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LIPOIDAL COMPOSITION AND BIOACTIVITY OF LEAVES AND BARKS OF SWIETENIA MAHOGANI AND SWIETENIA MACROPHYLLA GROWN IN EGYPT

Ola Mohamed Mousa¹*, Marwa Yousry Issa¹, Hesham Ibrahim El-Askary¹, Soheir Mohamed El Zalabani¹ and Amani A. Sleem²

¹Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr-El-Aini Street,

Cairo, Egypt, 11562

² Pharmacology Department, National Research Center, Dokki, Guiza, Egypt.

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*Author for Correspondence Dr. Ola Mohamed Mousa Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr-El-Aini Street, Cairo, Egypt, 11562

ABSTRACT

Ethanol (90%) extractives of leaves and stem barks of *Swietenia mahogani* and *Swietenia macrophylla* were fractionated and comparatively examined regarding yield, lipoidal composition and bioactivity. Bark ethanol extractives were obtained in higher amounts than those of leaves. Lipoids were detected in the petroleum ether and chloroform-soluble fractions of ethanol extracts. Unsaponifiable lipoids (USL) and fatty acid methyl esters (FAME) of petroleum ether extracts were analyzed by GC/FID. Despite close qualitative similarity, GC profiles revealed quantitative variability. Relative percentage of identified phytosterols in *S. macrophylla* samples exceeded that in *S.*

mahogani with prevalence of β -Sitosterol; meanwile *n*-Tricosane was the predominant hydrocarbon in all tested samples. Three known lipoids were isolated from *S. macrophylla* bark: *n*-nonacosane and β -sitosterol from the petroleum ether fraction of its ethanol (90%) extract and β -sitosterol-3-O- β -D-glucoside from the chloroform fraction. Identification of isolated compounds was through physicochemical, chromatographic and spectral data. *S. macrophylla* ethanol extracts were found more efficient as cytotoxic against the *in vitro*tested human cancer cell lines (liver, HEPG2; colon, HCT116 and brain, U251) with best efficiency exhibited on HCT116. Determined LD₅₀s demonstrated the safety of all extracts in the range of the orally given doses. Prolonged administration of all extracts significantly decreased the level of liver enzymes in CCl₄-intoxicated rats indicating a hepatoprotective activity. A moderate antipyretic effect was exerted by extracts of leaves only; and none of the samples exhibited significant anti-inflammatory effect. The highest potency was, in most cases, observed for *S. mahogani* leaves extract particularly as antiulcer, antioxidant and hepatoprotective.

Key words: *Swietenia mahogani*, *Swietenia macrophylla*, unsaponifiable lipoids, sterols, fatty acids, bioactivity.

INTRODUCTION

Swietenia species (Meliaceae or Mahogany family) are valuable trees of commercial and ethnopharmacological importance. They are, nowadays, considered as endangered species due to high exploitation of their woods as precious timbers ⁽¹⁻⁴⁾. *Swietenia* seeds are traditionally used as antihypertensive, antidiabetic and antimalarial; the stem bark decoctions are, as well, taken as potent febrifuge and antidiarrheal, and locally applied as wound astringent ⁽²⁻⁵⁾.

Swietenia mahogani L. Jacq. and *Swietenia macrophylla* King. have been successfully naturalized in Egypt ^(1-3, 5). The phytochemical profile of the leaves and stem bark of these locally cultivated plants revealed the presence of carbohydrates and/or glycosides, free and/or combined flavonoids, sterols and/or triterpenes, as well as tannins ⁽⁶⁾. HPLC quantitative analysis of the polyphenolic content of the two species revealed the prevalence of catechin in the stem barks and of flavonols in the leaves ⁽⁶⁾.

Among scientifically-based bioactivities reported, the methanol and aqueous extracts of *S*. *mahagoni* seeds were found to exert significant antioxidant activity *in-vitro*, the effect being attributed to phenolic components ⁽⁷⁾. Besides, the seed oil revealed a significant antidiabetic activity, when *in-vitro* assessed by the amylase inhibition assay ⁽⁸⁾. In a previous *in-vivo* study, the authors recorded a significant reduction in blood glucose level in Alloxan-diabetic rats receiving aqueous solutions of stem bark exudates of either *S. mahogani* or *S. macrophylla* ⁽⁹⁾.

Moreover, the proximate nutritional composition of the seed cake was determined and the fatty acid composition of the oil analyzed by Gas Chromatography resulting in identification of 48 compounds ⁽¹⁰⁾. Despite the numerous reports ⁽¹¹⁻¹⁵⁾ on isolation of tetranortriterpenoids (limonoids) from petroleum ether extracts of seeds and other organs of *S. mahogani* (L.) Jacq., as well as seed cake of *S. macrophylla* King, those concerning other lipoids were

scarce. This prompted the performance of the following investigation of the lipoid composition of the leaves and stem barks of *Swietenia mahogani* and *Swietenia macrophylla*. In addition, the biological activities of these plant organs were evaluated aiming to assess their potential as new herbal drugs; especially that, on economical scale, both are considered as waste products of these timber trees.

MATERIALS AND METHODS

Plant material

Leaves and stem barks of *Swietenia mahogani* (L.) Jacq. and *Swietenia macrophylla* King. were collected from plants cultivated at the Zoological Garden, Giza, Egypt. Authentication was achieved by Dr. Mohamed El-Gebaly, botanist specialist and voucher specimens kept at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo, Egypt. Samples were air-dried, powdered and saved for investigation.

Extracts for phytochemical investigation

For examination of extractives: powdered leaves and stem barks of *Swietenia mahogani* (L.) Jacq. and *Swietenia macrophylla* King. (100 g, each) were exhaustively extracted by cold maceration in ethanol (90%) and the solvent evaporated under vacuum. The dried ethanol extract was suspended in water and successively partitioned using petroleum ether, chloroform, ethyl acetate and *n*-butanol saturated with water. The percentage yields and organoleptic characteristics of the solvent-free extractives were recorded.

For investigation of lipoidal contents: exhaustive extraction of the four different samples (1000 g, each) was performed with petroleum ether. The solvent was distilled off and the sovent-free non-polar extractives weighed. Aliquot amounts were saponified and the resulting saponifiable and unsaponifiable lipoids subjected to GC analysis. The residual marc in case of *Swietenia macrophylla* stem bark was further percolated with cold ethanol (90%), the extract evaporated, the residue suspended in water and subjected to liquid-liquid fractionation with chloroform to extract any remaining semi-polar lipoids.

Extracts for biological evaluation

Ethanol extracts were prepared by exhaustive cold maceration of each of the four air-dried powdered samples (500 g) in ethanol (90%). The solvent was evaporated to dryness and extracts saved for further investigation.

Cell lines and experimental animals

Cancer Cell lines *viz.*, Hepatocellular (HEPG2), Colon (HCT116) and Brain (U251) carcinoma human cell lines were kindly provided from the National Cancer Institute (Kasr El Ainy Street, Cairo, Egypt).

Experimental animals were obtained from the animal house colony at the National Research Centre (Dokki, Guiza, Egypt). Albino mice (25-30 g) were used for determination of LD_{50} , and adult male albino rats of Sprague Dawley strain (120-150 g) were utilized for *in-vivo* assessment of the other bioactivities. The animals were kept on standard laboratory diet and water supplied *ad libitum*. All animal procedures were carried out according to the agreement of the Ethics Committee of the National Research Centre (Dokki, Guiza, Egypt) and in harmony with the recommendations of the Proper Care and Use of Laboratory Animals.

Dugs and laboratory kits

The following drugs were purchased from their respective sources: Carrageenan (Sigma, USA) for induction of inflammation; Alloxan (Sigma, USA) for induction of diabetes. Indomethacin, (Egyptian International Pharmaceutical Industries Co, EIPICO, under license of Merck & Co, Inc-Rahway, NJ, USA), as anti-inflammatory; Amiloride HCl-Hydrochlorothiazide (Moduretic[®], El Kahera Co, Egypt), as diuretic; Dipyron-Metamizol (Novalgin[®], Hoechst Origent, S.A.E., Cairo, under license of Hoechst AG Frankfort, Germany), as analgesic; Paracetamol (Paramol[®], Misr Co, Egypt), as antipyretic; Silymarin (Sedico Pharmaceutical Co, 6th of October City, Egypt), as hepatoprotective; Vitamin E (dl- α -tocopheryl acetate, Pharco Pharmaceutical Co), as antioxidant.

Glutathione kits (Wak-Chemie Medical GmbH, Steinbach/Ts., Germany) were used for measuring the antioxidant activity; and Transaminase Kits (Bio-Merieux Co, France) were utilized in assessing serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes.

Saponification of the lipoidal extract and preparation of fatty acid methyl esters (FAME)

The petroleum ether extract of the tested samples (1g, each) was saponified with alcoholic KOH (10%) ⁽¹⁶⁾. The alkaline hydrolysate was concentrated, suspended in water and the unsaponifiable lipoids (USL) extracted with ether. The extract was dehydrated (anhydrous Na₂SO₄), freed from the solvent, weighed and saved for GC analysis. The remaining aqueous

alkaline mother liquor was acidified with dilute HCl, exhaustively extracted with ether. The resulting extract was washed (distilled water), dehydrated and evaporated to dryness yielding the free fatty acids (FA) mixture which was weighed. Methylation of FA (in methanol) was performed by drop wise addition of an ethereal solution of diazomethane ⁽¹⁷⁾, the FAME were isolated and analyzed by GC.

Chromatographic procedures

Thin layer chromatography (TLC) of lipoids was carried out on precoated silica gel F_{254} plates, (20×20 cm, Sigma-Aldricth Chemicals-Germany) alongside with reference samples. Solvent systems: S₁, Hexane-Ethyl acetate (80:20 v/v), S₂, Chloroform-Methanol-Formic acid (95:5:0.2 v/v), S₃: Chloroform-Ethyl acetate-Formic acid (80:20:0.1 v/v) and S₄: Chloroform-Methanol-Formic acid (90:10:0.2 v/v) were used for developing the chromatograms. Spot visualization was achieved by *p*-anisaldehyde / H₂SO₄, chromogenic spray reagent ⁽¹⁸⁾.

Vacuum Liquid Chromatography (VLC) and Column Chromatography (CC) for isolation of lipoids of *Swietenia macrophylla* bark were, respectively, performed on Silica gel H 60 and Silica gel 60 (E-Merck, Darmstadt, Germany) by using appropriate eluents.

Gas chromatography (GC) of USL mixtures (2 μ L of 2% chloroform solution, each) was performed on a Hewlett-Packard HP-6890 N network GC system equipped with FID detector and HP-5-MS column (5% phenyl-methyl siloxane, 30 m × 0.32 mm \emptyset × 0.25 μ m film thickness) using N₂ (flow rate, 30 ml/min) as carrier gas, injector temperature was set at 250°C and detector temperature at 300°C (air flow rate, 300 ml/min and H₂ flow rate , 30 ml/min). Oven temperature programming was: initial temperature, 80°C, kept isothermal for 1 min, increased to 250°C by the rate of 8°C/min, and then kept isothermal for 20 min.

GC of FAME mixtures (2 μ L each, of 2% chloroform solution, each) was conducted on a Pye Unicam 304 series gas chromatograph equipped with an FID detector. Analysis was performed on a 10% PEGA (adsorbed on Chromosorb W-AW, 100-120 mesh) column (1.5 m × 4 mm \emptyset), by adopting the following operating conditions: carrier gas, N₂ (flow rate, 30 ml/min); injector temperature, 250°C; detector temperature, 300°C; air flow rate, 300 ml/min and H₂ flow rate, 33 ml/min; oven temperature programming: initial temperature, 70°C increased to 190°C by the rate of 8°C/min, then kept isothermal for 25 min.

Reference samples of hydrocarbons, sterols and triterpenes (E-Merck, Darmstadt, Germany) and of fatty acids methyl esters (FAME, available at the Central Laboratory of the Faculty of Agriculture, Cairo University, Guiza, Egypt) were utilized in TLC and GC.

Qualitative identification of components in GC was based on comparison of the individual peaks retention times with those of available authentic samples similarly analyzed. Peak area measurement was the basis of relative quantitation of the constituents.

Isolation and identification of major lipoids from Swietenia macrophylla stem bark

The petroleum ether extract of the stem bark of *Swietenia macrophylla* (8 g) was subjected to VLC (silica gel H 60 column, 150 g, 12.5 cm \times 7 cm \emptyset). Gradient elution was carried out using *n*-hexane, the polarity being gradually increased by 2 % stepwise addition of chloroform till 100 %. Fractions (200 ml) were collected, monitored by TLC (S₁, *p*-anisaldehyde/H₂SO₄) and pooled into 3 collective fractions (I-III). Fractions I (100 % *n*-hexane, 2.6 g) and III (6-10% CHCl₃ in *n*-hexane, 3 g) each showing one major spot (R_f values, S₁, 0.96 and 0.49) were refractionated on silica gel 60 columns (27 cm \times 2.7 cm \emptyset) to yield compounds **1** and **2**, respectively.

The chloroform-soluble fraction of the defatted ethanol extract of the same organ (5 g) was subjected to VLC (silica gel H 60 column; 100 g, 10 cm × 4 cm \emptyset); elution was started with hexane-chloroform mixture (50:50), followed by gradual increments of chloroform, ethyl acetate and methanol until a concentration of 10% methanol in ethyl acetate was reached. Fractions (200 ml) were combined on the basis of TLC profiles (S₂, *p*-anisaldehyde / H₂SO₄) to yield four collective fractions I-IV. Fraction IV (chloroform-ethyl acetate 20:80 up to ethyl acetate-methanol 90:10, 1.6 g) showing one major spot (R_f 0.16, violet with *p*-anisaldehyde / H₂SO₄) was purified by CC (silica gel 60 column; 16 cm × 2.7 cm \emptyset); gradient elution was with chloroform followed by chloroform-methanol mixtures. Individual fractions (10 ml, each) were screened by TLC (S₂). Those eluted with chloroform-methanol 50:50 up to 30:70, on pooling and concentration, afforded compound **3**.

Identification of isolated compounds was through physicochemical and spectral analysis. Melting points were uncorrected and determined on an Electrothermal 9100 (U.K.) apparatus. A Varian Mercury NMR-spectrometer (Japan) was used for recording ¹H-NMR, 300 MHz, ¹³C, 75 MHz spectra in DMSO using TMS as internal standard and chemical shift values

expressed in δ ppm. Mass spectra were run on a Varian Mat 711, Finnigan SS Q 7000 (USA) mass spectrometer.

Biological evaluation of the ethanol extracts

The ethanol (90 %) extracts of the leaves and stem barks of the two plants under investigation were comparatively subjected to evaluation of their biological efficiency. Tested samples were dissolved in bi-distilled water by the aid of few drops of Tween 80, except for the cytotoxic activity where DMSO was used as solvent.

Data obtained were analyzed using student's t- test where, means of the treated groups were compared to that of the control group for each variable ⁽¹⁹⁾.

In-vitro assessment of the cytotoxic activity

This was performed on three human cancer cell lines *viz.*, HEPG2 (liver), HCT116 (colon) and U251 (brain) by the Sulpho-Rhodamine-B (SRB) assay, adopting the method of Skehan *et al* ⁽²⁰⁾. Cells were plated in 96-multiwell plates (104 cells/well) for 24 hours before treatment with the extracts to allow attachment of the cells to the wall of the plate. Different concentrations (0, 1, 2.5, 5 and 10 μ g/ml in DMSO) of the tested samples were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extracts for 48 hours (at 37 °C in 5 % CO₂ atmosphere). After 48 hours, the cells were fixed, washed and stained with Sulpho-Rhodamine-B stain. Excess stain was washed with acetic acid and the attached stain recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and extract concentration was plotted to establish the survival curve of each cancer cell line after being treated with the specified extract and IC₅₀ (concentration that reduces cell survival to 50%) was deduced for each sample.

Acute toxicity studies

The median lethal doses ($LD_{50}s$) of the different ethanol (90%) extracts were determined by Karber's procedure ⁽²¹⁾ *via* oral administration to male albino mice (25-30 g). Preliminary experiments were carried out to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were selected in between these two doses; each dose was given to a group of six animals. The mice were observed for 24 hours and symptoms of toxicity and mortality rates in each group were recorded.

Assessment of analgesic, anti-inflammatory, antipyretic and diuretic potential

These were performed *in-vivo* according to published procedures ⁽²²⁻²⁵⁾ using male albino rats (120-150 g). Ethanol extracts (100 mg/kg b.wt., each) as well as, standard drugs (in appropriate doses), were orally administered, each to a group of 6 animals. A negative control group, in each experiment, received only saline.

The analgesic activity was tested according to the procedure described by Charlier *et al.* ⁽²²⁾ on male rats and using Novalgin[®] (20 mg/kg b.wt.) as standard drug. Electric current was applied as a noxious stimulus to rats' tails by means of a 515 Master Shocker (Lafayette Instruments Co).The minimal voltage required for the animal to emit a cry was recorded, prior treatment, at zero time (V₀) then after 1 and 2 hours intervals from drug administration for each of the treated groups (V_t). The percentage change was deduced from the following equation: % of change = (V_t-V₀) × 100/V₀

The acute anti-inflammatory effect was evaluated as compared to Indomethacin (20 mg/Kg b.wt.) by the carrageenan-induced rat paw oedema test, as described by Winter *et al.* ⁽²³⁾. Male animals were used; the percentage of oedema produced and that of oedema inhibition due to drug administration were, respectively, calculated as follows:

Oedema= (wt of right paw-wt. of left paw) \times 100 / wt. of left paw; and

% Oedema inhibition = $(Mc - Mt) \times 100/Mc$; where, Mc represents the mean oedema in control rats and Mt the mean oedema in drug-treated animals. The % of change was calculated by computation.

The antipyretic effect was assessed as compared to Paracetamol (20 mg/kg b.wt.) using the yeast-induced hyperthermia method of Tomazetti *et al.* ⁽²⁴⁾. Pyrexia was induced by intramuscular injection of a 44 % yeast suspension (1 mg/100 g b.wt.). The tested samples and standard were orally administered and a group of animals kept untreated (negative control). Rectal temperatures were recorded at zero time (T₀), prior treatment, and after 1 and 2 hours intervals (T_t) from dose administration for the treated animals. The percentage of change was calculated using the following equation: % of change = (T₀-T_t) × 100 / T₀.

The diuretic activity was assessed by holding the rats in the metabolic cages, fasted for 18 h, being supplied with water only. The first group received, orally, 1 ml saline and was kept as normal control, the second group Moduretic[®] (standard diuretic, 5 mg/kg b. wt., positive control), and the other four groups the alcohol extracts ⁽²⁵⁾.

Assessment of antiulcer potential

The antiulcer activity of the ethanol extracts was assessed according to Corell *et al.* ⁽²⁶⁾. Male albino rats of Sprague Dawley strain (130-140 g b.wt.) were divided into five groups (n=10). The positive control group received Indomethacin (20 mg/kg b.wt.); and each of the other 4 groups received Indomethacin (20 mg/kg b.wt.) in addition to one of the tested samples (100 mg/kg b.wt. Animals were starved for 18 hours, water being supplied *ad libitum*. Four hours later, they were sacrificed, the stomach removed, fixed in 10% formalin, dissected along the greater curvature and examined for any eroded or ulcerated areas by means of a magnifying lens. Number of ulcers were counted and compared to those observed in the control group.

Assessment of antioxidant potential

Diabetes was induced to male albino rats (120-150 g) by intraperitoneal (i.p.) injection of Alloxan (150 mg/kg b.wt.) as described by Eliasson and Samet ⁽²⁷⁾. Tested extracts (100 mg/kg b.wt., each) and Vitamin E (standard antioxidant, positive control, 7.5 mg/kg b.wt.) were orally administered to the experimental animals (n=6), followed by collection of blood samples, at intervals; a group of animal was kept untreated (non-diabetic control) and a diabetic group received only saline (diabetic control). The antioxidant activity was assessed by measuring the glutathione level (reduced due oxidative stress produced by diabetes) in the blood samples, according to Beutler *et al.* ⁽²⁸⁾. The restoration of blood glutathione levels was taken as a measure of antioxidant activity. The percentage change observed after dose administration was calculated as follows:

% of change = $(G - G_0) \times 100 / G_0$; where, G_0 represents the glutathione level in diabetic animals, prior administration of the samples and G that measured after.

Assessment of hepatoprotective potential

Liver damage was induced in adult male albino rats (130-140 gm, 6 groups, n=10) with carbon tetrachloride (CCl₄, 25 % in liquid paraffin) by i.p. injection (5 ml/kg b.wt.) ⁽²⁹⁾. Blood samples were withdrawn 72 hours after CCl₄ administration (liver damage). The ethanol extracts (100 mg/kg b.wt.) and Silymarin (standard hepatoprotective, 25 mg/kg b.wt.) were daily administered, for one month before and after liver damage. A group of animals was kept untreated (negative control) receiving only saline. Biochemical studies were carried out after an overnight fast. Whole blood samples were withdrawn from the retro-orbital venous plexus through the eye canthus of anaesthetized rats. Serum was isolated by centrifugation and divided for determination of levels of aspartate amino-transferase (AST), alanine amino-

transferase (ALT) ⁽³⁰⁾ and alkaline phosphatase (ALP) ⁽³¹⁾. Blood samples were collected at zero time, after one month of receiving the tested drugs, 72 hours after induction of liver damage then after a month of treatment with the tested samples and standard.

RESULTS AND DISCUSSION

Examination of extractives

The organoleptic characteristics of ethanol extractives of leaves and stem bark of *S. mahogani* were found almost similar to their analogues derived from *S. macrophylla*. Obviously, a higher yield of ethanol (90%) extractives was observed for stem barks as compared to leaves (Table 1). Besides, petroleum ether and chloroform extractives of leaves and stem bark in *S. macrophylla* were obtained in a higher yield than those of *S. mahogani*.

 Table (1): Percentage yield of solvent extractives of leaves and stem barks of Swietenia

 mahogani and Swietenia macrophylla

	Percentage yield					
Extractives	S. mah.	S. mah.	S. macr.	S. macr.		
	leaves	bark	leaves	bark		
Ethanol (90%)	35	37.3	33	38.5		
Petroleum ether	1.9	0.3	2.1	0.9		
Chloroform	2.0	0.4	3.3	0.7		
Ethyl-acetate	4.3	3.1	4.0	2.9		
n-Butanol	4.5	2.7	4.3	2.6		

S. mah. = Swietenia mahogani; S. macr. = Swietenia macrophylla

Investigation of lipoids

Upon saponification, the petroleum ether extracts of leaves and stem barks of *S. mahogani* and *S. macrophylla* yielded 69, 64, 62, and 72 % of yellowish white unsaponifiable lipoids (USL), respectively. Correspondingly, the isolated fatty acids (FA) represented 13, 8, 17, and 7 % of the parent extracts.

GC profiling of the USL samples (Table 2) revealed a close similarity in composition with obvious quantitative variation. The largest number of identified components was in the leaves of *S. mahogany*; while the highest total percentile of identified constituents was found in the stem bark of *S. macrophylla* being the lowest in the leaves of the same plant. The amount of identified phytosterols in *S. macrophylla* samples obviously exceeded that in those of *S. mahogani* with prevalence of β -Sitosterol; meanwhile, Campesterol was detected in appreciable amounts in the barks of the 2 species and dominated the sterol composition of that of *S. mahogani*. β -Amyrin was the only detected triterpenoid under the experimental

conditions adopted. Among hydrocarbons, *n*-Tricosane was prevalent in all the investigated samples.

GC analysis of FAME derived from the FA fractions of the different samples (Table 3) revealed an almost qualitative similarity together with quantitative variability. The total identified components represented higher percentages in *S. mahogani* leaves and stem bark as compared to those of *S. macrophylla*. The highest percentile of unsaturated FA was in *S. mahogani* leaves and that of the saturated ones in the bark of the same species. Among unsaturated FA, oleic and linoleic acids were detected in all the analyzed samples, these results are in agreement with those previously reported for the seeds ⁽³²⁻³⁶⁾. The collective percentage of these 2 acids was higher in the stem bark samples than in those of the leaves reaching (55.89 vs 20.56 %) in *S. mahogani* and (36.37 vs 11.66 %) in *S. macrophylla*, respectively. Palmitic acid was the predominant saturated fatty acid in all the samples, with the highest percentage recorded in the leaves of *S. mahogani*.

Table (2): Components identified by GC analysis of USL of leaves and stem barks of
Swietenia mahogani and Swietenia macrophylla

Identified		Rela	ative perce	ntage in U	SL of
	RR _t *	S. mah.	S. mah.	S. macr.	S. macr.
components		leaves	bark	leaves	bark
<i>n</i> -Decane	0.12	10.54	1.34	0.88	
<i>n</i> -Undecane	0.16	3.41	1.34	0.85	
<i>n</i> -Dodecane	0.23	9.84	1.87	2.25	0.43
<i>n</i> -Tridecane	0.29	1.47	2.10	1.45	0.73
<i>n</i> -Tetradecane	0.34	0.73	1.20	1.02	
<i>n</i> -Pentadecane	0.46	1.68	2.60	2.57	0.45
<i>n</i> -Hexadecane	0.49	0.63	2.50	2.57	1.21
<i>n</i> -Heptadecane	0.56	0.51		2.34	
<i>n</i> -Octadecane	0.64	5.38	3.21	2.20	1.10
<i>n</i> -Nonadecane	0.69	1.20	1.10	1.15	
<i>n</i> -Eicosane	0.72	3.46	16.08	2.26	3.84
<i>n</i> -Heneicosane	0.79	6.92	1.14	0.98	1.25
<i>n</i> -Docosane	0.84	4.63	5.20	0.91	2.60
<i>n</i> -Tricosane	1	22.62	18.75	27.32	34.56
<i>n</i> -Tetracosane	1.05	0.78	1.70	1.77	7.38
<i>n</i> -Pentacosane	1.10	0.18	4.25		1.14
<i>n</i> -Hexacosane	1.17	1.15	1.86	5.54	11.32
<i>n</i> -Heptacosane	1.21	0.11	1.12		0.69
<i>n</i> -Octacosane	1.25	0.19	3.48	0.96	1.65
<i>n</i> -Nonacosane	1.31	0.47			1.03
Campesterol	1.51	0.18	4.33	1.94	4.31
Stigmasterol	1.56	0.16			2.50

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β-Sitosterol	1.65	1.77	0.77	9.22	7.40
β-Amyrin	1.76	0.18			0.52
No of identified components		24	20	19	19
% identified components		78.19	75.94	68.18	84.11
% identified hydroc	arbons	75.9	70.84	57.02	69.38
% identified sterols &		2.29	5.1	11.16	14.73
triterpenes		2.29	5.1	11.10	14.75

*RR_t= Retention time relative to n-Tricosane (R_t =21.74 min).

S. mah. = Swietenia mahogani; S. macr. = Swietenia macrophylla.

In comparison, earlier studies revealed that: the major fatty acid components of *S. macrophylla* seeds were linoleic (37.50--39.21%), oleic (18.82-22.03%), stearic (16.57-17.65%) and palmitic (14.62-15.47%) acids ⁽³⁷⁾; while the seed oil of *S. mahagoni* was dominated by saturated fatty acids (about 98%), being mainly represented by palmitic, stearic, arachidic and myristic acids together with a very small amount of unsaturated fatty acid fatty acid site (38).

Table (3): Components identified by GC analysis of FAME of leaves and stem barks of
Swietenia mahogani and Swietenia macrophylla

Fatty acids corresponding	RR _t *	Relat	ive percen	tage in FA	ME of
Fatty acids corresponding to identified FAME	of	S. mah.	S. mah.	S. macr.	S. macr.
to identified FAME	FAME	leaves	bark	leaves	bark
Lauric acid (C ₁₂)	0.57	1.45	2.62	6.49	3.70
Myristic acid (C ₁₄)	0.73	1.42	4.20	14.48	8.60
Palmitic acid (C ₁₆)	1	64.10	28.28	34.53	29.37
Margaric acid (C ₁₇)	1.27		7.99	12.61	4.42
Stearic acid (C ₁₈)	1.50	3.11			
Oleic acid $(C_{18:1})$	1.57	2.09	32.96	3.56	26.81
Linoleic acid (C _{18:2})	1.75	18.47	22.93	8.10	9.56
Total % identified compo	onents	90.64	98.98	79.77	82.46
% saturated fatty acids		70.08	43.09	68.11	46.09
% unsaturated fatty ac	cids	20.56	55.89	11.66	36.37

FAME; fatty acid methyl ester; S. mah. = Swietenia mahogani; S. macr.= Swietenia macrophylla

*RR_t= Retention time relative to methyl palmitate ($R_t = 27.46 \text{ min}$)

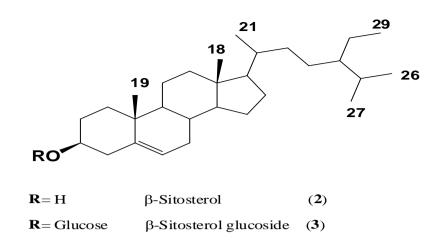
Based on chemical tests and TLC screening (S_1 and S_2), the petroleum ether and chloroform extracts of the stem bark of *S. macrophylla* were selected for isolation of the major lipoids (number of spots and response to visualizing agent). Column chromatographic fractionation of these two extracts allowed the isolation of three known lipoids, Compounds 1-3, which were identified through physicochemical and spectral analysis as well as by comparison with published data.

Compound 1: 20 mg, white plates, soluble in *n*-hexane, m.p. 52-53°C, negative tests for sterols and/or triterpenes. R_f values (0.91, S_1 and 0.96, S_2); color with *p*-anisaldehyde/H₂SO₄ (violet); EI-MS (70 ev, rel. int., m/z): 410 (M⁺), 57 (100%); IR (KBr) cm⁻¹: 2919, 2850 (CH), 1465, 721 (CH₂). By comparison with published data ⁽³⁹⁾, Compound **1** was identified as *n*-nonacosane (C₂₉H₆₀). As far as the available literature is concerned, this is the first report on its isolation from the stem bark of *Swietenia macrophylla* King.

Compound 2: 90 mg, white needle crystals, soluble in *n*-hexane, m.p. 140-141°C, positive tests for sterols and /or triterpenes. R_f values (0.49, S_1 and 0.69, S_3); color in *p*-anisaldehyde / H₂SO₄ (purplish-violet), identical to β -sitosterol. EI-MS (70 ev, rel. int., m/z): 414 (M⁺, 100%), 396 (10.7%), 329 (34.9%), 303 (21.9%), 273 (22.3%), 255 (26%)

From the above findings and by comparison with published data ⁽⁴⁰⁾, as well as m.p., m.m.p. and co-chromatography Compound **2** was identified as β -sitosterol. The isolation of this compound has been previously reported from the heartwood, bark and leaves of *Swietenia mahogani* (L.) Jacq. ^(41, 42), yet this is the first report of its isolation from the bark of *Swietenia macrophylla* King.

Compound 3: 150 mg, white amorphous powder, m.p. 270-273 °C, insoluble in chloroform and methanol, soluble in chloroform-methanol 1:1, positive response to tests for sterols and/or triterpenes, carbohydrates and/or glycosides. R_f values (0.16, S_2 and 0.38, S_4); color in *p*-anisaldehyde (violet), identical to β -sitosterol glucoside. ¹H-NMR (δ ppm, 300 MHz, DMSO *d*₆)): 0.65 (3H, *s*, H-18), 0.95 (3H, *s*, H-19), 0.79 (3H, *d*, J=6Hz, H-26), 0.83(3H, *d*, J=6.3Hz, H-27), 0.90 (3H, *d*, J=5.7Hz, H-21), 1.16 (3H, *t*, J= 6.3, 7.5Hz, H-29), 5.32 (1H, *brs*, H-6), 3.62 (1H, *m*, H-3), 4.21 (1H, *d*, J=7.8Hz, anomeric H-1`). By comparing this data with the published ones ^(40, 43-45), Compound **3** was identified as β -sitosterol-3-O- β -D-glucoside. Identity was confirmed by its m.p., m.m.p. and by co-chromatography alongside an authentic sample. This report is the first on isolation of this phytosterol glucoside from genus *Swietenia*.



Bioactivities of the ethanol extracts

Cytotoxic activity

The IC₅₀ recorded for the different ethanol extracts, on the three human carcinoma cell lines (HEPG2, HCT116 and U251) (Table 4), were deduced from the corresponding survival curves displayed in Figures 1-3, respectively. Obviously, the highest cytotoxic potential was observed for the extracts of the stem bark and leaves of *S. macrophylla* on colon carcinoma cell line (HCT116), respective IC₅₀s 0.43 and 0.67µg/ml. Meanwhile, that of the stem bark of *S. mahogani* showed moderate activity (IC₅₀ = 6.44 and 8.7 µg/ml) on the other two cell lines *viz.*, liver (HEPG2) and brain (U251) carcinoma.

The cytotoxic properties of the alcoholic extract of the seeds of *S. mahagoni* Jacq. have been previously reported; with the dichloromethane and petroleum ether soluble fractions exhibiting the most significant effect $^{(46, 47)}$.

Table(4): IC₅₀ of ethanol extracts of leaves and stem barks of *Swietenia mahogani* and *Swietenia macrophylla* on liver (HEPG2), colon (HCT116) and brain (U251) carcinoma cell lines

Alcohol extracts	HEPG2	HCT116	U251
S. mah. leaves	-	10	9.87
S. mah. bark	6.44	-	8.37
S. macr. leaves	7.85	0.67	5.37
S. macr. bark	4.23	0.43	4.63

S. mah. = *Swietenia mahogani; S. macr.* = *Swietenia macrophylla*.

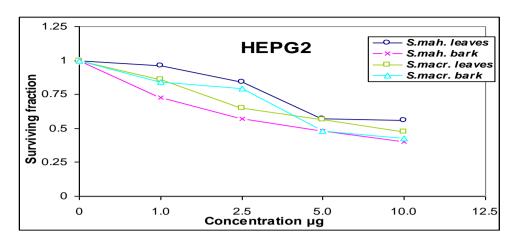


Figure (1): Effect of ethanol extracts of leaves and stem barks of *Swietenia mahogani* and *Swietenia macrophylla* on liver carcinoma cell line (HEPG2)

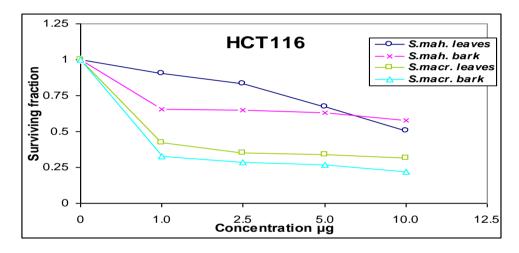


Figure (2): Effect of ethanol extracts of leaves and stem barks of *Swietenia mahogani* and *Swietenia macrophylla* on colon carcinoma cell line (HCT116)

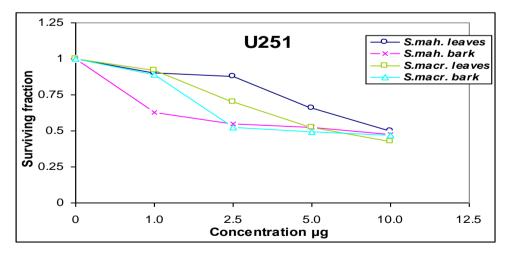


Figure (3): Effect of ethanol extracts of leaves and stem barks of *Swietenia mahogani* and *Swietenia macrophylla* on brain carcinoma cell line (U251)

Acute toxicity studies

Results of acute toxicity studies, as deduced through determination of LD_{50} s, revealed that all the tested ethanol extracts could be considered as safe in the range of the orally administered doses ⁽⁴⁷⁾. LD_{50} s recorded being 6.7 and 6.1 g / kg b.wt. for the leaves and stem bark extracts of *S. mahogani* and 5.8 and 5.4 g / kg b.wt. for those of *S. macrophylla*, respectively. Data reported for the seed extract of *S. mahogani* proved that it was also non-toxic ⁽⁴⁷⁻⁴⁹⁾.

Analgesic, anti-inflammatory, antipyretic and diuretic activities

The **analgesic** effect of the tested ethanol extracts (Table 5) was obvious as indicated by the significant increase in the voltage needed by the treated animal to emit a cry. The most active, in this respect, were those of the leaves with that of *S. mahogani* exhibiting the highest activity as compared to Novalgin.

On the other hand, none of the extracts produced an **anti-inflammatory** effect (Table 6), in comparison to Indomethacin, when evaluated adopting the procedure described by Winter *et* $al^{(23)}$.

Table (5): Analgesic activity of ethanol extracts of leaves and stem barks of *Swietenia mahogani* and *Swietenia macrophylla* (100 mg/kg b.wt.) as compared to Novalgin (50 mg/Kg b. wt)

	Voltage needed	Voltage needed after treatment $(V_t)^1$					
Animal group (n=6)	before treatment (V ₀)	After 1 hour		After 2 h	ours	Potency ²	
	Mean ± S.E.	Mean ± S.E.	% Change	Mean ± S.E.	% Change	i i	
Control (saline)	72.6 ± 2.3	73.8 ± 2.5		71.9 ± 2.6			
S.mah. leaves	78.2 ± 1.9	132.6 ±4.8*	69.56	149.7 ±5.1*	91.43	0.74	
S.mah. bark	76.1 ±2.1	116.2 ±3.7*	52.69	124.8 ±4.2*	63.99	0.52	
S macr. leaves	75.9 ± 2.3	$118.2 \pm 3.9*$	55.73	$136.4 \pm 5.2*$	79.7	0.65	
S. macr. bark	74.8 ± 1.5	112.3 ±4.8*	50.13	$128.9 \pm 5.7*$	72.33	0.59	
Novalgin	75.6 ± 1.4	$152.8 \pm 6.1*$	102.12	$168.9 \pm 4.8*$	123.41	1	

S. mah. = Swietenia mahogani; S. macr. = Swietenia macrophylla.

¹ Minimal voltage needed for the animal to emit a cry expressed in kilo volt as mean±S.E.

² Potency calculated with reference to the standard analgesic drug Novalgin

S.E. = standard error

*Statistically significant from zero time at p<0.01.

% of change is calculated with reference to the effect at zero time.

Animal group	Dose	% O	1	
(n=6)	(mg/kg. b.wt.)	(mg/kg. b.wt.) Mean ±S.E. %		Potency
Control	Saline (1 ml)	59.6±1.8		
S.mah. leaves	100	33.2±1.4	44	0.71
S. mah. bark	100	35.3±1.1	41	0.66
S. macr. leaves	100	36.4±1.5	39	0.63
S. macr. bark	100	38.1±1.3	36	0.58
Indomethacin	20	22.8±0.7*	62	1

Table (6): Acute anti-inflammatory activity of ethanol extracts of leaves and stem barksof Swietenia mahogani and Swietenia macrophylla as compared to Indomethacin

S. mah. = Swietenia mahogani; *S.* macr. = Swietenia macrophylla.

¹Potency calculated with reference to the standard anti-inflammatory drug Indomethacin

*Significantly different from control group at P<0.01.

S.E. = standard error

% of change calculated reference to the control group.

% of change is calculated with reference to the effect at zero time

Meanwhile, a moderate **antipyretic** activity was observed for the ethanol extracts of the leaves of the two species (Table 7) as compared to Paracetamol; the effect being more pronounced after two hours. However, extracts of stem barks exhibited no effect on the yeast-induced hyperthermia in rats; thus, not complying with the traditional use of the decoction of the bark as febrifuge $^{(50, 51)}$.

All tested ethanol extracts exerted a remarkable **diuretic** activity on comparing to Moduretic (Table 8); the highest increase in urine volume being recorded for the extract of *S. mahogani* leaves.

Table (7): Antipyretic activity of ethanol extracts of leaves and stem barks of Swietenia mahogani. and Swietenia macrophylla (100 mg/kg b.wt.) as compared to Paracetamol (20 mg/kg b.wt.).

		Bod	y temperatu	re change		
		1 hr after	treatment	2 hrs after treatment		
Animal group (n=6)	Induced rise in temperature T ₀ (°C)	T _t (°C, Mean±S.E)	% Change ¹	T _t (°C, Mean±S.E.)	% Change ¹	Potency ²
Control (Saline)	38.2±0.4	39.3±0.3		39.4±0.2		
S. mah. leaves	39.4±0.3	38.5±0.2*	2.3	37.8±0.1*	4	0.5
S. mah. bark	38.9±0.2	38.6±0.1	0.8	38.4±0.2	1.3	0.16
S. macr. leaves	39.7±0.2	38.9±0.3*	2	38.1±0.5*	4	0.5
S. macr. bark	39.2±0.3	38.8±0.2	1	38.6±0.1	1.5	0.18
Paracetamol	39.5±0.1	37.3±0.2*	5.6	36.4±0.1*	7.9	1

S. mah. = Swietenia mahogani; S. macr. = Swietenia macrophylla.

¹% Change calculated with reference to control (induced hyperthermia without treatment)

²Potency calculated as compared to the standard antipyretic drug Paracetamol

* Significantly different from zero time at P<0.01. S.E. = standard error

Table (8): Effect of ethanol extracts of leaves and stem barks of Swietenia mahogani and Swietenia macrophylla (100mg/kg b.wt.) on urine volume as compared to Moduretic (5 mg/kg. b.wt.)

Animal group	Urine volume (ml)				
(n=6)	After 2 hrs	After 3 hrs	After 24 hrs		
Control (Saline)	0.7 ± 0.02	1.8 ± 0.03	6.2 ± 0.3		
S. mah. leaves	$2.8\pm0.2^{\ast}$	$5.9 \pm 0.1*$	$13.1\pm0.6^*$		
S. mah. bark	2.1 ± 0.1	$4.0 \pm 0.1*$	$8.3\pm0.5*$		
S. macr. leaves	2.6 ± 0.4	$5.1 \pm 0.2*$	$11.3 \pm 2.1*$		
S. macr. bark	1.9 ± 0.3	$4.3\pm0.6*$	8.6 ± 1.1*		
Moduretic	4.5 ± 0.3	$7.6 \pm 0.5*$	$16.4 \pm 0.8*$		
S. mah. = Swietenia mahogani ; S. macr. = Swietenia macrophylla					

S. mah. = *Swietenia mahogani* ;

*Statistically significant from the control at p<0.1

Antiulcer activity

The ethanol extracts of the leaves and stem bark of the two species under investigation showed a remarkable antiulcer activity (Table 9) with a percentage protection ranging from 65.22-84.78 % against Indomethacin-induced stomach ulceration; the highest effect being observed for that of the leaves of S. mahogani. These results are in agreement with those previously reported for the ethanol extract of the same plant organ when evaluated against

Values are means of 6 observations

absolute ethanol-induced gastric ulcer in rats ⁽⁵²⁾; pretreatment of the animals with 500 mg/kg of the plant extract significantly suppressed the formation ulcers and protect the gastric mucosa.

Antioxidant activity

In the present investigation, the reduced blood glutathione level (due to oxidative stress) in Alloxan-diabetic rats, was greatly restored in animals receiving all of the tested ethanol extracts; the antioxidant potential of these was comparable to that of Vitamin E (7.5 mg/kg, % of change, 1.6) (Table 10). The ethanol extract of the leaves of *Swietenia mahogani* appeared the most active (% of change, 3). In fact, the antioxidant property of *S. mahagoni* seeds oil has been evaluated, earlier, by *in-vitro* models; the oil showed free radical scavenging as well as reducing property⁽⁸⁾.

 Table (9): Antiulcer activity of ethanol extracts of leaves and stem barks of Swietenia

 mahogani and Swietenia macrophylla in rats treated with Indomethacin

Animal group (n=10)	Dose (mg/kg b.wt.)	Number of gastric ulcers (Mean±S.E.)	% Protection
Indomethacin (positive control)	20	9.2 ± 0.8	
Indomethacin + S. mah. leaves	20 + 100	$1.4 \pm 0.3*$	84.78
Indomethacin + <i>S. mah.</i> bark	20 + 100	$2.1 \pm 0.6*$	77.2
Indomethacin + S. <i>macr</i> . leaves	20 + 100	$2.6 \pm 0.5*$	71.74
Indomethacin + S. macr. bark	20 + 100	3.2 ± 0.7	65.22

S. mah. = Swietenia mahogani ; S. macr. = Swietenia macrophylla

* Statistically significant from the control at p<0.1

rol at p<0.1 S.E. = standard error

% of protection calculated with reference to the positive control group

Table (10): Effect of ethanol extracts of the leaves and stem barks of *Swietenia mahogani* and *Swietenia macrophylla* on blood glutathione level of diabetic rats as compared to Vitamin E

Animal group (n=6)	Dose (mg/kg b. wt.)	Blood glutathione (mg % , Mean ± SE)	% Change from control
Control (normal non-diabetic)		36.8±1.2	—
Diabetic control		22.6±0.5*	38.6
Diabetic + S. mah. leaves	100	35.7±1.1	3.0
Diabetic + S. mah. bark	100	35.3±0.9	4.0
Diabetic + S. macr. leaves	100	$34.4{\pm}0.6$	6.5
Diabetic + S. macr. bark	100	34.1±0.8	7.3
Diabetic + Vitamin E	7.5	36.2±0.7	1.6

S. mah. = Swietenia mahogani ; S. macr. = Swietenia macrophylla

* Statistically significant from the control at p<0.1;

• Statistically significant from the control at p<0.05

% of change is calculated with reference to the control group. S.E. = standard error

Hepatoprotective activity

Results obtained upon evaluating the hepatoprotective activity of the ethanol extracts of the two species are recorded in Table (11). Obviously, prolonged administration of the extracts (daily oral dose, 100 mg/kg b.wt.) did not affect the serum level of liver enzymes (AST, ALT and ALP) in normal rats. Meanwhile, the rise in the enzyme levels due to CCl₄-induced liver damage was significantly reduced in animals pre-treated (for one month) with the tested extracts. Administration of these samples for another month led to a further decrease in the enzyme levels.

These data support the findings previously reported concerning the methanol extract of *S. mahagoni* bark ⁽⁵³⁾. The latter was found to exert a dose dependent potential hepatoprotection against Paracetamol-induced hepatic damage, normalizing biochemical parameters and liver histology in Wistar rats plausibly by modulating lipid peroxidation and increasing antioxidant defense systems.

Table (11): Effect of ethanol extracts of leaves and stem barks of *Swietenia mahogani* and *Swietenia macrophylla* (100 mg/kg b.wt.) on the level of serum enzymes in CCl₄-liver damaged rats (n=10), as compared to Silymarin (25 mg/kg b.wt.)

Enzyme	Time	Control	<i>Swietenia mahogani</i> extracts		<i>Swietenia macrophylla</i> extracts		Silymarin
			Leaves	Bark	Leaves	Bark	
AST (µ/L)	Zero	25.3±0.9	30.4±1.2	29.2±1.1	29.8±0.7	28.6±1.3	29.5±1.3
	30 days	26.1±0.8	29.8±0.9	28.3±1.2	28.2±0.6	26.2±1.2	26.8±1.2
	72 hours•	131.6 ± 5.9	62.3±2.1*	73.2±2.6*	52.4±1.3*	61.5±2.9*	51.3±2.7*
	30 days•	144.2 ± 6.1	35.1±1.4*°	38.4±1.5*°	$34.7 \pm 1.8^{*\circ}$	39.8±1.5*°	$24.9 \pm 1.1^{*\circ}$
ALT (µ /L)	Zero	28.6±1.1	29.7±0.9	32.1±0.9	27.6 ± 0.9	25.2±0.7	27.6±1.2
	30 days	26.8±1.2	30.1±1.2	31.6±0.5	27.1±0.8	26.1±0.6	26.3±1.4
	72 hours•	121.3±6.5	76.3±3.8*	81.5±4.2*	58.9±2.4*	68.5±2.1*°	48.5±2.1*
	30 days•	139.2±5.8	41.6±2.4*°	45.9±2.1*°	47.3±1.2*°	39.2±1.3*°	$29.2 \pm 0.9 *^{\circ}$
ALP (µ /L)	Zero	7.2±0.3	7.8±0.2	6.9±0.4	7.6±0.1	7.2±0.1	6.8±0.2
	30 days	7.1±0.2	7.5±0.3	7.1±0.2	7.4±0.2	7.1±0.1	6.6±0.1
	72 hours•	31.8±1.1	16.2±0.9*°	13.2±0.9*	19.2±0.5*	18.6±0.9*	17.9±0.7*
	30 days•	33.6±1.7	12.3±2.1*°	11.9±0.8*°	10.5±0.7*°	11.3±0.8*°	7.1±0.3*°

* Statistically significant from the control at p<0.1

*°Statistically significant from 72 hours at p<0.1

• After liver damage.

CONCLUSION

Analysis of the lipoids of the leaves and stems barks of *Swietenia mahogani* L. Jacq. and *Swietenia macrophylla* King. revealed a quantitative rather than qualitative variability in composition which could, however, be helpful for interspecies differentiation. Column chromatography of the non-polar and semi-polar fractions of the ethanol extract the stem bark of *Swietenia macrophylla* allowed the isolation of three known lipoids which are for the first time reported from the plant. The ethanol extracts of the leaves and stem barks of the two species could be considered as safe based on their $LD_{50}s$. Assessment of the different pharmacological potentialities of the extracts reflected their traditional medicinal usage; thus encouraging the local propagation of these species for both economical and pharmaceutical purposes. However, further incorporation in herbal formulations will necessitate accurate clinical trials.

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