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(Research Article)

Phytochemical Investigation and Standardization of Mahogany Tea Powder from *Swietenia mahagoni* Leaves

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ABSTRACT

Depending on the chemical constituents present, herbal tea can be used for therapeutic or nutritional purposes. The present study has been undertaken for evaluating phytochemical screening and standardization of Mahogany tea powder from Swietenia mahagoni leaves. Standardizations of the various medicinal plants used in traditional medicine is becoming more important today in view of the commercialization of formulations based on these plants. The standardization process include organoleptic characteristics, physicochemical parameters, nutritional values, toxic heavy metal contents, animal toxicity study, and microbiological contaminants of herbal tea from Swietenia mahagoni leaves. Phytochemical screening results showed the presence of carbohydrates, saponins, terpenoids, glycosides, flavonoids, and tannins, while the physicochemical parameters which includes moisture content, ash, acid-insoluble ash, water extractive, alcohol extractive matter, carbohydrate, protein, fat, crude fiber values were 6.55, 3.22, 1.96, 46.82, 29.56, 76.0,7.31,4.35 and 16.86, respectively. Atomic absorption spectroscopy (AAS) was used for toxic heavy metal analysis of Mahogany tea in which the contents were 0.20, 0.01, 0.18, 0.07, and 0.16 mg/kg for Lead (Pb), arsenic (As), nickel (Ni), cadmium (Cd), and chromium (Cr), respectively. The microbiological contaminants limit of herbal tea from Swietenia mahagoni leaves remained within the limit stated in WHO guideline's for alternative medicines. The total tannin content was calculated as quite high in water extract (308.58 mg/g of tannic acid equivalent). In acute toxicity study, at a higher dose of 6.0 g/kg body weight, no mortality or any toxic reaction was recorded in any group after 15 days of administering the extract to the rats. Therefore, the outcome of this study serves as an important contribution to knowledge in establishing quality parameters for the standardization of Mahogany herbal tea from S. mahagoni leaves and can be efficiently used as standardized herbal or alternative remedies for anti-diabetic or antioxidant supplements individually and in a polyherbal formulation.

Key Words: *Swietenia mahagoni*, Phytochemical investigation, Physicochemical, Nutritional, Heavy metal, 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[1]. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens[2]. 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[3]. 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[4]. 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[4]. Standardization of herbal raw drugs include passport data of raw plant drugs, botanical authentification, microscopic & molecular examination, identification of chemical composition by various chromatographic techniques and biological activity of the whole plant[5,6].

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[7].

Swietenia mahagoni seeds extract has high free radical scavenging and xanthine oxidase inhibitory activity[8]. Its seeds extract also has inhibitory effects on the growth of *Candida albicans, Staphylococcus aureus, Pseudomonas aeroginosa, Streptococcus faecalis* and *Proteus mirabillase*[9]. Its seeds extract has also been reported to have medicinal value for the treatment of hypertension, diabetes, and malaria[10], and it has also been reported to have medicinal value for treatment of cancer, amoebiasis, coughs, chest pains and intestinal parasitism[11]. The biologically active ingredients, tetranortriterpenoids and fatty acids are considered to be responsible for these therapeutic effects[12]. *Swietenia mahagoni* seeds extract is high in lipids, particularly neutral lipids, glycolipids and phospholipids, the most abundant of which is phosphatidylcholine.[13]

The main objective of this study was to evaluate phytochemical screening and standardization of Mahogani tea powder from *Swietenia mahagoni* leaves.

MATERIALS AND METHODS

Plant Material

The leaves of *Swietenia mahagoni* (L.) Jacq was collected at May, 2012 from Rampal, Bagerhat, southern district of Bangladesh and was identified by Taxonomist of BCSIR. The green leaves of Mahogani were freed from any of the foreign materials and washed with sufficient amount of tap water (two times) to remove any adhering foreign matters and then wash with warmed water (50°C) for two times. Then the plant materials were chopped and air-dried under shed temperature followed by drying in a hot air oven at 40º C. The dried leaves were then grounded into powder. Then the brown color leaf powder was ready for phytochemical screening and standardization process.

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.[14]

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 ($CHCl_3$) and wormed with concentrated H_2SO_4 (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.

Standardization Parameters

The various standardization parameters studied were organoleptic properties, physico□chemical investigations, successive extractive value, nutritional value, microbiological test, acute toxicity test, determination of pH, determination of moisture content, ash content, acid insoluble ash, crude fiber, bulk density and determination of total tannin content of Mahogany tea powder.

Organoleptic Evaluation

The organoleptic characters[15, 16] of the samples were evaluated based on the method described by Mukharjee *et al*. Organoleptic evaluation refers to evaluation of the formulation by color, odor, taste and texture etc.

Physico-chemical Investigations

AOAC methods were applied to carryout proximate analysis of the sample for moisture, total ash, crude fiber, crude fats, proteins and carbohydrates[17]. The moisture and ash were determined using weight difference method[18]. The determination of proteins in terms of nitrogen was done by micro Kjeldahl method involving digestions, distillation and finally titration of the sample [19]. The nitrogen value was converted to protein by multiplying to a factor of 6.25. The crude fat content of the samples was done using Soxhlet type of the direct solvent extraction method. The solvent

used was petroleum ether (boiling range 40-60 ºC). The crude fibre was also determined by the method described by[20]. The energy values (kcal/100 g) were determined by multiplying the values of carbohydrates, fat and proteins by a factor of 4, 9, and 4 respectively, and taking the sum expressed in kilocalories. The total carbohydrates were determined by difference method [100 - (proteins + fats + moisture + ash in percentage)]. All the proximate values were reported in percentage[21].

Preparation of Successive Extracts

Hot extraction processes were carried out successively using four solvents such as: pet-ether, chloroform, methanol and water[22]. 100 g of powdered sample was taken in thimble for pet-ether hot extraction and placed in the soxhlet extraction unit and the soxhlet extraction apparatus units were fitted in a water bath at 70^0 C temperature (boiling temperature of the solvent). The extraction process was continued for about eight hours for the sample. Extracted fat was collected and filtered. Then the solvent was evaporated and concentrated at reduced pressure using a rotary vacuum evaporator. The residue of pet-ether extraction of sample was dried and taken successively for chloroform, methanol and water hot extraction. Filtrate was dried as done before for pet-ether extraction. All the extracted parts were concentrated at reduced pressure using a rotary vacuum evaporator and dried in a vacuum drier.

Toxic Heavy Metal Analysis

The macronutrient contents including Pb, Cr, Cd, As and Ni of the sample were determined using Atomic Absorption Spectrometer (Shimadzu, AA-7000). The results were obtained while using a working standard of 1000 ppm for each of the species [21].

Microbiological Analysis of Sample

For the quantitative determination of total count of mesophilic bacteria, total fungal count, total coliform, faecal coliform, the standard procedure was followed [23]. 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.

Total Tannin Content Determination

The modified Folin-Ciocaltu method[24] followed to determine the total phenolic 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[25] 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.

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 methanol and water extract of *S. mahagoni* leaves.

Phytoconstituents	Methanol extract of *S. mahagoni*	Water extract of *S. mahagoni*
Alkaloid	-	-
Carbohydrate	+	+
Tannins	++	++
Glycosides	+	+
Flavonoids	+	+
Saponins	+	+
Steroid	-	-
Terpenoids	+	+

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

Acute Toxicity Test

In acute toxicity study, oral administration of graded doses (1500, 3000, and 6000 mg/kg, p.o.) of the tea powder (water 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.

Table-2: Acute toxicity study 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	Slightly decreased
	Activity	Normal activity observed
	Diarrhoea	Not observed
	Mortality	Nil
6000	Body weight	Slightly decreased
	Activity	Normal activity observed
	Diarrhoea	Not observed
	Mortality	Nil

Physio-chemical Characteristics

The organoleptic properties are mentioned in table 3. Mahogani tea powder was brown colored, astringent in taste, and had a characteristic odor.

Foreign materials: Herbal drugs should be made from the stated part of the plant and be devoid of other parts of the same plant or other plants. They should be entirely free from moulds or insec, including excreta and visible contaminant such as sand and stones, poisonous and harmful foreign matter and chemical residues. Animal matters such as insects and "invisible" microbial contaminants, which can produce toxins, are also among the potential contaminants of herbal medicines[26, 27].

Extractive value: Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. The water-soluble extractive value of Mahogani tea powder was 46.82% (table-3). The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying, or storage or formulating. The ether soluble extractive value signifies the presence of amounts of fats, lipids, and some steroids in the drug. The alcohol-soluble extractive value of powder was 29.56% and the pet-ether and chloroform soluble extractive values of powder were 1.92 and 3.08% respectively (table-3). It was observed that the percentage of water-soluble extractive value was higher than alcohol- and ether-soluble extractives.

Moisture content: Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The moisture content of leaves powder was 6.55% as shown in table 3. The level of moisture in herbal products can influence the susceptibility of microbial activities on the sample. Several literatures revealed that less moisture keeps the product microbiologically safe by preventing bacterial, fungal and yeast growth. If moisture content is very high, enzymatic activation may occur and result in loss of therapeutically active substance.

Bulk density: Study of bulk density is important as density of a powder defines its packaging, and is listed in table-3, for powder.

Ash values: A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing. The powder was found to have total ash values of 3.32% w/w (table-3). The value was found to be reasonably low indicating low contamination. Water-soluble ash is the part of the total ash content, which is soluble in water. It is a good indicator of either previous extraction of water-soluble salts in the drug or incorrect preparation. Thus, it is the difference in weight between the total ash and the residue obtained after treatment of total ash with water. The water-soluble ash value of the tea powder was 1.02% w/w. This shows a normal quality of the drugs. The acid-insoluble ash value of this product was also below 2.0%. This signifies the ash value determination as an important parameter to standardize the herbal drugs.

Nutritional value: Carbohydrates of the leaf powder was calculated as 76.0% and results are shown in table-3. Protein concentration of the powder was 7.31%, which is lower compared to other protein rich plants ranging between 23 - 33%. The fat and crude fiber contents were calculated as 4.35% and 16.86% respectively.

Table-3: Physiochemical characteristics of mahogany tea powder.

Sl. No.	Parameters	Percentage
1.	**Organoleptic properties**	
	Appearance	Brown color powder
	Odor	Characteristic
	Taste	astringent
	Texture	Fine
2.	**Extraneous material**	
	Foreign matter	Nil
	Sand and silica	Nil
3.	**Successive extractive value**	
	Petroleum ether extractive value (w/w %)	1.92 ± 0.03
	Chloroform extractive value (w/w %)	3.08 ± 0.29
	Alcohol soluble extractive value (w/w %)	29.56 ± 2.77
	Water soluble extractive value (w/w %)	46.82 ± 5.01
4.	**Physico-chemical properties**	
	Moisture content (w/w %)	6.55 ± 0.06
	pH (5% solution)	6.78 ± 0.09
	Ash (% w/w)	3.32 ± 0.08
	Water insoluble ash (% w/w)	1.02 ± 0.03
	Acid insoluble ash (% w/w)	1.96 ± 0.06
	Bulk density	0.512 ± 0.16 (g/ml)
	Material passing through 100 microns (% w/w)	98.12± 1.57
5.	**Nutritional value**	
	Carbohydrate (% w/w)	76.0 ± 6.69
	Protein (% w/w)	7.31 ± 2.05
	Fat (% w/w)	4.35 ± 0.92
	Crude fiber (% w/w)	16.86 ± 1.26
	Total calorie	349.0 ± 10.29 (Kcal/100 g)

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

Heavy Metals

Contamination by toxic metals can either be accidental or intentional. Contamination by heavy metals such as lead, copper, chromium, nickel, cadmium, and arsenic in herbal remedies can be attributed to many causes, including environmental pollution, and can pose clinically relevant dangers for the health of the user and should therefore be limited [28, 29]. The potential intake of the toxic metal can be estimated on the basis of the level of its presence in the product and the recommended or estimated dosage of the product. This potential exposure can then be put into a toxicological perspective by comparison with the so-called Provisional Tolerable Weekly Intake values (PTWI) for toxic metals, which have been established by the Food and Agriculture Organization of the World Health Organization (FAO-WHO) [30].

While the analysis of heavy metals Cr concentration was found to be 0.16 mg/kg (Table 4). It has been reported that for many plant species Cr proved to be toxic at 5 mg/L. In this regard, the studied plant powder has very lesser concentration of Cr as compared to that of recommended level for toxicity in plants [31]. In case of Pb concentration, the suggested concentration in plant species is 2 to 6 mg/kg [32], so the analyzed plant species carries very lesser level of Pb, which further clarifies their use. Again, lead (Pb) induces various toxic effects in humans at low doses with typical symptoms, such as colic, anaemia, headache, convulsions and chronic nephritis of the kidneys, brain damage and central nervous system disorders. WHO prescribed maximum limit for its contents in herbal medicine as 10 mg/kg, while the dietary intake limit of it per week as 3 mg/week. The values of arsenic, cadmium and nickel were of 0.01, 0.18 and 0.07 mg/kg respectively, also found below in the WHO prescribed maximum limit. The result of this work indicated that, the elemental content of the tea analyses were below the RDA (recommended daily allowance) per day.

Table-4: Heavy metal analysis of mahogany tea powder.

Parameters	mg/kg (ppm)
Lead	0.20 ± 0.02
Arsenic	0.01± 0.003
Nickel	0.18 ± 0.07
Cadmium	0.07 ± 0.009
Chromium	0.16 ± 0.05

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

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. 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 [33]. 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 Entero-bacteriaceae and *E. coli* for finished product must be within 10^5 (cfu/g), 10^3(cfu/g), and 10^1(MPN/g) respectively[33]. Total fungal count must be within 10^3 cfu/g.

Table-5: Microbiological test of mahogany tea powder.

Parameters	Results
Total viable aerobic count (CFU/g)	6900
Total fungal count (CFU/g)	50.0
Total Entero-bacteriaceae (CFU/g)	13.0
Coliform (MPN/g)	Absent
E. coli (MPN/g)	Absent
Salmonella (g/ml)	Absent

Phytochemical Analysis

Total Tannin Content

The amount of total tannin content was calculated as quite high in hot water extract of *S. mahagoni* leaves (308.58 ±5.08 mg/g of tannic acid equivalent) (Table-2).

Table-6: Total tannin content of water extract of *S. mahagoni* leaves.

Extract	Total tannin content (mg of tannic acid equivalent per g of dry extract)
Water extract of *S. mahagoni* leaves	308.58 ±5.08

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

Phytochemical components, especially phenolic compounds (such as flavonoids, phyenyl 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[34, 35]. 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[36].

CONCLUSION

In the present study it can concluded that the presence of flavonoids, glycoside, saponins, tannins, and triterpenes like phytoconstituents in *S. mahagoni* leaves are responsible for hypoglycemic and antioxidant activity[7]. The

physicochemical parameters of *S. mahagoni* leaves such as the water-soluble, alcohol-soluble, and ether-soluble extractive values, moisture content, bulk density, pH, water-soluble ash, acid-insoluble ash, organoleptic characteristics, nutritional values, toxic heavy metal contents, animal toxicity study, and microbiological contaminants limit remain within WHO guideline's for alternative medicines. Therefore the *S. mahagoni* leaves (Mahogany tea powder) can be efficiently used as standardized herbal or alternative remedies for anti-diabetic or antioxidant supplements individually and in a polyherbal formulation. The results obtained from the study could be utilized as a reference for setting limits for the reference standards for the quality control and quality assurance of Mahogany tea powder. However, extensive researches are necessary to search for active principles of *S. mahagoni* leaves through chromatographic and spectroscopic method.

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