

BIOACTIVE FRACTIONS AND POLYPHENOLS OF THE ETHANOL EXTRACT OF *SWIETENIA MAHOGANI* LEAVES

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ABSTRACT

The ethanol (90%) extract of *Swietenia mahogani* L. Jacq. leaves was fractionated with solvents of increasing polarities. The antioxidant and hepatoprotective activities of all successive extractives were evaluated. The highest efficiency was, in most cases, observed for the ethyl acetate-soluble fraction. Repeated column chromatography of this fraction and sub-fractions derived therefrom, on different stationary phases (Polyamide and Sephadex LH-20), allowed the isolation of six known polyphenols, previously unreported in the genus. Isolated compounds namely: (+) catechin, quercetin-3-O- β -D-glucopyranoside (isoquercitrin), 3-caffeoylquinic acid (neochlorogenic acid), quercetin-3-O- β -D-galactopyranoside (hyperoside), quercetin and kaempferol were identified through physico-chemical and spectral analyses and by comparison with published data. The antioxidant activity of the three

first isolates was also assessed and found significant.

KEYWORDS: *Swietenia mahogani*, leaves, antioxidant, hepatoprotective, polyphenols.

INTRODUCTION

Herbal formulations have reached widespread acceptability as therapeutic agents in treatment of diabetes, arthritis and liver diseases, as well as cough remedies, memory enhancers and adaptogens^[1]. Moreover, the use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies^[2]. However, some can cause health problems and others are either not effective or may interact with other drugs. Quality control

and assurance of herbal formulations is thus essential and necessitates identification of the bioactive constituents to allow standardization of the final product^[3, 4].

Swietenia mahogani L. Jacq. (Mahogany, Meliaceae) is traditionally used for treatment of fever, diabetes, malaria, hypertension and tuberculosis^[5, 6]; moreover, its extract showed ameliorative effects on diabetic mice and antimicrobial properties^[7, 8]. The bioactivities of the plant, particularly the seeds^[9-17], were attributed to the abundance of phenolics and terpenoids especially limonoids^[18]. From the economic standpoint, the plant is a reputed timber tree^[19], being also grown for purpose of shade and ornament^[20-23] in several countries including Egypt.

In a series of previous publications^[24-27], the authors comparatively investigated the aforementioned locally cultivated plant and the related species *Swietenia macrophylla* King. In this respect, taxonomic criteria were established *via* genetic and botanical profiling^[14]. Furthermore, the ethanol (90%) extracts of leaves and stem barks were subjected to acute toxicity studies and evaluation of several bioactivities viz., analgesic, anti-inflammatory, antipyretic, diuretic, antiulcer, antioxidant and hepatoprotective^[27]; in addition, the acaricidal effect was assessed against *Varroa destructor* mite^[25]. Leaf volatiles exhibited remarkable antibacterial activity against Gram-positive microorganisms and aqueous extracts of stem bark exudates significantly reduced blood glucose level in Alloxan-diabetic rats^[26]. Chemical investigations comprised qualitative and quantitative chromatographic analyses of polyphenols revealing the prevalence of catechin in stem barks and of flavonols in leaves^[25]; hydrodistilled leaf volatiles and stem bark exudates^[26]; as well as, lipoids in the two plant organs^[27]. Furthermore, three known lipoidal constituents namely; nonacosane, β -sitosterol and its glucoside were isolated from the stem bark of *S. macrophylla*^[27].

The present study was designed aiming to perform a deeper exploration of the ethanol (90%) extract of the leaves of *S. mahogani* which was found safe and demonstrated relatively higher antioxidant and hepatoprotective effects compared to its analogue derived from *S. macrophylla*^[27]. Consequently, a bioactivity-guided fractionation was carried out and trials performed to isolate the constituents from the most promising fraction(s). Special emphasis was made on phenolic components due their rather high abundance in the extract^[25] and reputed antioxidant and hepatoprotective efficiency^[10, 11, 15-17, 27, 28]. Moreover, the lack of publications on the plant leaves polyphenols compared to those of seeds^[11, 17, 18, 29] stimulated this investigation.

MATERIALS AND METHODS

Plant Material and Extracts

Leaves of *Swietenia mahogani* (L.) Jacq. were obtained from plants cultivated at the Zoological Garden, Giza, Egypt. Identification of the samples was kindly confirmed 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.

Ethanol (90%) extract was prepared from powdered leaf sample (1 kg) by exhaustive percolation at ambient temperature, followed by evaporation of the solvent (at 40°C, under reduced pressure) then lyophilization^[27]. An aliquot (180 g) of the solvent-free dark green semi-solid ethanol extractives was suspended in water (600 ml) and subjected to conventional sequential liquid-liquid fractionation using petroleum ether 60-80 °C (**PE**), chloroform (**CHCl₃**), ethyl acetate (**EtOAc**) and *n*-butanol saturated with water (**BuOH**). Successive dry extractives (21.1, 19.8, 43.1 and 45 g, respectively) obtained upon vacuum evaporation of the solvents^[27] were saved for further investigation. Prior biological evaluation, samples were separately, dissolved in bi-distilled water by the aid of few drops of Tween 80.

Material for Biological Evaluation

Experimental animals: were obtained from the animal house colony at the National Research Centre (Dokki, Giza, Egypt). Adult male albino rats of Sprague Dawley strain (120-150 g) were utilized for *in-vivo* bioactivities assessment. The animals were kept on standard laboratory diet composed of: vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2%), casein (10.5%) and starch (54.3%). Water was supplied *ad libitum*. All animal procedures were carried out according to the agreement of the Ethics Committee of the National Research Centre (Dokki, Giza, Egypt) and in harmony with the recommendations of the Proper Care and Use of Laboratory Animals.

Drugs and laboratory kits: Alloxan (Sigma, USA) was used for induction of diabetes, Silymarin (Sedico Pharmaceutical Co, 6th of October City, Egypt) as hepatoprotective, and 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.

Material for Phytochemical Investigation

Stationary phases for chromatography: Thin layer chromatography (TLC) was carried out on precoated silica gel F254 plates (20X20 cm, Sigma-Aldrich Chemicals-Germany) and sheets of Whatmann No. 1 filter paper (Whatmann, Ltd., Maidstone, Kent, England) were utilized for paper chromatography (PC). Meanwhile, various stationary phases were used for column chromatography (CC) viz., Polyamide (E-Merck Darmstadt, Germany), Sephadex LH 20 (Pharmacia Fine Chemicals AB Uppsala, Sweden), silica gel 60 H (70-230 mesh, Fluka, Sigma-Aldrich chemicals-Germany) and Silica gel RP-18 (70-230 mesh, Fluka, Sigma-Aldrich chemicals-Germany).

Solvents (of analytical grades) and **authentic reference samples** used in this study were all purchased from E-Merck (Darmstadt, Germany).

Solvent systems and spray reagents: S₁, Chloroform-Methanol-Formic acid (90:10:0.2 v/v), S₂, Chloroform-Methanol-Formic acid (80:20:0.2 v/v), S₃, Ethyl acetate-Methanol-Water-Formic acid (100:16.5:13.5:0.2 v/v) and S₄, *n*-Butanol-Acetic acid-Water (4:1:5 v/v) were utilised for developing the chromatograms. Spray reagents used for visualization of phenolics were: Aluminum chloride^[30], Ferric chloride^[31] and Natural Products-Polyethylene Glycol^[32] (NP/PEG); and Aniline phthalate^[32] for sugar moieties.

Shift reagents and chemicals for UV spectroscopic analysis: Sodium methoxide solution, Aluminium chloride solution, Hydrochloric acid, Sodium acetate and Boric acid were used according to published procedures^[33] for UV spectroscopic analysis of flavonoids.

Equipment and Apparatus for Phytochemical Investigation

Chromatographic jars and glass columns of different dimensions were used for TLC, PC and CC, respectively. Melting points were uncorrected and determined on an Electrothermal 9100 (U.K.) apparatus. UV lamp (λ_{max} = 254 and 330 nm, Shimadzu), a product of Hanovia lamps, was used for spot visualization on the chromatograms and a UV/Visible spectrophotometer, Shimadzu UV- 1650 PC for recording UV spectra. Varian Mercury NMR-spectrometers (Japan) were used: 300 MHz for recording ¹H-NMR and 75 MHz for ¹³C-NMR; spectra were recorded in DMSO using TMS as internal standard and chemical shift values expressed in δ ppm. Mass spectra were recorded by means of a Varian Mat 711, Finnigan SS Q 7000 mass spectrometer.

***In-vivo* Biological Evaluation**

The aqueous solutions of successive fractions of the ethanol (90%) extract of the leaves were subjected to *in-vivo* evaluation of their antioxidant and hepatoprotective potential. Results obtained were recorded in tables (1-4). Statistical analysis was carried out by using Student's t-test where, means of the treated groups were compared to that of the control group for each variable^[34].

Assessment of antioxidant potential: The antioxidant activity was evaluated, according to Beutler *et al.*^[35], by measuring the glutathione level (reduced due to oxidative stress produced by diabetes) in blood samples of diabetic animals; the restoration of blood glutathione levels was taken as a measure of antioxidant activity. Diabetes was induced to rats by intraperitoneal (i.p.) injection of Alloxan (150 mg/kg b.wt.) as described by Eliasson and Samet^[36]. The tested fractions (50 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 percentage change observed after dose administration was calculated as follows: % of change = $(G - G_0) \times 100 / G_0$; where, G_0 and G represent the glutathione level in diabetic animals, prior and after administration of the samples, respectively. Results including the potencies of the tested samples relative to Vitamin E were recorded in table (1).

Assessment of hepatoprotective potential: This was evaluated based on measuring the level of liver enzymes AST, ALT and ALP^[37, 38] by means of appropriate biochemical kits in blood samples of CCl₄-liver injured rats. The experimental animals were divided in 7 groups (n=10, each). The first group was kept untreated (negative control) receiving only saline (0.5 ml, p.o., daily) in addition to liquid paraffin (i.p., twice per week). Liver damage was induced in rats of groups 2-7 by means of carbon tetrachloride (CCl₄, i.p., 5 ml/kg b.wt. of 25 % solution in liquid paraffin)^[23]. Animals of Group 2 received only saline (positive control) and those of Group 3 were given Silymarin (standard hepatoprotective, 25 mg/kg b.wt.). Meanwhile, the remaining four animal groups (3-7) were treated with the successive fractions of the ethanol extract (100 mg/kg b.wt., each). All samples and standard were daily administered (p.o.) for a month before and a month after liver damage. Serum levels of biochemical parameters (AST, ALT and ALP) were determined in blood samples^[37, 38] withdrawn from the retro-orbital venous plexus through the eye canthus of anaesthetized rats. Measurements were performed a

month after intake of the tested samples, prior induction of liver injury; then after 72 hours and a month following liver injury and concomitant treatment with the samples. Results are displayed in tables (2-4).

Isolation and Identification of the Constituents of the Ethyl Acetate Extract

The ethyl acetate extract (20 g) dissolved in methanol was applied on a polyamide column (250 g, 70 X 5 cm). Elution was started with water, followed by gradual 10% increments of methanol until 100% methanol was reached. Fractions (150 ml, each) were pooled on the basis of TLC patterns (S_2 , spot visualization in visible and UV_{365nm} lights before and after treatment with NH_3 or $AlCl_3$). Three collective fractions **I-III** were obtained and concentrated under vacuum; the resulting residues were weighed and refractionated (CC) for isolation of individual components.

Fraction I: (4.5 g; TLC: 2 major spots, R_f values 0.76 and 0.46, S_2) was subjected to repeated CC on a sephadex LH-20 column (30 X 3 cm; isocratic elution: mixture of 50% methanol - H_2O) followed by a silica gel 60 H column (10 X 2 cm; gradient elution: starting with 95% $CHCl_3$ / MeOH followed by 2% increments of methanol). Two **compounds 1** and **2** were isolated upon concentration of the 95% $CHCl_3$ / MeOH and 93% $CHCl_3$ / MeOH, respectively.

Fraction II: (3.5 g; TLC: 2 major spots, R_f values 0.20 and 0.49, S_2). Upon re-chromatography on a sephadex LH-20 column (30 X 3 cm; elution: isocratic 50 % methanol - H_2O) afforded two main sub-fractions; the first revealed on TLC a major spot (R_f 0.20, S_2) and yielded on concentration **compound 3**; while, the second upon purification on a silica gel 100 C₁₈-RP column (10 X 1.5 cm) afforded **compound 4** (R_f 0.49 in S_2).

Fraction III: (0.5 g; TLC: 2 major spots, R_f values 0.79 and 0.86, in S_2 and 0.40 and 0.49 in S_1). CC of this fraction on 2 successive sephadex LH-20 columns (20 X 2 cm) using methanol-water (50 %) as eluent for the first and 100 % methanol for the second; this processing resulted in isolation of two **compounds 5** (R_f = 0.40, S_2) and **6** (R_f = 0.49, S_1).

Identification of the isolated compounds was performed through co-chromatography, as well as physicochemical and spectral analysis. Structure elucidation was based on interpretation of the spectral data including those of the UV (with or without addition of shift reagents), NMR (1H NMR, ^{13}C NMR and 2D NMR) and MS spectra.

RESULTS AND DISCUSSION

Antioxidant activity

The crude seeds oil of *S. mahogani* was reported to exert antioxidant and amylase inhibitory activities in *in-vitro* models; in addition, its free-radical chain reaction inhibitory effect and reducing properties were attributed to its phenolic content^[30]. Among phenolics those of reputed antioxidant potential are phenolic acids, flavonoids and tannins^[11]. Furthermore, when tested alongside its analogue derived from the stem bark and those of stem bark and leaves of the allied species *S. macrophylla*, the ethanol (90%) of *S. mahogani* was found to exert the highest antioxidant activity^[27]. These findings prompted the performance of the current bioactivity-guided fractionation of this extract. In the present study, the different fractions (PE, CHCl₃, EtOAc and BuOH) of the ethanol (90%) of the leaves of *S. mahogani* revealed variable although significant antioxidant efficacy and potency as compared to the reference drug vitamin E; the restoration of blood glutathione level (reduced due to induction of diabetes) was taken as a measure of antioxidant activity. Yet, the highest activity was observed for the ethyl acetate followed by butanol fraction (potencies, 0.92 and 0.67 relative to Vitamin E), while the chloroform fraction was the least active (table 1).

Table (1): Effect of the fractions of the ethanol extract of *Swietenia mahogani* leaves on blood glutathione level of Alloxan-diabetic rats as compared to vitamin E

Animal group (n=6)	Dose (mg/kg b. wt.)	Blood glutathione (mg%, Mean + S.E.)	Relative Potency
Normal	-----	36.8±1.2	—
Diabetic	-----	22.6±0.5*	—
Diabetic + Vitamin E	7.5	36.2±0.7	1.00
Diabetic + PE	50	29.6±0.7*	0.51
Diabetic + CHCl ₃	50	26.1±0.8*	0.26
Diabetic + EtOAc	50	35.1±1.1	0.92
Diabetic + BuOH	50	31.7±0.9 [•]	0.67

*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

In a previous study^[28], the defatted methanol extract of *S. mahogani* bark was evaluated for its protective effect on Paracetamol-induced liver damage in Wistar rats. A remarkable hepatoprotective effect was observed by normalizing the measured biochemical parameters viz. serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total serum protein, total bilirubin

content and was supported by histopathological examination of the isolated fresh liver tissues [28]. In this current investigation, a prolonged administration of all the tested fractions significantly decreased the level of liver enzymes (AST, ALT and ALP) in CCl₄-intoxicated rats (tables 2-4), thus suggesting their use as hepatoprotective.

Table (2): Effect of the fractions of the ethanol extract of *Swietenia mahogani* leaves on the level of serum AST in liver damaged rats (n=10) as compared to Silymarin

Group	Dose	Time of sample collection			
	mg/kg b. wt.	30 days	72 hours•	30 days•	Relative Potency
Normal	--	25.3±0.9	28.6±0.9	32.1±1.2	--
CCl ₄	--	26.1±0.8	131.6±5.9*	144.2±6.1*	--
Silymarin	25	26.8±1.2	51.3±2.7*	24.9±1.1*°	1.00
PE	100	28.4±0.8	98.6±4.2*	82.4±3.6*	0.52
CHCl ₃	100	28.1±0.9	85.3±3.9*	79.2±3.1*	0.54
EtOAc	100	31.6±1.4	58.4±2.3*	41.3±2.6*	0.86
BuOH	100	29.3±0.9	76.2±2.7*	54.8±2.1*	0.75

• After liver damage. * Statistically significant from the control at p<0.1

° Statistically significant from 72 hours at p<0.1

Table (3): Effect of the fractions of the ethanol extract of *Swietenia mahogani* leaves on the level of serum ALT in liver damaged rats (n=10) as compared to Silymarin

Group	Dose	Time of sample collection			
	mg/kg b. wt.	30 days	72 hours•	30 days•	Relative Potency
Normal	--	30.2±1.3	31.6±1.5	27.6±1.2	--
CCl ₄	--	26.8±1.2	121.3±6.5*	139.2±5.8*	--
Silymarin	25	26.3±1.4	48.5±2.1*	29.2±0.9*°	1.00
PE	100	28.9±0.8	91.7±3.6*	86.4±4.1*	0.48
CHCl ₃	100	31.2±0.7	104.3±4.8*	94.2±4.2*	0.41
EtOAc	100	30.4±0.7	53.9±1.8*	44.8±1.5*	0.86
BuOH	100	31.4±1.1	79.8±2.3*	61.2±2.4*	0.71

• After liver damage. * Statistically significant from the control at p<0.1

° Statistically significant from 72 hours at p<0.1

The ethyl acetate was obviously the most active fraction as depicted by its high efficiency in normalizing the elevated levels of all tested enzymes (AST, ALT and ALP; potencies relative to Silymarin: 0.86, 0.86 and 0.81, respectively), and was followed by the butanol fraction (0.75, 0.71 and 0.67, respectively). These findings correlate with those of the evaluated antioxidant activity; which is recognized as one of the main routes to achieve hepatoprotection.^[39]

Table (4): Effect of the fractions of the ethanol extract of *Swietenia mahogani* leaves on the level of serum ALP in liver damaged rats (n=10) as compared to Silymarin

Group	Dose	Time of sample collection			
	mg/kg b. wt.	30 days	72 hours•	30 days•	Relative Potency
Normal	--	7.2±0.3	7.1±0.2	6.8±0.2	--
CCl ₄	--	7.1±0.2	31.8±1.1*	33.6±1.7*	--
Silymarin	25	6.6±0.1	7.9±0.7*	7.1±0.3*°	1.00
PE	100	7.2±0.2	22.6±0.9*	20.1±1.2*	0.51
CHCl ₃	100	6.9±0.2	24.7±1.3*	23.4±1.1*	0.38
EtOAc	100	7.5±0.1	14.6±0.9*	12.1±0.6*	0.81
BuOH	100	7.8±0.2	19.4±1.1*	15.8±1.2*	0.67

• After liver damage. * Statistically significant from the control at p<0.1

° Statistically significant from 72 hours at p<0.1

Phytochemical Investigation of the Ethyl Acetate Extract

TLC screening of the bioactive ethyl acetate extract (S₁-S₃) revealed the presence of a series of polyphenols (at least 11 in S₂, Chloroform-Methanol-Formic acid 80: 20: 0.2 v/v). Attempts to isolate the components responsible of the extract activity were performed through normal and/ reversed column chromatography. Fractionation of the extract afforded six known phenolics (**Compounds 1-6**), which as far as the available literature is concerned are for the first time reported from the plant. Structure elucidation was based on physicochemical and spectral analysis as well as by comparison with published data. Structures of isolated compounds are represented in **Figure (1)**.

Characterization of the isolated compounds

Compound 1: White powder, 150 mg, m.p. 175-177 °C, R_f: 0.76 in S₂; UV λ_{max} nm: MeOH, 276, 288; NaOMe, 300, 310 sh; AlCl₃, 280, 300 sh; AlCl₃ / HCl, 275, 288 sh; NaOAc, 276, 310 sh; NaOAc / H₃BO₃, 282, 310 sh; ¹H NMR δ ppm (300 MHz, DMSO), 8.92 (br s, free OH), 6.76 (1H, d, J=1.5Hz, H-2'), 6.73 (1H, dd, J'=1.5, 8.1 Hz, H-6'), 6.63 (1H, d J=8.1 Hz, H-5), 5.93 (1H, br s, H-8), 5.75 (1H, br s, H-6), 4.54 (1H, d, J=7.2 Hz, H-2), 3.78 (1H, m, H-3), 3.66 (1H, br s, 3-OH), 2.70 (1H, dd, J=4.5, 15.9_(gem) Hz, H-4 ax), 2.40 (1H, dd, J=7.5, 15.9_(gem) Hz, H-4eq); ¹³C NMR δ ppm (75 MHz, DMSO), 156.65 (C-7), 156.38 (C-5), 155.57 (C-9), 145.03 (C-3'), 144.69 (C-4'), 130.89 (C-1'), 118.73 (C-6'), 115.39 (C-5'), 114.7 (C-2'), 99.35 (C-10), 95.42 (C-6), 94.19 (C-8), 81.17 (C-2), 66.57 (C-3), 27.93 (C-4), CI-MS (m/z, %), 291 [M⁺] (10), 273 (5), 181 (7), 153 (100), 138 (11), 127 (42), 111 (7). Based on the obtained spectral data^[29, 34-35], co-chromatography, m.p. and m.m.p. and by comparison with published data^[40] **compound 1** was identified as **Catechin**.

Compound 2: Yellow powder, 190 mg, m.p. 240-242°C, R_f : 0.46 in S_2 ; UV λ_{\max} nm: MeOH, 257, 362; NaOMe, 272, 327sh, 409; $AlCl_3$, 275, 305sh, 438; $AlCl_3$ / HCl, 270, 350sh, 395; NaOAc, 273, 324sh, 380; NaOAc / H_3BO_3 , 262, 298sh, 377; 1H NMR δ ppm (300 MHz, DMSO), *Aglycone: 7.57 (1H, d, $J=8$ Hz, H-6'), 7.53 (1H, d, $J=1.8$ Hz, H-2'), 6.84 (1H, d, $J=9$ Hz, H-5'), 6.4 (1H, d, $J=1.8$ H-8), 6.2 (1H, d, $J=1.5$ H-6). *Sugar: 5.4 (1H, d, $J=6.8$ Hz, H-1''); ^{13}C NMR δ ppm (75 MHz, DMSO), 177.43 (C-4), 164.39 (C-7), 161.26 (C-5), 156.38 (C-9), 156.18 (C-2), 148.52 (C-4'), 144.85 (C-3'), 133.37 (C-3), 121.63 (C-1'), 121.2 (C-6'), 116.25 (C-5'), 115.26 (C-2'), 103.94 (C-10), 100.98 (C-1''), 98.77 (C-6), 93.58 (C-8), 77.59 (C-5''), 76.57 (C-3''), 74.16 (C-2''), 70 (C-4''), 61.04 (C-6''). Based on results of physicochemical and spectral analysis, and reported data [30-33, 41-43] compound **2** was identified as: **Quercetin-3-O- β -D-glucopyranoside (Isoquercitrin)**.

Compound 3: White powder, 105 mg, chars on melting, R_f : 0.65 in S_3 ; UV λ_{\max} nm: MeOH, 303 sh, 327; 1H NMR δ ppm (300 MHz, DMSO), Quinic acid: 1.78 (2H, m, H-2 ax, eq), 1.59 (1H, d, $J=15$ Hz, H-6 ax), 1.94 (1H, dd, $J=13$ Hz, 9 Hz, H-6 eq), 5.14 (1H, m, H-3), 3.83 (1H, s, H-4), 3.95 (1H, s, H-5). Caffeic acid: 7.40 (1H, d, $J=15.9$ Hz, H-7'), 7.04 (1H, br s., H-2'), 6.94 (1H, dd, $J=8.1$ Hz, 2 Hz, H-6'), 6.73 (1H, d, $J=6.6$ Hz, H-5'), 6.18 (1H, d, $J=15.6$ Hz, H-8'). From the above data and by comparison with those published [44] compound **3** was identified as: **3-Caffeoylquinic acid (Neochlorogenic acid)**.

Compound 4: Yellow powder, 15 mg, m.p. 215-217 °C, R_f : 0.46 in S_2 ; UV λ_{\max} nm: MeOH, 258, 268 sh, 298 sh, 362; NaOMe, 272, 328 sh, 410; $AlCl_3$ 274, 305 sh, 335 sh, 435; $AlCl_3$ / HCl, 269, 300 sh, 360, 403; NaOAc, 273, 324 sh, 390; NaOAc / H_3BO_3 , 260, 295 sh, 376; 1H NMR δ ppm (300 MHz, DMSO), Aglycone: 7.65 (1H, d, $J=8.4$ Hz, H-6'), 7.51 (1H, d, $J=2.1$ Hz, H-2'), 6.84 (1H, d, $J=8.4$ Hz, H-5'), 6.39 (1H, d, $J=1.8$ H-8), 6.19 (1H, d, $J=2.4$ H-6). Sugar: 5.27 (1H, d, $J=5.1$ Hz, H-1'). The UV spectral data of this compound were in accordance with those of 3-OH substituted quercetin derivatives. [30-33] Acid hydrolysis and chromatographic examination of the hydrolysate of **4** showed that the R_f value of the aglycone coincides with that of quercetin (TLC); in addition, the sugar moiety was identified by co-chromatography (PC) as galactose. From the above findings and through comparison with reported data [33] compound **4** was tentatively identified as **Quercetin-3-O- β -D-galactopyranoside (Hyperoside)** ($C_{21}H_{20}O_{12}$).

Compound 5: Yellow crystals, 20 mg, m.p. 315-317°C, R_f : 0.40 in S_1 and 0.79 in S_2 ; UV λ_{max} nm: MeOH, 256, 301 sh, 368; NaOMe, 247, 330 sh, 406; $AlCl_3$, 269, 457; $AlCl_3 / HCl$, 266, 303 sh, 352, 430; NaOAc, 272, 329 sh, 390; NaOAc / H_3BO_3 , 260, 384; 1H NMR δ ppm (300 MHz, DMSO), 7.67 (1H, d, $J=1.5$ Hz, H-2'), 7.56 (1H, d, $J=8.1$ Hz, H-6') 6.88 (1H, d, $J=8.1$ Hz, H-5'), 6.40 (1H, d, $J=1.5$ Hz, H-8), 6.18 (1H, d, $J=1.5$ Hz, H-6). From the previous UV spectral data and by comparison with published data^[30-33, 41], compound **5** was identified as **Quercetin** ($C_{15}H_{10}O_7$).

Compound 6: Yellow powder, 10 mg, m.p. 276-278 °C. R_f : 0.49 in S_1 and 0.86 in S_2 ; UV λ_{max} nm: MeOH, 265, 292sh, 329sh, 366; NaOMe, 280, 322sh, 418; $AlCl_3$, 269, 304sh, 347, 425; $AlCl_3 / HCl$, 269, 303sh, 350, 424; NaOAc, 274, 303, 390; NaOAc / H_3BO_3 , 267, 295sh, 368; 1H NMR δ ppm (300 MHz, DMSO), 8.03 (2H, d, $J=7.5$ Hz, H-2', 6'), 6.92 (2H, d, $J=7.8$ Hz, H-3', 5'), 6.41 (1H, d, $J=1.8$ Hz, H-8), 6.17 (1H, d, $J=1.8$ Hz, H-6). From the previous spectral data and by comparison with published data^[30-33, 41], compound **6** was established as the 3, 5, 7, 3', tetrahydroxy flavone, **Kaempferol** ($C_{15}H_{10}O_6$). This was confirmed through direct comparison with an authentic sample (m.p., m.m.p. and co-TLC).

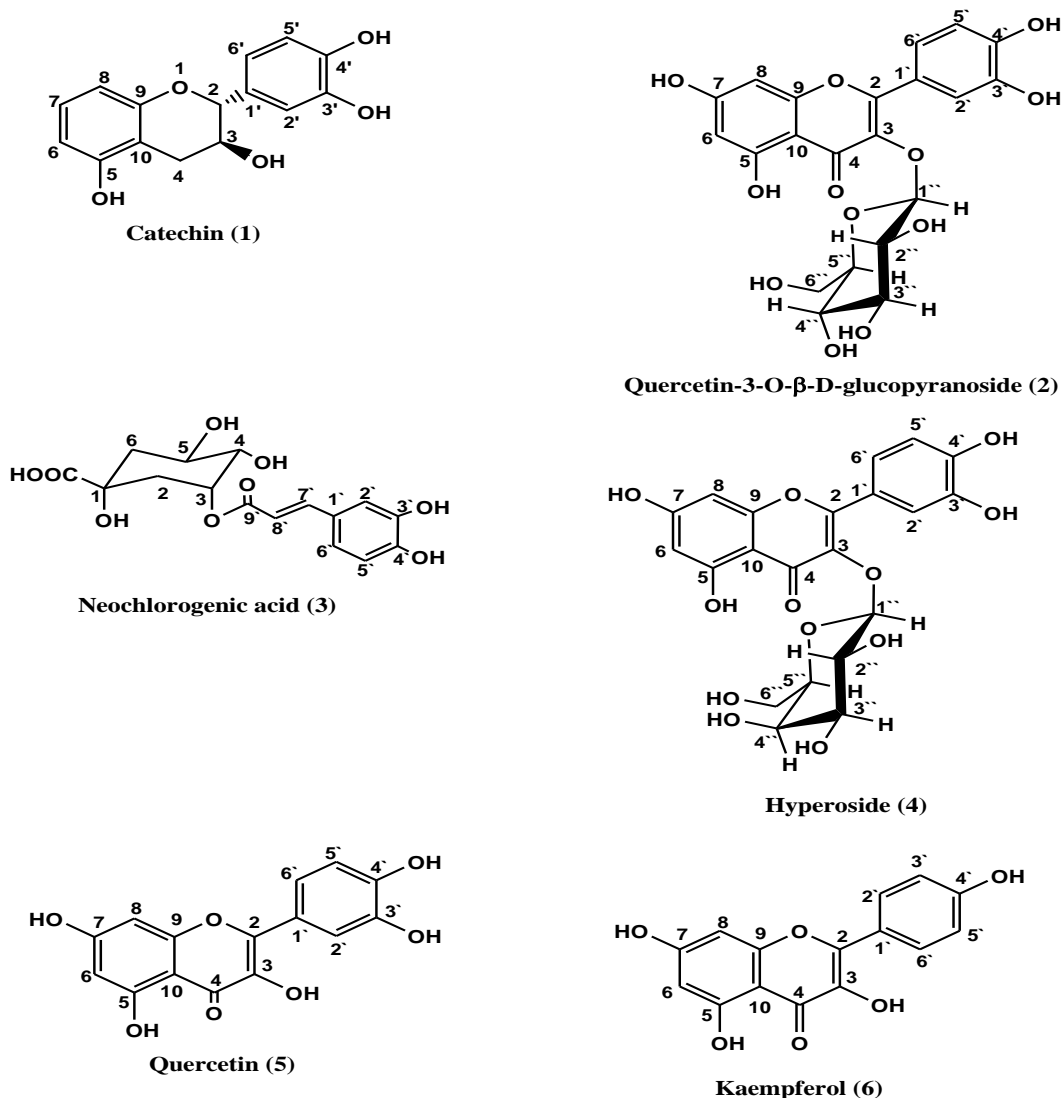


Figure 1: Compounds isolated from the leaves of *S. mahogani*

Antioxidant activity of the isolated major compounds

Finally, the three major isolates *viz.*, catechin, isoquercitrin and neochlorogenic acid (yields 150, 190 and 105 mg) revealed a significant antioxidant activity almost equivalent to that of the standard drug. The restoration of blood glutathione levels (reduced due to induction of diabetes) calculated as potencies relative to vitamin E were 0.89, 0.92 and 0.92, respectively.

CONCLUSION

In conclusion, the more polar fractions of the ethanol extract of the leaves of *Swietenia mahogani* L. Jacq. exerted remarkable antioxidant and hepatoprotective effects through biochemical evaluation. The highest potency in all cases was exhibited by the ethyl acetate fraction. Repeated column chromatography of this fraction afforded six polyphenols, which were not previously reported, including: a flavanol, a phenolic acid, 2 flavonol glycosides and

2 flavonol aglycones. The antioxidant activity of the compounds isolated in sufficient amounts was also evaluated and found significant. The hepatoprotective effect of the plant extract and isolates could be correlated to its antioxidant activity especially due to its phenolic components. Assessment of the different pharmacological potentialities of the fractionated extracts probably reflect the traditional medicinal usage of the plant; thus encouraging the local propagation of this species for both economical and pharmaceutical purposes and suggests its incorporation in herbal formulations after necessary clinical trials.

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