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Composition of the essential oil from leaves of *Smallanthus quichensis* (Asteraceae) from Costa Rica

[Composición química del aceite esencial de las hojas de *Smallanthus quichensis* (Asteraceae) de Costa Rica]

Carlos CHAVERRI & José F. CICCIO

*Escuela de Química and Centro de Investigaciones en Productos Naturales (CIPRONA),
Universidad de Costa Rica, 11501-2060, Costa Rica*

Contactos / Contacts: José F. Cicció - E-mail address: jfciccio@gmail.com

Abstract: *Smallanthus* is a genus of flowering plants in the Asteraceae family, which has about 24 species, ranging mostly from southern Mexico and Central America to the Andes in South America. The aim of the present study was to identify the chemical composition of leaf essential oil of *S. quichensis*, growing wild in Costa Rica. The extraction of the oils was carried out by the hydrodistillation method, using a modified Clevenger type apparatus. The chemical composition of the oils was analyzed by capillary GC-FID and GC-MS using the retention indices on DB-5 type capillary column. A total of 100 compounds were identified, accounting for about 90% of the total amount of the oils. *Smallanthus quichensis* leaf produced a monoterpenoid-rich oil, whose composition was dominated by α -pinene (64.5%) and 1,8-cineole (9.7%) or, in a different sample, by α -pinene (35.5%) with moderate amounts of p-cymene (11.5%), β -phellandrene (9.2%), α -phellandrene (9.0%) and limonene (5.8%). This is the first report of the chemical composition of the essential oil obtained from this plant species.

Keywords: *Smallanthus quichensis*, Asteraceae, Costa Rica, essential oil, GC-FID, GC-MS, α -pinene, p-cymene, 1,8-cineole, β -phellandrene, α -phellandrene, limonene

Resumen: *Smallanthus* es un género de plantas perteneciente a la familia Asteraceae que contiene aproximadamente 24 especies, la mayoría ubicadas desde el sur de México, América Central y hasta la cordillera de los Andes en América del Sur. El objetivo del presente estudio fue el de identificar la composición química del aceite esencial de las hojas de *S. quichensis*. La extracción se realizó mediante el método de hidrodestilación, empleando un instrumento de tipo Clevenger. Se analizó la composición de los aceites mediante CG-FID y CG-EM, utilizando índices de retención obtenidos en una columna capilar tipo DB-5. Se identificaron 100 compuestos, correspondientes a un 90% de los constituyentes totales. Los aceites están constituidos principalmente por monoterpenoides (ca. 85%). Los componentes mayoritarios se identificaron como α -pineno (64.5%), acompañado por 1,8-cineol (9.7%) o, en otra muestra distinta, α -pineno (35.5%) junto a cantidades moderadas de p-cimeno (11.5%), β -felandreno (9.2%), α -felandreno (9.0%) y limoneno (5.8%). Este es el primer informe acerca de la composición química de aceites esenciales obtenidos de *S. quichensis*.

Palabras clave: *Smallanthus quichensis*, Asteraceae, Costa Rica, aceite esencial, CG-FID, CG-EM, α -pineno, p-cimeno, 1,8-cineol, β -felandreno, α -felandreno, limoneno

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INTRODUCTION

Asteraceae (Compositae) is the largest flowering plant family on Earth with a cosmopolitan distribution including 1600-1700 genera and about 24000 accepted species (Funk *et al.*, 2009). This family has a considerable ecological and economic importance, as several species are used as food source [*Helianthus annuus* L., sunflower, *Lactuca sativa* L., lettuce, *Cynara cardunculus* L., artichoke, *Smallanthus sonchifolius* (Poepp.) H. Rob., yacon, *Eruca sativa* Lam., arugula], some are used as spices (*Artemisia dracunculus* L., tarragon, *Tagetes lucida* Cav., *pericón*), as herbal medicines [*Matricaria chamomilla* L., chamomile, *Echinacea purpurea* (L.) Moench, purple coneflower, *Arnica montana* L., arnica]. Also many Asteraceae are important in horticulture as ornamentals (*Chrysanthemum* spp., *Dahlia* spp., *Gaillardia* spp., *Gerbera* spp., *Tagetes* spp., *Zinnia* spp.), and as a source of insecticides [*Tanacetum cinerariifolium* (Trevir.) Sch. Bip., pyrethrum].

Studies of the Heliantheae (Asteraceae) subtribe Melampodiinae, concluded that *Smallanthus* is a valid genus (Robinson, 1978) segregated from *Polymnia* L., a genus proposed by Mackenzie (1933). Actually, *Smallanthus* includes about 24 species, ranging mostly from southern Mexico and Central America to the Andes in South America (Grau & Rea, 1997; Tropicos, 2014; Vitali & Viera-Barreto, 2014). The genus is constituted by perennial herbs or shrubs or less frequently small trees and only rarely annuals. In Costa Rica the genus is represented by *Smallanthus maculatus* (Cav.) H. Rob., and *S. quichensis* (J. M. Coult.) H. Rob.

Smallanthus quichensis is an herb (1-2 m tall) growing on moist thickets and edges and is uncommon in Costa Rica. The dentate leaves (ca. 5-20 cm long) are ample and opposite, ovate-lanceolate, tapering at base to a short petiole, acuminate, and are scabrous above and somewhat pubescent beneath, specially upon the conspicuous reticulations. The flowers are yellow, solitary or few in a terminal corymb. This species was described originally as a *Polymnia* L. (Compositae, Heliantheae, subtribe Melampodiinae) (Coulter, 1895; Standley & Steyermark, 1940). Apparently, *S. quichensis* is distributed only in Guatemala and Costa Rica.

There is scarce information about popular and ethnomedical uses of plants of the genus

Smallanthus except for commercial yacon (*S. sonchifolius*) the most important and best known species. In Alta Verapaz Guatemala, a dried, powdered leaves of *S. maculatus* are used on wounds and ulcers of the legs (Morton, 1981) and, in Highlands Mayas of Chiapas, Mexico, is taken for the treatment of gastrointestinal diseases (Ríos & León, 2006). American Cherokee people used the aqueous extract of the dry root of *S. uvedalia* (hairy leafcup) as emmenagogue and to expel afterbirth (Olbrechts, 1931). Aqueous extracts of dry leaves of *S. pyramidalis* are also used in Colombia as emmenagogue (García-Barriga, 1975). Yacon tuberous roots are classified in the Andes markets as a “fruit” with agreeable sweet flavor and they are consumed in Bolivia by people with diabetes and digestive disorders (Grau & Rea, 1997). The potential of this plant to treat hyperglycemia generated great interest. In Brazil, the dried leaves are used to prepare a medicinal infusion for diabetics (Kakihara *et al.*, 1996, cited in Grau & Rea, 1997) and its hypoglycemic activity has been demonstrated in a rat experimental model by Volpato *et al.* 1997 (as cited in Grau & Rea, 1997). Several extracts of the dried leaves showed significant hypoglycemic effect (Aybar *et al.*, 2001; Miura *et al.*, 2004; Miura, 2007; Baroni *et al.*, 2008; Genta *et al.*, 2010; Serra-Barcellona *et al.*, 2014). Some uses and biological activities of yacon were listed and reviewed by Grau *et al.* (2007), Ojansivu *et al.* (2011) and Choque Delgado *et al.* (2013). To our knowledge, there is no precedent for the use of *S. quichensis* in Central America traditional medicine.

Several members of *Smallanthus* have been investigated chemically and are characterized by the occurrence of sesquiterpene lactones of the melampolide type (Herz & Bhat, 1973; Le Van & Fischer, 1979; Bohlman *et al.*, 1979; Bohlman *et al.*, 1980a; Bohlman *et al.*, 1980b; Bohlman *et al.*, 1985; Malcolm & Fischer, 1987; Castro *et al.*, 1989; Inoue *et al.*, 1995; De Pedro *et al.*, 2003; Lin *et al.*, 2003; Bach *et al.*, 2007; Coll Aráoz *et al.*, 2007; Hong *et al.*, 2008; Serra-Barcellona *et al.*, 2014) and kaurenoic acid derivatives (Bohlman & Zdero, 1977; Bohlman *et al.*, 1979; Bohlman *et al.*, 1980a; Bohlman *et al.*, 1980b; Bohlman *et al.*, 1985; Le Van & Fischer, 1979; Calle *et al.*, 1988; Kakuta *et al.*, 1992; Ríos & León, 2006; Qiu *et al.*, 2008; Coll-Aráoz *et al.*, 2010; Dou *et al.*, 2010). Also, the chemical investigation of several *Smallanthus*

species afforded some polyacetylenes, monoterpenes, thymol derivatives, cinnamate esters, flavanones (Bohlmann & Zdero, 1977; Bohlmann *et al.*, 1979; Bohlman *et al.*, 1980b), flavones (Beutler *et al.*, 1993), caffeic acid derivatives (Takenaka *et al.*, 2003; Simonovska *et al.*, 2003), derivatives of octulosonic acid (Takenaka & Ono, 2003), homogteranyl nerol derivatives (Bohlman *et al.*, 1985), cadinene compounds (Dominguez *et al.*, 1988) and smallanthaditerpenic acids (Dou *et al.*, 2008; Xiang *et al.*, 2014). Goto *et al.* (1995) studied the oligosaccharides of yacon (*S. sonchifolius*) tuberous roots and demonstrated that there are inulin-type oligofructans.

Little is known about the composition of the volatile oils of *Smallanthus* species. Previous work with *S. maculatus* from Costa Rica (Ciccío, 2004) revealed the presence of α -pinene and β -pinene as main monoterpene constituents and germacrene D, β -caryophyllene and bicyclogermacrene as major sesquiterpene components of the leaf oil. Adam *et al.* (2005) reported that β -pinene, β -caryophyllene and γ -cadinene were the predominant constituents of the leaf oil from *S. sonchifolius* (Poepp.) H. Rob. (yacon). Miyazawa & Tamura (2008) reported the constituents of the essential oil from yacon tubers, and the main components of the oil were α -pinene (33.5%) and the diterpenic labdanes *neo*-abienol, *cis*-abienol, *trans*-abienol and 8,12-epoxy-14-labden-13-ol.

To the best of our knowledge, the chemical composition of the essential oil of *S. quichensis* has not been previously reported.

MATERIAL AND METHODS

Plant material

Aerial parts of *Smallanthus quichensis* (Asteraceae) growing wild, were collected in March, 2001 (sample 1) and June, 2005 (sample 2), in Southern slope of Turrialba Volcano, at an elevation about 2200 m, Province of Cartago, Costa Rica. A voucher specimen was kept at the Herbarium of the University of Costa Rica (USJ 77458).

Oil isolation

Leaves were dried and subjected to hydrodistillation for three hours using a modified Clevenger-type glass apparatus. The distilled oils were collected and dried over anhydrous sodium sulfate, and stored under N₂ in sealed amber vials, at -10° C to 0° C, for further

analysis. The yield (v/w) of the light yellowish oil from the leaf was 0.1%.

General analytical procedures

GC-FID analysis

The oils of *S. quichensis* were analyzed by GC-FID (gas chromatography with flame ionization detector) using a Shimadzu GC-2014 gas chromatograph. The data were obtained on a poly (5% phenyl-95% methylsiloxane) fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 μ m), (MDN-5S), with a LabSolutions, Shimadzu GC Solution, Chromatography Data System, software version 2.3. Operating conditions were: carrier gas N₂, flow 1.4 mL min⁻¹; oven temperature program: 60-280° C at 3° C min⁻¹, 280° C (2 min); sample injection port temperature 250° C; detector temperature 280° C; split 1:60.

GC-MS analysis

The analyses by gas chromatography coupled to mass selective detector were performed using a Shimadzu GC-17A gas chromatograph coupled with a GCMS-QP5000 apparatus and CLASS 5000 software with Wiley 139 and NIST computer databases. The data were obtained on a poly (5% phenyl-95% methylsiloxane) fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 μ m), (MDN-5S). Operating conditions were: carrier gas He, flow 1.0 mL min⁻¹; oven temperature program: 60-280° C at 3° C min⁻¹; sample injection port temperature 250° C; detector temperature 260° C; ionization voltage: 70 eV; ionization current 60 μ A; scanning speed 0.5 s over 38-400 amu range; split 1:70.

Identification

The oil components were identified using the retention indices (RI) on DB-5 type column (van den Dool & Kratz, 1963), and by comparison of their mass spectra with those published in the literature (Adams, 2007) or those of the author's database. Integration of the total chromatogram (GC-FID), expressed as area percent, has been used to obtain quantitative compositional data without FID response factor correction.

RESULTS AND DISCUSSION

From the hydrodistilled oils, a total of 100 compounds were identified using GC-FID and GC-MS, accounting for about 90% of the total

composition of the essential oils. The compounds identified in the oils are shown in Table 1 with their

percentage compositions and were listed in order of elution on a MDN-5S column.

Table 1
Percentage composition of the essential oils of *Smallanthus quichensis* leaves

Compound ^a	R.I. ^b	Sample 1 %	Sample 2 %	Identification Method ^c
2-Methyl-butanoic acid	832	-	0.1	1, 2
(3E)-Hexenol	844	-	t	1, 2
(2E)-Hexenal	847	t	-	1, 2
Tricyclene	921	t	t	1, 2
α -Thujene	924	1.8	0.2	1, 2
α -Pinene	935	35.5	64.5	1, 2, 3
α -Fenchene	949	t	t	1, 2
Camphene	951	0.8	0.1	1, 2
Thuja-2,4(10)-diene	954	t	t	1, 2
Sabinene	972	3.4	0.3	1, 2
β -Pinene	978	4.4	1.2	1, 2, 3
6-Methyl-5-hepten-2-one	982	-	t	1, 2
Myrcene	988	1.2	0.1	1, 2
Mesytilene	994	-	t	1, 2
α -Phellandrene	1005	9.0	0.1	1, 2
<i>p</i> -Mentha-1(7),8-diene	1005	0.1	-	1, 2
α -Terpinene	1018	0.1	-	1, 2
<i>p</i> -Cymene	1025	11.5	0.1	1, 2
Limonene	1029	5.8	2.1	1, 2, 3
β -Phellandrene	1030	9.2	-	1, 2
1,8-Cineole	1034	-	9.7	1, 2, 3
(Z)- β -Ocimene	1034	t	-	1, 2
Benzene acetaldehyde	1044	t	-	1, 2
(E)- β -Ocimene	1044	0.1	-	1, 2
γ -Terpinene	1054	0.2	-	1, 2
<i>cis</i> -Linalool oxide (furanoid)	1070	-	0.1	1, 2
<i>trans</i> -Linalool oxide (furanoid)	1086	-	0.1	1, 2
<i>p</i> -Mentha-2,4(8)-diene	1086	0.2	-	1, 2
<i>p</i> -Cymenene	1090	t	0.1	1, 2
Pinene oxide	1098	-	t	1, 2
Linalool	1099	t	1.2	1, 2, 3
Nonanal	1102	0.2	-	1, 2
Endofenchol	1120	-	t	1, 2
<i>cis-p</i> -Menth-2-en-1-ol	1122	t	t	1, 2
α -Campholenal	1127	t	0.1	1, 2
(Z)-Myroxide	1131	t	-	1, 2
<i>cis-p</i> -Menth-2,8-dien-1-ol	1137	-	t	1, 2
<i>trans</i> -Pinocarveol	1141	-	0.4	1, 2
Geijerene	1143	0.3	-	1, 2
<i>trans</i> -Verbenol	1146	t	0.7	1, 2
Camphene hydrate	1147	-	t	1, 2

<i>trans</i> -Pinocamphone	1158	-	t	1, 2
Pinocarvone	1163	-	t	1, 2
δ -Terpineol	1170	-	0.1	1, 2
Borneol	1173	t	0.1	1, 2
<i>p</i> -Mentha-1,5-dien-8-ol	1174	t	-	1, 2
Terpinen-4-ol	1181	0.9	0.1	1, 2, 3
<i>p</i> -Cymen-8-ol	1187	-	0.1	1, 2
Criptone	1186	0.3	-	1, 2
α -Terpineol	1196	0.2	1.4	1, 2
<i>cis</i> -Piperitol	1198	t	-	1, 2
<i>cis</i> -Sabinol	1202	0.1	-	1, 2
Verbenone	1209	t	0.2	1, 2
<i>trans</i> -Piperitol	1211	0.1	-	1, 2
<i>trans</i> -Carveol	1219	t	0.1	1, 2
<i>cis</i> -Carveol	1233	-	t	1, 2
Thymol methyl ether	1230	0.1	-	1, 2
Carvone	1236	-	t	1, 2
Geraniol	1249	0.3	-	1, 2
Pregeijerene B	1263	t	-	1, 2
Cogeijerene	1283	t	-	1, 2
<i>p</i> -Cymen-7-ol	1286	t	-	1, 2
Thymol	1292	0.2	-	1, 2
Carvacrol	1299	0.2	-	1, 2
<i>p</i> -Mentha-1,4-dien-7-ol	1321	-	t	1, 2
Myrtenyl acetate	1325	-	t	1, 2
7- <i>epi</i> -Silphiperfol-5-ene	1343	0.2	-	1, 2
β -Elemene	1387	0.2	-	1, 2
Decyl acetate	1410	0.1	-	1, 2
β -Funebrene	1413	-	t	1, 2
β -Caryophyllene	1418	0.5	0.5	1, 2, 3
Dictamnol	1433	0.5	-	1, 2
Aromadendrene	1437	-	0.1	1, 2
α -Humulene	1455	0.1	0.9	1, 2
<i>Allo</i> -Aromadendrene	1459	-	t	1, 2
Germacrene D	1479	0.5	-	1, 2
Viridiflorene	1488	-	t	1, 2
α -Muurolene	1500	-	t	1, 2
γ -Cadinene	1512	-	t	1, 2
<i>trans</i> -Calamene	1522	-	0.1	1, 2
α -Calacorene	1538	-	t	1, 2
Hedycaryol	1548	0.1	-	1, 2
Germacrene B	1557	0.1	-	1, 2
(<i>E</i>)-Nerolidol	1559	-	0.2	1, 2
Maaliol	1567	-	t	1, 2
Spathulenol	1574	t	0.9	1, 2
Caryophyllene oxide	1579	0.3	2.0	1, 2
Globulol	1589	-	t	1, 2
Viridiflorol	1594	-	0.1	1, 2
Humulene oxide	1606	-	2.0	1, 2
<i>epi</i> - α -Cadinol	1639	-	t	1, 2

α -Eudesmol	1654	0.3	-	1, 2
α -Cadinol	1654	-	t	1, 2
<i>cis</i> -Calamenen-10-ol	1663	-	0.1	1, 2
Cadalene	1669	-	0.2	1, 2
Eudesma-4(15),7-dien-1 β -ol	1685	0.1	-	1, 2
10- <i>nor</i> -Calamenen-10-one	1700	-	0.2	1, 2
(2 <i>E</i> ,6 <i>Z</i>)-Farnesol	1715	-	t	1, 2
Benzylbenzoate	1762	-	t	1, 2
Baেকেol	1868	-	t	1, 2
Compound classes				
Monoterpene hydrocarbons		83.3	68.8	
Oxygenated monoterpenes		2.4	14.4	
Sesquiterpene hydrocarbons		1.9	1.8	
Oxygenated sesquiterpenes		1.3	5.5	
Others		0.3	0.1	
Total Oil Composition		89.2	90.6	

a Compounds listed in order of elution from poly(5% phenyl-95% methylsiloxane) column.

b RI= Retention index relative to a homologous series of n-alkanes.

c Method: 1 = Retention index on poly(5% phenyl-95% methylsiloxane) column; 2 = MS spectra; 3 = Standard; t = traces (< 0.05%)

Smallanthus quichensis leaves gave oils which were predominantly terpenoid in nature (composed mainly of monoterpenoids, accounting *ca.* 85%). The leaf oil of sample 1 was rich in α -pinene (64.5%) and 1,8-cineole (9.7%). Other monoterpenoids were not particularly abundant, with the most prominent members being limonene (2.1%), α -terpineol (1.4%), β -pinene (1.2%) and linalool (1.2%). The composition of the oil from sample 2 was dominated also by α -pinene (35.5%) with moderate amounts of *p*-cymene (11.5%), β -phellandrene (9.2%), α -phellandrene (9.0%), limonene (5.8%), β -pinene (4.4%) and sabinene (3.4%). This sample was richer in monoterpene hydrocarbons (83.3%) than sample 1 (68.8%). The sample 1 presented more quantity of oxygenated compounds (14.4% of monoterpenoids and 5.5% of sesquiterpenoids). The oil of sample 2 contained only 1.9% of sesquiterpene hydrocarbons and 0.8% of the sesquiterpene-derivatives dictamnol (0.5%), geijerene (0.3%) and traces of pregeijerene B and cogeijerene that are not present in sample 1.

Among the constituents identified α -pinene was found to be the major component (35.5-64.5%). Significant amounts of 1,8-cineole (9.7%) or β -phellandrene (9.2%), α -phellandrene (9.0%), limonene (5.8%), β -pinene (4.4%) and sabinene (3.4%) were also found. The oil of *S. maculatus*

studied previously (Ciccío, 2004) was also of terpenoid character with α -pinene (32.9%) as major constituent, accompanied by β -pinene (7.1%), camphene (5.4%), sabinene (4.8%) and limonene (2.9%). Unlike *S. quichensis*, this had a higher proportion of the sesquiterpene hydrocarbons germacrene D (13.7%), β -caryophyllene (10.7%) and bicyclgermacrene (6.6%).

CONCLUSION

From these results, it may be concluded that the leaves of *S. quichensis* produce terpenoid-rich essential oils and the composition of both investigated samples was dominated by α -pinene. The previous studied *S. maculatus* leaf essential oil was terpenoid in nature and also α -pinene was the main constituent (Ciccío, 2004). Despite the fact that the oils presented some qualitative differences in composition, it could be said that they have a similar terpenoid nature. These findings suggest that there may be a close relationship between both species of plants, which seems to agree with the results of molecular phylogenetic study conducted by Rauscher (2002), who proposed one lineage which includes *S. meridensis* and *S. riparius* from northwestern South America and *S. maculatus* and *S. quichensis* from Central America. However, the lack of compositional

data from other species makes, at this point, any conclusion impossible.

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