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LEAF VOLATILES AND STEM BARK EXUDATES OF TWO *SWIETENIA* SPECIES: COMPOSITION AND BIOACTIVITY

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ABSTRACT

This study comprises a comparative physico-chemical investigation of the hydrodistilled leaf volatiles and stem bark exudates of two *Swietenia* species grown in Egypt *viz*., *Swietenia mahogani* (L.) Jacq. and *Swietenia macrophylla* King. The physical characters were described, and chemical composition determined *via* chromatographic analyses (PC, GLC and GC/MS). Moreover, the antimicrobial potential of all samples was assessed and the long-term antihyperglycemic activity of gum exudates evaluated. Hydrodistilled leaf volatiles (0.15 vs. 0.10% v/dry wt. in *S. mahogani* and *S. macrophylla*, respectively) were dominated by sesquiterpenoids among which hydrocarbons prevailed (75.51 vs. 80.95%), as evidenced by GC/MS analysis. *Trans*-caryophyllene (33.89%) dominated the *S. mahogani* sample and *α*-humulene (39.64%) that of *S. macrophylla*. Oxygenated constituents were minor in both, being mainly represented by sesquiterpenoids, with elemol (6.13%) as major in *S. mahogani* and E-nerolidol (10.18%) in *S. macrophylla*. Analytical parameters (moisture content and total ash) of the exudates and mineral composition of ashes were determined. GLC analysis of the sylilated exudate hydrolysates revealed that galactose dominated the sugar composition of the samples (57.99 vs. 59.57%) followed by xylose (8.24 vs. 8.37%). In addition, traces of glucuronic acid were detected in both samples. The volatiles were found effective against all tested Gram-positive bacteria meanwhile stem bark exudates inhibited mycobacterial growth only, and yeast was not affected by any of the samples. Minimum inhibitory concentrations were determined. A significant reduction in blood glucose level was recorded in Alloxan-diabetic rats treated with the aqueous solutions of the stem bark exudates of both species.

Key words: *Swietenia* species, leaf volatiles, stem bark exudates, antimicrobial, antihyperglycemic.

INTRODUCTION

Swietenia species (Meliaceae) constitute a small genus of tropical American forest trees. *Swietenia mahogani* (L.) Jacq. provides the original "American mahogany" wood; supplies have, however, become very rare due to over-harvesting and the majority of the trade is currently from the faster growing *Swietenia macrophylla* King [1, 2]. Both species are, as well, grown for shade and ornament [1-4] and were, in this respect, naturalized in Egypt. The genetic and botanical profiling of the two Egyptian plants indicated a relatively high degree of taxonomical similarity, despite providing distinct criteria for discrimination [5]. Limonoids isolated from various meliaceous are reputed as potent insect antifeedants and growth regulators [6]. The low toxicity of these natural products to non-targeted organisms has prompted extensive trials for their isolation [6]. Secondary metabolites of certain American and Asian *Swietenia* spp. have been extensively investigated as a source of useful non-wood forest products, especially the antifeedant tetranortriterpenoids [6] and the seed oil [7]. *Swietenia* seeds are traditionally used as antihypertensive, antidiabetic and antimalarial; the stem bark decoctions are, in addition, taken as potent febrifuge and antidiarrheal, and applied as wound astringent [1-2, 4]. Furthermore, the acaricidal activity of the ethanol extracts of the leaves and stem bark of the locally cultivated *Swietenia mahogani* and *Swietenia macrophylla* were tested against *Varroa destructor* mite, a parasite with marked economic impact on the beekeeping industry [8]. A pronounced miticidal activity was noticed without almost affecting the bees; thus suggesting the use of either the plants extracts or products derived there from as valuable ecofriendly biodegradable agents for controlling *Varroa* mite [8].

The scarcity of reports concerned with the leaf volatiles and stem bark exudates of the selected species stimulated the performance of this study. The present comparative investigation aimed to throw light on the composition of the leaf volatiles and stem bark exudates of the locally cultivated *Swietenia mahogani* and *Swietenia macrophylla* in view to provide possible chemotaxonomical criteria for interspecies differentiation. In addition, the antimicrobial potential of these plant products and anti-hyperglycemic activity of the exudates was evaluated intending further implementation in national drug industry.

MATERIALS AND METHODS

Plant material

Leaves and stem barks of *Swietenia mahogani* (L.) Jacq., and *Swietenia macrophylla* King. 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.

Leaf volatiles

Fresh leaves (1kg) were gathered in January (winter) from of each of the plants under investigation. Each sample was individually subjected to hydrodistillation in a Clavenger's apparatus [9]. The isolated volatiles were dried over anhydrous sodium sulfate and samples saved in a refrigerator for further analysis.

Stem bark exudates

The dried exudates were collected after a lapse of three weeks from incisions made in the barks of the plants, at the trunk level and saved for chemical and biological investigation.

Microorganisms and experimental animals

A set of bacterial and fungal strains (available in stock cultures at the Microbiology Department, Faculty of Pharmacy, Cairo University) was used for evaluation of the antimicrobial activity. This comprises *Staphylococcus aureus* (ATCC 4175), *Sarcina lutea* (Laboratory collection strains) and *Bacillus subtilis* (NCTC 6633) as representative Gram-positive bacteria; *Escherichia coli* (ATCC 10536), *Proteus vulgaris* (NCTC 4175) and *Pseudomonas aeruginosa* (CNCM A21) as Gram-negative ones; and *Mycobacterium phlei* (Laboratory collection strains) as a type of acid fast bacilli. The yeast *Candida albicans* (ATCC 60193) was the tested fungal strain.

Adult male albino rats of Sprague Dawley strain (120-150 g, obtained from the animal house colony at the National Research in Egypt) were utilized for assessment of the long-term anti-hyperglycemic activity. 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 the animal procedures were carried out according to the agreement of the Ethics Committee of The National Research Centre, Egypt and in harmony with the recommendations of the proper Care and Use of Laboratory Animals.

Reference samples, solvent systems and chemicals

Authentic reference sugars used for PC and GLC analysis of the hydrolysates of the stem bark exudates were purchased from E-Merck, (Darmstadt, Germany). Solvent systems used for PC were: *n*-butanol-pyridine-water 6:4:3 v/v (S_1) and *n*-butanol-acetic acid-water 4:1:5 v/v (S_2). All chemicals utilized in this study were of analytical grade.

Drugs and kits

Ofloxacin and Amphotericin B (Bristol-Myers Squibb, Switzerland) were utilized as standard antibacterial and antifungal, respectively. Alloxan (Sigma, USA) solution (10 mg/0.1 ml) was used by intraperitoneal route for induction of diabetes; Metformin (Cidophage®, Chemical Industries Development Co., CID Co., Giza, Egypt) was used as standard antidiabetic and Bio-Merieux kits were employed for measuring blood glucose levels (Bio-Merieux Co, France).

Characterization and GC/MS analysis of the hydrodistilled volatiles

The percentage yield of the hydrodistilled volatiles was calculated on dry weight basis, and organoleptic characters described. The samples were then subjected to chromatographic analysis on a GC/MS system (Hewlett Packard G1800A GCD coupled to an HP automatic injector 7673A) operated in an electron impact mode. Separation was achieved on an HP-5-MS capillary column (30 m × 0.25 mm∅, 0.25µm film thickness) by adopting the following conditions: injector temperature, 220^{o}C; electron ionization detector temperature, 280^{o}C; carrier gas, He (1 ml/min); oven temperature program: initial temperature 40^{o}C, increased to 160^{o}C at a rate of 4^{o}C/min, isotherm for 3 min, increased to 280^{o}C at a rate of 10^{o}C/min and kept isotherm for 4 min i.e. ramp function programming. Mass spectra were taken at 70eV. Mass range was from m/z 40-500. Library search was carried out using a Willey 275 L GC-MS data base. A series of authentic *n*-alkanes (C8-C22, Poly Science Inc., Niles, USA) was subjected to GLC analysis under the same experimental conditions and the retention indices (Kovat's indices, KI) of the oil constituents computed by logarithmic interpolation between bracketing alkanes. Identification of individual components was confirmed by comparison of their retention indices and MS fragments patterns with published data [10, 11]. Relative

percentage amounts were calculated from the Total Ion Chromatograms by a computerized integrator.

Characterization of the stem bark exudates

Organoleptic characteristics (condition, color and odor) of the exudates were examined. The solubility in different solvents as well as distilled water was determined at 25°C, and optical activity (of 1% solution in distilled water) was measured, at 25°C in a 1 dm tube using a Polyscience Div. Preaton Ind. Inc polarimeter. The two samples were then subjected to chemical tests for carbohydrates, proteins, tannins and oxidase enzymes [9, 12-14]. Analytical parameters including moisture and total ash contents were determined in triplicates [15, 16].The moisture content (expressed as %) was determined after heating 1gm samples of air-dried exudates in an air-oven at 120 °C for 2 hours followed by keeping in a desiccator till constant weight. The total ash content (calculated as %) was determined by gradual heating the oven-dried samples (1 gm, each), in an ignition crucible, up to 1100°C then temperature maintained isothermal at 1100°C for at least 1 hour.

Determination of the mineral composition of the stem bark exudates

The mineral composition of the acid-soluble and acid-insoluble ashes of each of the two exudates was determined. The cationic components of the acid-soluble ash were estimated in the filtrates obtained upon boiling weighed amounts of the total ash samples for 3 to 5 min in 1:1 HNO_3; the analysis was performed by atomic absorption spectrometry (at 800 °C) in a Perkin Elmer 2380 atomic absorption spectrometer, equipped with an acetylene-air flame. The mineral content of the acid-insoluble ash was determined gravimetrically (as µg silicon/g ash) after incineration of the nitric-acid insoluble residue in an ignition crucible at 1100°C, as processed for total ash determination.

Analysis of the sugar composition of the stem bark exudates

The monosaccharide composition of the acid-hydrolysates of the exudates was analysed by both paper and gas-liquid chromatography (PC and GLC).

Preparation of the samples: For PC; aliquots (0.5 gm) of the exudates were hydrolysed by heating with 2N H_2SO_4 (boiling water bath for 24 hours), hydrolysates filtered purified by treatment with $BaCO_3$ and monosacharides extracted from the dried filtrates with hot pyridine, freed from the solvent then redissolved in 10% isopropanol to be used as spotting liquids. **For GLC**; samples (0.1 gm) were heated with 1N HCl (10 ml), for 5 hours on a

boiling water bath [17]; the neutralized hydrolysates (0.5 ml, each) were evaporated to dryness under a stream of nitrogen at 40°C, in a small screw-stopped septum vial, 0.5 ml isopropanol was then added to each sample and the solvent completely removed under a stream of nitrogen; the septum was then screwed on and 0.5 ml of 2.5% hydroxylamine hydrochloride in pyridine injected into the vial; the resulting solution was mixed, heated for 30 minutes at 80°C, then allowed to cool; silylation of the hydrolysates was performed by using a mixture of trimethylchlorosilane and N,O-*bis*-(trimethylsilyl) acetamide, 1:5 v/v, the silylating reagent (1 ml) was injected in the sample solution, mixed, heated for 30 minutes at 80°C and then cooled; for GLC analysis, samples (1µl, each) of the silylated hydrolysates were used.

Chromatographic analysis: PC of the hydrolysates was performed on Whatmann No. 1 sheets alongside with available authentic sugars (development technique, ascending; solvent systems, S_1 and S_2; visualization, aniline phthalate spray reagent [19] and heated at 105°C for 5 min). **GLC** of silylated hydrolysates was performed on a Hewlett-Packard HP 6890 N network GC system equipped with a ZB-1701 capillary column (30 m × 0.25 mm ∅, 0.25 µm film thickness) and conducted under the following operating conditions: injector temperature, 250° C; FID detector, temperature 270°C, air flow rate 45 ml/min, H_2 flow rate 40 ml/min; carrier gas, He (1.2 ml/min); oven temperature program: initial temperature 150°C, isotherm for 2 min, increased to 200°C at a rate of 7°C/min, then kept isotherm for 20 min. Identification of the components was based on comparison of their retention times with those of authentic samples similarly analyzed.

Assessment of the antimicrobial activity

The antimicrobial activity of the hydrodistilled volatiles and stem bark exudates, was tested against the selected bacterial and fungal strains. The Minimum Inhibitory Concentrations (MICs) of the samples exhibiting significant activity against specific strains were further determined. The agar diffusion method from cups [20, 21] was adopted for evaluation of the antimicrobial activity. Tripticase soy agar (Difco) was used as culture medium. Cups (0.5 cm in diameter) were made using a no.3 cork borer. The samples were dissolved in DMSO at a concentration of 100 mg/ml for each the volatiles and stem bark exudates. Aliquots of 50 µl of each of the tested samples (equivalent to 5 mg) were, separately, aseptically added to the cups of the inoculated plates (previously prepared). The plates were incubated while inverted, at 37°C for 24 hours in case of bacteria and at 25°C for 48 hours in case of fungi (yeasts). DMSO (50 µl) was used as a negative control and cups of Ofloxacin and Amphotericin B (5

µg/cup, each), were used as positive controls. After incubation, zones of inhibition were measured and diameters less than 5 mm were considered as an indication of no growth inhibitory effect. The percentage potency as compared to the appropriate reference drug was, in each case, calculated. Minimum inhibitory concentrations (MIC) were determined using the dilution method [20]. Several dilutions of each active sample were incubated, as previously described, with each of the microorganisms towards which it exhibited a significant growth inhibitory effect. A curve representing the relationship between the bacterial count (colony/ml sample) and the concentration of the sample (µl/ml) was plotted and minimum inhibitory concentrations deduced.

Assessment of the long-term anti-hyperglycemic activity

Diabetes was induced to male albino rats of Sprague Dawley strain (120-150 g) by intraperitoneal injection of Alloxan (150 mg/kg b.wt.), as described by Eliasson and Samet [22]. The experimental animals were divided in four groups, each of 10 animals. Samples of the stem bark exudates and standard antihyperglycemic drug, Metformin (150 mg/kg b.wt., each) were administered orally, followed by collection of blood samples, at intervals, for determination of blood glucose levels. The long-term anti-hyperglycemic activity was evaluated adopting the method described by Trainder [23]. Glucose levels were measured in blood samples collected at zero time (G_0, *prior* treatment) and after 4 and 8 weeks intervals from administration of the tested samples (in appropriate doses) in case of treated animals (G_t). The percentage change in blood glucose level from initial glycemia was, in each case, calculated according to the following equation: % of change = $(G_0–G_t) \times 100/G_0$.

The 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 .

RESULTS AND DISCUSSION

Yield, physical characteristics and composition of the leaf volatiles

The volatiles isolated by hydrodistillation from fresh leaves of *Swietenia mahogani* (L.) Jacq. amounted to 0.02% v/w (calculated on dry weight basis); being higher in those of *Swietenia macrophylla* King., reaching 0.03 % v/w. The two samples exhibited nearly the same physical characters being oily, pale yellow in color, with a characteristic woodsy balsamic aromatic odor and readily soluble in ethanol 70%. Components identified by GC/MS analysis of the isolated volatiles, their Kovat's indices, relative percentages and mass spectral data are listed in tables (1 and 2) and represented in figs. (1-3).

Data of GC/MS analysis revealed a qualitative and quantitative variability in composition between the examined volatiles. The total number of constituents identified under the adopted operating conditions was 42 among which 18 components were common in the two samples. Components identified were 27 in number in the volatiles of *S. mahogani* and 33 in *S. macrophylla* representing 95.54% vs 96.88% of the total composition. Hydrocarbons dominated the chromatographic profiles of the two volatiles (76.63% vs 82.75% in *S. mahogani* and *S. macrophylla*, respectively) with prevalent sesquiterpenoids (75.51% vs. 80.95%). *Trans*-caryophyllene was the major in *S. mahogani* (33.89%), reaching only (29.12%) in *S. macrophylla* being exceeded by α-humelene (39.64%) in that sample. Oxygenated constituents were minor in both *S. mahogani* and *S. macrophylla* (18.91% vs 14.13%, respectively) and are mainly sesquiterpenoid in nature (18.24% vs 13.03%). Alcohols (12.35% vs 10.45%) were prevalent with major elemol in *S. mahogani* (6.13 %) and E-nerolidol in *S. macrophylla* (10.18%). Oxides were detected in appreciable amounts (5.89% vs 2.68%). The variability in composition among the volatiles of these two closely related species could serve as a helpful tool for chemotaxonomical discrimination. The oils appeared to be rich in insect attracting pheromones such as *trans*-caryophyllene and *α*-humulene [25]. As a matter of fact, the prevalence of sequiterpenoid hydrocarbons in the volatiles of the leaves of *S. macrophylla* was previously reported (major component germacrene D, 58.5-66.5%) [26]; yet, a noticeable qualitative variation is, here, recorded for the Egyptian sample. This may be attributed to climatic and/or geographical factors.

Table (1): Identified components in the hydrodistilled volatiles of the leaves of *Swietenia mahogani* and *Swietenia macrophylla*

Peak #	Identified Component	KI Adams	Relative percentage (Observed KI)		M^+	B
			S. mah.	*S. macr.*		
1	*n*-Decane	999	0.52 (990)	0.57 (989)	142	57
2	*n*-Octanal	1001	0.21 (996)	—	128	41
3	Limonene	1031	0.17 (1020)	—	136	67
4	*n*-Undecane	1099	0.05 (1085)	0.12 (1084)	156	57
5	*n*-Nonanal	1102	0.33 (10930	0.21 (1094)	142	57
6	Pinocarvone	1162	—	0.36 (1155)	150	53
7	*trans*-*β*-Terpineol	1163	—	0.10 (1163)	154	43
8	(Z)-3-Hexenyl butyrate	1186	0.13 (1179)	0.28 (1178)	170	67
9	*n*-Dodecane	1199	—	0.22 (1190)	170	57
10	Myrtenyl acetate	1235	—	0.15 (1230)	194	43
11	*n*-Tridecane	1299	—	0.14 (1287)	184	57

12	*δ*-Elemene	1339	0.27 (1337)	0.03 (1331)	204	93
13	*α*-Cubebene	1351	—	0.13 (1335)	204	105
14	*α*-Copaene	1376	—	3.82 (1371)	204	105
15	*β*-Bourbonene	1384	2.06 (1378)	1.63 (1379)	204	81
16	*cis*-Caryophyllene	1404	1.20 (1400)	0.83 (1398)	204	41
17	*trans*-Caryophyllene	1418	**33.89** (1416)	**29.12** (1415)	204	41
18	*α*-Humulene	1454	**6.56** (1459)	**39.64** (1460)	204	93
19	*γ*-Muurolene	1477	—	0.14 (1473)	204	161
20	Germacrene D	1480	**26.77** (1486)	0.71 (1484)	204	161
21	*β*-Selinene	1485	—	1.18 (1485)	204	93
22	*cis*-β-Guaiene	1490	2.15 (1495)	—	204	105
23	*α*-Farnesene (E,E)	1508	—	2.87 (1504)	204	93
24	*γ*-Cadinene	1513	—	0.73 (1515)	204	161

Table (1): Identified components in the hydrodistilled volatiles of the leaves of *Swietenia mahogani* and *Swietenia macrophylla* (continued)

Peak #	Identified Component	KI Adams	Relative percentage (Observed KI))		M^+	B
			S. mah.	*S. macr.*		
25	*δ*-Cadinene	1524	2.38 (1526)	0.12 (1529)	204	119
26	*α*-Cadinene	1538	0.23 (1534)	—	204	105
27	Elemol	1549	**6.13** (1555)	—	222	59
28	E-Nerolidol	1564	—	**10.18** (1566)	222	41
29	*β*-Caryophyllene oxide	1581	**5.31** (1585)	1.58 (1581)	220	41
30	Humulene epoxide II	1606	0.58 (1610)	1.10 (1607)	220	67
31	10-*epi*-*γ*-Eudesmol	1619	0.91 (1630)	—	222	161
32	*t*-Muurolol	1641	1.18(1650)	0.13 (1646)	222	43
33	Torreyol	1645	—	0.04 (1650)	222	161
34	*α*-Cadinol	1653	3.72 (1661)	—	222	43
35	Khusinol	1674	0.41 (1683)	—	220	41
36	Heptadecane	1700	—	0.23 (1698)	240	57
37	*n*-Octadecane	1800	0.11 (1796)	0.17 (1797)	254	57
38	*n*-Nonadecane	1900	0.07 (1896)	0.16 (1892)	268	57
39	*n*-Eicosane	2000	—	0.07 (1995)	282	57
40	*n*-Heneicosane	2100	0.09 (2097)	0.07 (2096)	296	57
41	*n*-Docosane	2200	0.07 (2195)	—	310	57
42	*n*-Tricosane	2300	0.04 (2298)	0.05 (2295)	324	57
Total number of identified constituents			**27**	**33**		
Total percentage of identified constituents			**95.54**	**96.88**		

S. mah.: Swietenia mahogani (L.) Jacq. (Volatiles of the leaves)
S. macr.: Swietenia macrophylla King. (Volatiles of the leaves)
KI Adams: Kovat's indices according to Adams (1995); Observed KI: Observed Kovat's indices
M^+: molecular weight. B: base peak

Table (2): Relative percentages of the different classes of constituents identified in the volatiles of the leaves of *Swietenia mahogani* and *Swietenia macrophylla*

Constituents	Relative percentage	
	S. mah.	***S. macr.***
Non-oxygenated constituents (Hydrocarbons):		
Aliphatics	0.95	1.8
Monoterpenoids	0.17	0
Sesquiterpenoids	75.51	80.95
Total non-oxygenated constituents	**76.63**	**82.75**
Oxygenated constituents:		
Aliphatics	0.67	0.49
Monoterpenoids	0	0.61
Sesquiterpenoids	18.24	13.03
Total oxygenated constituents	**18.91**	**14.13**
Alcohols	12.35	10.45
Aldehydes	0.54	0.21
Ketones	0	0.36
Esters	0.13	0.43
Oxides	5.89	2.68

S. mah.: Swietenia mahogani (L.) Jacq. (Volatiles of the leaves)

S. macr.: Swietenia macrophylla King. (Volatiles of the leaves)

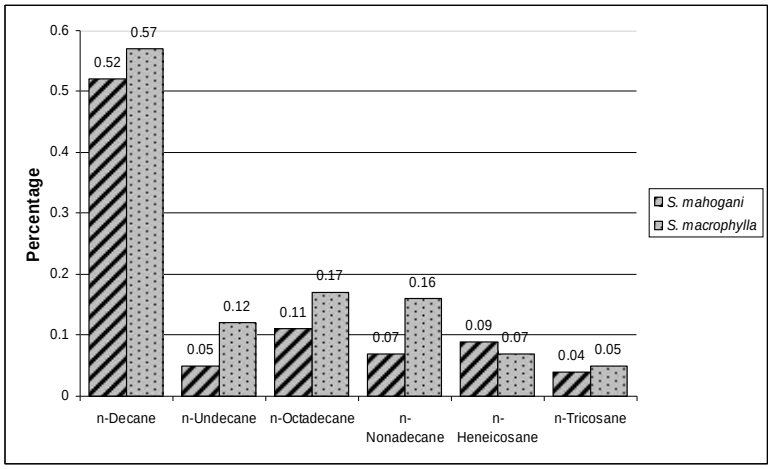

Fig. (1): Histogram representing the quantitative variability among the aliphatic hydrocarbons identified in the volatiles of the leaves of *Swietenia mahogani* and *Swietenia macrophylla*

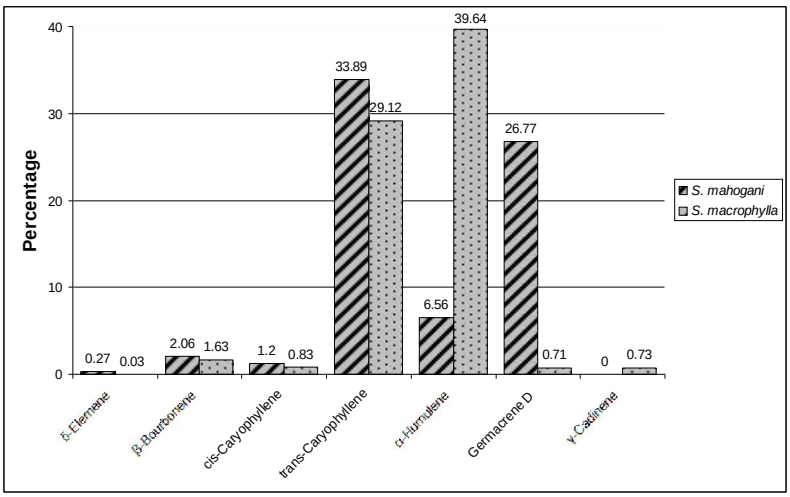

Fig. (2): Histogram representing the quantitative variability among the sesquiterpenoid hydrocarbons identified in the volatiles of the leaves of *Swietenia mahogani* and *Swietenia macrophylla*

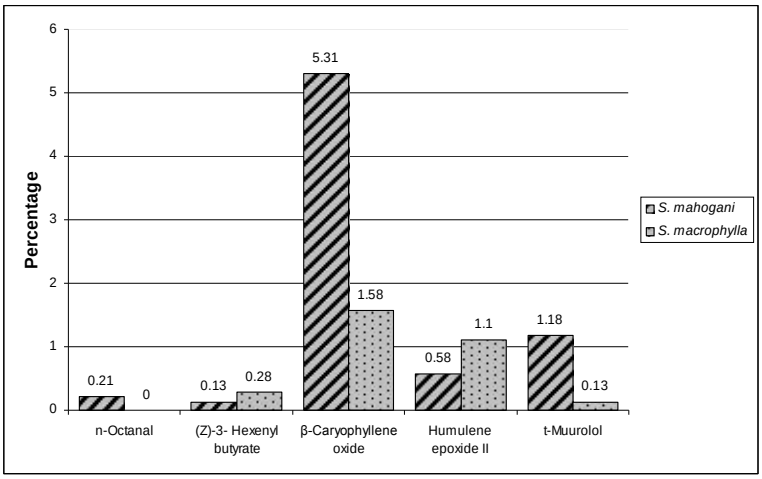

Fig. (3): Histogram representing the quantitative variability among the oxygenated constituents identified in the volatiles of the leaves of *Swietenia mahogani* and*Swieteniamacrophylla*

Characterization and analysis of stem bark exudates

The exudates collected after incision of the stem bark of the two locally cultivated species were obtained as nearly odorless solids usually polyhedral in shape; being transparent and light yellow in case of *S. mahogani*, and more or less translucent and amber-colored in case of *S. macrophylla*. The analytical parameters (solubility in H_2O, optical activity, moisture and ash contents) and response to chemical tests are listed in table (3).

Table (3): Analytical parameters and response to chemical tests of the exudates of *Swietenia mahogani* and *Swietenia macrophylla*

Analytical parameters / Tested constituents	Stem bark exudates	
	S. mahogani	***S. macrophylla***
Solubility in H_2O (25°C)	1: 83	1: 104
Optical activity (25°C)	+ 8.5	+ 11.6
Moisture content (%)	5.02	6.64
Ash content (%)	1.39	0.53
Volatiles	Not detected	Not detected
Carbohydrates	Detected	Detected
Oxidase enzymes	Detected in traces	Detected in traces
Proteins	Detected in traces	Detected in traces
Tannins	Detected in traces	Detected in traces

The exudates were found soluble in water (dextrorotatory solution), but insoluble in alcohol, ether or chloroform. They gave similar response to chemical tests indicating their carbohydrate nature. Proteins, tannins and oxidase enzymes were detected in traces, while hydrodistillable volatiles were absent. The analytical parameters were however different. The mineral composition of the acid-insoluble ash as determined by Atomic Absorption Spectrometry (cations concentrations, µg/g ash) is displayed in table (4).

Table (4): Mineral composition of the acid-soluble ash of the exudates of *Swietenia mahogany* and *Swietenia macrophylla*

Cations	Concentration (µg/g ash , 800°C)	
	Swietenia mahogani	***Swietenia macrophylla***
Calcium	683130	225510
Chromium	Nil	Nil
Iron	21430	9680
Magnesium	60260	237830
Manganese	720	610
Potassium	63200	376860
Sodium	93330	372270
Zinc	70	458

Results of table (4) revealed the absence of Cr in both samples. Meanwhile, Zn was detected in the lowest amount and Ca in the highest. In addition, Na concentration exceeded that of K in the *S. mahogani* exudate, the order being reversed in *S. macrophylla*. The mineral content

of the acid-insoluble ash (gravimetrically determined in terms of µg/g Silicon) was higher (620) in *S. macrophylla* than in *S. mahogani* (450).

Results of PC analysis of the sugar components of the exudate hydrolysates as compared to authentic samples are represented in table (5). Meanwhile, those obtained on GLC analysis of the silylated derivatives of the hydrolysates are represented in table (6). The sensitivity of the GLC technique is obvious since it allowed the identification of a larger number of components as compared to PC (7 vs. only 3), Galactose, xylose and rhamnose being detected by both techniques. The components identified under the GC analytical conditions adopted reached about 73% of the total composition in the two samples; where galactose was detected as the major followed by xylose, while glucuronic acid was the minor. Relative amounts of all components were almost similar in the two hydrolysates except for arabinose which was higher in the *S. macrophylla* sample and L-rhamnose in that of *S. mahogani*.

Table (5): Results of PC analysis of the exudate hydrolysates of *Swietenia mahogani* and *Swietenia macrophylla*

Authentic samples	R_f values		Color with aniline phthalate	Exudate hydrolysates	
	S_1	S_2		*S. mahogani*	*S.macrophylla*
L-Rhamnose	0.63	0.37	Yellowish-brown	±	–
Xylose	0.52	0.28	Reddish-violet	+	+
Arabinose	0.48	0.25	Reddish-brown	–	–
Fructose	0.46	0.20	Brown	–	–
Mannose	0.43	0.19	Brown	–	–
Glucose	0.40	0.23	Brown	–	–
Galactose	0.36	0.17	Pale brown	+	+
Glucuronic acid	0.1	0.15	Pale brown	–	–
Galacturonic acid	0.06	0.12	Pale brown	–	–

S_1 *n*-butanol-pyridine-water 6:4:3 v/v , S_2 *n*-butanol-acetic acid-water 4:1:5 v/v, (+): detected, (±): faint, (-): not detected

Table (6): Components identified by GLC in the exudate hydrolysates of *Swietenia mahogani* and *Swietenia macrophylla*

Identified compounds	RR_t (min)	Relative % in exudate hydrolysates	
		S. mahogani	*S. macrophylla*
Xylose	0.65	8.24	8.37
Arabinose	0.67	1.05	1.81
Ribose	0.69	0.02	0.03
L-rhamnose	0.75	3.66	0.92
Galactose	1	57.99	59.57
Glucose	1.01	1.57	1.46
Glucuronic acid	1.32	0.56	0.42

RR_t= Retention time relative to galactose (R_t=11.29 min)

The monosaccharide composition of the hydrolysates seemed in agreement with previous data on the constitution of the polysaccharide of the gum exudates of *Swietenia* species growing abroad which was reported to be mainly formed of galactose .

Anti-microbial activity

The growth inhibitory activity of the leaf volatiles on the selected bacterial and fungal strains (diameter of zone of inhibition and % potency) are displayed in table (7) and MIC values listed in table (8).

Table (7): Antimicrobial activity of the volatiles of the leaves of *Swietenia mahogani* and *Swietenia macrophylla*

Tested Microorganisms	Diameter of zone of inhibition (mm) (% Potency)*			
	S. mah.	*S. macr.*	Ofx.	Amp. B
Escherichia coli ATCC 10536	-	-	29 (100)	-
Proteus vulgaris NCTC 4175	-	-	38(100)	-
Pseudomonas aeruginosa CNCM A21	-	-	29(100)	-
Staphylococcus aureus ATCC 4175	25(78.13)	20(62.5)	32(100)	-
*Sarcina lutea***	20(66.67)	17(56.67)	30(100)	-
Bacillus subtilis NCTC 6633	11(30.56)	12(33.33)	36(100)	-
*Mycobacterium phlei***	20(80)	17(68)	25(100)	-
Candida albicans ATCC 60193	-	-	-	25(100)

*Percentage of Potency as compared to standard drug; **: Laboratory collection strains
S. mah.: Swietenia mahogani (L.) Jacq. sample
S. macr.: Swietenia macrophylla King. sample

Ofx.: Ofloxacin (5µg/cup) Amp. B: Amphotericin B (5µg/cup); - : no inhibition zone

Table (8): Minimum inhibitory concentrations (MIC, µl/ml) of the volatiles of the leaves of S*wietenia mahogani* and S*wietenia macrophylla*

Tested Microorganisms	MIC (µl/ml)	
	S. mah.	*S. macr.*
Staphylococcus aureus ATCC 4175	6	6
Sarcina lutea	6	12.5
Bacillus subtilis NCTC 6633	12.5	12.5
Mycobacterium phlei	6	12.5

S. mah.: Swietenia mahogani (L.) Jacq. sample

S. macr.: Swietenia macrophylla King. sample

A significant antibacterial activity was observed for the two volatiles against the tested Gram-positive bacteria and the acid-fast *Mycobacterium phlei*, while no effect was recorded on either the selected Gram-negative rods or the fungus *Candida albicans*; MICs of the volatiles against Gram-positive bacteria as well as *Mycobacterium phlei* (table 8) varied from 6-12.5 µl/ml, indicating a high antibacterial activity against these microorganisms. Mean while, the two stem bark exudates revealed only a moderate anti-mycobacterial activity while failing to exert any effect on the other tested microorganisms.

Long-term anti-hyperglycemic activity

Results obtained on assessing the long-term anti-hyperglycemic effect (table 9) revealed a significant reduction in blood glucose level in Alloxan-diabetic rats treated with aqueous solutions of the stem bark exudates. The aqueous extract of the stem bark exudate of *Swietenia mahogani*, orally given at a dose of 150 mg/kg b.wt., exhibited a slightly higher activity than that of *Swietenia macrophylla* as compared to the standard drug Metformin administrated at the same dose level (69 vs 62% potency).

Table (9): Long term antihyperglycemic activity of the aqueous solutions of the stem bark exudates of *Swietenia mahogani* and *Swietenia macrophylla* in diabetic rats (n=10).

Animal group (n=10)	Blood glucose level (mg/dl)					Potency
	Zero time	After 4 weeks		After 8 weeks		
	Mean±S.E.	Mean±S.E.	% change	Mean±S.E.	% change	
Diabetic	261.4±9.8	265.6±11.3	—	271.5±13.2	—	—
***S. mah.* exudate**	251.3±11.2	173.4±5.1*	31	134.2±5.8*	46.6	0.69
***S. macr.* exudate**	262.8±14.5	192.6±7.3*	26.7	153.7±6.1*	41.5	0.62
Metformin 150 mg/kg b.wt.	265.9±12.8	147.1±6.2*	44.7	87.4±3.5*	67.1	1

Statistically significant from the control at $p<0.1$ S.E. = standard error

% of change is calculated as regards to the control group

S. mah.: *Swietenia mahogani* aqueous solution; *S. macr.*: *Swietenia macrophylla* aqueous solution

CONCLUSION

Studies on the Egyptian *Swietenia* cultivars were mainly of agrochemical interest and focused on isolation and establishment of structure-activity relationship of their insecticidal limonoid components. The objective of this work was targeted towards assessing the efficacy of the locally cultivated *Swietenia mahogani* (L.) Jacq. and *Swietenia macrophylla* King., as a source of potential medicinals in order to further increase their propagation. The yield of hydrodistilled leaf volatiles was found higher in *S. mahogani* than *S. macrophylla*. Non-oxygenated and oxygenated sesquiterpenoids were prevalent with hydrocarbons constituting the major make up of the oils. Moreover, the oils appeared to be rich in insect attracting pheromones such as *Trans*-caryophyllene (in *S. mahogani*) and α-humulene (in *S. macrophylla*). The apparent variability in composition could provide useful taxonomical criteria for interspecies differentiation. The physico-chemical characteristics of the stem bark exudates were established including organoleptic features, analytical parameters, and mineral and carbohydrate composition. The exudates were devoid of Cr, but rich in Ca and galactose and contained traces of glucuronic acid. The volatiles were found effective against a set of Gram-positive bacteria while stem bark exudates inhibited mycobacterial growth only and yeast was not affected by any of the samples. A significant reduction in blood glucose level

was recorded in Alloxan-diabetic rats treated with the aqueous solutions of the stem bark exudates of both species.

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