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Qualitative and quantitative analysis of phytochemicals in crude extract of big – Leaf mahogany (*Swietenia macrophylla* King.)

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Abstract
The present study investigates the qualitative and quantitative analysis of major bioactive constituents of medicinally important tree, *Swietenia macrophylla* in its methanol extract of leaf, seed and central-fruit-axis. The qualitative phytochemical tests exhibited the presences of common phyto-compounds including alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, and volatile oils as major active constituents. Alkaloids, terpenoids and carbohydrates are major compounds in both leaf extract and seed extract. Flavonoids and fixed oil are absent in leaf extract. Tannins and glycosides are not found in the seed extract. Tannin is major chemical compound in leaf and central-fruit-axis. Steroids and amino acids are found in all the extracts. The high content of tannins, alkaloids and flavonoids was found in seed extract where as high phenols was recorded in leaf extract.

Keywords: Mahogany, swietenia, phytochemical, seed, leaf, analysis

1. Introduction
Nature provides everything for the well-being of mankind over the years, including the tools for the first attempts at therapeutic intervention. In ancient times, people rely on plants for the treatment of various ailments. Even today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or used as templates for the development of new therapeutic agents, food additives, agrochemicals and industrial chemicals [1]. The photochemical is natural bioactive compound(s) found in plants which as act as a defense system against diseases. Based on the functions in plant metabolism, phytochemicals are two kinds viz., primary and secondary constituents. Primary constituents comprise common sugars, amino acids, proteins e family *Meliaceae.* It is exotic species introduced in India by then British in 1795. As its fruit seem and many more such as flavonoids and tannins etc. Among the 2, 50,000 - 5, 00,000 plant species in the world, only a small percentage has been investigated phytochemically [2]. So the systematic screening of plant species with the purpose of discovering new bioactive compounds can help us to cure many fungal and bacterial diseases of economically important crops and animals including human being. The value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. These are superior to synthetic pesticides in a number of ways like low mammalian toxicity, target specificity and biodegradability [3].
A Big- leaf mahogany (*Swietenia macrophylla* King.) is one of the highly prized timber in the world belong to the family *Meliaceae*. It is exotic species introduced in India by then British in 1795. As its fruit seem to point upwards to the sky, it is commonly called as “sky fruit” [4]. It grows usually taller than 30 m, with straight trunk and cylindrical with 100 to 200cm at breast height. The bark is dark reddish brown, entirely rosy, thick and deeply furrowed. The leaves are alternate with leaflets opposite or occasionally changed. The fruit is woody with 10 to 14 winged seeds [5]. *S. macrophylla* has been widely used in the common folk communities around the world. Each part of this plant have many uses and beneficial to humans whether as a medicine or other purposes [4]. The traditional practice by chewing and then swallowing the seeds of *S. macrophylla* by the natives in Malaysia is believed to cure high blood pressure [6], hypertension [7] and also to relieve pain [4]. Skin ailments and wounds also can be treated using the decoction of the crushed seeds of this tree [8]. Further, the local healers of the East Midnapore, West Bengal (India) use the seeds of *S. macrophylla* for curing diarrhea traditionally [9].

S. macrophylla contained various phyto-chemical compounds, of which limonoids and their derivatives have been identified as major constituents of this plant[7] and some fatty acids and terpenoids too isolated from the seeds [10]. The systematic screening of plants with the purpose of discovering new bioactive compounds is a routine activity in the field biomedical research. Therefore, crude extract of leaf, seed and central- fruit-axis of *S. macrophylla was* used for qualitative and quantify analysis of photochemical compounds.

2. Materials and methods

2.1 Plant Material

Disease free fresh leaf, seed and central-fruit-axis samples of *Swietenia Macrophylla* were collected from Mullur, Kothagiri, Tamil Nadu, India during the month of November, 2015. The samples were washed with tap water to remove dust and other inert materials. The cleaned samples were shade dried on the clean floor for 2 days. These shade dried samples were powdered using electrical blender.

2.2 Crude Extract Reparation

Thirty gram of dry powdered leaf, seed and central-fruit-axis materials were subjected to successive organic solvent extraction by refluxing in the Soxhlet apparatus each for 10 hours. In this study, the aqueous methanol (80 %) was used as solvent [11] (Sukanya *et al*., 2009). All the extracts were concentrated by oven-drying [12] (Azhari *et al*., 2012). Each fraction was collected when no further elution of compounds was observed. The collected extracts were subject to distillation and drying in incubator. The dried extracts were stored in sterile containers in the refrigerator till further analysis.

2.3 Phyto-chemical Screening

The condensed *S. macrophylla* crude methanolic extract (SMCM) of leaf, seed and central- fruit–axis were used for preliminary qualitative screening of phytochemicals such as alkaloids (Dragendorff test & Mayer's test), tannins (Ferric chloride test), flavonoids (HCL test and Lead acetate test [13], glycosides (Fehling's test and Glacial acetic acid test), terpenoids and steroids (H_2SO_4 test), saponins (Foam test), fixed oil (Spot test), Amino acids and proteins (Ninhydrin test and copper sulphate test) and terpenes (Liberman-Burchard) and tannins [14].

2.4 Quantification of phyto-chemicals in crude extract of *S. macrophylla*

2.4.1 Alkaloids

One gram each of SMCM leaf, seed and central-fruit-axis extract was added into dimethyl sulphoxide (DMSO) and dissolved it. To this mixture, 1ml of 2N HCl added and filtered. Then, 5 ml each of bromocresol green and phosphate buffer was added to the filtrate. These mixtures were transferred to volumetric flasks (10ml) and shaken well after adding 1, 2, 3 and 4 ml of chloroform. Then, volume of these flasks was made to the mark with chloroform. A set of standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described above. Absorbance for test and standard solutions was recorded against blank at 470nm with an UV/Visible spectrophotometer. The total alkaloid content was calculated from standard curve against absorbance and expressed as mg of AE/g of extract [15] (Mallikarjuna *et al*., 2012).

2.4.2 Flavonoids

The Aluminium Chloride Colorimetric Assay was employed for quantifying flavonoids in the crude extracts. One ml of each extract and 4 ml of distilled water were taken into a 10ml volumetric flask. To this flask, 0.3 ml of 5 % sodium nitrite was added and 0.3ml of 10 % aluminium chloride was mixed, after 5min. Thereafter, 2 ml of 1M sodium hydroxide was treated after 5min. and the content was diluted to 10 ml with distilled water. A standard curve was prepared with quercetin solution (20, 40, 60, 80 and 100 µg/ml) as the procedure described earlier. The absorbance readings were recorded for test and standard solutions against blank at 510 nm in UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract [16] (Hanane *et al*., 2010)

2.4.3 Phenols

The Folin - Ciocalteu Spectrophotometric method was used for determination of total phenolic content in plant extracts of big–leaf mahogany. To a 25 ml volumetric flask, 1 ml of extract and 9 ml of distilled water was taken. One ml of Folin-Ciocalteu phenol reagent was added to this mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate solution was added to the mixture. The volume of the same was made to 25 ml with distilled water. A standard curve was developed using different concentrations of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml). Incubated for 90 minutes at room temperature and the absorbance values for test and standard solutions were noted against blank at 550 nm with a UV /Visible Spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract [17, 18].

2.4.4 Tannins

Total tannin content was determined by using Folin - Ciocalteu Spectrophotometric method. Plant extract 0.1 ml was added to a volumetric flask containing 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu-phenol reagent and 1 ml of 35 % Na_2CO_3 solution and the content was diluted with 10 ml distilled water. The mixture was shaken well and kept at room temperature for 30 min. Using a set standard solutions of gallic acid, a standard curve was prepared. Absorbance for test and standard solutions was measured against blank at 725 nm with a UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract [19].

3. Results and Discussion

3.1 Phyto-chemical screening test

The qualitative analyses of bioactive compounds for the three crude extracts of *S. macrophylla* have been analyzed in this study and there is wide range of phytochemical compounds present in these extracts given in the Table 1. The data reveals that the strong positive results were found for alkaloids, terpenoids and carbohydrate in both methanolic seed and leaf extracts. The strong positive results for tannins were found in leaf and central-fruit axis. Steroids and Amino acids were positively found in all three extracts. The positive result was found in seed and central-fruit-axis for flavonoids where as saponin was found in seed and leaf extract only. The positive result for glycosides and fixed oil was found in leaf and seed, respectively.

Table 1: Phyto-chemicals in the crude extracts of *S. macrophylla*

Sl No	Phyto-constituent	Leaf	Seed	Central-fruit-axis
1	Alkaloids	++	++	+
2	Tannins	++	+	++
3	Steroids	+	+	+
4	Terpenoids	++	++	_
5	Flavonoids	_	+	+
6	Saponins	+	+	_
7	Carbohydrate	++	++	_
8	Glycosides	+	_	_
9	Amino acid and Proteins	+	+	+
10	Oil	_	+	_

(- Negative result; + Positive result and ++ Strong positive result)

3.2 Quantification of phyto-chemicals in crude extract of *S. macrophylla*

The quantitative estimation of phenols, tannins, alkaloids and flavonoids contents in the crude extract of leaf, seed and central-fruit-axis of *S. macrophylla* has been undertaken as per methods reported in literature and the results have been reported in Table 2. The highest phenols (24 mg GA/g) followed by central-fruit-axis and seed. The total tannins content of leaf extract was found between 18.67 and 90.33 mg QE/g dry weight of crude extract. The methanolic seed extract found to have high tannins content (11.5 mg of GA/g) followed by leaf and central–fruit-axis. The alkaloid contents was examined in plant extracts and expressed in terms of atropine equivalent as mg of AE/g of extract. The high content of alkaloids was 27.25 mg of AE/g recorded in seed extract and low content of the same was 14.5 mg of AE/g noted in leaf extract. The total flavonoids content of *S. macrophylla* leaf extract ranges from 8.75 to 19.5QE/g dry weight of extract. The high flavonoids content was 19.5 mg QE/g estimated in seed extract followed by central-fruit-axis extract and leaf extract Table 2). Thus, the results extracted from our research are in agreement with the studies associated with other workers in the same field [20-23].

Table 2: Content of phyto-chemicals in the plant extracts

Sl No.	Plant parts	Phyto-chemicals (mg)			
		Phenols	Tannins	Alkaloids	Flavonoids
1	Leaf	24.00	5.75	14.50	14.25
2	Seed	4.50	11.50	27.25	19.50
3	Central – fruit-axis	6.50	4.00	17.00	8.75

4. Conclusion

As the methanol extract of leaf, seed and central-fruit-axis of *S. macrophylla* evaluated in this work has different varieties of phytochemicals that could be considered as responsible for their therapeutic effects, antimicrobial and anti-oxidant activities. Alkaloids are the most significant compounds play a metabolic role in the living systems and are involved in the protective function in animals. Flavonoids have been used against the cancer causing tumors and it inhibits the promotion of growth and progression of tumors [23]. Phenols when mixed with the flavonoid compounds in plants are reported to show multiple activities like antioxidant, anticarcinogenic, anti-inflammatory, etc. [24]. Tannins inhibit the pathogenic fungi and antimicrobial activity of extracts showed better activity by the presence of tannins [25]. The plant based compounds have the effective dosage response and minimal side effects when compared to the synthetic compounds. Further, the plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health [26].

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