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Estimation of Some Phytochemicals in *Swietenia macrophylla* Leaves

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ABSTRACT

Extract of the leaf for the detection of the phytochemicals were obtained by soaking 100g of the sample in 250ml ethanol for forty-eight hours with frequent agitation. The phytochemical screening of *Swietenia macrophylla* showed that tannin, phenol, flavonoid, terpenoids and alkaloids are present in the leaf extract. Quantitative determination of the detected secondary metabolites was carried out to know their percentages in the *S. macrophylla* leaves.The quantitative estimation of phytochemicals revealed that the various phytochemical constituents present in the leaf extract. In leaf extract of *Swietenia macrophylla*, the alkaloid content was 0.045 (9%), flavonoids content was 0.062 (12.4%), phenol content was 0.032 (6.4%) and tannin content was 0.043 (8.6%).

Keywords: Estimation, Phytochemicals, *Swietenia macrophylla* Leaves

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Introduction

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing (Karande *et al.*, 2016). Nature has always remained a great source of medicinal agents and medicinal system of the world has used plant-based medicines from time immemorial (Mudasir *et al*., 2011). Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, terpenoids, steroids and flavonoids etc. (Edeoga *et al.,* 2005; Mann, 1978). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan, 1999). These are non-nutritive chemicals that have protective or disease preventive property (Subhashini *et al.,* 2010).

In order to understand these plants, know their medicinal value and for the plants to reach rightful role in contributing to affordable healthcare, these plants must be assessed through scientific point of view that ensures predictable chemical consistency, therapeutically benefits and proof of safety based on well designed and controlled studies using phytochemical screening and antimicrobial activities. *S. macrophylla* is one of the plants which have been used in traditional medicine for many years. To the best of our knowledge little or no work has been done on the estimation of phytochemicals in *S. macrophylla* in Wukari, Taraba State. The main objective of the research work was to check the presence and quantity of the phytochemical constituents in the medicinal plant and to enrich the available scientific data on the phytochemistry of *S macrophylla* leaves.

Materials and Methods

Sampling and Extraction

The leaf of *S. macrophylla* were collected from its natural habitat in Wukari local government area of Taraba state and was identified according to Shomkegh *et al*., 2016 and confirmed by Mr. Damasius Idiege of Forestry Department of Federal University Wukari. The sample was air dried for two weeks then milled into fine powder using milling machine. The extract of the leaf was prepared by soaking 100g of the sample in 250ml ethanol for forty-eight hours with frequent agitation. The resulting mixture was filtered using filter paper and the filtrate was concentrated by evaporation using rotary evaporator, kept in a vacuum oven over a night at room temperature to remove all the solvent and weighed. The extract was kept in the refrigerator until required for testing.

Phytochemical Screening Assay

Phytochemical examinations were carried out for all the extracts using standard procedures to identify the constituents. Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989), Harborne (1988).and Ushie *et al*., 2012

Test for Tannins

A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A blue solution indicated the absence of tannins in distilled water and dark green colour indicating presence in methanol.

Test for Saponins

About 0.2g of plant extract was mixed with distilled water and heated to boil. Frothing (appearance of creamy mix of small bubbles) showed the presence of Saponins in Methanol while red in Distilled water.

Test for Terpenoids

The extract (0.2g) was mixed with 2ml of chloroform, and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown interface was formed which indicated the presence of terpenoids on both extract.

Test for Steroids

Acetic anhydride (2 ml) was added to 0.5g of the extract in a test tube. It was then followed by the addition of 2 ml of sulfuric acid. A colour change from violet to blue or green indicated the presence of steroids on both extract.

Test for Flavonoids

About 0.2g of the extract was dissolved in dilute sodium hydroxide solution, and equal amount of hydrochloric acid was added. A yellow solution that turned colourless indicated the presence of flavonoids on both extract.

Test for Alkaloids

The aqueous (3ml) was stirred with (3ml) of 1% HCl on a steam bath. Meyer's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as positive evidence of alkaloids

Test for phlobatannins

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid. Disposition of red precipitate determines the presence of phlobatannins.

Test for Anthraquinones

About 0.5g of the extract was boiled with 2ml of 10% HCl for few minutes in a water bath. The resultant solution was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH_3 solution was added to the mixture and heated. Formation of rose pink colour indicated the presence of anthraquinones on both extract.

Test for Cardiac glycosides

10 cm_3 of 50%H_2S0_4 was heated in boiling water for 5 min. 10 cm_3 of Fehlings solution (5 cm_3 of each solution A and B) was added and boiled. A brick red precipitate indicating presence of glycoside was observed.

Detection of Phenol

To 1ml of leaf extract 2ml of distilled water was added followed by a few drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for Steroids

Exactly 5 drops of concentrated H_2SO4 was added to 1 mL of each extract in a test tube. The solutions were observed for a red colouration indicating the presence of steroids in the extracts.

Quantitative Phytochemical Screening

Quantitative determination of the detected secondary metabolites was carried out to know their percentages in the *S. macrophylla* leaves by the methods described by Iqbal, *et al.* (2011), Mudasir, (2012) and Sathya (2013).

Test for alkaloids

0.5g of the leaves sample was dissolved in 96% ethanol, 20% tetraoxosulphate (vi) acid (1:1) 1ml of the filtrate was added to 5ml of 60% tetraoxosulphate (vi) acid and allowed to stand for 5mins. Then 5ml of 0.5% formaldehyde was added and allowed to stand for 3hrs. The reading was taken at absorbance of 565nm.

Test for flavonoids

The determination of flavonoids on the leaves sample was done by acid hydrolysis of spectrophotometric method. 0.5g of the processed leaves sample was mixed with 5ml of dilute hydrochloric acid and boiled for 30mins. The boiled extract was allowed to cool and filter. 1ml of the filtrate was added to 5ml of ethyl acetate and 5ml of 1% ammonium. This was then scanned from 420nm and 520nm for the absorbance.

Test for phenols

The quantity of phenol is determined using spectrophotometric method. The leaves sample is boiled with 50ml diethyl ether or petroleum spirit. 5ml of the boiled sample is then pipette into 50ml flask and 10ml of distilled water is added. After the addition of the distilled water, 2ml of ammonium hydroxide solution and 5ml

of concentrated pentanol is added to the mixture. The leaves sample was made up to mark and left for 30mins to react for colour development and measure at 505nm wavelength using a spectrophotometric method.

Test for tannins

The quantity of tannins is determined by using spectrophotometric method. 0.5g of the leaves sample is weighed into plastic bottle; 50ml of distilled water is added and stirred for 1hr. The sample is filtered into a 50ml flask and made up to mark. 5ml of the filtered sample is then pipette out into test tube and mixed with 2ml of 0.1M HCl and 0.008M $K_4Fe(CN)_6.3H_2O$. The absorbance is measured with a spectrophotometer at 395nm wavelength within 10mins.

Results and Discussions

Table 1: Preliminary Pytochemical Screening of Ethanolic Leaves Extract of *Swietenia macrophylla*

S/N	Phytochemicals	Results
1	Tannin	+
2	Phenol	+
3	Steroid	-
4	Saponin	-
5	Terpenoid	+
6	Flavonoids	+
7	Phlobatanin	-
8	Glycosides	-
9	Alkaloid	+
10	Anthraquinone	-

Table 2: Result of Quantification of Phytochemicals Leaf of *Swietenia macrophylla*

S/N	Test (sample)	Concentration(Abs)	% Calculated
1	Tannin	0.043	8.60
2	Phenol	0.032	6.40
3	Flavonoid	0.062	12.40
4	Alkaloid	0.045	9. 00

The phytochemical screening of *Swietenia macrophylla* showed that tannin, phenol, flavonoid, terpenoids and alkaloids are present in the leaf extract (Table-1).The quantitative estimation of phytochemicals revealed that the various phytochemical constituents present in the leaf extract (Table-2). In leaf extract of *Swietenia macrophylla*, the alkaloid content was 0.045 (9%), flavonoids content was 0.062 (12.4%), phenol content was 0.032 (6.4%), tannin content was 0.043 (8.6%). These phytochemicals are reported to have many biological and therapeutic properties (Narender *et al*., 2012). Ushie *et al* 2016 pointed out that *S. macrophylla* can be used as anti-inflammatory, antispasmodic, antianalgesic and diuretic properties which can be attributed to their high flavonoids, alkaloids, steroids,

glycosides and saponins (Savithramma *et al.,* 2011). The habitual intake of flavonoids from food sources such as tea may lead to a lower risk of atherosclerosis and coronary heart disease, and also protect against stroke (Keli *et al.,* 1996).

Flavonoids have received considerable attention because of their beneficial effects as antioxidants in the prevention of human diseases such as cancer and cardiovascular diseases, and some pathological disorders of gastric and duodenal ulcers, allergies, vascular fragility, and viral and bacterial infections (Jenkins *et al.,* 2002). In vitro and animal studies have demonstrated that flavonoids have antioxidant and antimutagenic activities (Aherne *et al.,* 2002) and may reduce the risk of cardiovascular disease and stroke (Duthie *et al.,*2000). Flavonoids have been demonstrated to reduce carcinogenesis in animal models and to modulate enzymes implicated in the carcinogenic process (Yao *et al.,* 2004). Flavonoid intake may reduce the risk of death from coronary heart disease (Knekt *et al.,* 1996). Application of flavonoids may provide a means for industries in developing anti-HIV agents (Yao, 2004).

Diarrhea is treated with an effective astringent tannins that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine (Cheng *et al.,* 2002). Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more. Tannins are also beneficial when applied to the mucosal lining of the mouth (Stéphane *et al.,* 2004). Tannins can also be effective in protecting the kidneys. Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers. Tannins can cause regression of tumors that are already present in tissue, but if used excessively over time, they can cause tumors in healthy tissue. They have been also reported to have anti-viral (Lin *et al.,* 2004) antibacterial (Akiyama *et al.,* 2001) and antiparasitic effects (Kolodziej, *et al.,* 2005). Tannins can also be effective in protecting the kidneys. Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers. Tannins can cause regression of tumors that are already present in tissue, but if used excessively over time, they can cause tumors in healthy tissue. They have been also reported to have anti-viral (Lin, *et al.,* 2004) antibacterial (Akiyama, *et al.,* 2001) and antiparasitic effects (Kolodziej *et al.,* 2005). When incubated with red grape juice and red wines with a high content of condensed tannins, the polio virus, herpes simplex virus, and various enteric viruses are inactivated (Bajaj, 1999).

Alkaloids play an important role in the defense systems against pathogens and animals and the applications of alkaloids are not limited to biological control of herbivores but also have pharmacological, veterinary and medical importance (Patel *et al* 2012). Alkaloids belonging to beta-carboline group possess antimicrobial, anti-HIV and antiparasitic activities (Bouayad *et al.,* 2011). In some cases, alkaloids obtained from plants may cause serious illness, injury or even death (Patel *et al* 2012). Natural phenolic compounds play an important role in cancer prevention and treatment (Huang *et al.,* 2010). Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways (Huang *et al.,* 2010).

Conclusion

Extract of the leaf for the detection of the phytochemicals were obtained by soaking 100g of the sample in 250ml ethanol for forty-eight hours with frequent agitation. The phytochemical screening of *Swietenia macrophylla* showed that tannin, phenol, flavonoid, terpenoids and alkaloids are present in the leaf extract. Quantitative determination of the detected secondary metabolites was carried out to know their percentages in the *S. macrophylla* leaves. The quantitative estimation of phytochemicals revealed that the various phytochemical constituents present in the leaf extract. In leaf extract of *S. macrophylla*, the alkaloid content was 0.045 (9%), flavonoids content was 0.062 (12.4%), phenol content was 0.032 (6.4%) and tannin content was 0.043 (8.6%). *S. macrophylla* can be used as anti-inflammatory, antispasmodic, analgesic and diuretic properties which can be attributed to their high flavonoids, alkaloids, tannins and phenols.

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