

Antibacterial, Cytotoxic and Antioxidant activities of *Madhuca indica*

Israt Jahan Bulbul

Assistant Professor, Department of Pharmacy, Southeast University, Banani.
Dhaka-1213, Bangladesh
israt_jahanb872@yahoo.com

Yesmin Begum

Senior Lecturer, Department of Pharmacy, Southeast University, Banani.
Dhaka-1213, Bangladesh
yesumyta@gmail.com

Abstract- The research work was conducted on the evaluation of antibacterial, cytotoxic and antioxidant activities of the methanolic extracts of leaf and bark of *Madhuca indica* (Family-Sapotaceae). Disc diffusion technique was used for in vitro antibacterial screening using kanamycin as standard. Zone of inhibition was observed in disc diffusion against four gram-positive and eight gram-negative pathogenic bacteria. The leaf and bark extracts showed average zone of inhibition ranged from 7-10 mm. Maximum zone of inhibition was observed 10 mm against *Bacillus megaterium*, *Salmonella paratyphi*, *Vibrio parahemolyticus* for barks and *Vibrio mimicus* for leaves. The cytotoxic activities of crude extracts were determined using Brine shrimp lethality using Vincristine sulfate as standard with LC₅₀ of 8.84 µg/ml hence the crude extracts of leaves and barks showed significant cytotoxicity with LC₅₀ of 17.09 µg/ml and 45.96 µg/ml respectively. Antioxidant activity test of the crude extracts was assessed by means of DPPH free radical scavenging method where ascorbic acid was used as standard with IC₅₀ value 45.738 µg/ml. Leaves and barks of *Madhuca indica* showed significant antioxidant activity with IC₅₀ value 61.832 µg/ml & 66.342 µg/ml respectively. The phenolic content was found in leaf 62.43 mg of GAE / gm of extractives and the amount of phenolic content was 61.08 mg of GAE / gm of extractives for bark which correlated with good antioxidant potentiality.

Index Terms—*Madhuca indica*, Antibacterial, Cytotoxicity and Antioxidant Activity.

I. INTRODUCTION

Madhuca indica belongs to the family Sapotaceae is commonly known as mahua, mahuva, ippe, poonam, moha, mohoka, illupai. The tree is indigenous to the Central India. It is common in sub-mountainous regions of the Himalayas, and is, at certain places, a chief constituent of the forest vegetation. Traditionally it is used as astringents, laxative, tonic, aphrodisiac and stimulant. It is useful in burning sensation in body, debility emaciation, respiratory diseases and rheumatism and in snake bite and fish poison. Bark is useful in bleeding gums and ulcers, and also useful in the diabetes. Flowers are useful in the cough and seeds are useful in making of soaps and roots are useful in skin diseases. The honey from the flowers is used in the treatment of eye diseases.

Leaves contain a glycoside saponin, protobassic acid and traces of an alkaloid. [1], [2], [3] Flowers are a good source sugars, calcium, phosphorus and protein. [4] They contain good quantity of sugar, enzymes, yeast and albuminoids. [5], [6] Seeds contain 43.3% fat, 16.9% protein, 51.5% oil & a saponin, a saprogenic & basic acid. [7], [8] Bark contains tannins and saponins and sterols. Beta-amyrin, beta-amyrin acetate, beta-amyrin cinnamate, beta-amyrin decanoate, beta-amyrinone, betulinic acid, triedelin, hederagenin, isoarborinal, ursolic acid, alpha-sapinasterol and alpha-spinasterol-beta-D-glucoside have been isolated from the bark and timber. [9] The stem bark of *M. indica* is devoid of tannins. [10] *M. indica* include fatty acids, sapogenins, carbohydrates, triterpenoids, steroids, saponins, flavonoids, and glycosides. Madhucic acid, a pentacyclic triterpenoid, madhushazone, an untypical isoflavone, and madhusalmone, a bis(isoflavone) were isolated from the fruit coat. [11] The structures of madhucosides were isolated from the bark of *M. indica*. Two important protobassic acids were isolated that showed significant inhibitory effects on both superoxide release from polymorphonuclear cells and hypochlorous acid generation from neutrophils assessed in a luminol-enhanced chemiluminescence assay. [12] Distilled spirit of the flowers is astringent, tonic, & appetizing, decoction is used in colds, coughs and bronchitis and honey of the flowers is edible and used for eye diseases. [13] Flowers are also taken to increase production of breast milk. [3] Seed oil has emollient properties & is used in skin diseases, rheumatism and headache. It also acts as emetic & laxative, & is used in habitual constipation, piles & hemorrhoids. Seeds are galactagogue & barks are astringent and tonic. The leaves are applied as a poultice to relieve eczema. [3] The leaf saponin protobassic acid showed moderate spasmolytic activity. [2] The bark is used traditionally in the treatment of rheumatism, ulcers, tonsillitis and diabetes mellitus. It is also useful in the treatment of helminthes, acute and chronic tonsillitis, pharyngitis as well as bronchitis. [14] It has anti-inflammatory, anti ulcer and hypoglycemic effect of ethanol extract & crude alkaloid extract of seed cake on albino rats. [15] 50% alcoholic extract of stem bark of *M. indica* reveal hypotensive activity

and devoid of diuretic and anticancer property and LD₅₀ was 1000 mg/kg in albino mice. [16]

II. MATERIALS AND METHODS

A. Plant material

The fresh leaves and barks of *M. indicawere* collected during 2012 in the month of May from the area of Jahangirnagar University, Savar, Dhaka and identified by Dr. M. A. Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Dhaka, Bangladesh.

B. Plant materials extraction

The each ground leaves and barks 100gm were extracted with 3 times methanol of their weight in a round bottom flask container with 1:2 sample and solvent ratio at room temperature through occasional shaking and stirring for 7 days. After 7 days the extracts were filtered through the cotton and then filter paper (Double filter paper 102, 11.0cm). Then the liquid extract was dried with room temperature (37°C) to achieve a greenish mass.

C. Antibacterial assay

The disc diffusion method [17] was used to test antimicrobial activity against eleven bacteria. In this method, solutions of known concentration (500 µg /disc) of the test samples were made by dissolving measured amount of the samples (50 mg) in 1 ml of solvents. Then sterile filter paper discs (5 mm diameters) were impregnated with known test substances and dried. Dried and sterilized filter paper discs were then impregnated with known amounts of the test substances using micropipette. Discs containing the test materials were placed on nutrient agar medium uniformly seeded with the pathogenic test microorganisms. Standard antibiotic discs (Kanamycin 30µg/disc) and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4°C) for 24 hrs to allow maximum diffusion. The plates were then incubated at 37°C for 24 hrs to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out three times and the mean of the reading is required. [17]

D. Cytotoxicity Screening

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds. [18], [19] Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hrs to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method. [18] The test samples (extracts) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations of 5, 10, 20, 40, and 80 µg/ml. A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard Vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 hours, the vials were inspected using a magnifying glass and the number

of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality [20] was plotted on the graph paper and the values of LC₅₀ were calculated using Microsoft Excel 2003 (Figure 1).

E. Screening for Antioxidant activity

Antioxidant activities of methanolic extracts of leaf and bark of *M. indica* were determined on the basis of their scavenging potential of the stable DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay by the method of Blois (1958). DPPH offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants. [21] DPPH solution was prepared in 95% methanol. The crude extracts of bark and leaf were mixed with 95% methanol to prepare the stock solution (5 mg 50mL⁻¹). The concentration of the sample solutions was 100µg mL⁻¹. The test samples were prepared from stock solution by dilution with methanol to attain a concentration of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively. Freshly prepared DPPH solution was added in each of these test samples and after 20 min, the absorbance was taken at 517 nm. Ascorbic acid was used as a positive control. The DPPH solution without sample solution was used as control. 95% methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation-

$$\% \text{ DPPH radical scavenging (\%)} = [1 - (As/Ac)] \times 100$$

Here, Ac = absorbance of control,

As = absorbance of sample solution.

Then % inhibitions were plotted against respective concentrations used and from the graph IC₅₀ was calculated (Figure-2).

F. Determination of total phenolics

Total phenolic contents were measured employing the method as described by Skerget *et al.*(2005) [22] involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard. [23] Gallic acid was used here as standard. Different Gallic acid solution were prepared having a concentration ranging from 0 µg / ml to 100 µg / ml . 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na₂CO₃ (7.5 % w/v) solution was added to 0.5 ml of Gallic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples (Figure -3). To 0.5 ml of extract solution (conc. 2 mg/ml) of leaf and bark 2.5 ml of Folin-Ciocalteu reagent and 2.0 ml of Na₂CO₃ (7.5 % w/v) solution were added. After 20 minutes incubation the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared from Gallic acid solution with different concentration, the total phenols content of the sample was measured. The phenolic contents of the sample were expressed as mg of GAE (Gallic acid equivalent) / gm of the extract (Table-2).

III. RESULTS AND DISCUSSIONS

A. Antibacterial Screening

The methanol extract of barks & leaves of *M. indica* possess antibacterial activity with average zone of inhibition 7-10 mm against eleven human pathogenic bacteria including both gram positive and gram negative bacteria using Kanamycin disc (30 µg/disc) as reference standard. The maximum zone of inhibition showed 10 mm against *Bacillus megaterium*, *Salmonella paratyphi*, *Vibrio parahemolyticus* for bark and 10 mm against *Vibrio mimicus* for leaves. The extracts showed mild activity against the bacteria.

B. Cytotoxicity Test

Following the procedure of Meyer (Meyer *et al.*, 1982) the lethality of the crude methanol extract of bark and leaf of *M. indica* were evaluated to brine shrimp using vincristine sulfate as standard (Figure 1). The LC₅₀ values for standard vincristine sulfate, leaves and barks of *M. indica* were found to be 8.84 µg/ml, 17.09 µg/ml, and 45.96 µg/ml, respectively. The leaf extract showed significant cytotoxic activity. This significant lethality of the crude plant extracts to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigation. Other cytotoxicity tests and specific bioassays may be done on the isolated bioactive compounds later.

C. Antioxidant activity using DPPH free radical scavenging capacity

Methanol extracts leaf and bark of *M. indica* was evaluated for the antioxidant activity using ascorbic acid as reference standard. The IC₅₀ value of Ascorbic acid was obtained 45.738µg/ml. The parts of the plant leaves and barks revealed antioxidant activity with IC₅₀ of 61.832µg/ml and 66.342µg/ml respectively. Many herbal plants contain antioxidants compounds and these compounds protect cells against the

damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxy radicals, hydroxyl radicals and peroxynitrite.^[24] Natural anti-oxidative compounds from plants have aroused much attention due to concerns about the safety of synthetic antioxidants. [25]

D. Antioxidant activity by determining total phenolic content (TPC)

The methanolic extract of leaves and barks of *M. indica* were subjected for the determination of the total phenolic content. Based on the absorbance values, the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of Gallic Acid (table 2) equivalents. Total phenolic content of the samples are expressed as mg of GAE (gallic acid equivalent)/ gm of extractives. The phenolic content was found in leaf 62.43mg of GAE / gm of extractives and the amount of phenolic content was 61.08mg of GAE / gm of extractives for bark. Medicinal plant parts are potentially rich sources of natural antioxidants in polyphenols.

IV. CONCLUSION

The present study revealed that the methanol extract of *M. indica* has got profound antioxidant activity. So the plants may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to free radical. In comparison with the standard (Gallic Acid) leaves and barks of *M. indica* have significant total phenolic contents. The parts of leaves and barks showed mild to antibacterial activity against most of the tested bacteria and possessing significant cytotoxic activity.

In addition, the results confirm the use of the plant in traditional medicine. Now our study will be directed to explore the lead compound responsible for aforementioned activity from this plant.

TABLE I. IN VITRO ANTIBACTERIAL ACTIVITY OF THE EXTRACTS OF *MADHUCA INDICA*

Test organism	Diameter of zone of inhibition (mm)		
	Barks(mm) 500µg/disc	Leaves (mm) 500µg/disc	Kanamycin (30µg/disc)
Gram positive bacteria			
<i>Bacillus subtilis</i>	9	-	30
<i>Bacillus megaterium</i>	10	9	32
<i>Sarcina lutea</i>	-	-	25
<i>Staphylococcus aureus</i>	7	-	33
Gram negative bacteria			
<i>Salmonella paratyphi</i>	10	8	31
<i>Vibrio mimicus</i>	9	10	30
<i>Salmonella typhi</i>	8	-	32
<i>Vibrio parahemolyticus</i>	10	9	32
<i>Shigella boydii</i>	9	8	30
<i>Pseudomonas aeruginosa</i>	10	9	28
<i>Escherichia coli</i>	-	-	22
<i>Shigella dysenteriae</i>	7	7	25

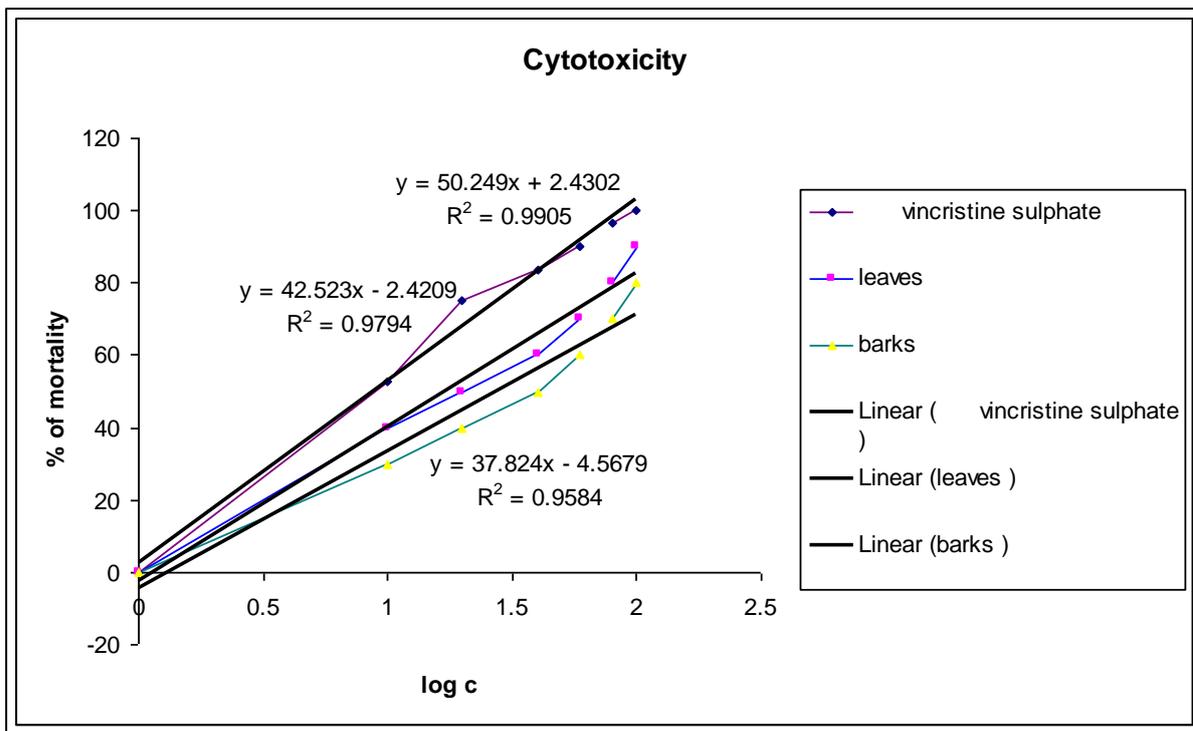


Fig. 1. Determination of LC50 values for standard and methanol extract of leaves and barks of *Madhuca indica* from linear correlation between logarithms of concentration versus percentage of mortality

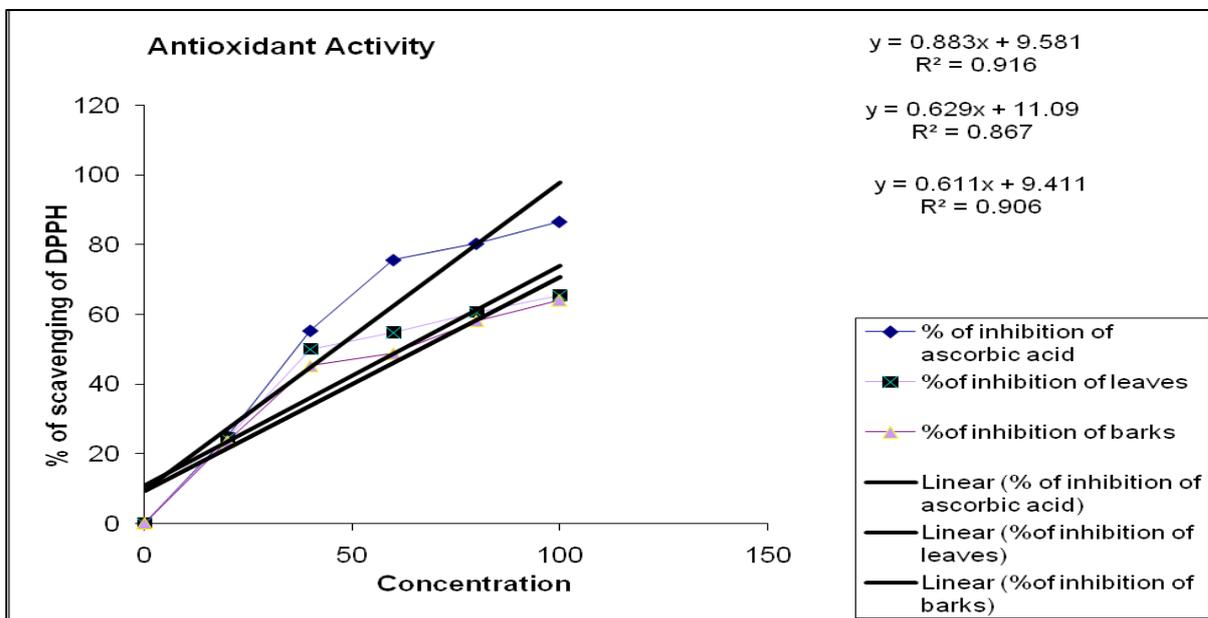
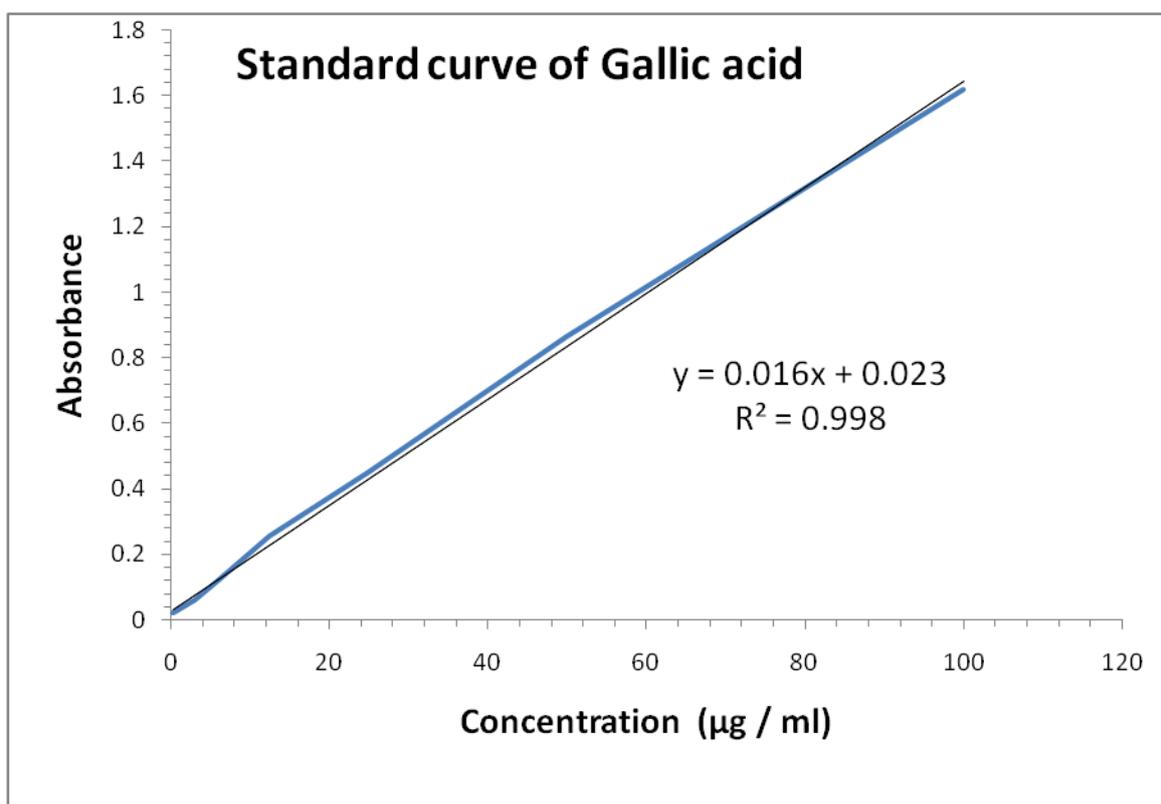


Fig. 2. Determination of IC50 value for standard and methanol extract of leaves and barks of *Madhuca indica* from linear correlation between concentrations ($\mu\text{g/ml}$) versus percentage of scavenging of DPPH.

TABLE II. STANDARD CURVE PREPARATION BY USING GALLIC ACID

SL. No.	Conc. Of the Standard (µg / ml)	Absorbance	Regression line	R ²
1	100	1.620	$y = 0.0162x + 0.0215$	0.9985
2	50	0.866		
3	25	0.450		
4	12.5	0.253		
5	6.25	0.120		
6	3.125	0.059		
7	1.5625	0.034		
8	0.78125	0.022		
9	0.3906	0.020		
10	0	0.011		

Fig. 3. Standard curve of Gallic Acid for total phenolic determination



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