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Volatile constituents of the leaves of *Siparuna thecaphora* (Siparunaceae) from Turrialba, Costa Rica

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Abstract: The composition of the essential oil from leaves of *Siparuna thecaphora* (Poepp. et Endl.) A. DC. collected in Turrialba, Costa Rica, was determined by capillary GC/MS. Seventy-six compounds were identified corresponding to *ca.* 95% of the oil. The major components were germacrene D (32.7%), α-pinene (16.3%), β-pinene (13.8%) and β-caryophyllene (4.1%). Thirty-one minor compounds were identified for the first time in this genus of plants.

Key Words: *Siparuna thecaphora*, Siparunaceae, essential oil composition, germacrene D, α-pinene, β-pinene, β-caryophyllene.

The family Siparunaceae comprises two genera, the monotypic West African genus *Glossocalyx* and the Neotropical genus *Siparuna* (formerly placed in the family Monimiaceae), which contains about 72 species of shrubs, straggling shrubs, and trees (Renner *et al*. 1997). Up to now, six species of *Siparuna* from Costa Rica have been described.

Siparuna thecaphora (Poepp. et Endl.) A. DC. is an aromatic shrub or small tree (3-5 m tall), with opposite, simple leaves, and unisexual flowers. The fruits are fleshy and reddish when ripe, with arilate seeds. Due to its highly variable morphology, a great number of synonyms are found in the literature (e.g., *S. nicaraguensis*, *S. andina*, *S. gilgiana*). This plant is a widespread species in Costa Rica. It can be found in both Pacific and Caribbean sides of the Country, from near sea level up to close to 2 000 m elevation. It is distributed from southern Mexico throughout Central America to Brazil and Bolivia. It is called colloquially "limoncillo" (little lemon) probably because of the strong lemon-scented fruits.

In Panama, the infusion of crushed leaves is used to treat colds and rheumatism (Duke 1972). In Guatemala, the fresh leaves are placed on the forehead to relieve headaches (Morton 1980).

To the best of our knowledge, no reports have been made regarding the essential oil composition from this genus of plants in Costa Rica.

Early phytochemical studies of the genus *Siparuna* revealed the presence of aporphine, oxoaporphine and morphinandienone alkaloids (Braz *et al*. 1976, Chiu *et al*. 1982, Gerard *et al*. 1986, Lopez *et al*. 1988, 1990, 1993); cadinane sesquiterpenes (El-Seedi *et al*. 1994) and kaempherol glycosides (Leitão *et al.* 2000). Reports of the leaf essential oils constituents

obtained from the Panamanian and Brazilian *S. guianensis* were varied. They range from those containing as main constituents curzerenone and curzerenone derivatives (Antonio *et al*. 1976), epi-α-bisabolol, spathulenol, selin-11-en-4α-ol, elemol, β-eudesmol, atractylone and germacrone (Zoghbi *et al*. 1998) or (*E*)-nerolidol (Fernandez-Machado *et al*. 1998).

Some chemical studies of *S. thecaphora* have been done previously. The oxoaporphine alkaloid liriodenine was isolated from the twigs (under the synonym *S. nicaraguensis*) (Gerard *et al.* 1986). The alkaloids liriodenine and oxonantenine, reported for the first time in the genus, were isolated from the roots (under the synonym *S. gilgiana*) (Chiu *et al.* 1982). The volatile oil of the fruits (under the synonym *S. nicaraguensis*) was studied previously (Manjarrez and Mendoza 1967). The main constituents of the oil were β-elemene, citral and β-ionone.

MATERIALS AND METHODS

Plant Material: Leaves of *Siparuna thecaphora* from Costa Rica, were collected in Santa Cruz, Turrialba, province of Cartago, in 1998. A voucher specimen was deposited in the Herbarium of the University of Costa Rica at the School of Biology (USJ 68612).

Essential Oil Isolation: Fresh leaves were subjected to hydrodistillation for 3 hr using a modified Clevenger-type apparatus. The distilled oil was collected as a colorless liquid, dried over anhydrous sodium sulfate, and stored at -10ºC [0.2% (v/w) yield].

General Analytical Procedures: The oil was analyzed by gas chromatography/mass spectrometry (GC/MS). The mass spectra were obtained using a Hewlett-Packard G1800A GCMSD System, employing a fused silica (30 m x 0.25 mm) column coated with a 5% phenyl methyl silicone (film thickness 0.25 µm). Operation conditions were: carrier gas: He (1.0 mL/min); oven temperature program: 75ºC (4 min), 75º-200ºC at 3ºC/min, 200ºC (8 min); sample injection port temperature: 250ºC; detector temperature: 260ºC; ionization voltage: 70eV; scanning over 38-400 amu range; split injection 1:20. Integration of the total ion chromatogram, expressed as area percent, has been used to obtain quantitative compositional data.

Identification: Identification of the components of the oil was performed using the retention indices on a phenyl methyl silicone column, and by comparison of their mass spectra with those published in the literature (Stenhagen *et al*. 1974, Swigar and Silverstein 1981, McLafferty 1993, Adams 1995, 2001) or those of our own database.

RESULTS

The oil is constituted mainly by hydrocarbons (89.0%) whereas the oxygenated compounds accounts only for 6.4% (Table 1). Among the 76 compounds identified, sesquiterpenoids represented 56.7% and monoterpenoids 38.7%.

Germacrene D (32.7%), α-pinene (16.3%), β-pinene (13.8%) followed by the widespread sesquiterpene β-caryophyllene (4.1%) were identified as the main constituents of the oil. Among the oxygenated compounds α-cadinol (1.6%), epi-α-muurolol (1.0%) and 1,8-cineole (0.8%) were the major ones. The other constituents were 69 compounds found in minor or trace amounts. Of these ones, thirty-one are newly identified compounds in the genus *Siparuna* (see Table 1).

TABLE 1
Percentage composition of the Siparuna thecaphora *leaf oil from Costa Rica*

[a]Compound	[b]RI	Leaves	[d]Method
α-thujene*	930	0.3	1, 2
α-pinene	939	16.3	1, 2, 3
α-fenchene*	953	[c]t	1, 2
camphene	954	0.4	1, 2
sabinene*	975	1.3	1, 2
β-pinene	979	13.8	1, 2, 3

myrcene	991	2.5	1, 2
α-phellandrene	1003	t	1, 2
α-terpinene*	1017	0.1	1, 2
p-cymene	1025	t	1, 2
limonene	1029	0.4	1, 2, 3
β-phellandrene	1030	0.3	1, 2
1, 8-cineole	1031	0.8	1, 2, 3
(*Z*)-β-ocimene	1037	1.0	1, 2
(*E*)-β-ocimene*	1050	0.2	1, 2
γ-terpinene	1060	0.2	1, 2
cis-sabinene hydrate*	1070	t	1, 2
p-mentha-3,8-diene*	1073	t	1, 2
terpinolene	1089	0.1	1, 2
linalool	1097	0.2	1, 2, 3
n-nonanal*	1101	t	1, 2
endo-fenchol*	1117	t	1, 2
cis-*p*-menth-2-en-1-ol*	1122	t	1, 2
allo-ocimene*	1132	t	1, 2
trans-pinocarveol*	1139	t	1, 2
trans-*p*-menth-2-en-1-ol*	1141	0.2	1, 2
pinocarvone*	1165	t	1, 2
terpinen-4-ol*	1177	0.4	1, 2
α-terpineol	1189	0.1	1, 2
myrtenal*	1196	t	1, 2
trans-piperitol*	1208	t	1, 2
pulegone*	1237	0.1	1, 2
neral	1238	t	1, 2
geraniol	1253	t	1, 2
geranial	1267	t	1, 2
bornyl acetate*	1289	t	1, 2
δ-elemene	1338	2.3	1, 2
α-cubebene	1351	0.2	1, 2
cyclosativene	1371	0.1	1, 2
longicyclene*	1374	0.1	1, 2
α-ylangene	1375	0.3	1, 2
α-copaene	1377	1.8	1, 2
β-bourbonene	1385	0.2	1, 2
β-cubebene	1388	0.3	1, 2
β-elemene	1391	0.8	1, 2
β-caryophyllene	1419	4.1	1, 2, 3
β-copaene*	1432	0.5	1, 2
γ-elemene	1437	0.8	1, 2
α-humulene	1455	0.6	1, 2
alloaromadendrene	1460	0.1	1, 2
cis-muurola-4(14),5-diene*	1467	0.1	1, 2
germacrene D	1485	32.7	1, 2
β-selinene	1490	0.6	1, 2
trans-muurola-4(14),5-diene*	1494	0.4	1, 2
epi-cubebol	1494	0.5	1, 2
bicyclogermacrene	1500	1.2	1, 2
α-muurolene	1500	0.5	1, 2
germacrene A	1509	0.1	1, 2
δ-amorphene*	1512	0.5	1, 2
γ-cadinene	1514	0.3	1, 2
cubebol	1515	0.3	1, 2
δ-cadinene	1523	2.0	1, 2
trans-cadina-1(2),4-diene*	1535	0.1	1, 2
α-cadinene*	1539	0.1	1, 2
α-calacorene	1546	t	1, 2
germacrene B	1561	1.3	1, 2
E-nerolidol	1564	0.3	1, 2
β-calacorene*	1566	t	1, 2
germacrene D-4-ol*	1576	0.1	1, 2
spathulenol	1578	0.1	1, 2
caryophyllene oxide	1583	0.2	1, 2
epi-a-muurolol*	1640	1.0	1, 2
cubenol	1647	0.3	1, 2
β-eudesmol	1651	0.2	1, 2
α-cadinol*	1654	1.6	1, 2
mint sulfide*	1741	t	1, 2
Monoterpene hydrocarbons	36.9		
Oxygenated monoterpenes	1.8		
Sesquiterpene hydrocarbons	52.1		
Oxygenated sesquiterpenes	4.6		

[a] Compounds are listed by elution order in a 5% phenyl methyl silicone column; [b] RI = Retention indices (Adams 2001); [c] t = trace (<0.05%); [d] Method: 1 = Retention Indices on 5% methyl phenyl silicone column; 2 = MS spectra; 3 = standard.

* Newly identified constituents in the genus *Siparuna.*

DISCUSSION

These results differ markedly from those obtained with oils isolated from *S. guianensis.* Antonio *et al*. (1976) reported that the leaf oil of plants collected in Panama, contains as major constituents curzerenone and curzerenone type compounds or products decomposing to curzerenone. Zoghbi *et al.* (1998) reported the composition of three types of oils from *S. guianensis* collected at different locations of the Amazon, Brazil: type A, with epi-α-bisabolol and spathulenol as main constituents; type B, with spathulenol, selin-11-en-4α-ol, β-eudesmol and elemol; and type C, with germacrone, germacrene D, bicyclogermacrene, germacrene B and atractylone as major constituents. Fernandez-Machado *et al*. (1998) reported that (*E*)-nerolidol was the main component of the leaf oil of *S. guianensis*, during flowering. They observed a large seasonal variation of nerolidol.

Our results also differ from those obtained by Manjarrez and Mendoza (1967) from the fruits of *S. thecaphora* from Mexico (under the synonym *S. nicaraguensis*). The oil was

characterized by the presence of β-elemene, citral and β-ionone.

In the current study of *S. thecaphora*, Germacrene D (32.7%), α-pinene (16.3%) and β-pinene (13.8%) were identified as the main constituents of the oil. Preliminary studies (still in progress) concerning the composition of the oil of *S. thecaphora* from three locations in Costa Rica also revealed chemical differences (Chavarría 2002). This chemical differences could be attributed to the effect of geographical location and microenvironment or to the effect of interspecific and infraspecific differences or to the phenological stage of the plant.

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RESUMEN

La composición química del aceite esencial de las hojas de *Siparuna thecaphora* (Poepp. et Endl.) A. DC., recolectada en Turrialba, Costa Rica, se estudió mediante la técnica de cromatografía capilar gaseoso-líquida, acoplada a un espectrómetro de masas (GC/MS). Se identificaron 76 compuestos que correspondieron a cerca del 95% del aceite. Los componentes mayoritarios fueron germacreno D (32.7%), α-pineno (16.3%), β-pineno (13.8%) y β-cariofileno (4.1%). Dentro de los compuestos minoritarios, treinta y uno se describen por primera vez como constituyentes de este género de plantas.

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