Short communication

Major chemical constituents of *Leucaena leucocephala* and *Salix babylonica* leaf extracts

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Abstract

Chemical constituents in the extracts of *Leucaena leucocephala* (Lamk.) de Wit. and *Salix babylonica* L. foliage were identified by gas chromatography-mass spectrometry and their relative concentrations determined. *L.leucocephala* extract contained 44 compounds: 2(H)-benzofuranone-5,6,7,7a-tetrahydro-4,4,7a-trimethyl (23.1%), pentadecanoic acid-14-methyl-methyl ester (8.2%), and 6,10,14-trimethyl-2-pentadecanone a ketone (4.2%) were the principal constituents. Fifty nine compounds were identified in *S. babylonica* leaves, but the main constituents were tritetracontane (15.2%), octadecenoic acid-1,2,3-propanetriyl ester (11.1%), hexadecanoic acid-methyl ester (10.5%), and 1,3-dioxane-4-(hexadecyloxy)-2-pentadecyl (10.3%). Results suggest that the alcohol extract is mainly composed of oxygenated hydrocarbons of C_{10} to C_{60} compounds and contains predominantly phenolic hydrocarbons.

Keywords: Alcohol extract, Gas chromatography, Oxygenated hydrocarbons, Phenolic hydrocarbons.

Leucaena leucocephala (Lamk.) de Wit. is a tropical mimosoid multipurpose tree species native to southern Mexico and northern Central America. It has medicinal properties ranging from control of stomach diseases to contraception and abortion. The flavonoid quercetin was isolated from the extracts of *L. leucocephala* leaves (Adekunle and Aderogba, 2008). Seeds of *L. leucocephala* also contain mimosine, an imino acid known to be toxic to ruminants. Digestibility and intake of *L. leucocephala* ranged from 50 to 71% and from 58 to 85 g kg^{-1} live weight respectively (Jones, 1979). Compounds such as ficaprenol-11 (polyprenol), squalene, lupeol, β-sitostenone, trans-coumaric acid, cis-coumaric acid, pheophytin-a, pheophorbide a methyl ester, methyl-1,3,2-hydroxy-(1,3,2-S)-pheophorbide-b and aristophyll-C were also isolated from the whole plants of *L. leucocephala* (Chen and Wang, 2010). Among them, ficaprenol-11 (polyprenol) and squalene were identified for the first time from this species.

Salix babylonica L. (Babylon willow; var. *babylonica*) is a species of willow native to the dry areas of northern China. Willows have salicylate compounds which are related to the phenolic glycosides based on the structure of salicin (Ruuhola and Julkunen-Tiitto, 2000). The naturally occurring benzyl ester of gentisic acid 2'-*O*-acetyl β-D-glucoside has been isolated, along with the known compounds trichocarpin, salicin, kaempferol-7-*O*-glucoside, apigenin-7-*O*-galactoside, luteolin-4'-*O*-glucoside, and an ester of terephthalic acid from the leaves of *S. babylonica* (Khatoon et al., 1988). Three flavonoid compounds were obtained from *S. babylonica* and identified as luteolin-7-*O*-β-D-glucopyranoside, luteolin, and chrysoeriol. This study aimed to evaluate the quantity

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and the chemical constituents of *L. leucocephala* and *S. babylonica* extracts and their mixture.

Samples of young and mature leaves of *L. leucocephala* and *S. babylonica* were collected from several locations in the south of the Estado de México and three individual samples (~5 kg each pooled from at least seven trees) were randomly collected at different sampling times and for each tree species during August/September of 2010. The samples were dried in a forced air oven at 23 to 25°C until constant weights and were ground to pass through a 1 mm sieve using a small laboratory mill (Wiley, UK) and stored in plastic bags under darkness until laboratory analysis. Subsamples (100 g) were soaked in 150 mL each of methanol, acetone, and hexane (1:1:1) solvent at room temperature for 24 h. The crude extract was filtered through Whatman No.1 and over active charcoal to remove chlorophyll. Extracts were concentrated in vacuum to 20 ml and then lyophilized to obtain dried extract, which was stored in a refrigerator until use. Ten milliliter from each extract was mixed and kept at 4°C until GC-MS analysis. The chemical constituents of the leaf extracts (20 mg) were dissolved in dichloromethane and analyzed by GC-MS (Varian Saturn 2100T 3900 GC/MS mass selective detector connected to a RTX 6890 Gas Chromatograph). Sepa-ration was done in a capillary column, RTX 5MS (5% phenyl methyl polysiloxane) 30 m long, 0.25 mm internal diameter, and 0.25 ìm film thickness. The column temperature was kept at 50°C for 6 min and programmed to increase to 300°C at a rate of 5°C per min. The flow rate of He (the carrier gas) was 1 mL.min^{-1} in the split 20 mL.min^{-1} of 0 to 0.01. Aliquots (1 µL) of the solvent containing the extracts of *L. leucocephala*, *S. babylonica* and their mixture (1:1, v/v) were injected into the GC column with the injector heater at 300°C. The MS was operated in full scan mode (40 to 650 *m/z* at a rate of two scans per second) with electron impact ionization (EI mode) at 70 electron volts (eV) at an ion source tempe-rature of 230°C. The relative proportion of constituents was expressed as mg g^{-1} of peak area normalization. Identification of extract components was based on direct comparison of the retention times and mass spectral data, and computer search matching with the NIST MS Search 2.0 library, as well as by comparison of the fragmentation patterns of mass spectra data with those reported in the literature MS.

A total of 44 compounds were identified in the foliar extracts of *L. leucocephala* (Table 1). The principal chemical constituents were 2(H)-benzofuranone-5,6,7,7a-tetrahydro-4,4,7a-trimethyl (a volatile terpene: 23.1%) and pentadecanoic acid-14-methyl-methyl ester (a monomethyl branched acid: 8.2%). Minor constituents included 6,10,14-trimethyl-2-pentadecanone (a phytone ketone: 4.2%), phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) an acyclic terpenoid (1.9%), and 1-nonadecanol (a phenolic compound with monofunctional alkanes or 1-alkanols: 1.4%).

Retention time and mass spectral comparison identified 59 compounds in *S. babylonica*. The main chemicals were tritetracontane (an aliphatic hydrocarbon: 15.2%), 9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E, a trioleoylglycerol: 11.1%), hexadecanoic acid-methyl ester (a saturated fatty acid: 10.5%), 1,3-dioxane-4-(hexadecyloxy)-2-pentadecyl (a heterocyclic organic compound: 10.3%) and phytol (3,7,11,15-tetramethyl-

Table 1. Principal chemical components identified in *L. leucocephala* leaves extract by GC/MS analysis.

Compound	Retention time (min)		Molecular wt.	Chemical	Concentration
	Measured	Authentic	(g mol^{-1})	formula	(mg g^{-1})[a]
2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (r)-	12.6	12.7	180.2	$C_{11}H_{16}O_2$	230.9
1-Nonadecane	14.3	14.3	268.0	$C_{19}H_{40}$	13.6
3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol)	15.7	15.7	296.5	$C_{20}H_{40}O$	19.1
6,10,14-trimethyl 2-pentadecanone (phytone ketone)	15.7	15.7	268.5	$C_{18}H_{36}O$	41.7
Pentadecanoic acid, 14-methyl-,methyl ester	16.3	16.5	270.1	$C_{17}H_{34}O_2$	82.0

[a]Concentration based on the total areas of the identified peaks.

Table 2. Principal chemical components identified in *S. babylonica* leaves extract by GC/MS analysis.

Compound	Retention time (min)		Chemical formula	Molecular wt. (g mol^{-1})	Concentration (mg g^{-1})[a]
	Measured	Authentic			
2-hidroxy-6-methyl- benzaldehyde	7.7	7.8	$C_8H_8O_2$	136.2	9.9
2-Methoxy-4-vinylphenol	9.8	9.8	$C_9H_{10}O_2$	150.0	3.6
Hexatriacontane	13.2	13.2	$C_{36}H_{74}$	507.0	7.7
Nonadecane	14.3	14.3	$C_{19}H_{40}$	268.5	11.7
Tridecanoic acid, 12-methyl, methyl ester	14.5	14.5	$C_{15}H_{30}O_2$	242.4	6.7
3,7,11,15-tetramethyl-2-hexadecen-1-ol(phytol)	15.7	15.6	$C_{20}H_{40}O$	296.5	97.2
Hexadecanoic acid (palmitic acid, methyl ester)	16.5	16.5	$C_{17}H_{34}O_2$	270.5	149.7
9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	18.0	18.0	$C_{57}H_{104}O_6$	885.4	110.5
Octadecanoic acid, methyl ester	18.2	18.2	$C_{19}H_{36}O_2$	296.5	16.8
1,3-dioxane, 4-(hexadecyloxy)-2-pentadecyl	18.7	18.7	$C_{35}H_{49}O_3$	517.0	103.3
Tritetracontane	19.5	19.5	$C_{43}H_{88}$	605.2	152.1
1-pentacontanol	21.6	21.6	$C_{50}H_{102}O$	719.3	9.7

[a]Concentration based on the total areas of the identified peaks.

2-hexadecen-1-ol: 9.7%). There were also some aliphatic hydrocarbons such as nonadecane (1.2%) and hexatriacontane (0.8%). Overall, C_{10} to C_{60} compounds predominated in the *L. leucocephala* and *S. babylonica* leaf extracts. Examples include fatty acids and their methyl esters such as hexadecanoic acid, octadecatrienoic acid, octadecenoic acid and pentadecanoic acid, which are relatively common essential oils in higher plants (Shabi et al., 2010). There was also carboxylic acid in the form of oxygenated hydrocarbons.

The chemical analysis of the mixture of *L. leucocephala* and *S. babylonica* leaf extract using GC-MS showed 60 compounds, some of which are in Table 3. The main compound in the extract was 9,12,15-octadecatrienoic acid-ethyl ester (Z,Z,Z) with concentration of 29.4%

Table 3. Principal chemical components identified in the mixture of *L. leucocephala* and *S. babylonica* leaves extract by GC/MS analysis.

Compound	Retention time(min)		Chemical formula	Molecular wt. (g mol^{-1})	Concentration (mg g^{-1})[a]
	Measured	Authentic			
9-Oxononanoic acid methyl ester	11.3	11.3	$C_{10}H_{18}O_3$	186.2	7.7
7,10-Hexadecadienoic acid, methyl ester	12.7	12.7	$C_{17}H_{30}O_2$	266.4	3.07
3,7,11, 15-Tetramethyl-2-hexadecen-1-ol	15.6	15.6	$C_{20}H_{40}O$	296.5	9.8
6,10,14-Trimethyl, 2-pentadecanone	15.7	15.7	$C_{18}H_{36}O$	268.5	4.6
9-Hexadecenoic acid, methyl ester	16.3	16.3	$C_{17}H_{34}O_2$	268.4	2.9
Hexadecanoic acid, methyl ester	16.4	16.5	$C_{17}H_{34}O_2$	270.2	90.2
9,12-Octadecadienoic acid methyl ester	17.9	17.9	$C_{19}H_{34}O_2$	294.5	112.4
9,12,15-Octadecatrienoic acid, ethylester (Z,Z,Z)	18.0	18.0	$C_{20}H_{34}O_2$	306.5	294.0
Octadecanoic acid, 16, methyl, methyl ester	18.2	18.2	$C_{20}H_{40}O_2$	312.3	9.9
5,8,11,14,17-Eicosapentaenoic acid, methyl ester	18.4	18.3	$C_{22}H_{34}O_2$	330.5	20.3
Nonanoic acid, 9-(o-propylphenyl), methyl ester	18.4	18.4	$C_{19}H_{30}O_2$	290.2	5.0
Oxacyclotetradeca-4,11-diyne	18.5	18.5	C13H18O	190.0	0.9
2-Pentadecyl-4-(hexadecyloxy)-1,3-dioxane	18.7	18.7	$C_{35}H_{70}O_3$	538.0	128.7
Hexacontanoic acid	20.2	20.2	$C_{60}H_{122}O_2$	874.0	20.9
Tritetracontane	20.9	20.9	$C_{43}H_{88}$	605.2	32.0

[a]Concentration based on the total areas of the identified peaks.

followed by 9,12-octadecadienoic acid-methyl ester (11.2%), and hexadecanoic acid-methyl ester a saturated fatty acid with concentration 9.0%. Hexacontanoic acid (2.1%), 5,8,11,14,17-eicosapentaenoic acid-methyl ester (2.0%) and octadecanoic acid-16-methyl-methyl ester (1.0%) were the major fatty acids. However, there was 12.9% 1,3-dioxane, 4-(hexadecyloxy)-2-pentadecyl and the aliphatic hydrocarbon was in the tritetracontane form at 3.2%. The GC-MS analyses of *L. leucocephala* and *S. babylonica* leaf extracts and their mixture 1:1 (v/v) revealed that the alcohol extract is mainly composed of oxygenated hydrocarbons of C_{10} to C_{60} compounds and contains predominantly phenolic hydrocarbons.

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