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Original Research Article

ANTIMICROBIAL EVALUATION AND DETERMINATION OF TOTAL PHENOLIC CONTENT OF *MELIA AZEDARACH*

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Abstract

Melia azedarach used traditionally for various diseases and infections as per the klowledge of ayurveda. The acetone extraction was carried out of the plant. The phytochemical groups were identified by the tests of characterization and then quantified by the test of total phenolics. Total phenolics were determined using Folin–Ciocalteu reagent procedure. The total polyphenolic content and flavonoid content in the extract were found to be 71.13±0.11 mg/g and 11.14±0.12 mg/g respectively; since antimicrobial activity is related to phenolic compounds contained thus extract was also evaluated for the antibacterial activity. Result of study suggested that *Melia azedarach* having great potential as antibacterial agent. The extract at dose level of 200 mcg/ml showed highest inhibition against *P.* aerugunosa, *S. aureus* and E. *coli*.

Keywords: *Melia azedarach*, Total phenolics, Total flavonoids, Antimicrobial.

Introduction:

Melia azedarach L. is an ornamental fast growing tree of worldwide distribution[1]. Common names of *Melia azedarach* include chinaberry, persian lilac, white cedar, texas umbrella, bead-tree, cape lilac, ceylon cedar, cride of India. It grows to a height of 6-12m, some rainforest varieties reaching 30-45m[2-3]. The plant is used in some countries for medicinal purposes; it has been used as antihelmintic, tonic, antipyretic and also for the treatment of leprosy, eczema and asthma[4]. The leaves have insecticidal properties. In some area it is the only tree that is not eaten by the grasshoppers, for that reason, it has been frequently planted around the fields in several countries[5-6]. Seasoning is relatively simple in that plant dry without cracking or warping and are resistant to fungal infection[7]; in this fact, the aim of the present work was to evaluate the relationships between total phenolic contents

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and antibacterial potential of *Melia azedarach.*

Materials and Methods

Chemicals such as Quercetin and Gallic acid were obtained from Sigma Ltd., Nutrient broth procured from the Hi-media, Mumbai. All other reagents and solvents were of analytical grade.

Plant materials

The Plant materials were collected locally and allowed to shade dry, pulverized and preserved in plastic sachets safe from the light.

Preparation of extract

The shaded dry plant material was powdered coarsely with the help of grinder. A 100gm of powdered was extracted with organic solvents by using Soxhlet apparatus. Successive extraction was performed with petroleum ether, ethyl acetate and then acetone. The obtained extract was further filtered with Whatmman filter paper and then allowed to dry.

Preliminary phytochemical screening

The plants may be consisting of many chemical constituents like alkaloids, glycosides, carbohydrates, volatile oils, tannins, saponins, flavonoids etc. These chemical constituents are called as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of these secondary metabolites extract was subjected to chemical tests[8].

Total polyphenolic content

Total phenolic content of extract was determined using Folin–Ciocalteu reagent[9]. In this method, the blue colour formed due to the polyphenol present in the extract was measured using UV spectrophotometer. Sample solution of extract was prepared by dissolving 10 mg of the extract in 100 ml of methanol to give 100 µg/ml solutions. The extract (0.1ml) was mixed with the Folin-Ciocalteu phenol reagent (0.2 ml), water (2 ml) and sodium carbonate (15 % w/v, 1 ml) and absorbance was measured at 660 nm spectrophotometerically (Shimadzu 2405 spectrophotometer) after incubation at 50 ^{0} C. All the experiment was performed in triplicate. The total phenolic content was expressed as Gallic Acid Equivalents (GAE)**.**

Total flavonoid content

Total flavonoid content of extract was determined using previously reported method[9]. Sample solution was prepared by dissolving 10 mg extract in 100 ml of methanol to give 100µg/ml solution. Sample solution of extract (0.5 ml), ethanol (1.5 ml), $Al(NO_3)_3$ (0.1 ml, 10%), CH_3COONa (0.1 ml, 1 M) and water (2.8 ml) were thoroughly mixed and kept at ambient temperature for 40 min. The absorbance of reaction mixture was measured at 415 nm spectrophotometerically (Shimadzu 2405 spectrophotometer). All the experiment was performed in triplicate. Total flavonoid content was calculated according to a standard curve established with Quercetin. The total flavonoid content was expressed as Quercetin Equivalents (QE).

Antibacterial activity

Antibacterial activity of *Melia azedarach* extract was performed using both Gram negative and Gram positive bacterial strains; *Escherichia coli* as Gram negative strain while *Pseudomonas aeruginosa* and *Staphylococcus aureus* as Gram positive strains were used. Penicillin, Ampicillin and Ciprofloxacin were used as reference standard.

Preparation of inoculums

The active cultures required for the experiment were prepared by transferring one loop full of microorganisms from the stock cultures to the test tubes containing nutrient broth and were incubated for 24 hrs at 37^{0}C in incubator.

Preparation of nutrient agar

Generally nutrient agar is used as the media for the growth of the bacteria. Media were prepared by dissolving the given amount in the distilled water; sterilized it with the help of autoclave at 121^{0}C for 15 min.

Determination of zone of inhibition

The zone of inhibition was determined by the agar well diffusion method by using the nutrient agar plates seeded with the testing microorganisms' i.e; *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. Coli.* The

nutrient agar plates were prepared by pouring the 20 ml of sterilized nutrient agar solution in to the sterilized petri plates, the plates were allowed to solidify. The bacterial suspension was swabbed on the solidified nutrient agar, make hole in solidify plates with the help of borer having diameter 6 mm. Pour the 0.5 ml of testing solution of different concentration separately into separate cup and allow to stand for 10 min in refrigerator, then plates were kept in incubator at 37 ^{0} C for 24 hrs.

Determination of Minimum Inhibitory Concentration (MIC):

The extract of *Melia azedarach* was initially dissolved in DMSO and tested at the different concentration of 50 to 250 mcg/ml. Nutrient agar sterile plates were prepared and the inoculums of test microorganisms were spread uniformly. Wells were prepared by using sterile borer having the diameter of 6mm. About 100µl of test solution; standard antibiotic solution and solvent control were added in each well separately. The plates were kept at 40^{0}C for 1 hour for the diffusion of the test solution and then place them in the incubator for 24 hrs. at 37 ^{0} C. Each experiment was repeated three times. The MIC was defined as a lower concentration of the extract at which the bacteria do not demonstrate the visible growth.

Results and Discussion

Preliminary phytochemical screening

Preliminary phytochemical screening of acetone extract revealed the presence of chemical constituents like Tannins, Flavonoids, Carbohydrates, Saponins and Amino acids etc.

Total polyphenolic and flavonoid content

Melia azedarach are the good sources of natural antioxidants and might be useful in treating the diseases associated with oxidative stress. The floavonoid and polyphenolic were reported to be responsible for the antioxidant activity. The results of quantitative analysis indicate the presence of significant amount of polyphenol and flavonoids in the extract of plant with respect to Gallic Acid and Quercetin respectively. The total polyphenolic content and flavonoid content in the extract were found to be 71.13±0.11 mg/g and 11.14±0.12 mg/g respectively.

Antimicrobial activity

Results of determination of the bacterial inhibiting activity and the minimal inhibitory concentration of the extracts were represented in **Tables 1 and 2** respectively, extract was found to be active against all the tested strains of bacteria, with significant inhibition of diameters (zone of growth) comparable to the reference antibiotics for which strains were sensitive. Zone of inhibition increased gradually as we increased concentration of extract; significant inhibition observed even at low level also i.e; zone of inhibition ranging from 8±0.5 mm to 10±0.3 mm for tested species at the dose level of 50 mcg/ml. marked inhibition observed at the dose level of 200 mcg/ml; at this concentration the zone of inhibition were observed 18±0.7 mm, 20±0.4 mm and 24±0.7 mm for *P. aerugInosa*, *S. aureus* and *E.coli* respectively. The inhibition of bacterial growth at minimal concentration of extract indicates the antibacterial potential of extract even at low dose.

Table 1: Antibacterial activity of *Melia azedarach* extract (Diameter of zone in mm)

	Zone of Inhibition (mm)		
Concentration (mcg/ml) of Extract	***P. aerugInosa***	***S. aureus***	***E.coli***
20	11±0.3	9±0.1	6±0.2
50	10±0.3	10±0.5	8±0.5
100	14±0.1	13±0.4	12±0.6
150	20±0.4	16±0.3	15±0.6
200	24±0.7	20±0.4	18±0.7
Penicillin	17±0.2	-	-
Ampicillin	19±0.11	-	-
Ciprofoxacin	43±0.11	22±0.1	31±0.3

Values are Mean ± SEM (n=3)

Minimal inhibitory concentration

In most cases the MIC was found to be 0.65 mg/ml against most bacteria. *E. Coli*. was appeared the most resistant bacterial strain as it has highest MIC values in comparison with other species. There was no significant growth was observed in bacterial culture of microorganisms; which indicates *Melia azedarach* extract having significant antibacterial activity against the tested microorganisms. The results of the present study support the traditional usage of *Melia azedarach* and it can be recommended as newer antimicrobial agent for the treatment of infectious disease caused by the tested microorganism.

Table 2: Minimal inhibitory concentrations of extract of *Melia azedarach*

Microbial Strain	Minimum Inhibitory Concentration of Extract (mg/ml)
P. aerugInosa	0.65
S. aureus	0.65
E.coli	1.5

Conclusion

Our results indicate that the extracts of the plant of *Melia azedarach* contain polyphenols. This acetone extract was also able to inhibit the bacterial growth. The spectrum of action of the extracts is broad because it covers the Gram negative and positive bacteria. Based on the results, it can be concluded that the *Melia azedarach* extracts have great potential as antimicrobial agent and it can be used in the treatment of infectious diseases caused by resistant microorganisms as used in experiment.

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