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Antifungal, Antioxidant and Phytochemical Screening of *Melia azedarach* Flower Extracts by Using Different Solvents

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Authors' contributions

This work was carried out in collaboration between all authors. Author MKA designed this research study, performed the research activities in collaboration with co-authors and wrote the first draft of the manuscript. Author MA has supervised the designed research study and evaluated results critically. Author KB managed the analyses of the study, evaluation of results and guided about protocols of thesis writing. Author NA collected the literature review data. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To investigate the antifungal, antioxidant and phytochemical screening of *Melia azedarach* flowers by using different solvents.
Materials and Methods: Antifungal activity was experimented against three fungi (*Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus*). Antifungal activity of extract was measured by using agar well diffusion and serial microdilution methods. Antioxidant activity has been measured by 2, 2-diphenyl-1-picryl-hydrezyl [DPPH] free radical scavenging assay. Phytochemical screening was done by doing different tests for presence of saponins, anthraquninone glycosides,

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cardioactive glycosides, phenolic glycosides, cynogenic glycosides, phenols, alkaloids, tannins and flavonoids.

Results: Methanolic extract of *Melia azedarach* flowers showed highest antifungal activity against *Aspergillus flavus* fungus with zone of inhibition of 19.6±0.66 mm. Dichloromethane, ethyl acetate and n-butanol extracts showed significant antifungal activity with 18±0.57 mm, 18.6±0.88 mm and 18±0.66 mm zone of inhibition, respectively. N-butanol extract showed lower antifungal activity against *Aspergillus niger* with 13.6±0.88 mm zone of inhibition. Dichloromethane and methanolic extracts exhibited excellent minimum inhibitory concentration against *Aspergillus flavus* (13.909±1.5 µg/ml). Antioxidant activity has been measured by DPPH free radical scavenging assay. Only ethanolic extract of *Melia azedarach* flowers showed antioxidant activity with value of IC_{50} = 50±2.0. While dichloromethane, ethyl acetate, n-butanol and methanol showed no antioxidant activity. Phytochemical screening showed presence of saponins, anthraquninone glycosides, cardiaoactive glycosides, phenolic glycosides, cynogenic glycosides, phenols, alkaloids, tannins and flavonoids.

Conclusion: These findings demonstrate that leaf extracts of *Melia azedarach* extracts possess excellent antifungal activity and have potential to reduce problems of fungal pathogens.

Keywords: Antifungal; antioxidant; phytochemical analysis; agar well diffusion; MIC; DPPH assay.

1. INTRODUCTION

Plants have been used as source of medicine since the beginning of human civilization. Immemorial people believed that plants have healing power. Earliest uses of plants were found from era of Babylonian circa 1770 BC and ancient Egypt circa 1550 BC. Medicinal plants also have been found from Gaza pyramids and are present in the dark corner of Cairo Museum [1].

Pakistan has a particular biodiversity in the world. Approximately, 6000 plants species are found in Pakistan and used as traditional Unani medicines. Pakistan is one of those countries in which Unani medicines are properly used by large population. Unani medicines first originated in Greece. Ancient Greek Philosophers were founders of Unani medicine and this was documented by muslims during Islamic civilization [2].

Melia azedarach is the botanical name of white cedar and it belongs to the family *Meliaceae* [3, 4]. *Melia azedarach* has many common names as Pride of India, China berry, Pride of China, Persian Lilac, Umbrella China berry, Indian lilac, Umbrella tree. It has many local names which vary from country to country [5]. Worldwide name of *Melia azedarach* is White Cedar [6]. *Melia azedarach* is native to tropical Asia. It is widely distributed in Pakistan, Australia, Indonesia, India, Persia, Southeast Asia, South Korea and Japan. It is also cultivated in Brazil, Philippines, Argentine and United States of America. It is also cultivated in many Arab and African Countries. Nowadays, it is also cultivated in Manila and South Europe in parks and gardens [4,5,7,8]. *Melia azedarach* is a medium size deciduous tree. The height of the plant is 5-15 metres and 30-60 cm in diameter. It has wide, dense and dark green crown [4].

The purpose of study was to evaluate antifungal, antioxidant activity and phytochemical analysis of *Melia azedarach* flowers' extracts by using different solvents to know which solvent is better for extraction and best activity. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. The existing requirement is to introduce new herbal antifungal and antioxidant formulations in order to reduce the risk of drug resistance. The current study aims to reveal the antifungal and antioxidant potentials of *Melia azedarach* flowers by using different solvent extracts. Flowers of *Melia azedarach* were collected from Rahim Yar Khan. The flowers were then identified from Department of Life Sciences, the Islamia University of Bahawalpur. The voucher specimen (2105/DLS/IUB) was deposited in herbarium Department of Life Sciences, Islamia University of Bahawalpur.

2. MATERIALS AND METHODS

2.1 Plant Sample Preparation

Fresh flowers of *Melia azedarach* were separated, cleaned carefully and washed with distilled water. Flowers were shade dried at room temperature of 21-30ºC for 30 days. Fungitoxic chemical constituents of the plants are thermolabile. They lost their fungitoxic activty when they were dried at normal temperature. [9,10]. After grinding, all plant materials were

crushed and then milled to fine powder with electrical grinder. The powder was passed through sieve to get fine powder and then stored in air tight container [3].

2.2 Preparation of Extract

300 gm powder of the *Melia azedarach* flowers was taken in amber glass bottle having capacity of 2.5 liter and 900 ml of methanol (Merck, Germany, 99.8%), was added, soaked for 5 days with occasional stringing and shaking. After 5 days, soaked material was first filtered through several layers of muslin cloth to separate out coarse material from the solvent. Then solvent was filtered with Whatman No. 1 filter paper by using funnel and filtrate was obtained. Then remaining coarse material was soaked again in the same solvent to obtain maximum quantity of chemical constituents present in the plant material. This procedure was repeated three times and filtrates obtained were combined. This same procedure was done with remaining four solvents ethanol (Merck, Germany, 99.8%), ethyl acetate (Merck Germany, 99.5%), n-butanol (UK, 99.4%) and dichloromethane (Dongying Rich Chemical Co.Ltd, China, 99.8%) [11]. All the solvents used were of analytical grade.

2.3 Fungal Strains

Aspergillus niger (0002)*, Aspergillus fumigatus* (0013) and *Aspergillus flavus* (0051) were provided in form of stock culture agar by the First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences (IAGS), the University of Punjab, Lahore.

2.4 Preparation of Inoculum

Fungal inoculums were prepared from 3 day old cultures plates by taking few colonies of the respective fungus and transferred into 5ml sterile saline solution and the turbidity was adjusted to 0.5 McFarland turbidity standards. From this solution 1:10 dilution was performed three times with growth medium to get inoculums' density of $1\text{-}5x10^3$ CFU/ml [12].

2.5 Preparation of Sabouraud's Dextrose Agar

Sabouraud's dextrose agar 65 g was mixed with 1 litre distilled water and sterilized in autoclave at 121ºC at 15 Psi for 15 minutes.

2.6 Antifungal Assay

Petri dishes were sterilized in hot air oven at 180ºC for 30 minutes and then for aseptic environment put into laminar flow hood. Sterilized Sabouraud's dextrose agar medium was cooled to room temperature. 20 ml of the Sabouraud's dextrose agar was poured into petri dishes and then allowed to solidify at room temperature. Then with sterile cork borer 8 mm well were formed. 60 µl inoculums were poured into petri dish and spreading was done with sterilized L-shaped rod. 30 µl of 1, 10, 20, 50 and 100 mg/ml extract were transferred into the wells and the fungal culture petri dishes were put into refrigerator for the diffusion and then transferred into incubator for incubation at 27℃ for 48 hours. Fluconazole was used as positive controls 5 mg/ml per well concentration and DMSO as negative control to compare antifungal effect. Then zone of inhibition is measured in mm. All the procedures were done in triplicate and the mean values were taken [12].

2.7 Minimum Inhibitory Concentration (MIC) of Fungus

Serial dilution method was used to determine the minimum inhibitory concentration. 5 mg stock solution was prepared and further serial dilutions were made of 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 mg/ml. For the preparation of inoculums RPMI 1640 was supplemented with glucose to make a final concentration of 2% and used. Inoculums were prepared by counting the spores in haemocytometer chamber under microscope to make $1\text{-}5\times10^5$ CFU/ml spores of the fungus. In this method 96-well sterile microplates were used. The 100 µl of the inoculum and 40 µl of test drug were added in each well of the 96-well microplate. Then pre-read absorbance was measured at 530 nm and plates were incubated at 37℃ for 48 hours. After 48 hours at 530 nm after-read was taken and difference between pre-read and post read was taken as an index of fungal growth. All procedures were done in triplicate and mean of the reading were taken, respectively. In this fluconazole was taken as positive control and DMSO as negative control [13].

2.8 Antioxidant Activity

Method used for antioxidant activity was DPPH free radical scavenging assay.

2.9 DPPH Free Radical Scavenging Assay

To determine antioxidant activity 2, 2-diphenyl-1-picryl-hydrezyl (DPPH) was used as free radical. 100 µM concentration of DPPH was used in methanol. Serial dilutions were made to check the IC50. In 96-well microplate total volume was 100 µl which consisted of 90 µl of DPPH solution and 10 µl of the test solution. The contents were mixed and incubated for 30 minutes at 37℃. To determine the absorbance at 517 nm synergy HT BioTEk USA microplate reader were used. Ascorbic acid was used as standard antioxidant. All readings were taken in triplicate [14].

Ez-fit-5 Perrella Scientic Inc., Amherst USA software was used to calculate the IC50. Decrease in absorbance indicated increased radical scavenging activity which was determined by following formula:

$$\text{Inhibition}(\%) = \frac{(\text{abs.of control} - \text{abs.of test solution}) \times 100}{\text{Abs.of control}}$$

Where,

Absorbance of control = Total radical activity without inhibitor.

Absorbance of test = Activity in the presence of test compound [15].

3. PHYTOCHEMICAL SCREENING

The phytochemical screening was done to know the secondary metabolites present in the different solvents extracts which are responsible for the antimicrobial and antioxidant activity.

3.1 Test for Saponin

2 g of the *Melia azedarach* flowers' extract was boiled in 20ml of distilled water and filtered. 5 ml of distilled water was mixed with 10 ml of filtrate and vigorously shaken for stable persistent froth. Froth was mixed with 3 drops of olive oil, shaken vigorously and observed for the formation of emulsion which shows the presence of saponin [16].

3.2 Test for Tannins

3.2.1 Ferric chloride test

0.5 g of the extract was boiled in 10 ml of distilled water and filtered. 5 ml of filtrate taken in test tube and few drops of 0.1% ferric chloride was added and observed for color change. Formation of brownish or blue-black coloration shows the presence of tannins in extract [17].

3.2.2 Sodium nitrite test

In 3 ml filtrate of extract was taken in test tube and few crystals of sodium nitrite were added. In test tube no color change showed absence of tannins [18].

3.2.3 Bromine water test

In the filtrate of extract by adding few drops of bromine water there was precipitation. The formation of the precipitation shows the presence of tannins [18].

3.3 Test for Fats and Oils

Small quantity of *Melia azedarach* flowers' extract was taken and pressed between two filter papers. A stain on filter paper indicates the presences of oil [19].

3.4 Test for Phenols

5 ml of extract was taken in test tube and 2 ml of 5% sodium nitrate and 2 ml of 5% glacial acetic acid was added and observed for color change [19].

3.5 Test for Alkaloids

3.5.1 Wagner's test

5 ml of the extract was taken in the test tube and a few drops of the Wagner's reagent was added. Formation of reddish brown precipitate show the presence of alkaloids [19].

200 mg extract was taken in test tube; 10 ml methanol was added in test tube and stirrer and filtered. 2 ml of filtrate was taken in test tube and 1% HCl was added, in a test tube, boiled and filtered. 1 ml of the filtrate was taken and 6 drops of the Wagner's reagent were added in test tube. The formation of the brownish red precipitate shows the presence of alkaloids [20].

3.6 Test for Terpenoids

200 mg extract was mixed with 10 ml chloroform in a test tube and filtered. 2 ml of filtrate was taken in test tube and 2 ml of acetic anhydride and concentrated H_2SO_4 was added in it. Blue green ring formation shows the presence of terpenoids [20].

3.7 Test of Flavonoids

200 mg extract was taken and 10 ml ethanol was added, mixed and filtered. 2 ml of filtrate was

taken in test tube and few drops of concentrated H_2SO_4 and magnesium ribbon was added. The formation of the pink tomato red color shows the presences of flavonoids [20].

3.8 Test for Glycosides

3.8.1 Keller Kiliani test

200 mg plant extract was dissolved in distilled water and filtered. 2 ml of filtrate was taken and 1 ml glacial acetic acid, $FeCl_3$ and concentrated H_2SO_4 was added to it. Formation of the blue green color shows the presence of cardiac glycosides [20].

3.8.2 Guignard's test

2 gm of the plant extract was taken in a test tube and 5 ml of distilled water was added in it. With the help of the cork, sodium picrate paper strip was hung in the test tube above the extract. The test tube was incubated in the water bath at temperature up to 40℃. Appearance of reddish or brick red color shows the presence of cardiac glycosides [18].

3.8.3 Erdmann's test

200 gm extract was taken and mixed with methanol and filtered. The residual portion was taken and 3 drops of Erdmann's reagents were added into it. Appearance of the bright red color shows the presence of phenolic glycosides [18].

3.8.4 Borntrager's test

Filtrate was shaken with chloroform and the organic layer was separated. Organic layer was taken in test tube and dilute ammonia solution was added in it. Formation of the pink color in the lower layer shows the presence of glycosides [18].

3.9 Test for Anthraquinones Glycosides

0.5 g of the extract was taken in test tube, boiled with 10 ml of sulphuric acid and filtered. Filtrate was taken and shaken with 5 ml of chloroform. With help of the pipette chloroform layer was transferred into another test tube and 1 ml of diluted ammonia was added in it and observed for color change [17].

4. RESULTS

4.1 Antifungal Activity

Methanolic extract of *Melia azedarach* flowers showed highest antifungal activity against *Aspergillus flavus* (19.6±0.66 mm diameter zone of inhibition) as compared to fluconazole (24.6±1.20 mm) which was used as positive control. This is due to its high polarity and absorbent power to absorb most of the phytochemical constituents, which are proven due to phytochemical screening tests in current study. Dichloromethane, ethyl acetate and n-butanol extracts showed significant antifungal activity with 18±0.57 mm, 18.6±0.88 mm and 18±0.66 mm zone of inhibition, respectively with respect to fluconazole 24.6±0.66 mm. N-butanol extract showed lower antifungal activity against *Aspergillus niger* with 13.6±0.88 mm zone of inhibition. All these results are given below in Table 1 and Fig. 1.

4.2 Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) against fungus was also determined through serial dilution method [21]. Methanolic extracts exhibited excellent minimum inhibitory concentration against *Aspergillus flavus* (13.909±1.5 µg/ml). Ethanolic and Dichloromethane extract exhibited excellent minimum inhibitory concentration against *Aspergillus niger* with value of 14.266±1.0 and 14.409±1.1, respectively as shown in Table 2 and Figs. 2 and 3.

4.3 Antioxidant Activity

Antioxidant activity has been measured by DPPH free radical scavenging assay. Only ethanolic extract of *Melia azedarach* flowers showed antioxidant activity with value of IC_{50} = 50±2.0. While dichloromethane, ethyl acetate, n-butanol and methanol gave no antioxidant activity, which are shown in Table 3 and Fig. 4.

4.4 Phytochemical Analysis

Different tests were performed for phytochemical screening. Results showed presence of saponins, anthraquninone glycosides, cardio-active glycosides, phenolic glycosides, cynogenic glycosides, phenols, alkaloids, tannins, and flavonoids as given in Table 4.

Table 1. Antifungal activity of different extracts of *Melia azedarach* flowers

Fungus	Extract conc.	Dichloro-methane	Ethyl acetate	N-butanol	Methanol	Ethanol	Fluconazole
Aspergillus flavus	1	10±0.57	8.3±0.33	8.6±0.33	9.6±0.33	9.6±0.33	
	10	11±0.57	10.6±0.33	10.3±0.33	11.6±0.33	11±0.57	
	20	12.6±0.33	12.3±0.33	11±0.57	15.3±0.33	12.3±0.33	24.6±1.20
	50	14.3±0.33	13.6±0.66	12.6±0.33	16±1.73	13.3±0.33	
	100	17±0.57	15.3±0.33	14.6±0.33	19.6±0.66	13.3±0.33	
Aspergillus niger	1	12±1.00	0±00	0±00	10.3±0.33	9.6±0.88	
	10	13±1.15	9.3±0.33	0±00	11.3±0.33	12.6±0.33	
	20	15±0.57	12±0.57	10±0.57	12.6±0.33	14.6±0.33	23.6±0.66
	50	15.6±0.33	13.3±0.33	11.3±0.88	14.6±0.33	16.3±0.6 6	
	100	17.6±0.33	14.6±0.33	13.6±0.88	15.3±0.66	17±0.33	
Aspergillus fumigatus	1	11.3±0.66	11.6±0.33	13.3±0.88	11.3±0.66	10.6±0.66	
	10	12±1.00	13.6±0.33	14±0.57	12.6±0.33	11.3±0.33	
	20	14.6±0.33	14.6±0.33	15.6±0.33	13.6±0.33	12.3±0.3 3	24.3±0.66
	50	15.6±0.33	15.6±0.33	17.6±0.88	15±0.57	14±0.57	
	100	18±0.57	18.6±0.88	18.6±0.66	16±0.57	17±1.15	

**Zone of inhibition is measured in mm. ± S.E.M. Mean difference is significant at 0.05 level (p-value <0.05). The size of well was 6 mm*

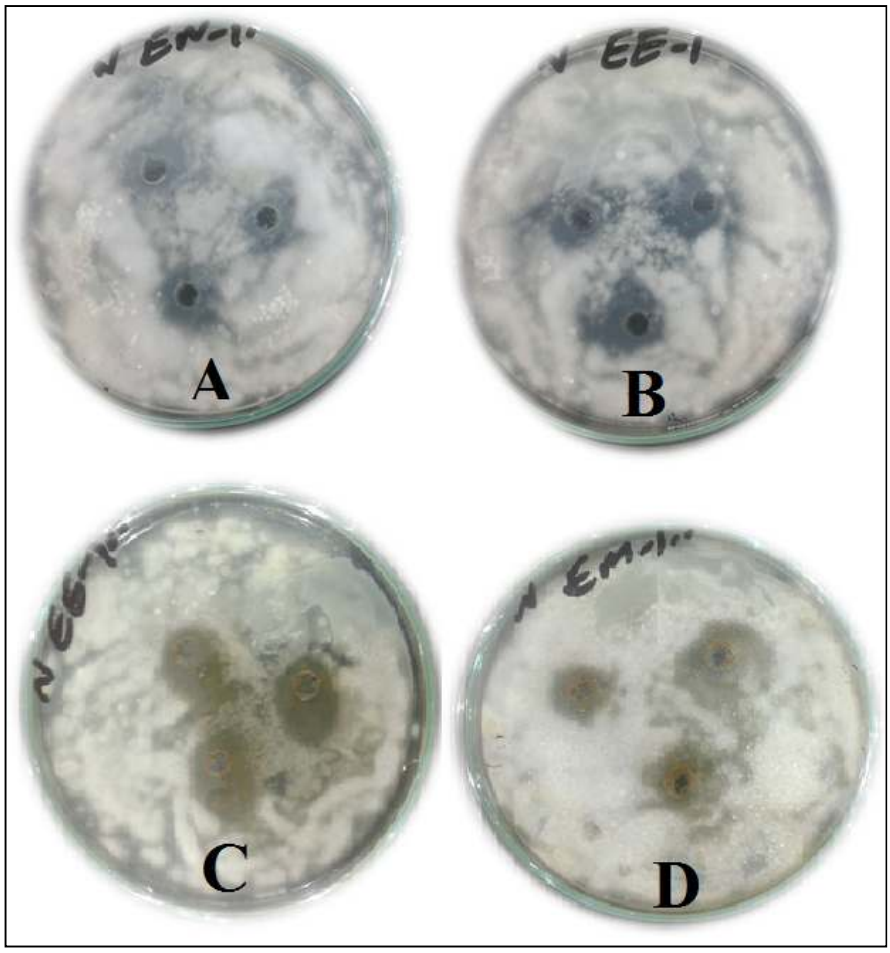

Fig. 1. A, B, C, D showing zone of inhibition against different fungus

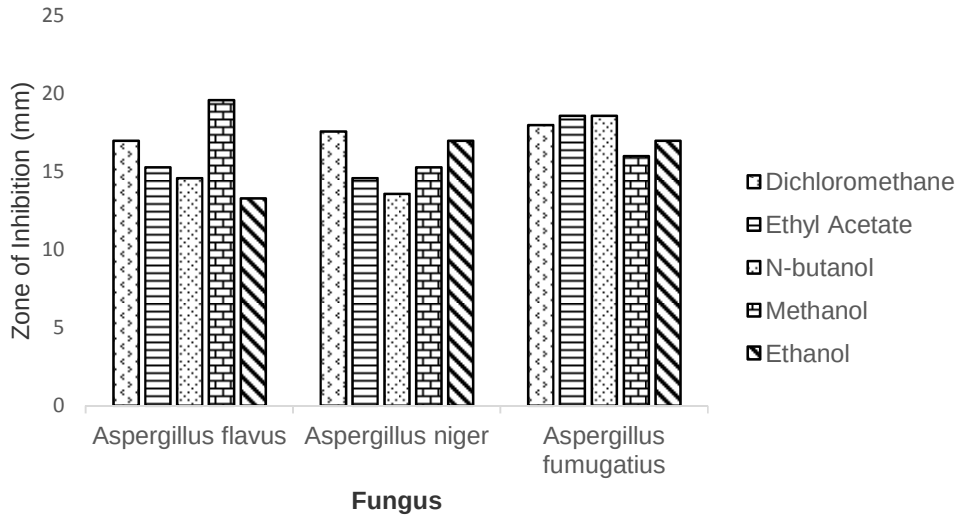

Fig. 2. Antifungal activity of 100 mg/ml dose of plant extract against *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus*

Table 2. Minimum inhibitory concentration of different extracts of *Melia azedarach* flowers

Extracts	Tests fungus		
	Aspergillus flavus	***Aspergillus niger***	***Aspergillus fumigatus***
	MIC (µg/ml)		
Dichloromethane	15.038±1.3	14.409±1.1	15.494±1.5
Ethyl acetate	21.215±1.0	23.803±1.5	16.001±1.0
N-butanol	17.446±1.1	26.461±1.0	20.567±1.3
Methanol	13.909±1.5	18.684±1.1	18.992±1.1
Ethanol	15.958±1.0	14.266±1.0	15.306±1.0
Fluconazole	4.65±0.79	4.65±0.79	4.65±0.79

**Zone of inhibition is measured in mm. ± S.E.M. Mean difference is significant at 0.05 level (p-value <0.05). The size of well was 6 mm*

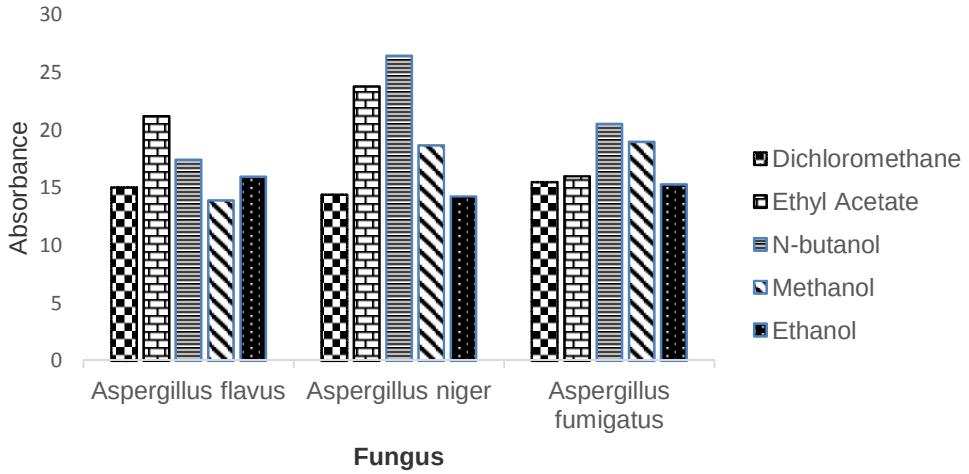

Fig. 3. Minimum inhibitory concentration of plant extract against *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus*

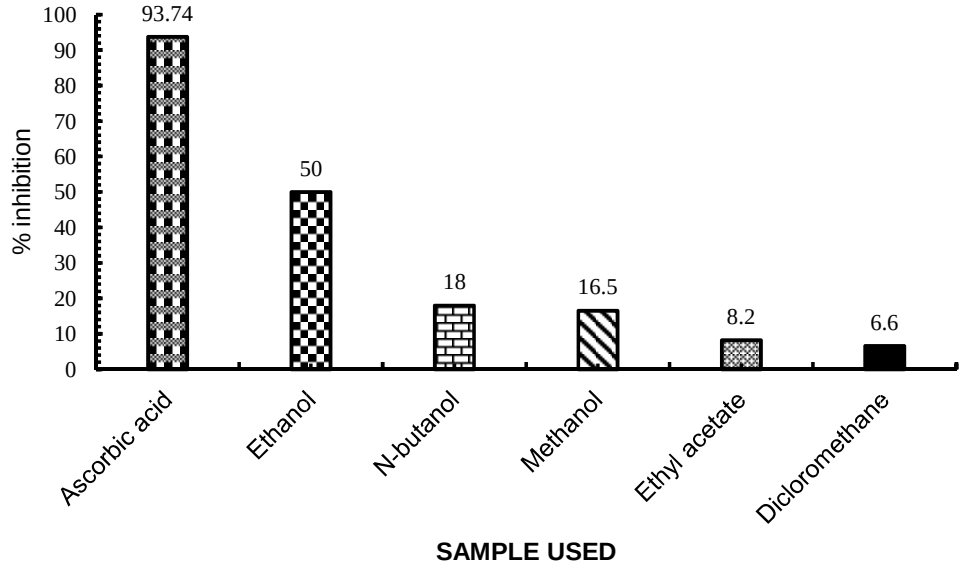

Fig. 4. Antioxidant activity of *Melia azedarach* flower's extracts by DPPH assay

Table 3. Antioxidant activity of *Melia azedarach* flower's extracts by DPPH assay

Extracts	Concentration (mg/ml)	% inhibition	IC_{50} (mg/ml)
Dichloromethane	5	6.6±0.95	-
Ethyl acetate	5	8.2±0.2	-
N-butanol	5	18±3.0	-
Methanol	5	16.5±0.5	-
Ethanol	5	50±2.0	5
Ascorbic acid	0.5 (mmol/ml)	93.74±0.12	-

± S.E.M. Mean difference is significant at 0.05 level (p-value <0.05)

Table 4. Phytochemical analysis of *Melia azedarach* flowers' extracts

Chemical groups	Dichloromethane	Ethyl acetate	N-butanol	Methanol	Ethanol
Saponin	+	+	++	+++	+++
Anthraquinone glycosides	-	-	+	+	+
Cyanogenic glycoside	+	+	++	+++	+++
Phenolic glycoside	-	-	-	+	+
Cardioactive Glycosides	-	-	-	+	+
Phenols	+	+	+	+	+
Alkaloids	+	+	+	-	-
Tannins	-	-	+	+	+
Flavonoids	-	-	+	+	+

** (+) Present, moderate (++), (+++) highly present, (-) absent*

5. DISCUSSION

Plants have been used in the treatment of many diseases by many ethnic groups in different parts of the world from ancient times. But they were unable to evaluate their medicinal effects which were used for treatment of many diseases without any scientific evidence. To find out these, phytochemicals, pharmacological studies have been done by many scientists all over the world [22]. Medicinal plants are preferred natural source of medicines. Antimicrobial agents from natural sources have gained immense consideration nowadays. Efforts have been made to find out such compounds with antimicrobial activities that can replace synthetic drugs. Phytochemicals obtained from plants are used as prototypes to evolve much adequate and less toxic drugs which can be used to treat several etiologies viz. human pathogens as well as fungus, virus or bacteria. Many studies have been conducted on various extracts of plants to find out new antimicrobial entities. Due to such attractive abilities, medicinal plants are taking their place in food supplements, neutraceuticals and pharmaceuticals [23-25].

In the current study, different extracts of *Melia azedarach* flowers were investigated to explore their antifungal activity, antioxidant activity and phytochemical screening. Different solvents i.e., methanol, ethanol, dichloromethane, n-butanol and ethyl acetate of varying polarity were used for extraction. Antifungal activity was experimented against three fungi (*Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus*) utilizing different concentrations i.e.,1, 10, 20, 50 and 100 mg/ml. Antifungal activity of extracts was measured by using agar well diffusion and serial microdilution methods.

Dichloromethane, ethyl acetate, n-butanol, ethanol and methanol extracts of *Melia azedarach* flowers were active against all tested human pathogens but pathogens showed less susceptibility at small doses i.e., 1, 10 and 20 mg/ml while their susceptibility was increased with the increase of drug concentration i.e., from 50 to 100 mg/ml as shown in Table 1. From results of this study it was revealed that difference in zone of inhibition in growth of microorganisms was significant at small doses i.e., 1, 10 and 20 mg/ml of extracts as compared to standard drug but difference decreases as the drug concentration increases i.e., 50 mg/ml and 100 mg/ml as given in Table 1 as compared against fluconazole.

In another study, ethanolic extract of ripe fruits was investigated for their fungicidal and

fungistatic activity. Ethanolic extract showed excellent fungicidal activity with value of minimum inhibitory concentration of 50 mg/ml and minimum fungicide concentration with value of 60 mg/ml against *Micrisporum canis.* Ethanolic extract showed significant fungistatic and fungicidal effects against different pathogenic fungi [26]. These results are comparable with the results of present study in which ethanolic extract of *Melia azedarach* flowers showed minimum inhibitory concentration of 15.306 ±1.0 µg/ml, 14.266 ±1.0 µg/ml and 15.958±1.0 µg/ml against *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus*, respectively.

The results of current study indicate presence of compounds possessing antioxidant activity in ethanolic extract. Ethanolic extract have ability to scavenge DPPH free radical then initial purple color of solution changes into yellow due to formation of diphenyl picryl hydrazine [27]. Amount of antioxidant components that can be extracted from plant material is mainly affected by procedure of extraction, which may differ sample to sample and to solvent which is used for extraction with other factors [28]. Antioxidant activity is due to the presence of total phenolic and flavonoids compounds in extracts [29]. Phytochemical analysis of *Melia azedarach* flowers extracts showed presence of phenols and flavonoids in extracts. Ethanolic extract showed highest percentage of free radical inhibition due to which it is revealed that ethanolic extract of *Melia azedarach* can be used as antioxidant due to its antioxidant potential.

The presence of different phytochemicals in extracts shows that the polar solvents are much efficient in degradation of cell wall, which has unipolar character and cause phenols, polyphenols or anthocyanins to be released from cells [30]. Previous studies of phytochemical screening of different parts of *Melia azedarach* showed that plant contains 7 carboxylic acids, 96 limonoids and terpenoids, 3 anthraquiones and 5 flavonoids [31]. These compounds have long history of possessing both medicinal and physiological properties e.g., anti-inflammatory, antibacterial, antifungal, hypolipidemic, antioxidant, anti-carcinogenic, anti-mutagenic effect and digestive stimulation action [3,21,27, 15,32-35].

6. CONCLUSION

In current study, I have determined antifungal activity by agar well diffusion method and micro serial dilution method. Antioxidant activity was measured by free radical scavenging assay. Phytochemical analysis of *Melia azedarach* flowers' extract was performed by performing different identification tests for tannins, saponins, phenols, glycosides, alkaloids, flavonoids and assessed that *Melia azedarach* flowers' extracts have excellent antimicrobial and antioxidant activities. So there is necessity for further studies for isolation and characterization of active chemical constituents and clinical studies to standardize their pharmacodynamics and pharmacokinetics.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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