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Author(s): T. Munir
A. Mohyuddin
Z. Khan
R. Haq

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Exploration of Antibacterial Potential of *Melia Azedarach* L.

Munir, T.[1*], Mohyuddin, A.[1], Khan, Z.[2] and Haq, R.[3]

[1]Department of Chemistry, University of Management and Technology

[2]Department of Botany, G. C. University Lahore,

[3]Department of Biotechnology, Lahore College for Woman University, Lahore.

[*] mrswaseemkhan@gmail.com,

Abstract

Melia azedarach L. belongs to one of the most versatile medicinal plants family meliaceae (mahogany) which has great attraction for researchers. The plant was selected for research because it was one of the least explored members. The presence of saponin, alkaloids, tannins and flavonoids in the leaves extracts of plant indicated its medicinal value. These compounds have pharmacological effects against cancer, viral and malarial infections that are one of the main causes of deaths. With passage of time most of bacterial strains develop resistance against traditional medicines so they are needed to be upgraded or replaced. There is a need to develop antimicrobial agents with more effectiveness and minimum side effects. There are some reports from last two decades that Melia azedarach is a potential source of novel antibodies. Its extracts have both antioxidant and antimicrobial activities. Powdered leaves of M. azedarach were extracted with methanol and extract was preliminary examined by phytochemical tests and thin layer chromatography. The different concentrations of extracts showed good antibacterial activities against three pathogenic bacterial strains Staphylococcus aureus, Escherichia coli and Bacillus thuringiensis. The results indicated that M. azedarach L. could be an effective source of herbal medicines against infectious diseases.

Keywords: *meliaceae, melia azedarach L, antibacterial activity.*

Introduction

The medicinal plants are major source of raw materials for treatment of various diseases in Ayurveda, Siddah and Unani traditional systems. In the modern medicine system the 25% of drugs are extracted from the plants. *Azadirachta indica* (former *Melia indica*) is widely used in herbal medicinal system. *Melia azedarach* L. (*M. azedarach*), although a close family relative, has been ignored in indigenous and modern system of medicine. The classification of two plants is presented in Table1 show their relevance with each other. The plant is native to Asia and is widely spread and naturalized in most of the subtropical and tropics parts of the world [1-4]. Deciduous tree of *M. azedarach* is small to medium in size. Maximum height is 15m and diameter is about 60cm.The plant has

characteristic dark green dense and spreading crowned with dark brown bark. Dark green, pale leaves are short stalked, hairless and thin and white flowers having purple stripes and fragrance (1, 2). It has yellow round, smooth and fleshy berries with four to five seeds [5].

Table1.
Classification of M. azedarach and A. indica

Category	***Melia azedarach* L. *(dhraik)***	***Azadirachta Indica*(neem)**
Kingdom	Plantea	Plantea
Bionomial Name	*Melia azedarach* L.	*Azadirachta Indica*
Division	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida
Order	Sapindales	Sapindales
Family	Maliaceae	Maliaceae
Genus	*Melia*	*Azadirachta*
Species	*M.azedarach*	*M.Indica*
Synonyms	*Melia australis sweet*	*Melia indica*

The extracts of leaves, fruits have antioxidant, analgesic and antimicrobial activities. The plants also showed antifungal, antimalarial & cytotoxic activities. Alkaloids, flavonoids, glycosides, saponins & tannins present in the methanolic extract of plant [5]. It has been used as a bitter tonic, fuel, an anthelminthic, astringent and an antiseptic agent [6]. It has been shown curing properties for diseases such as rheumatism, leprosy, rashes etc [7]. It is revealed from a literature survey that limonoides, limonin, nomilin and obacunone have been discovered [8]. The oil of *M. azedarach* contains sulfur containing limonoids which has been used in soap & cosmetic industries [9].

Figure1. Leaves of *Melia Azedarach* Linn

Figure2. Flowers of *Melia Azedarach* Linn

2. Materials

2.1. Collection and Identification of Plant Material

The plant material of *M. azedarach* L. was collected from garden of G.C. University of Lahore. Taxonomist Prof. Dr. Zaheer-Ud-Din Khan of Govt. College University Lahore identified the plants. Further the plant specimen was deposited at herbarium of G.C University.

Family : *Meliaceae*

Species : *Melia Azedarach* Linn.

Voucher No.: G.C. Herb Bot. 2908

2.2. Drying Of Plant Material

The fresh plants leaves were placed in clean stainless tray and kept under shade at temperature of 20°C to 25°C for one week. After drying the leaves were powdered by mechanical means.

3. Methodology

3.1. Soxhlet Extraction

An average of 40g (500g total) of dried and powdered plant leaves were taken at a time in the thimble and put into the Soxhlet apparatus. Plant material was extracted with 350ml of methanol for 4 to 6 hours. The crude extract obtained was filtered with filter paper and the filtrate was then analyzed further. The extract was concentrated in a rotary evaporator for complete removal of methanol [7].

3.2. Detection of Secondary Metabolites in Crude Plant Extract

For detection of secondary metabolites, 4g of powdered plant leaves were extracted with methanol (50ml), boiled it for 5 minutes, cooled and filtered. In the filtrate added small amount of Lead acetate to make solution. Filtered and divided in several parts. Different tests were applied for the Tannins, Flavonoids, Alkaloids and Saponins according to standard procedures [10].

3.3. Thin Layer Chromatography

The TLC was carried out with BAW (Butanol, Acetic Acid CH_3COOH, H_2O in ratio 4:1:5) as solvent. After drying, the chromatogram was observed under UV lamp. The wave length 366 nm was used for observation. Distribution of components was good and different colored spots were observed. Pattern of colored spots was recorded and Rf value of each spot was calculated.

3.4. Antibacterial activity

The antibacterial investigation was carried out by well diffusion method against three pathogenic bacterial strains. *Bacillus thuringiensis* (Ref. # BL- Bt6), *Staphylococcus aureus* (ATCC # 6633) and *Escherichia coli* (ATCC # 25922). The concentrated methanolic extract of leaves of *M. azedarach* L was dissolved in distilled water and different concentrations were prepared.

Nutrient broth was dissolved in distilled water and heated. Adjust the PH to 7.4 and then sterilized it in an autoclave for 25 minutes. Added some agar in it and heated again. Bacterial cultures were taken from stock slants. The bacterial cultures were incubated at 37°C for one day. These cultures were then added to conical flask which consists of freshly prepared nutrient broth and placed in a shaker at 37°C and incubated for one day. The nutrient agar in molten form was poured as a basal layer in petri dishes. Plates were inoculated with respective organism. After solidification, wells were bore which were then fill with control and extracts of different concentrations, and incubated again for 24 hours at 37 °C. The diameters of zones of inhibition around the wells were recorded. The antibacterial activity of plant extracts were estimated by evaluation of Minimum inhibitory concentration (MIC) [11-13].

4. Results and Discussions

4.1. Phytochemical Analysis

Phytochemical analysis of *M. azedarach* methanol leaves extracts was carried out to investigate the absence or presence of alkaloids, flavonoids, saponins, glycosides and tannins in leaves and the results are presented in the Table2. The results supported the previous studies that plant contained Alkaloids, Tannins, Steroids Saponins [14].

Test results revealed that plant leaves contain bioactive compounds like Alkaloids, Steroids, Saponins, Tannins, and flavonoids. These compounds are responsible for the antimicrobial efficacy of plant. However the sample extract did not show the presence of Anthraquinones.

Table2.
Phytochemical analysis of methanolic extract of leaves

Experiment Test for Alkaloids:	**Observation**	**Inference**
Mayer's test	Yellow ppt.	Alkaloid present
Wagner's test	Reddish Brown ppt.	Alkaloid present
Hagner's test	Yellow ppt.	Alkaloid present
Dragendorff test	Cloudy Appearance	Alkaloid present
Test for Tannins:		

5ml extract + 6 drop $FeCl_3$ and allowed to stand for 5 minutes	Bluish Green ppt.	Tannins present
Test for Flavonoids:		
Plant extracts+2 ml $AlCl_3$.	Yellow Fluorescence	Flavonoids present
Test for Steroids:		
Leiberman-Burchard test	Violet Color	Steroids present
Test for Saponnins:		
Shook the aqueous plant vigorously	Persistent Froth	Saponnins present
Test for Anthraquinones:		
Plant extract + few drops of *N, N*-dimethyleanaline	No red color	Anthraquinones absent

4.2. Thin Layer Chromatography

TLC on prepared TLC card (0.2 mm) was used for the initial investigation of bioactive compounds present in methanolic leaves extracts of *M. azedarach*. The chromatogram showed three differently colored spots under UV lamp (Table3). The Rf values of spots were compared with the standard data from Harborne [15] for general indication of classes of compounds present. The Rf values indicated the existence of Anthocyanidin-3-glycosides, Anthocyanidin 3, 5–diglycosides or isoquercetin and rutinosides.

Table3.

TLC of the methanolic extract using BAW

No. of spot	Color in UV light (366nm)	Rf value	Expected compounds
1	Orange	0.674	Anthocyanidin-3-glycosides
2	Red	0.584	Anthocyanidin 3,5–diglycosides or isoquercetin
3	Green	0.426	Rutinosides

4.4. Antibacterial Activity

M. azedarach L was reported to have antibacterial properties against bacterial strains other than those used in this study [16,17]. The antibacterial activity of *M. azedarach* L was investigated by using three concentrations of concentrated methanolic leaves extract in methanol as 100 mg/ml (D2), 50 mg/ml (D2), and 10 mg/ml (D1). Control used was Streptomycin for whom average diameter of zone of inhibition was 27.5mm. The extracts showed significant inhibition against Gram positive as well as Gram negative bacteria as shown in Table4.

The maximum inhibition was observed by concentration D1 in case of *Escherichia coli* while *Bacillus THURINGIENSIS* AND *Staphylococcus aureus* gave maximum reduction on concentration D2. This indicated the different response of Gram positive and Gram negative bacteria against bioactive compounds.

Table4.
Antibacterial activity of *M. azedarach* leaves extract

Bacterial Strain	Type of Bacteria	Diameter of zone of inhibition(mm)			
		Methanol	D1[a]	D2[b]	D3[c]
Bacillus thuringiensis	Gram positive	0	27	28	11
Staphylococcus aureus	Gram positive	0	27	31	17
Escherichia coli	Gram negative	0	39	32	12

[a] D1 showed concentration 100 mg/ml

[b] D2 showed concentration 50 mg/ml

[c] D3 showed concentration 50 mg/ml

A Comparison of MIC value of three bacteria is demonstrated in Figure3. It showed that methanolic extract of *M. azedarach* is most potent against *Staphylococcus aureus*. Therefore in view of previous reports and present study, *M. azedarach* was found to be much potent and having bioactive compounds [18-21].

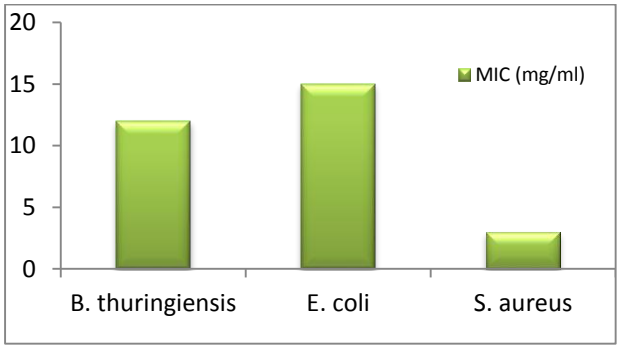

Figure3. Comparison of MIC of three bacteria

5. Conclusion

The *M. azedarach* is famous for its important alkaloid content. Phytochemical tests and TLC showed that the plant leaves extract contains large variety of secondary metabolites such as alkaloids, flavonoids, saponins, tannins and glycosides. Due to presence of such compounds, the plant exhibited significant antimicrobial activity against pathogenic bacteria. The leaves extract of plant showed high antibacterial potential and gave significant zones of

inhibition. The study indicated that *M. azedarach* can contribute well in the herbal medicinal system so it can be concluded that there is a need to explore and accept its medicinal value. The bioactivity spectrum of plant against pathogenic bacteria is worth noticing. Since the bioactive compounds present in the leaves extract of plant are responsible for the significant antimicrobial activity against the tested microorganisms so this plant need to be explored further for its medical potency.

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