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***In-vitro* Antibacterial and Brine Shrimp Lethality of Methanolic extract of *Melia Azedarach* linn.**

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ABSTRACT

The study was designed to determine the *in-vitro* antibacterial and cytotoxic activities of methanolic extract of *Melia azedarach* leaves. Dried powder of leaves of *Melia azedarach* was extracted with methanol. The *Melia azedarach* extract was assayed for antibacterial and cytotoxic activities using agar well diffusion technique and brine shrimp lethality test, respectively. The methanolic extract of *Melia azedarach* showed moderate antibacterial activity against three bacterial strains such as *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. The methanolic extract of the plant exhibited strong cytotoxicity in the brine shrimp lethality bioassay test. These results indicated the use of *Melia azedarach* to treat infections with emphasis to isolate and characterize the active principle responsible for antibacterial and cytotoxic activities and its exploitation as therapeutic agent.

Keywords: *Melia azedarach*, antibacterial activity, cytotoxic activity, brine shrimp lethality test.

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INTRODUCTION

Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional medicine relatively cheaper than modern medicine ¹. Medicinal plants contain pharmacologically active principles which, over the years, have been explored in traditional medical practice for the treatment of various ailments ².

Melia azedarach, commonly known as chinaberry tree, bead-tree or Cape lilac, is a species of deciduous tree in the mahogany, family Meliaceae, that is native to Pakistan, India, Southeast Asia and Australia. Different phytochemicals have been isolated from fruit include melianoninol (I), melianol (II), melianone (III), meliandiol (IV), vanillin (V) and vanillic acid (VI) ³. Leaves have been shown to contain nimbinene, meliacin, quercetrin, quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, rutin and kaempferol-3-O-rhamno-Dglucoside ^{4,5}. Hot methanolic extract of *Melia azedarach* leaves contain dipentadecyl ketone, glycerol 1, 3-bisundec-9-enoate 2-dodec-9-enoate and glycerol tris-tridec-9-enoate ⁶. The plant is traditionally used for the treatment of leprosy, inflammations, and cardiac disorders. Its fruits extracts possess ovicidal ⁷ and larvicidal activity ⁸. The leaf extracts also possess antiviral ⁹ and anti-fertility activity ¹⁰. With this background the present study was aimed to explore the antibacterial and cytotoxic activities of methanolic leaf extract of *Melia azedarach*.

MATERIALS AND METHOD

Plant Collection and Identification

The Leaves of plant *Melia azedarach* Linn, free from disease were collected from mummdivaram, East Godavari District, Andhra Pradesh, India on January 5th of 2012. The plant was identified and authenticated by Mr.P.V.Prasanna, Scientist-‘E’-in charge Botanical Survey of India, Deccan regional centre, Hyderabad-500048 where a voucher specimen has been deposited. The leaves were washed thoroughly 2-3 times under running water and with sterile distilled water. Leaves were then air-dried under shade by blotting them with a sterile paper.

Preparation of *Melia Azedarach* Leaf Extract

The plant samples were collected in cotton bags and dried under shade for one month. The material was ground using a grinder into a fine powder. One kilogram of ground material was soaked in 2 L of methanol for one week and filtered. The percolates were dried under vacuum in a rotary evaporator to obtain methanol extract of *Melia azedarach*.

Determination of Phytochemical Constituents

The preliminary qualitative phytochemical studies were performed for testing the different

chemical constituents such as alkaloids, steroids, tannins, phenols, flavonoids, glycosides, saponins and anthraquinone glycosides^{11,12}.

Antibacterial Assays

Test Organisms

Two strains of Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and one strain of Gram negative bacteria *Escherichia coli* were used to evaluate the antibacterial activity.

Antibacterial Activity

Agar well - diffusion method¹³ was followed to determine the anti- microbial activity. Nutrient agar plates were swabbed with sterile cotton swabs. Wells of 10mm diameter and about 2cm apart were made in each of these plates using sterile cork borer. The plant extract of various concentrations was added to the wells and the cultured Petri plates were incubated for 24 hrs at 37⁰ c. The antibacterial spectrum of extract then determined in terms of zone sizes [Inhibition Zone Diameter]. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones indicate the presence of antimicrobial activity.

Brine Shrimp Lethality Bioassay¹⁴

Brine Solution:

38 gms of NaCl was weighed and dissolved in 1000 ml of distilled water and autoclaved at 15 lbs pressure for 15-20 minutes and after sterilization p^H was adjusted to 8.5 by using 1 M NaOH solution.

Hatching of shrimps:

100 mg of *Artemia salina* eggs was added to 200 mL of sterile brine solution & aerated for 38 hrs.

Test items preparation:

Appropriate dilutions of test items (2.5µg/ml to 100 µg/ml) were prepared.

Standard: Podophyllotoxin

Experimental Method

5 ml of sterile brine solution was pipette into each test tube and from that 1000µl was taken out. 100 µl of different concentrations of the test substances were added to the test tube with control in triplicates and the solution was vortexed thoroughly. 10 shrimps were added to each test tube and the surviving larvae of test and control test tubes were observed after 24 hrs. Replicas were maintained for accurate results.

Statistical Analysis

Percentage lethality was determined by comparing the mean surviving larvae of test and control

tubes. The LC₅₀ values were obtained using Fenny probed analysis software at 95 % confidence limits from observed data.

RESULTS AND DISCUSSION

The *Melia azedarach* Linn methanol extract was subjected to phytochemical analysis and the results revealed that the leaves contain alkaloids, steroids, tannins, phenols, flavonoids, glycosides, saponins and anthraquinone glycosides (Table 1).

Table 1: Preliminary phytochemical evaluation of methanol extracts of *Melia azaderech*.

Phytochemical constituents	Test/ Reagents	Result
Alkaloids	Dragendorff's test	+
	Wagner test	+
Anthraquinones	Borntrager's test	+
Cardiac glycosides	Kellar – Kiliani test	+
Flavonoids	Shinoda test	+
Phenols	Phenol test	+
Reducing sugars	Fehling test	-
Saponins	Frothing test/ Foam test	+
Steroids	Liebermann-Burchardt test	+
Tannins	Braemer's test	+

+ =Present, - =Absent.

The extract was screened for antibacterial activity using *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* as experimental models. The highest zone of inhibition (21 mm for 500 µg/ml) was found against *Staphylococcus aureus* with the methanol extract of *Melia azedarach*. This was followed by 18mm and 15 mm (for 500 µg/ml) by the same extract against *Bacillus subtilis* and *Escherichia coli*, respectively (Table 1). So, the methanolic extract was showed significant antibacterial activity.

The methanol extract is screened for the *in vitro* cytostatic activity by brine shrimp lethality test. The extract showed potent cytotoxicity comparing to the standards (Table 2). The LC₅₀ of the methanolic extract of *Melia azedarach* was 28.62 µg/ml. The standard, Podophyllotoxin, produced LC₅₀ 2.72 µg/ml, which was compared with the extract.

Table 2: Antibacterial Activity (Zone of Inhibition) of Methanolic Extract of *Melia Azaderech*

Extract	Conc.(µg/ml)	inhibition zone, mm		
		S.A.	B.S.	E.C.
Methanolic extract of <i>Melia azaderech</i>	50	5	5	4
	100	7	7	6
	200	12	12	9
	400	16	17	12
	500	21	18	15
Ciprofloxacin	5	24	23	22

S.A.: *Staphylococcus aureus*, B.S.: *Bacillus subtilis*, E.C.: *Escherichia coli*.

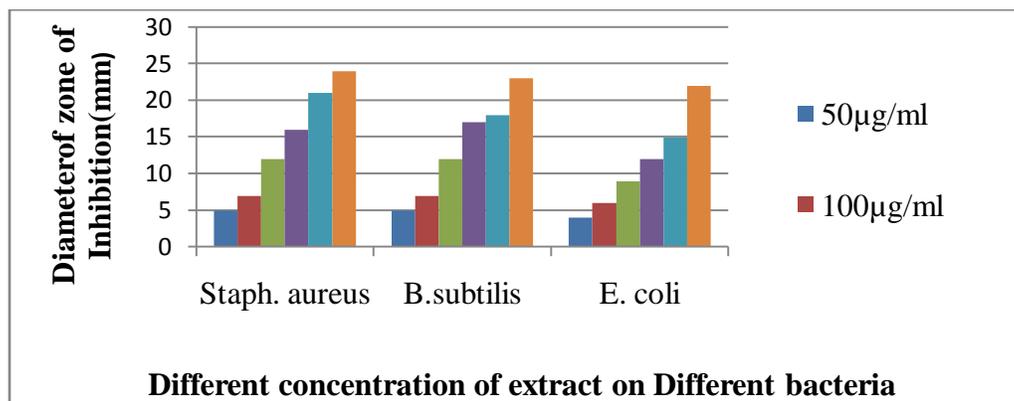


Figure. 1: Antibacterial activity of methanol extract of *Melia azaderech*

Table 3: Cytotoxic Activity of *Melia Azaderech* By Brine Shrimp Lethality Test

Test items	LC ₅₀ (µg/ml)	Degree(s) of freedom	UCL	LCL
Methanol extract	28.62	0.4933	37.71	16.32
Standard	LC₅₀ (µg/ml)	Degree(s) of freedom	UCL	LCL
Podophyllotoxin	2.72	0.4788	3.30	1.96

UCL = Upper confidence limit, LCL = Lower confidence limit.

CONCLUSION

Based on the result in the study, it was concluded that extract of *Melia azaderech* leaves are found to be having strong cytotoxic and moderate antibacterial activities. Further studies are required to identify specific active principles of this plant for these significant pharmacological effects.

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