

Research Article

Effects of Indian lilac (*Melia azedarach* L.) extract fractions against resilient documented hospital pathogens

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Abstract

A plant's response to the pioneer biological activities is the first step that it is used for therapeutic purposes. The antibacterial and antifungal properties of *Melia azedarach* L. (Indian Lilac) were investigated. As Indian Lilac is known for centuries as an antibacterial and antifungal agent, the main purpose of this study was to find is it effective against the most resilient human bacterial and fungal pathogens, which were collected from the different hospitals of Khyber Pakhtunkhwa. The crude extract was fractionated into five parts i.e. aqueous, chloroform, crude methanolic, ethyl acetate, and n-hexane. The fractions were then checked against most resistant strains of bacteria and fungi i.e. MRSA (Methicilline resistance *Staphylococcus aureus*), *Serratia marcescense*, *Staphylococcus aureus*, and *Streptococcus mutans*, for antibacterial activity and *Alternaria alternate*, *Aspergillus flavous*, *Fusarium oxysporum*, and *polyspondylium pallidum* for antifungal activity. Among all fractions, ethyl acetate was most effective against the selected pathogens showing the activity of i.e. (61-39%) and lowermost effectiveness shown by an aqueous fraction (47-30%) for bacterial pathogens. Regarding the antifungal activity, the most effective fraction was crude methanolic extracts (65-30%), and the least effective was fractions (20-10%). While the effectiveness of other selected fractions was in between the two. In conclusions of our experimentation, it is intensely supported, that *Melia azedarach* L. is even significant against most resilient strains of bacteria and fungi.

Keywords: Antibacterial; antifungal; antimicrobial; Indian Lilac; *Melia azedarach*

Introduction The use of plants as traditional medicine had been dated since the arrival of humans on planet earth. Humans have no other options in case of their diseases but to

utilize either plants or animals for cure purposes. Men of ancient times used plants for food and as traditional medicines but with time and advancements, the usage has been

improved as well. E.g. traditional and indigenous knowledge is slowly replaced by homeopathic and allopathic medicines [1, 2]. People belonging to different religions and living in different parts of the world used plants for treatment purposes independently. Almost three by fourth parts of plants have specific compounds that can cure a wide range of diseases [3, 4]. These compounds are the result of secondary metabolism and mostly include alkaloids, glycosides, terpenoids, flavonoids, saponins, tannins, and amino acids, etc. which can fight germs in the animal body and can cause deterioration against them. The discoveries transpired mainly in two disciplines i.e. science and technology in the last centuries had enabled the scientists to perform more accurate and advanced experiments which on success-ding produce new natural and synthetic drugs helping to reduce and eradicate various diseases. According to the WHO (world health organization), the annual death rate occurring throughout the world is mainly contributed by infectious diseases and in tropical countries, it may reach up to 50%. Reports disclosed that developing countries still rely on indigenous knowledge of plants and use 80% medicinal plants as therapeutic agents against numerous human diseases [5, 6]. Due to such shortcomings, scientists and researchers started working to process and develop safe herbal medicines and to isolate active therapeutic compounds used in both herbal and derived medicines to cope with germs [7, 8].

Melia azedarach L. an active therapeutic compound containing a plant appearing like a tree with 15-25-meter height with deciduous nature having dense canopy cover [9, 10]. It has its place in the family Meliaceae and it has originated from lower and humid countries of Asia [11, 12]. Its distribution is cosmopolitan regarding tropical countries and the areas of various countries falling in the tropical zone;

however, it also prevails in sub-tropical regions as well. Different vernacular names of this medicinal plant also exist as used in traditional medicinal purposes long ago, so it can be called Chinaberry, Persian lilac, Tulip cedar, and white cedar [13, 14]. Its use in indigenous and traditional systems of medicines from past decades reveals its medicinal properties and with advanced scientific researches, it has proven to stop malaria, cancer, inflammation, stomachache, and analgesic up to a certain extent. Similarly, it can be utilized as a diuretic, astringent, and anthelmintic as well. In the focused experiment, five different solvents have been used to dissolve parts used of *M. azedarach* and were checked against four bacterial and four fungal strains respectively to investigate the anti-microbial activity.

Material and Methods

Antibacterial activity

Collection and processing of plant specimen

Several visits have been conducted to different areas of the Khyber Pakhtunkhwa for the collection of *Melia azedarach* L. The collected plant material was rinsed and dried in shadow after which it was dried in an oven. The fully dried plant parts were pulverized by using a grinder. Then the powder is stored at a cool place before extraction.

Preparation of extraction and fractions

The cold maceration method was used for the extraction of the active metabolites. One and half of the powdered plant material were dipped in two liter of ethanol and incubated for 5 days at 40°C. The material filtered thrice and a clear filtrate was obtained. The filtrate was subjected to evaporation via a rotary evaporator at 40°C. The obtained extract was dried and then dissolved in 100ml distilled water. The solution was the fractionate of dissimilar diluents comprising methanol, n-hexane ethanol, ethyl acetate, and chloroform by using a separating funnel. All the fractions thus obtained were

concentrated by rotary evaporator and designate for that solvent fraction.

Media preparation

The anti-bacterial activity in this experiment was checked by the agar well diffusion method. One liter of distilled water was used to liquefy 25 g of Luria Broth, PH of Miller powder was put at 7.0. The media put in an autoclave in a 250ml flask. The selected four bacterial strains were introduced into the flask and kept overnight at 150RPM at 37°C. After that agar was converted into solid form, five holes were excavated into the agar through a borer. The inoculum was introduced into the tunnel. The bacterial and fungal species were selected due to their frequent occurrence in local hospitals of Khyber Pakhtunkhwa (KPK) and also showing resistance to different drugs.

Test for bacterial strains

Among the four selected bacterial strains, three were gram-positive and one strain is gram-negative i.e. MRSA (*Methicillin resistance staphylococcus aureus*), *Streptococcus mutans*, and *Staphylococcus aureus*. And the gram-negative is *Serratia marcescens*.

Measurement of zones of inhibition

Dimethyl sulfoxide 20mg/ml had been utilized as a negative control in which acted as a solvent. Cefotaxime (standard antibiotics) was used as a positive controller. The plant fractions of about 75µl were introduced into the wells of the petri dish and the Petri dishes were retained at 37°C in the incubator for 24 hours. When the incubation period was completed then the growth area of each transparent region was analyzed. The analyses were repeated thrice to calculate the standard data [7, 8].

Test for fungal strains

During the antifungal activity, the four fungal strains were selected i.e. *Fusarium oxysporum*, *Aspergillus flavus*, *polyspondylium pallidum*, and *Alternaria alternate*.

Statistical analysis

The statistical analysis was done through Excel MS Office 2013 while ANOVA were done through Excel two factor variation without replication.

Results

Antibacterial activity of *Melia azedarach* L.

Regarding check the effectiveness of *Melia azedarach* L. as an anti-bacterial agent, five fractions were utilized and four bacterial strains i.e. *Streptococcus mutans*, *Staphylococcus aureus*, MRSA (Methicillin resistance *Staphylococcus aureus*), and *Serratia marcescens* were analyzed. The selection of these species for experimental purposes is because of their pathogenic nature and the pathogen are reported frequently during the investigation from hospitals of Khyber Pakhtunkhwa. All these species are the causative agent for different human diseases and therefore we tested our extracts against these pathogenic species to determine their pathogenicity and find out cheap and efficacious treatment against these bacterial strains. Results shown in the table revealed the effectiveness of all the fractions by taking 6 mg/ml of selected and checked crude fraction of plant against the observed bacterial strains.

Antibacterial potential of the crude extract of *Melia Azedarach*

Crude methanolic extract inhibited the growth of MRSA with highest percentage of 52% followed by *S. marcescens* up to 47%, *S. aureus* with 46%, and *S. mutans* up to 39%. *n*-hexane fraction highly repressed the growth of *S. marcescens* with 52% which was followed by MRSA with 40%, *S. mutans*, and *S. aureus* with 35% and 34% respectively. The chloroform fraction was found to be the most active extract which showed the highest activity against the *S. marcescens* with 61% while its lowest activity is observed against *S. aureus* with 38%. In Ethyl acetate fraction the growth

inhibition was recorded in order of *S. marcescens* (52%), *S. aureus* (46%), MRSA (44%), and *S. mutans* (32%) respectively. The aqueous extracted fraction inhibited the growth of *S. marcescens* with the highest

inhibition zone (10.0±0.54 mm zone and 47%) while in the same fraction the lowest efficacy was obtained for *S. aureus* which is up to 30%. See (Table 1, 2; Fig. 1).

Table 1. Antibacterial Potential of the Crude Extract of *M. Azedarach*

Bacteria K2	Standard	Cr. Met. Ext		<i>n</i> - hexane		CHCl ₃		EtOAc		Aqueous	
		Zone	%	Zone	%	Zone	%	Zone	%	Zone	%
<i>S. aureus</i>	26.0±0.34	12.0±0.67	46.15	09.0±0.33	34.62	10.0±0.99	38.46	12.0±0.55	46.15	08.0±0.56	30.77
<i>S. mutans</i>	28.0±0.45	11.0±0.56	39.29	10.0±0.55	35.71	13.0±0.93	46.43	09.0±0.40	32.14	09.0±0.35	32.14
<i>S. marcescens</i>	21.0±0.22	10.0±0.36	47.62	11.0±0.45	52.38	13.0±0.45	61.90	11.0±0.44	52.38	10.0±0.54	47.62
MRSA	25.0±0.34	13.0±0.35	52.00	10.0±0.65	40.00	11.0±0.65	44.00	11.0±0.64	44.00	10.0±0.66	40.00

Table 2. ANOVA (two factors) of antibacterial potential of *M. azedarach*

Summary	Count	Sum	Average	Variance		
<i>S. aureus</i>	6	77	12.83333	44.16667		
<i>S. mutans</i>	6	80	13.33333	53.86667		
<i>S. marcescens</i>	6	76	12.66667	17.86667		
MRSA	6	80	13.33333	33.86667		
Standard	4	100	25	8.666667		
Cr. Met. Ext	4	46	11.5	1.666667		
<i>n</i> - hexane	4	40	10	0.666667		
CHCl ₃	4	47	11.75	2.25		
EtOAc	4	43	10.75	1.583333		
Aqueous	4	37	9.25	0.916667		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	2.125	3	0.708333	0.235457	0.870249	3.287382
Columns	703.7083	5	140.7417	46.78393	1.27E-08	2.901295
Error	45.125	15	3.008333	-	-	-
Total	750.9583	23		-	-	-

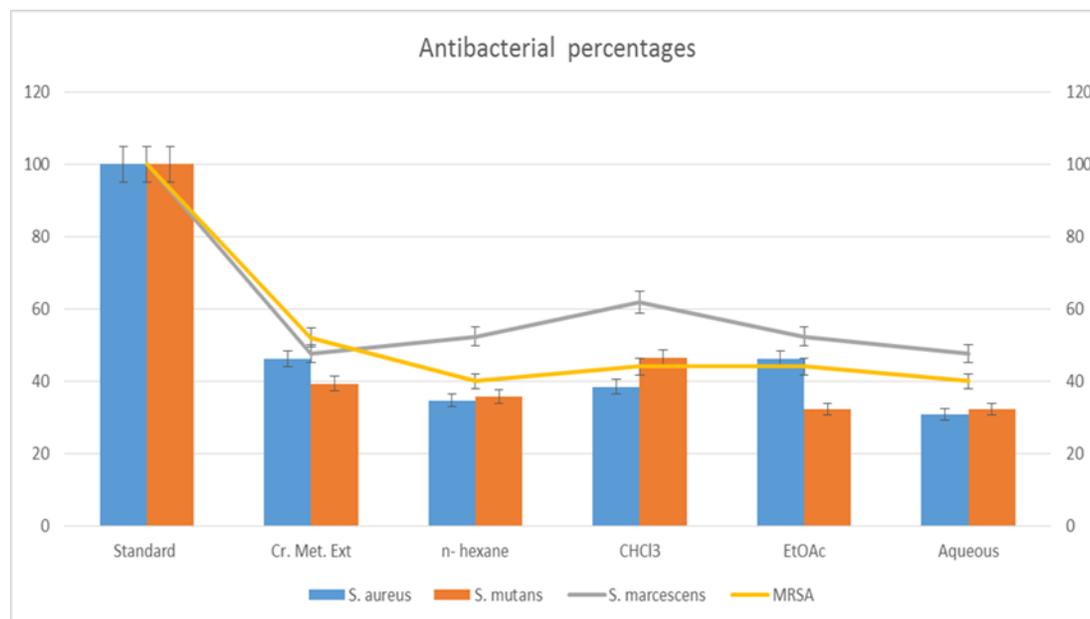


Figure 1. Antibacterial activity of *Melia azedarach*

Anti-fungal activity of *Melia azedarach* L.

Four fungal strains i.e. *Fusarium oxysporum*, *Aspergillus flavus*, *Polyspondylium pallidum* and *Alternaria alternate* were utilized to obtain the efficacy of the plant in five different solvents. The overall results of anti-fungal activities were significant except for aqueous extract. Crude methanolic extract proved to be very resistant against *A. alternate* and prevented its growth up to 65% followed by *P. pallidum* with 55% and was least resistant against *F. oxysporum* with 30%. N-hexane fraction repressed the growth of *P. pallidum* with 60% followed by *A. flavus* with 50%, *A. alternate* with 45%, and *F. oxysporum* with 40% which is the least for this fraction. Chloroform proved to be most active against *A. alternate* and prevented the growth of said fungus with the highest

percentage up to 60%, followed by *P. pallidum* with 55%, *A. flavus* with 45%, and was least active against *F. oxysporum* 40% respectively. *A. alternate* was also sensitive to ethyl acetate as well and was stopped by the fraction up to 55%, for which *P. pallidum* and *A. flavus* fall second each with 40%, and *F. oxysporum* was slightest sensitive with 35%. The aqueous extract was less active against all fungal lines, and its effectiveness was greater against *A. flavus* with 20% following *A. alternate* and *F. oxysporum* with 15% each and *P. pallidum* with 10% respectively. See (Table 3, 4; Fig. 2). From the above experiment, it is quite clear that *Alternaria alternate* and *Polyspondylium pallidum* appeared to be the most affected species by the action of extracts.

Table 3. Anti-fungal activity of *Melia azedarach* L.

Fungi	Standard	Cr. Met. Ext		<i>n</i> -hexane		CHCl ₃		EtOAc		Aqueous	
		Area	%	Area	%	Area	%	Area	%	Area	%
<i>A. flavus</i>	100.0±0.00	45.0±0.32	45.00	50.0±0.99	50.00	45.0±0.42	45.00	40.0±0.45	40.00	20.0±0.77	20.00
<i>A. alternate</i>	100.0±0.00	65.0±0.45	65.00	45.0±0.97	45.00	60.0±0.32	60.00	55.0±0.50	55.00	15.0±0.56	15.00
<i>F. oxysporum</i>	100.0±0.00	30.0±0.67	30.00	40.0±0.44	40.00	40.0±0.21	40.00	35.0±0.99	35.00	15.0±0.78	15.00
<i>P. pallidum</i>	100.0±0.00	55.0±0.66	55.00	60.0±0.50	60.00	55.0±0.22	55.00	40.0±0.56	40.00	10.0±0.98	10.00

Table 4. ANOVA (two factors) of Antifungal Potential of *M. Azedarach*

Summary	Count	Sum	Average	Variance		
<i>A. flavus</i>	6	300	50	710		
<i>A. alternate</i>	6	340	56.66667	766.6667		
<i>F. oxysporum</i>	6	260	43.33333	856.6667		
<i>P. pallidum</i>	6	320	53.33333	856.6667		
Standard	4	400	100	0		
Cr. Met. Ext	4	195	48.75	222.9167		
<i>n</i> -hexane	4	195	48.75	72.91667		
CHCl ₃	4	200	50	83.33333		
EtOAc	4	170	42.5	75		
Aqueous	4	60	15	16.66667		
ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Rows	583.3333	3	194.4444	3.517588	0.041343	3.287382
Columns	15120.83	5	3024.167	54.70854	4.24E-09	2.901295
Error	829.1667	15	55.27778	-	-	-
Total	16533.33	23		-	-	-

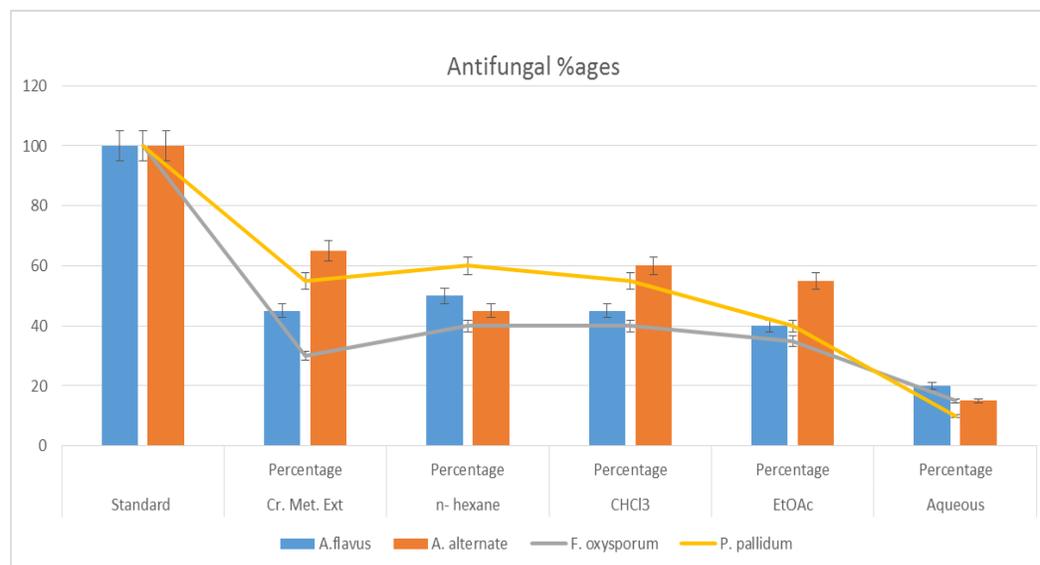


Figure 2. Antifungal Activity of *Melia azedarach*

Discussion

Medicinal plants are used against microbial species for many centuries and are a usual practice for the human being. These antimicrobial tests have been carried out at a regular interval of time because it produces useful compounds that prevent their pathogenic activity. Among the microbial pathogens, Bacteria and Fungi have the most disastrous impact on the health of plants and are contagious to animals resulting in huge yearly damages to crops, harmful effects on quality control, and reducing storing capabilities of cultivated crops. To deal with such kinds of fatal pathogens, active ingredients from plant or natural sources become the utmost importance of the modern era [15, 16]. These adaptable fungi when gets entry the human body result in several diseases that can be fatal but they can be prevented by medicines having no side effects [17]. For developing such natural drugs medicinal plants are the main spotlight [18, 19]. Plants contain several active compounds that have been separated before and resulted in effective prevention against

different diseases. As the knowledge of plants along with their discoveries increases, the value of drugs from plant's origin also increases and is at a peak due to their negligible side effects as compared to allopathic medicines. In the emerging areas of the world, the standards and quantity of allopathic and laboratory prepared drugs are less than their demands [20, 21], also the prices of these synthetic drugs are far from accessibility, for those people plants can be the ultimate solution because of its cheapness and no extra effects. Indigenous knowledge of medicinal plants is still an important way to cure diseases and is used in developing next-level drugs and chemical substances to interact with new diseases regarding safety [22, 23]. In this attempt, a medicinal plant i.e. *Melia azedarach* was applied in five fractions (Crude methanolic extract, n-Hexane, trichloromethane, ethyl acetate, and Aqueous extract) and optimized against four pathogenic bacteria (*Staphylococcus mutans*, *Serratia marcescens*, MRSA, and *Streptococcus aureus*) and fungal strains (*Fusarium oxysporum*, *Aspergillus flavous*,

Polysphondylium pallidum and *Alternaria alternata*) collected from hospitals of Khyber Pakhtunkhwa.

The plant extract in all solvents exhibited significant action against both pathogenic lines. *S. marcescens* and MRSA exhibited to be very sensitive against the plant extracts in different solvents regarding bacterial strains while *S. mutans* and *S. aureus* shows resistance to some extent but still these results can be prominent by increasing the concentration of extracts which is in agreement with [24, 25].

Conclusion

The overall antifungal activity was notable and significant except only aqueous extract which seems not to affect the growth of fungal strains. *Polysphondylium pallidum* and *Alternaria alternata* showed less resistance and were affected by the plant's extracts in all solvents and their average activity falls between 45% to 65%. *Fusarium oxysporum* and *Aspergillus flavus* showed high resistance and their average percentage also falls low. The overall results were very significant with p-value for antibacterial activity = 1.57×10^{-06} and $p = 1.19 \times 10^{-07}$ for antifungal activities.

Authors' contributions

Conceived and designed the experiments: M Hamayun, Performed the experiments: M Hamayun, T Yaseen & KU Rehman, Analyzed the data: KU Rehman & S Ullah, Contributed materials/ analysis/ tools: M Noor & AA Khan, Wrote the paper: KU Rehman, S Ullah & SS Khan.

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