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PHYTOCHEMICAL, PROXIMITE COMPOSITION AND ANTIOXIDANT POTENTIAL OF *SWIETENIA MAHAGONI* LEAVES

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ABSTRACT

Swietenia mahagoni is a medicinal plant traditionally used all over the world. The present study evaluates the phytochemical and proximate composition of the leaf and also assesses antioxidant potential of the leaf**.** The proximate composition of the plant was evaluated by the standard methods described by AOAC**.** The phytochemical analysis indicates the saponins as major components in leaf (198 mg/g). Tannins, flavanoids and alkaloids were also found to be in a significant amount 94 (µg/mg), 154 (µg/mg) and 91 (µg/mg) respectively. Proximate analysis indicates the presence of significant amount of fiber (in the form of both soluble and insoluble). Elemental analysis indicates presence of magnesium, calcium and sodium at a significant level. Mahagoni leaf powder is also shown many beneficial physical properties. Antioxidant assays indicates the mahagoni leaf has potential anti-oxidant potential by the virtue of electron donating, free radical scavenging properties. The results of the study indicate mahagoni leaf as potential source of bio-actives and as powerful anti-oxidant.

Key words: *Mahagoni* leaf, Antioxidant, Phytochemicals, Proximate analysis and Physical properties.

INTRODUCTION

Swietenia mahagoni Jacq. is a small leafy, medium sized tree native to west indies. The plant is commonly called as West Indies mahogany, caoba, caoba dominicana or acajou. It is one of the species of genus Swietenia which belongs to chinaberry family, meliacea. *Swietenia mahagoni* was once the most sought after cabinet wood in the world and now it is very famous for its wood [1-3]. The parts of the plant have been used to treat many human ailments such as malaria, diabetes, diarrhea, astringent, hypertension etc. locally. The fruit of the plant is used as powerful anti-hyperglycemic drug. The seed oil is being used as an alternative body ointment therapy for a range of skin cuts, itches and wounds to ameliorate the healing process in African countries. Decoction of bark is used to increase appetite, as an energizer in case of tuberculosis, to treat anemia, diarrhea, dysentery, fever and toothache. The decoction of leaf is used to treat nerve disorders, the infusion of seed to relieve from chest pain [4]. Mahogany seeds have potential in controlling amoebiasis, coughs and intestinal parasitism [5]. Many important phytochemicals have been isolated and characterized from various parts of the plant. Since thousands of year medicinal plants forms the complementary and alternative treatment for various ailments faced by humans. Even though medicinal plants have immense medicinal value, there is a lack of literature on the nutrient, phytochemical and physical properties. *Swietenia mahagoni* is one such medicinal plant where it is used in treatment of various ailments, but however, limited literature is available on the nutritional, phytochemical and physical properties. The present study was aimed to evaluate the nutrient, phytochemical composition and physical properties of the leaves of the plant.

METHODS

Proximate Analysis

Proximate composition of the leaves was determined by the official method of the Association of Official Analytical Chemists as follows: Moisture (section 926.08 and 925.09), Protein (section 955.04C and 979.09), Fat (section 922.06 and 954.02), ash (section 923.03) and crude fiber (section 962.09). Carbohydrate was calculated by difference [6].

Mineral analysis

For the analysis, in an electric furnace 3 g of the

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sample was dry-ashed at 550 ^{o} C for 24 hours. The resulting ash was cooled in a desiccator and weighed. The ash was dissolved in 2 ml of concentrated HCl and to the resultant solution few drops of concentrated HNO_3 was added. Then the solution was evaporated to almost dryness by placing on a boiling water bath. The content was made up to 100 ml with de-ionized water in a volumetric flask. Appropriate dilutions were made for each element before analysis. Calcium, magnesium and iron contents were quantified using atomic absorption spectrophotometer as described in the official method of the Association of official Analytical Chemists.

Functional properties

Bulk density of the sample was determined according to the method by Narayana and Narasinga [8]. In brief, 3 g of the sample was taken into a 10 ml measuring cylinder, gently taped over a soft surface, volume occupied by the sample after settling was noted in ml and expressed as g/cc. The water absorption capacity was determined by the method of Beuchat [9]. In brief, one gram of the sample was taken in 15 mL centrifuge tube; 5 ml of distilled water was added and mixed well with a glass rod. The tubes were allowed to stand for 30 min, centrifuged at 3000 ×g (20 min) and the supernatant was measured in measuring cylinder. Amount of water absorbed by the sample is calculated and expressed as g/g.

Swelling power and solubility

A known amount of sample with known moisture content was mixed with a measured volume of distilled water and heated at varying temperatures of 55, 65, 75, 85 and 95°C respectively on a temperature controlled water bath for 30 min with intermittent stirring. Thus obtained slurry was centrifuged (3000 g; 20 min) and supernatant was drawn off and evaporated to dryness on steam bath to obtain a measure of the dissolved solids. The sediment flour obtained after centrifugation was weighed to get the weight of the swollen flour particles. The values were expressed as percentages of total dissolved solids (solubility) and total swollen flour particles (swelling power) with respect to the weight of the flour sample used [10].

Phytochemical estimation

Total phenolics

Total phenolic content of the plant leaf was assayed by the method described by Folin Ciocalteu [11]. In brief, various aliquots of aqueous methonolic extract (10 mg/ml) were mixed with 5 ml Folin–Ciocaleu reagent and 4 ml of sodium carbonate (75 g/l). The resulting solution was vortexed and incubated at 40 ^{0}C for 30 min. The absorption was read at 765 nm. A calibration curve was prepared by using gallic acid as standard. All determinations were performed in triplicate. The total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$$A=0.980\ C+9.925\ X\ 10^{-3}$$

Where, A is the absorbance and C is the Concentration as gallic acid equivalents (µg/ml)

Flavonoids

The content of flavonoids was determined by a pharmacopeia method [12] using rutin as a reference compound. For brief, One ml of aqueous methanolic extract in methanol (10 mg/ml) was mixed with 1 ml aluminium trichloride in ethanol (20 g/l) and diluted with ethanol to 25 ml. The absorption at 415 nm was read after 40 min at 20 C. Blank samples were prepared from 1 ml plant extract and 1 drop acetic acid, and diluted to 25 ml. A standard graph was constructed using rurtin as the reference standard using the above method. All determinations were carried out in triplicate. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula.

$$X = \frac{A \times mo \times 10}{Ao \times m}$$

Where, X- Flavanoids content (mg/g) plant extract in rutin equivalents, A-Absorbance of the Sample, A_{o}- Absorbance of the standard, m- Weight of the sample in mg, m_o- Weight of rutin in the solution

Estimation of Total Alkaloids

Alkaloids were estimated by the gravimetric method [13]. The sample **(**0.5 gm) was taken in 250 ml beaker and 200 ml of acetic acid (10%) in ethanol was added and incubated at room temperature for 10 h. The resulting solution was filtered and the extract was concentrated on a water bath to one quarter of the original volume. To the Concentrate, concentrated ammonium hydroxide was added drop wise until the precipitation was complete. The above solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and filtered. Thus resulting alkaloid precipitate was dried, weighed and expressed in alkaloids per gm of sample taken.

Saponins

The Saponin content was analyzed according to gravimetric method [14]. In brief, 250 ml of 20% ethanol was added to 10 g of the pulverized leaf powder and stirred at 55° C using a magnetic stirrer for 12 h. The resulting solution was filtered using Whatman No 1 filter paper and reduced to 40 ml under vacuum and 20 ml Diethyl ether was added in a separating funnel and shaken vigorously. The pH of the aqueous layer was adjusted to 4.5 by adding HCl, where as the ether layer was discarded. To the pH adjusted aqueous part was extracted with 60 ml of n-butanol. The Butanol extract was washed twice with 10ml

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of 5 % NaCl and evaporated to dryness to give a crude saponin which was weighed and expresses in mg/g sample.

Tannins

Tannins were estimated according to spectroscopic method described by Trease & Evans [15]. 5 g of the leaf powder was extracted with 50 ml of boiling water and filtered. 0.5ml of the filtrate was added to 0.5 ml of 0.5M ferric solution in an alkaline medium and allowed to stand for 30 minutes for color development. The absorbance was read at 760 nm and the amount of tannin was extrapolated from a standard calibration curve for tannic acid.

Antioxidant activity

Preparation of solvent extracts

15g dehydrated leaf powder was extracted with 100 ml each of solvents [de-ionized water and 100% methanol] for 6 h in a mechanical shaker. The extracts were filtered through whatman filter paper. The filtrate of aqueous extract was freeze dried in freeze drier (Thermo freeze drier, USA) and the filtrate of methanol extract was evaporated at 50º C under reduced pressure to dryness in a rotary evaporator (Superfit, India). Both the extracts were stored in airtight container at 4ºC until further use.

2,2-diphenyl-1-picrylhydrazyl. (DPPH) Radical scavenging activity

The ability of the extracts to scavenge DPPH radicals was determined according to the method of Blois [16].

Reducing power assay

The ability of extracts to reduce iron (III) to iron (II) was determined as per the method of Yildirim et. al [17].

Ferric Reducing Antioxidant Power (FRAP)

Measurement of ferric reducing antioxidant power of the herbal extract was carried out based on Benzie and Strain procedure [18].

Preparation of Microsomes

A healthy adult rat was sacrificed to get fresh liver. The procedure followed for microsomes preparation was as previously reported [19]. After the sacrifice, liver was removed immediately into cold buffer (0.1 M triethanolamine.HC1, 0.02 M EDTA, and 2.0mM dithiothreitol (0-4°C) at pH 7.4). The liver tissue was minced into small pieced with scissors and homogenized with six strokes of a smooth-walled, glass Remi homogenizer and centrifuged for 10 min at 12,000 g to remove cell debris and mitochondria.

The supernatant was carefully removed and again centrifuged for 10 min at 12,000 g to ensure removal of mitochondria, it was then centrifuged at 60,000 g for 60 min. The 60,000g microsomal pellet was then rinsed with buffer (0.1 M triethanolamine buffer, containing 0.02M EDTA and 10mM dithiothreitol, pH 7.4,) and frozen in a freezer (-20°C) . Frozen microsomes were resuspended in the same buffer and allowed to stand for 60 min and packed in ice. The resuspended microsomes were were diluted with buffer to give a protein concentration of 5-10 mg/ml to use in anti-oxidant assay.

Antioxidant activity in Microsomes

Liver microsomes solution (equivalent to 1mg protein) and extracts at various concentrations were added with Fentons reagent and incubated at 50°C for 2 hrs. After incubation 1ml TCA 10% and 1ml TBA was added and heated in boiling water bath for 15 min and cooled in ice bath immediately. After cooling 2ml of butanol was added and the developed pink color was read at 532nm. A control was run without samples.

RESULTS

Physical properties and antioxidant potential:

Physical properties such as bulk density and water binding capacity are presented in Table 1 and Figure 1. Phytochemical composition of the bark is presented in the table 2. Leaf has significant amount of the polyphenols and saponins. Alkaloids and tannins were also present in the major amount. Mineral composition is given in the table 3. Proximate composition leaf is presented in the table 4. The leaf is composed of higher percentage of carbohydrates and is a good source of fiber (soluble and insoluble).

Anti-oxidant properties

Anti-oxidant potential of aqueous and methanol extracts of leaf are presented in Figure 2-5. From the values it can be observed that mahagoni leaf extracts have potential antioxidant potency and the methanolic extract has more prominent anti-oxidant activity.

Table 1. Physical properties

Property	Value
Bulk density (g/cc)	0.568 ± 0.01
Water Binding capacity (ml/g)	2.9 ± 0.05

Table 2. Phytochemical composition

Group	Amount
Total polyphenols (mg/g)	258 ±0.9
Flavanoids (µg/mg)	154±0.5
Saponins (mg/g)	198±1.8

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Tannins (µg/mg)	94±1.8
Alkaloids (µg/mg)	91±0.4
α-Tocopherol (µg/g)	80±1.2
β-Carotene(µg/mg)	3.4±0.6

The values expressed are the average of the triplicate values with the standard deviation.

Table 3. Mineral Content Of the leaf

Mineral	Quantity present ppm
Sodium	44
Calcium	343
Manganese	BDL
Zinc	0.28
Copper	BDL
Magnesium	339
Potassium	575
Iron	1.27

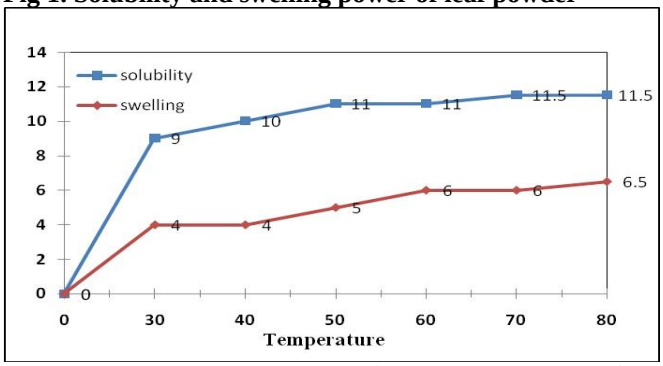
*BDL= below detectable limit.

Table 4. Proximate composition of leaf (Dry basis)

Parameter	Content
Moisture content	5.8 ± 0.16
Fat	1.49±0.15
Total Ash	9.5±0.4
Total protein	1.93%
Total Dietary Fiber	6.9±0.18
Total carbohydrates	73±1.2

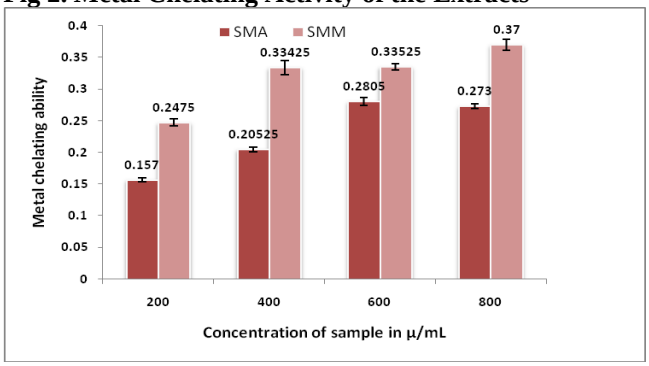
The values expressed are the average of the triplicate values with the standard deviation.

Fig 1. Solubility and swelling power of leaf powder



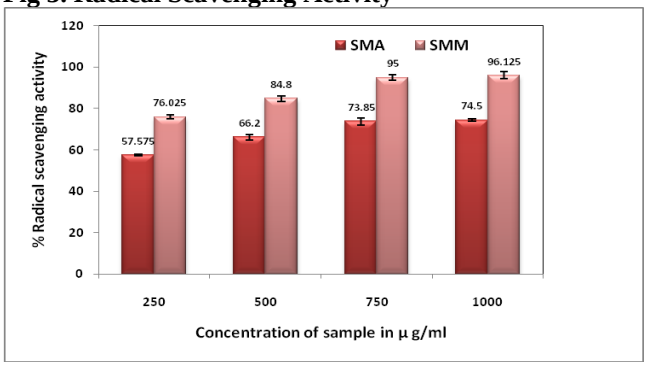
The values on Y-axis indicates the % of swelling and solubility of the *Sweitenia mahagoni* leaf powder in various temperatures

Fig 2. Metal Chelating Activity of the Extracts



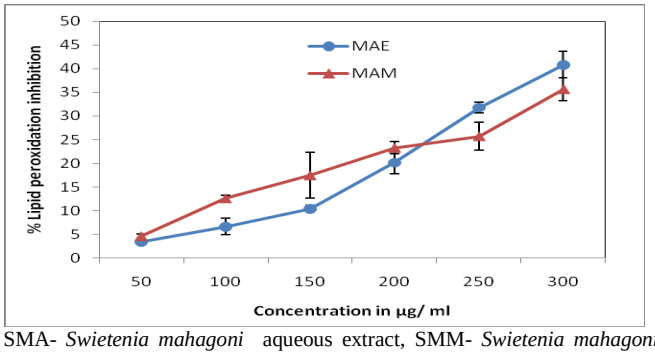
SMA- *Swietenia mahagoni* aqueous extract, SMM- *Swietenia mahagoni* methanol extract.

Fig 3. Radical Scavenging Activity



SMA- *Swietenia mahagoni* aqueous extract, SMM- *Swietenia mahagoni* methanol extract. The values indicate DPPH free radical scavenging activity of the extracts when compared to normal control.

Fig 4. Inhibition of Lipid Peroxidation In Microsomes By TBARS Method



SMA- *Swietenia mahagoni* aqueous extract, SMM- *Swietenia mahagoni* methanol extract. The values indicate percentage inhibition by the extracts when compared to normal control.

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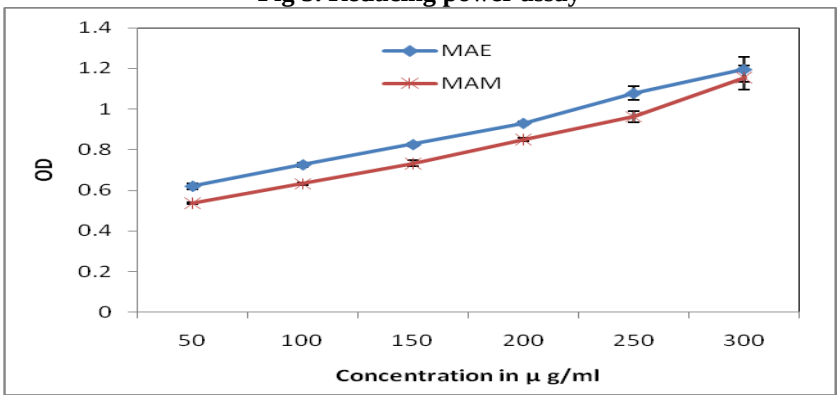
Fig 5. Reducing power assay



SMA- *Swietenia mahagoni* aqueous extract, SMM- *Swietenia mahagoni* methanol extract. The reducing power of the extract is directly proportional to absorbance of the assay mixture after incubation.

DISCUSSION

Swietenia mahagoni is a widely used as traditional medicine to treat various ailments. Previous studies have indicated the presence of important phytochemicals in the plant leaf by qualitative assays. The present study focuses on quntitation of constituent phytochemical groups and assaying the antioxidant potential of the plant. Polyphenols are ubiquitous metabolic intermediates found in plants. Regular consumption has been shown to reduce the risk of a number of chronic diseases, including cancer, cardiovascular disease (CVD) and neurodegenerative disorders [20]. Flavonoids are a class of plant secondary metabolites which were referred to as Vitamin P from the mid-1930s to early 50s. They have been found to play a key role in many beneficial physiological actions such as anti-oxidant, anti-atherosclerotic, anti-inflammatory, antitumor, anti-osteoporotic and antiviral activities. They have been found to show insignificant toxic effects in animal models [21]. The saponins are naturally occurring surface-active glycosides, which have been shown immunostimulant, hypocholesterolaemic and anticarcinogenic potency in both *In-vitro* and *In-vivo* models [22]. Presence of significant amount of all the above class of phytochemicals in the leaf reflects itself pharmacological value as evidenced by previous studies [23].

Minerals are naturally occurring element the body uses, to help and perform certain bio-chemical reactions. For most of the enzymatic reactions minerals such as Zn, Fe, Co acts as co-enzymes and also they form an integral part of functionally important organic compounds such as iron (Fe) in hemoglobin and cytochrome or zinc (Zn) in insulin [24, 25]. The elements such as K, Ca, Na are required to maintain osmolarity, nerve conduction and even in the absorption of certain nutrients. Calcium, potassium and magnesium are required for repair of worn out cells, strong bones and teeth in humans, building of red blood cells and other mechanisms in the body of organisms [2]. *Swietenia mahagoni* is a good source of iron, calcium, phosphorus and magnesium, beside serving as a nutraceuticals to treat various ailments, the leaf powder can add up for mineral supplementation.

Lipid peroxidation is a oxidative process in which unsaturated groups in the lipids undergo oxidation resulting in lipid derived radicals such as alkoxy and peroxyl radicals. In the normal physiology antioxidants are capable of stabilizing or deactivating free radicals before they attack cells [27]. Mahagoni extracts have potential ameliorating affect on the TBA generated by the oxidative-enzymes of the microsomes. The results of various antioxidant assays indicate mahagoni extracts exhibit electron donating and free radical scavenging property thus it can be extrapolated that the mahagoni treatment/supplementation in disorders/diseases involving oxidative stress can ameliorate the oxidative stress and thus the consequent results of oxidative stress.

CONCLUSION

Swietenia mahagony leaf contains biologically important antioxidant components that scavenge free radicals, chelate metal ions, and prevent lipid peroxidation, hence, can be used in the herbal formation to relieve oxidative stress associated with diabetes and CVD.

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