

Leaves are used in leprosy, scrofula, anthelmintic, antilithic, diuretic, deobstruent, and resolvent. Roots are effectively used as resolvent and deobstruent. Seed oil is the most active medicinal product of the plant and used as an 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

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malaria fever and leprosy. There are many traditional uses in Northern India. Fresh leaf extract is applied externally for burns. Fresh leaf extract is used as mouth wash for gingivitis (Inflamed bleeding gums). Stem bark infusion 30-50 mL is administered orally twice a day for onorrhea. Leaf extract 5 mL is administered orally thrice a day for piles. Leaf extract 5-10 mL is administered orally twice a day for 7 days for pyrexia[7].

Chemical constituents of the seeds including β-sitosterol, vanillin, benzoic acid, vanillic acid, daucosterol, α-D-glucopyranose, limonoid glycoside *viz* 6,11-diacetoxy-7-oxo-14beta, 15beta-epoxymeliacin (1,5-diene-3-O-beta-D-glucopyranoside) and scopoletin, a hydroxyl coumaramin, melianol meliacin, meliacarpin, meliartenin vanillin, hydroxyl-3-methoxcinnamaldehyde and (+-) pinoresinol have been reported earlier from the seeds of *M. azedarac*h[8,9].

Seeds of *M. azedarac*h L. have been scientifically reported to exert antimalarial, antifungal[9,10], ovicidal[11], insecticidal[12], antifeedant[13], rodenticidal activities[14] *in vitro* and *in vivo* studies.

In view of the ethnobotanical uses and medicinal properties of *M. azedarac*h, it is conjectured that this plant might possess antibacterial properties. Therefore, the present study was aimed to evaluate the antibacterial activity of seed extracts of *M. azedarach* against some hospital isolated pathogenic bacterial strains.

2. Materials and methods

2.1. Plant material

Matured seeds of *M. azedarac*h L. were collected from different localities of Aligarh district, India. Voucher specimen number [AV24, AV206] of the plant was deposited in the Herbarium of Department of Botany, Faculty of Life Sciences, Aligarh Muslim University, Aligarh-202002, U.P., India.

2.2. Preparation of extracts

Freshly dried and healthy plant material was pulverized into fine powder using an electric grinder. Powder was stored in a desiccator. 500 g plant powder was refluxed with 95% methyl alcohol (MeOH) in a round bottom flask on a hot water bath for 10 h. Mother liquor (crude MeOH extract) was filtered out and residual plant material was again refluxed with 95% MeOH for 10 h. The entire process was repeated four times to obtain maximum yield of MeOH extract. The extract was evaporated to dryness at 35 ℃ under reduced pressure. Dried methanol extract was refluxed with light petrol [(60-80) ℃] for 5 h. After filtration, the residual methanol extract was again refluxed with petrol for 5 h and filtered. This process was repeated five times. Petrol was evaporated under reduced pressure to obtain petrol soluble extract. Petrol insoluble fraction of methanol extract was refluxed with benzene for 5 h. Thereafter, it was filtered and refluxed again with benzene for 5 h and filtered. The process was repeated five times. Benzene was evaporated under reduced pressure to obtain benzene soluble extract. Benzene insoluble fraction was refluxed with ethyl acetate for 5 h. Thereafter, it was filtered and refluxed again with ethyl acetate for 5 h and filtered. The process was repeated five times. Ethyl acetate was evaporated under reduced pressure to obtain ethyl acetate soluble extract. Ethyl acetate insoluble fraction was refluxed with methyl alcohol (95%) for 5 h, filtered and was repeatedly refluxed for five times with methanol. The methanol soluble fraction was evaporated under reduced pressure to obtain methanol extract, while methanol insoluble residue was discarded.

2.3. Preparation of aqueous extract of the seeds

Shade dried plant material (500 g) was ground to a fine powder then it was poured with double distilled water, and left for 72 h at room temperature. The flask was then refluxed over hot water bath for 10 h and the mother liquor was filtered. The distilled water was again refluxed and filtered. This process was repeated for four times. The filtrate was evaporated to complete dryness under reduced pressure on a water bath. Thus the residue was aqueous plant extract.

2.4. Microorganisms

The bacteria were obtained from the bacterial stock, Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh, India. The bacterial cultures were maintained at 4 ℃ on nutrient agar.

2.5. Antibacterial activity

The agar disk diffusion method was used to determine the susceptibility of plant extracts against chosen bacteria[15,16]. Standardized inoculums ($1-2 \times 10^7$ CFU/mL 0.5 McFarland standard) were introduced on the surface of the plates containing Mueller Hinton agar (MHA), which was spread evenly with a glass spreader. The paper disk (6 mm in diameter; Whatman filter paper no 1) containing 1, 2, 5, 10 and 15 mg/mL plant extracts was dried and placed on the agar surface with the help of a sterile forcep. Finally, paper discs were placed with forceps to make complete contact with the surface of the medium. Plates were allowed to stand at room temperature for 30 min (Pre diffusion time) and then incubated aerobically at 37 ℃ and examined for the zone of inhibitions after 24 h. The experiments were repeated thrice.

3. Results

A result of the *in vitro* antibacterial activity of *M. azedarach* seed extracts was described in Table 1. The petrol extract of the seeds was found to be effective against six gram-positive and nine gram-negative pathogenic bacteria. Benzene extract inhibited the growth of three gram-positive and nine gram-negative pathogens. Ethyl acetate, methanol and aqueous extracts displayed inhibitory activity against all the tested pathogens.

Table 1
Inhibition zone of the seed extracts of *M. azedarach* L. against some hospital isolated human pathogenic bacterial strain (mm).

Extract (mg/mL)	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 extract (1)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Petrol extract (2)	8	–	–	10	8	–	10	–	–	–	10	–	–	7	7	–	10	–
Petrol extract (5)	10	7	7	16	11	–	13	7	12	–	14	9	11	11	11	–	14	11
Petrol extract (10)	16	11	12	17	16	–	19	9	17	–	17	11	12	15	15	–	19	14
Petrol extract (15)	21	18	17	22	18	–	23	13	22	–	21	13	17	19	20	–	22	17
Benzene extract (1)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Benzene extract (2)	8	–	–	–	8	–	7	–	–	–	10	–	–	7	7	–	10	11
Benzene extract (5)	10	–	–	–	11	–	10	11	11	–	14	9	11	11	11	–	14	14
Benzene extract (10)	16	–	–	–	16	–	15	14	16	–	17	11	12	15	15	–	19	18
Benzene extract (15)	21	–	–	–	18	–	19	19	21	–	21	13	17	19	20	–	22	21
Ethyl acetate extract (1)	9	–	–	–	–	7	9	–	7	–	7	7	9	–	–	7	9	7
Ethyl acetate extract (2)	12	7	11	10	9	10	12	10	11	11	12	12	12	12	9	10	13	11
Ethyl acetate extract (5)	19	11	17	16	16	15	17	13	17	16	17	17	18	17	16	17	18	19
Ethyl acetate extract (10)	22	16	19	19	20	18	20	17	19	18	22	22	23	19	20	22	22	23
Ethyl acetate extract (15)	26	21	23	23	26	23	26	20	23	23	25	27	26	24	24	27	26	27
Methanol extract (1)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	–	7
Methanol extract (2)	7	–	–	10	9	9	7	7	–	–	9	10	7	7	8	10	7	11
Methanol extract (5)	12	–	11	16	16	15	12	9	8	9	14	15	10	12	12	16	12	16
Methanol extract (10)	16	10	15	19	20	19	17	13	13	14	18	19	13	16	18	20	18	19
Methanol extract (15)	21	16	20	23	26	21	22	17	17	18	22	23	19	21	21	23	22	21
Aqueous extract (1)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aqueous extract (2)	7	–	7	10	9	–	7	–	9	–	–	–	–	–	–	–	7	7
Aqueous extract (5)	10	–	11	12	13	9	10	9	11	8	9	11	8	7	11	10	12	11
Aqueous extract (10)	15	8	15	16	18	11	15	11	14	12	14	15	11	12	14	12	15	16
Aqueous extract (15)	18	11	19	19	20	16	19	13	16	13	18	19	15	17	19	16	20	19
Chloramphenicol (30 mg/disk)	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*; 2. *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*.
–: no inhibition.

4. Discussion

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization[17]. Although a significant number of studies have been used to obtain purified phytochemicals, a very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants[18-36]. The results of the present study are encouraging as all the tested extracts revealed antibacterial potential, although the inhibitory activity was strain specific and concentration dependent. Petrol fraction showed maximum inhibition against *Bacillus subtilis*, *Proteus mirabilis* and *Shigella flexneri*. Benzene extract inhibited the growth of twelve tested pathogens and the maximum inhibition zone was recorded against *Proteus mirabilis* and *Shigella flexneri*. All the tested pathogens showed sensitivity against the ethyl acetate extract and the most affected bacteria were *Staphylococcus aureus*, *Bacillus subtilis* (gram positive) and *Pseudomonas aeruginosa* and *Shigella flexneri* (gram negative). Methanol extract was also found to be effective against all the strains and maximum inhibition was recorded against *Shigella dysenteriae* and *Plesiomonas shigelloides*. Aqueous extract showed moderate degree of sensitivity against all tested pathogenic bacteria.

From the results it is clear that seeds extracts of *M. azedarach* L. are effective in controlling 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 methanol and aqueous extracts which inhibited the growth of all the tested human pathogens. The petrol and benzene extracts, as compared with the polar extracts, showed antimicrobial action against fifteen and twelve pathogens, respectively. The above results show that seed extracts can be effective antibiotics, both in controlling Gram positive and Gram negative human pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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