



Evaluation of Antibacterial, Anti-Oxidant and Cytotoxic Activity of Organic Extracts of Mahogany Seeds

Assaduzzaman¹, Md. Ziaul Amin¹, Mohammad Habibur Rahman¹,
Md. Rasel Uddin¹, Md. Shohanuzzaman², Pritylata Mandal³,
Bristy Amy Karmoker⁴, Md. Raihan Dipu⁴ and Md. Rashed Nejum^{5*}

¹Genetic Engineering and Biotechnology, Jessore University of Science and Technology, Bangladesh.

²Environmental Science and Disaster Management, Bangabandhu Sheikh Mujibur Rahman Science & Technology University, Bangladesh.

³Department of Biotechnology, North South University, Dhaka, Bangladesh.

⁴Department of Microbiology, Stamford University, Dhaka, Bangladesh.

⁵Department of Biotechnology and Genetic Engineering, Islamic University, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. Author MRN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Assaduzzaman and MZA, MHR and MRU managed the analyses of the study. Authors MS, PM, BAK and MRD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The perception of normal medicine is changing, and the use of traditional or herbal medicine increasing worldwide due to our harmonious nature of the biological system. Many parts of the plants possess an impressive array of medicinal benefits, mahogany seed is one of them. The aim of this study is to evaluate the antimicrobial, anti-oxidant and cytotoxic activity of organic extracts of

*Corresponding author: E-mail: nejum.rashed@gmail.com;

mahogany seeds. For determination of antimicrobial and cytotoxic activity disk diffusion and brine shrimp lethality bioassay were used. DPPH free radical test were performed for evaluation of antioxidant activity. Ethanol, n-hexane and aqueous extracts were examined with the total number of 10 bacterial strain. Here, ethanol extract showed higher antimicrobial activity than n-hexane and aqueous extract for both gram positive and negative bacteria. In brine shrimp lethality bioassay LC₅₀ values were 82 µg/ml, 95 µg/ml and 93 µg/ml for ethanol, n-hexane and aqueous extracts respectively. The scavenging DPPH free radicals were found to be concentration dependent. The LC₅₀ value was measured at 140µg/ml, 160µg/ml and 180µg/ml for ethanol, n-hexane and aqueous extract. The results of this study indicated that the mahogany seeds can be used as an easily accessible source of natural antioxidants and Cytotoxic compounds which might be helpful in preventing the progress of various oxidative stresses and cell culture assay.

Keywords: *Antibacterial; anti-oxidant; shrimp; anti-inflammatory; ethnopharmacological profile; organic extracts; microbiology; antimicrobial activity.*

1. INTRODUCTION

Plant parts have been used as traditional medicine since prehistoric times. Medicinal properties derived from plants can come from many different parts of the plant including leaves, roots, bark, seeds, flowers and fruits [1]. According to United States department of agriculture, in one plants different part of the plant contains different ingredients. Generally, it may happen that one part of the plant could be toxic while another portion of the same plant could be harmless [2]. Therefore, the modern society has been interested in drugs of natural origin due to their harmonious nature with our biological system [3]. However, medicinal plants would be the best source to obtain a variety of drugs [4]. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants which shows that the trend of using natural products is getting increased [5,6].

Mahogany (*Swietenia mahagoni*) is a common plant in Bangladesh basically used for the wood purposes [7] but the planet is expected as a massive pharmacy filled with a dizzying collection of all-natural remedies for numerous diseases and sicknesses [8]. The mahogany seed comes from the fruit; sometimes called sky fruit are the ones that possess an impressive array of medicinal benefits [9]. A study reported that the plant has been used in ethno-medicine which have reach source of substances for the treatment of infectious diseases [10]. The seed of *S. mahagoni* has been reported for its anti-inflammatory, antimutagenicity, and antitumor activities [11]. The plant extracts have been accounted to possess antibacterial and antifungal activities and used for the treatment of diabetes [12] and usually used as well as

insecticides, larvicides, nematicides, antipyretic and fungicides [13]. The fruit of *S. macrophylla* has been used commercially in health care products for the improvement of blood circulation and skin condition [14]. The seeds are used as an anthelmintic and antidiarrhoeic and also used for the treatment of biliousness and syphilis [15]. In Malaysia, the seeds are used traditionally to treat hypertension, diabetes, and relieve pain [16]. It has been reported to have anti-inflammatory, antimutagenicity and antitumor activity. A Bolivian Amazonian ethnic group has used the seeds for leishmaniasis and as an abortion medicine [17]. In Indonesia, *S. macrophylla* seeds have been used as folk medicine for the treatment of diabetes, hypertension, and malaria [18]. Although several studies have been reported on the ethnopharmacological profile of *Swietenia mahagoni*, nevertheless, the study to take regional variety in our country is limited so that strong evidence has been generated for the use of several parts of the plant. Hence, the aim of this study is to evaluate the antimicrobial, anti-oxidant and cytotoxic activity of organic extracts of mahogany seeds.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Samples

The seeds of *Swietenia mahagoni* (Linn.) were collected from the local region of Jhikargacha, Jessore. The seeds of the collected fruits were separated from each other very carefully and cut into small pieces and dried under shade. The dried chips were ground into coarse powder using the blender. Then the powder was stored for further use and extraction [19].

2.2 Preparation of Organic Extracts

Preparations of plant seed organic extracts cold infusion method are used with modification. Ten grams of seed powder were taken in clean 250ml conical flasks and soaked in 100 ml of distilled water, ethanol, n-hexane extract. The flasks were kept in a mechanical shaker at room temperature for 48 hours. After that the extract was filtered by whatman filter paper. By using rotary evaporator (Rotary pump evaporator, RE300/MS, Barloworld, UK) the extract was evaporated at 55°C and dried. For water the extract was evaporated at 90°C. This process yielded ethanol (1.5gm), hexane (1gm) and water (1.2gm) respectively. Solvent (analytical grade) for extraction were collected from local supplier (Merck KGaA, Darmstadt, Germany). The dried extract was kept at 4°C until further use [20].

2.3 Test Organisms

The test organism was taken from the microbiology laboratory of Jessore University of Science & Technology, Jessore, Bangladesh. The total number of 10 bacterial strains were used for antimicrobial activity test among which 5 are Gram positive namely; *Bacillus subtilis*, *Sarcina luteae*, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus megaterium* while 5 are Gram negative namely; *Salmonella paratyphi*, *Enterococcus faecium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*.

2.4 Scavenging Effect of Extract on DPPH Radicals

The electron payment capabilities of the pure compound and equivalent extracts were measured by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH). The process used was almost the same as one used by another's but was slightly modified in detail. To perform this experiment different concentration of extracts (2ml) and standards (ascorbic acid and butylated hydroxytoluene) was prepared by added DPPH solution. To prepare DPPH solution (0.0004% w/v) was prepared in 95% methanol in the absence of light. The mixture was shaken dynamically and incubated at room temperature for 30 min. Absorbance was measured at 517nm in a spectrophotometer (UV Spectrophotometer, 1240V, Shimadzu, Japan). The percentage of inhibition (I %) was calculated with the following equation [21].

$$\text{DPPH radical concentration (\%)} = (\text{A Control} - \text{A Sample}) / \text{A Control} \times 100$$

Here, control represents the absorbance of blank (containing all reagents except the test sample) and A sample represents the absorbance of extracts and standard samples. The 50% inhibition (IC₅₀) was calculated from the graph plotted inhibition percentage against extract concentration.

2.5 Brine Shrimp Lethality Assay

The brine shrimp lethality assay was used to predict the cytotoxic activity [22] of the n-hexane, ethanol and aqueous extracts of the seed. The eggs of brine shrimp (*Artemio Schnur*) were collected from local market and hatched carefully by maintaining proper environment for 48h and resulting nauplii (larvae) were used for further test. The test samples were prepared by dissolving 40 mg of each of the crude extract in 4ml dimethyl sulfoxide (DMSO) and diluted with sea water to make concentrations 200, 100, 60, 45, 30 and 15µg/ml, respectively. The resulting samples were transferred to sterile vials where 10 live brine shrimp were kept in each vial with 5ml simulated seawater. After 24 h, the vial was inspected using a magnifying glass and the number of survived nauplii in each vial was counted. The percent of lethality of the brine shrimp nauplii for each concentration and control after 24 hours of incubation was calculated by the following equation

$$\% \text{ of mortality} = (\text{No. of death nauplii} / \text{initial no. of live nauplii}) \times 100.$$

The LC₅₀ was determined by plotting the log of concentration versus percent of mortality. Tests for each concentration and control experiment containing only DMSO performed for three times.

3. RESULTS AND DISCUSSION

Antibacterial activities of the samples against the tested bacteria assayed by measuring the zone of inhibition generated for each sample is shown in the Table 1. According to the results all the tested organisms were slightly active the concentration of 500µg, except *Vibrio parahaemolyticus*, *Salmonella paratyphi*, *Sarcina luteae* and *Bacillus cereus* bacteria. Comparatively the greater activity was found in ethanol extract than n-hexane and aqueous extracts for both gram positive and gram negative bacteria. In previous study similar result

has been found indicated that extract of polar compounds has more potential antimicrobial activity than non-polar compounds [23,24,25]. Why polar compound in more potential is not clear but it may be due to the extraction of active compounds effective for antimicrobial activity. The highest zone of inhibition ($20\pm 0.6\text{mm}$) was found against *Staphylococcus aureus* and the lowest zone of inhibition ($7\pm 0.2\text{mm}$) was found against *Escherichia coli*. No zone of inhibition was found against two gram-negative (*Salmonella paratyphi* & *Vibrio parahaemolyticus*) and two gram-positive (*Sarcina luteae* & *Bacillus cereus*) respectively. Small zone of inhibition of the standard kanamycin indicate that these bacteria may indicates resistant to antibiotics as well as extract compounds. In this study we found that various extracts of mahogany seeds are slightly inhibited the growth of some human infected pathogens which confirm the applications with other extracts in the pharmaceutical industry.

Antioxidant activity of various plants extracts standard ascorbic acid and butylated hydroxytoluene (BHT) examined at various concentrations of $30\mu\text{g/ml}$ to $1000\mu\text{g/ml}$. The scavenging DPPH radicals were found to be concentration dependent i. e, the inhibition activity was increased by the concentration. LC_{50} value was measured at $140\mu\text{g/ml}$, $160\mu\text{g/ml}$ and $180\mu\text{g/ml}$ for ethanol, n-hexane and aqueous extract respectively. While LC_{50} value of

the standards, BHT and ascorbic acid were determined at $63\mu\text{g/ml}$ and $55\mu\text{g/ml}$. The value obtains here elucidated that ethanol extract seems to be more scavenging than aqueous and n-hexane extracts which are consistent with previous study [26]. Why ethanol extract of this plant seed is higher antioxidant activity is not clear but it may be due to increased extraction of phenolic compounds.

Lethality bioassay of ethanol, n-hexane and aqueous extracts on brine shrimp nauplii was performed at concentrations of 15, 30, 45, 60, 100 and $200\mu\text{g/ml}$. Mortality was observed at the lower concentration at $6\mu\text{g/ml}$ (data not shown) and 100% mortality at highest concentration $200\mu\text{g/ml}$ as shown in Table 2. Our findings indicated that the extracts were cytotoxic and their activity was not altered by changing the concentration. The LC_{50} value of ethanol, n-hexane and aqueous extract were shown in Fig. 2. Among the sample, ethanol extract showed the lowest LC_{50} clarify its potency among other extracts. This means that it will take $82\mu\text{g/ml}$ of extract to kill half of the total individuals of the tested nauplii. In a previous study it has been shown that methanolic extract of *Swietenia mahagoni* seeds exhibits moderate cytotoxic activity at a high concentration where LC_{50} was $680\mu\text{g/ml}$ [27]. In the present research the order of cytotoxic potentiality of *Swietenia mahagoni* seeds extracts was ethanol>aqueous>n-hexane.

Table 1. In vitro antimicrobial activity of ethanol, n-hexane, aqueous extracts and standard kanamycin

Name of the test organism	Diameter of zone of inhibition (mm \pm SD)			
	Ethanol (500 μg /disc)	n-hexane (500 μg /disc)	Aqueous (500 μg /disc)	Kanamycin (30 μg /disc)
Gram negative bacteria				
<i>Salmonella paratyphi</i>	-	-	-	17 \pm 0.2
<i>Enterococcus faecium</i>	14 \pm 0.4	14 \pm 0.6	8 \pm 0.2	22 \pm 0.4
<i>Escherichia coli</i>	18 \pm 0.6	12 \pm 0.3	7 \pm 0.2	20 \pm 0.3
<i>Pseudomonas aeruginosa</i>	12 \pm 0.3	10 \pm 0.2	10 \pm 0.4	19 \pm 0.2
<i>Vibrio parahaemolyticus</i>	-	-	-	18 \pm 0.2
Gram positive bacteria				
<i>Bacillus subtilis</i>	19 \pm 0.3	15 \pm 0.2	9 \pm 0.2	23 \pm 0.4
<i>Sarcina luteae</i>	-	-	-	20 \pm 0.2
<i>Bacillus cereus</i>	-	-	-	18 \pm 0.3
<i>Staphylococcus aureus</i>	20 \pm 0.6	17 \pm 0.4	8 \pm 0.2	22 \pm 0.6
<i>Bacillus megaterium</i>	18 \pm 0.6	12 \pm 0.6	7 \pm 0.3	25 \pm 0.4

Table 2. Effect of ethanol, n-hexane and aqueous extract of brine shrimp nauplii

Conc. (µg/ml)	Mortality %			
	Log C	Ethanol	n-hexane	Aqueous
15	1.6	17	12	10
30	1.7	21	18	12
45	1.9	27	22	20
60	2	33	27	40
100	2.3	80	60	62
200	2.6	100	100	100

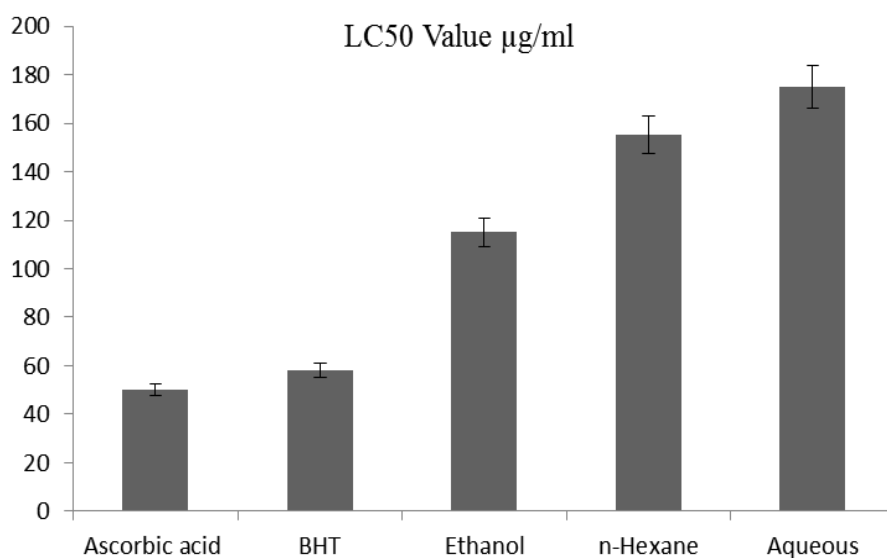


Fig. 1. LC₅₀ value of ethanol, n-hexane, aqueous extracts and standard ascorbic acid and butylated hydroxy toluene

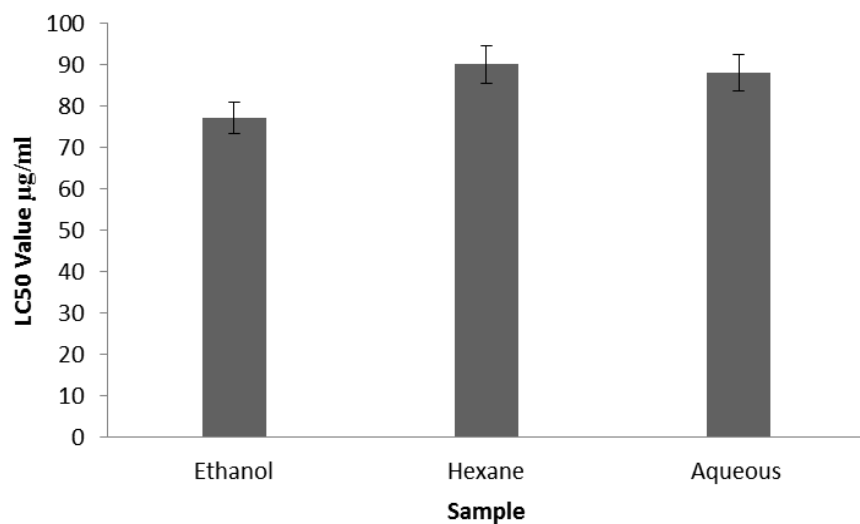


Fig. 2. Indicates the LC₅₀ value of ethanol, hexane and aqueous extract of mahogany seeds

4. CONCLUSION

This study indicated that the extract of *Swietenia mahagoni* seeds exhibits good cytotoxic and antioxidant activity as well as slight antibacterial effects on some gram-positive and gram-negative bacteria. The results of this study show that the mahogany seeds can be used as an easily accessible source of natural antioxidants and cytotoxic compounds which might be helpful in preventing the progress of various oxidative stresses and cell culture assay, respectively. However, the compounds having specific medicinal effects are still unclear. Therefore, further investigations are needed for isolation, identification and purification of the pure moiety responsible for this activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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