

## Research Article

# Total Tannin Content, Microbiological Investigation and Acute Toxicity Studies of Ethanolic Extract of *Swietenia mahagoni* Leaves

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## ABSTRACT

The present study was designed to investigate the total tannin content as secondary metabolite and to evaluate the microbiological contaminants and acute toxicity studies of ethanolic extract of *Swietenia mahagoni* leaves. Investigation on secondary metabolites showed the presence of carbohydrates, saponins, terpenoids, glycosides, flavonoids, and tannins. The total tannin content was calculated as quite high in ethanol extract (325.91 mg/g of tannic acid equivalent). In acute toxicity study, no mortality or any toxic reaction was recorded in any group after 15 days of administering the extract to the rats. Acute toxicity test showed that the leaves might be safe for pharmacological uses. The microbiological contaminants limit of ethanolic extract from *Swietenia mahagoni* leaves remained within the limit stated in WHO guideline's for alternative medicines. Therefore, the obtained results tend to suggest that the extract contains tannin content in high amount as well as does not possess microbiological contaminants and safe for pharmacological uses. Thus the outcome of this study serves as an important contribution to knowledge in establishing some quality parameters for the standardization of Mahogany herbal tea extract from *S. mahagoni* leaves.

**Keywords:** *Swietenia mahagoni*, Total tannin content, Acute toxicity studies, Microbiological test.

## INTRODUCTION

According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs<sup>1</sup>. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens<sup>2</sup>. As per WHO definition, there are three kinds of herbal medicines: raw plant material, processed plant material and medicinal herbal products. The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies<sup>3</sup>. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants<sup>4</sup>. Herbals are traditionally considered harmless and increasingly being consumed by people without prescription. However, some can cause health problems,

some are not effective and some may interact with other drugs. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles<sup>4</sup>. Standardization of herbal raw drugs include passport data of raw plant drugs, botanical authentication, microscopic & molecular examination, identification of chemical composition by various chromatographic techniques and biological activity of the whole plant<sup>5,6</sup>.

*Swietenia mahagoni* (L.) Jacq (Family: Meliaceae) locally known as 'Mahogany' found almost all parts of Bangladesh. Previous studies have shown that its bark contains significant hypoglycemic and antioxidant activity<sup>7</sup>. *Swietenia mahagoni* seeds extract has high free radical scavenging and xanthine oxidase inhibitory activity<sup>8</sup>. Its seeds extract also has inhibitory effects on the growth of *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Proteus mirabilis*<sup>9</sup>. Its seeds

extract has also been reported to have medicinal value for the treatment of hypertension, diabetes, and malaria<sup>10</sup>, and it has also been reported to have medicinal value for treatment of cancer, amoebiasis, coughs, chest pains and intestinal parasitism<sup>11</sup>. The biologically active ingredients, tetranortriterpenoids and fatty acids are considered to be responsible for these therapeutic effects<sup>12</sup>. *Swietenia mahagoni* seeds extract is high in lipids, particularly neutral lipids, glycolipids and phospholipids, the most abundant of which is phosphatidylcholine.<sup>13</sup>

Since no literature is currently available to substantiate total tannin content determination and acute toxicity studies of the leaves of *S. mahagoni*, the present study was designed to evaluate the total tannin content as secondary metabolite and to examine the microbiological contaminants and acute toxicity studies from ethanolic extract of *Swietenia mahagoni* leaves.

## MATERIALS AND METHODS

### Plant material

The leaves of *Swietenia mahagoni* (L.) Jacq were collected at May, 2012 from Satkhira, southern district of Bangladesh and was identified by Sarder Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. A voucher specimen (DACB: 36328) has been deposited in the Herbarium for further reference.

### Preparation of ethanolic extract

The green leaves of *Swietenia mahagoni* any were freed from any of the foreign materials and chopped and air-dried under shed temperature followed by drying in a hot air oven at 50°C. The dried leaves were then grounded into powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powdered sample was stored in an airtight container and kept in a cool, dark, and dry place until analysis commenced. About 600 g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1.8 L of ethanol. The container along with its contents was sealed and kept for 5 days at 25 ± 2°C with occasional shaking or stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. It was then filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated by using rotary vacuum evaporator at reduced pressure and dried. It rendered an 81 g of gummy concentrate

(13.50%) and was designated as crude ethanol extract for tannin content, microbiological test and acute toxicity studies.

### Test Animals

For the screening of acute toxicity activity male rats of Wister strain weighing 175-202 g were used. The animals were housed under standard Laboratory (at Pharmacology Laboratory of BCSIR, Chittagong) conditions maintained at 25±1°C and under 12/12 h light/dark cycle and feed with Balanced Trusty Chunts and water *ad libitum*. All experimental protocols were in compliance with BCSIR Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

### Phytochemical screening

The freshly prepared methanol and water extracts were qualitatively tested for the presence of chemical constituents, by using the different reagents and chemicals. In each test, 10% (w/v) solution of each extract in ethanol was used in individual test.<sup>14</sup>

### Tests for carbohydrate

**Benedict's test:** 0.5 ml of the extract was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

### Tests for alkaloids

**Mayer's test:** 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1ml of Mayer's reagent was added to it.

**Dragendroff's test:** 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and then 1 ml Dragendroff's reagent was added.

### Tests for tannins

**Ferric Chloride Test:** 5 ml of the extract was placed in a test tube and then 1 ml of 5% Ferric chloride solution was added to it.

### Test for flavonoids

5 ml extract was dissolved in 2 ml HCl. A yellow solution that turns colorless indicates the presence of flavonoids.

### Test for Glycosides

The extracts were hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of glycosides.

**Test for Terpenoids**

Salkowski Test: 5 ml extract was mixed with 2 ml of chloroform (CHCl<sub>3</sub>) and wormed with concentrated H<sub>2</sub>SO<sub>4</sub> (3ml) was carefully added form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

**Test for saponins**

1 ml of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

**Tests for steroids**

Libermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Libermann- Burchard reagent was added to it. Sulphuric acid test: 1 ml of the extract was placed in a test tube and 1 ml sulphuric acid was added to it.

**Total tannin content determination**

The modified Folin-Ciocaltu method<sup>15</sup> followed to determine the total tannin content of the extract of leaves of *S. mahagoni*. A 0.5 ml of each extract (1 mg/ml) was mixed with 5 ml Folin-Ciocaltu reagent (1:10 v/v distilled water) and 4 ml (75g/l) of Sodium carbonate and the mixture was then vortexed for 15 second for the development of color the mixture was allowed to stand for 30 min at 40°C. Then the absorbance was read at 765 nm with the same spectrophotometer. Total phenolic content was calculated as mg of tannic acid equivalent per gram using the equation obtained from a standard tannic acid calibration curve  $y=6.2548x - 0.0925$ ,  $R^2=0.9962$ .

**Acute toxicity test**

The acute toxicity of *S. mahagoni* leaves powder was determined in male rats of Wister strain according to the method of Hilaly et al<sup>16</sup> with slight modifications. Rats fasted for 16 h were randomly divided into groups of five rats per group. Graded doses of the extract (1500, 3000 and 6000 mg/kg p.o.) were separately

administered to the rats in each of the groups by means of bulbed steel needle. All the animals were then allowed free access to food and water and observed over a period of 72 h for signs of acute toxicity and next 12 days for any delayed effects. The number of deaths and behavioral changes within this period was recorded.

**Microbiological analysis of ethanolic extract**

For the quantitative determination of total count of mesophilic bacteria, total fungal count, total coliform, faecal coliform, the standard procedure was followed<sup>17</sup>. Aerobic plate count (APC) was performed by pour plate method using plate count agar (PCA), which was incubated at 35±10C for 48±2h. Lauryl tryptose broth was used for isolation of *Escherichia coli*. Gassing tube was selected for *E.coli* enumeration using most probable number (MPN) method. Enumeration of fungi was performed on Potato Dextrose Agar medium. For the isolation of *Salmonella* species, pre-enrichment was done by lactose broth followed by selective enrichment and finally confirmed using the standard method.

**Statistical Analysis**

For antioxidant determination, data were presented as mean ± Standard deviation (S.D). Statistical analysis was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. *p* values < 0.05 were considered to be statistically significant (*p* indicates probability).

**RESULTS AND DISCUSSION****Chemical group test**

Results of different chemical tests on the methanol and water extracts of *S. mahagoni* leaves showed the presence of carbohydrates, saponins, glycosides, flavonoids, terpenoids, and significantly presence of tannins (Table-1).

**Table 1: Results of different group tests of ethanolic extract of *S. mahagoni* leaves**

Phytoconstituents	Ethanol extract of <i>S. mahagoni</i>
Alkaloid	-
Carbohydrate	+
Tannins	++
Glycosides	+
Flavonoids	+
Saponins	+
Steroid	-
Terpenoids	+

+: Positive result; -: Negative result; ++: significantly positive

**Determination of total tannin content**

The amount of total tannin content was calculated as quite high in ethanol extract of *S.*

*mahagoni* leaves (325.91 ± 7.28 mg/g of tannic acid equivalent) (Table-2).

**Table 2: Total tannin content of water extract of *S. mahagoni* leaves**

Extract	Total tannin content (mg of tannic acid equivalent per g of dry extract)
Ethanol extract of <i>S. mahagoni</i> leaves	325.91 ± 7.28

The values are expressed as mean ± standard deviation (n=3)

Phytochemical components, especially phenolic compounds (such as flavonoids, phenyl propanoids, phenolic acids, tannins etc.) are very important components for the free radical scavenging and antioxidant activities of plants. Polyphenols are generally of the chemical patterns; phenolic groups react as hydrogen donors and neutralize the free radicals<sup>18, 19</sup>. In the present study the total amount of phenolic compounds was calculated as quite high in the ethanol extract of *S. mahagoni* leaves. The result of present study revealed that the presence of high concentration of phenolic components in the extract might cause the high inhibition value of the extract. Phenols are important components of plants. It is reported that the hydroxyl group

of the phenolic compounds to eliminate radicals and they contribute directly to antioxidant effect of the system<sup>20</sup>.

**Acute toxicity test**

In acute toxicity study, oral administration of graded doses (1500, 3000, and 6000 mg/kg, p.o.) of the ethanolic extract of *S. mahagoni* to rats did not produce any significant changes in weight, behaviour, breathing, activity or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any group after 15 days of administering the extract to the animals as shown in table 3.

**Table 3: Acute toxicity study of the ethanolic extract of *S. mahagoni* leaves**

Dose (mg/kg, p.o.)	Parameters	Observation
1500	Body weight	Unchanged
	Activity	Normal activity observed
	Diarrhoea	Not observed
	Mortality	Nil
3000	Body weight	Unchanged
	Activity	Normal activity observed
	Diarrhoea	Not observed
	Mortality	Nil
6000	Body weight	Slightly decreased
	Activity	Normal activity observed
	Diarrhoea	Not observed
	Mortality	Nil

**Determination of microbial contaminants**

Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi, and viruses. Inevitably, this microbiological background depends on several environmental factors and exerts an important impact on the overall

quality of herbal products and preparations. Microbiological contaminant test of the ethanolic extract of *S. mahagoni* leaves were done for standardization of Mahogany extract as herbal tea on the various bacteria and fungi as shown in table 4.

**Table 4: Microbiological test of the ethanolic extract of *S. mahagoni* leaves**

Parameters	Results
Total viable aerobic count (CFU/g)	6900
Total fungal count (CFU/g)	50.0
Total Enterobacteriaceae (CFU/g)	13.0
Coliform (MPN/g)	Absent
<i>E. coli</i> (MPN/g)	Absent
<i>Salmonella</i> (g/ml)	Absent

Risk assessment of the microbial load of medicinal plants has therefore become an important subject in the establishment of modern Hazard Analysis and Critical Control Point (HACCP) schemes. Herbal drugs normally carry a number of bacteria and molds, often originating in the soil. Poor methods of harvesting, cleaning, drying, handling, and storage may also cause additional contamination, as may be the case with *Escherichia coli* or *Salmonella spp.* while a large range of bacteria and fungi are from naturally occurring microflora, aerobic spore-forming bacteria that frequently predominate. Laboratory procedures investigating microbial contaminations are laid down in the well-known pharmacopeias, as well as, in the WHO guidelines<sup>21</sup>. The European Pharmacopoeia also specifies that *E. coli* and *Salmonella spp.* should be absent from herbal preparations. According to WHO guidelines, total viable aerobic count, total Enterobacteriaceae and *E. coli* for finished product must be within  $10^5$  (cfu/g),  $10^3$  (cfu/g), and  $10^1$  (MPN/g) respectively<sup>33</sup>. Total fungal count must be within  $10^3$  cfu/g.

#### CONCLUSION

In conclusion it can be revealed that the ethanolic extract of *S. mahagoni* leaves possess significant amount of tannins. Microbiological contaminant limit remains within WHO guidelines for alternative medicines. Again, no mortality was recorded in the acute toxicity test; it has been showed that the leaves *S. mahagoni* might be safe for use. The presence of flavonoids, glycoside, saponins, tannins, and triterpenes like phytoconstituents in *S. mahagoni* leaves might be responsible for hypoglycemic and antioxidant activity<sup>7</sup>. The results obtained from the study could be utilized for setting limits for the reference standards for the quality control and quality assurance of Mahogany tea extract from *S. mahagoni* leaves. However, extensive researches are necessary to search for other reference parameters (physicochemical, nutritional, heavy metals, pharmacological studies), and active principles of *S. mahagoni* leaves through chromatographic and spectroscopic method for developing tea extracts from Mahogany as alternative remedies.

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