

Volatile compounds of grosella (*Phyllanthus acidus* [L.] Skeels) fruit

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Key words: Tahitian gooseberry, volatile compounds, GC-MS, epi- α -muurolol, α -cadinol, hexadecanoic acid.

RESUMEN. *Phyllanthus acidus* (L.) Skeels es un árbol tropical pequeño, que alcanza hasta 12 m, que produce frutas grasosas de color amarillo claro en abundancia en las ramas, los que son comestibles y de sabor ácido. Esta especie es uno de los pocos miembros de la familia Euphorbiaceae que produce frutas comestibles. La pulpa ácida es usada como saborizante y para hacer jaleas. Los constituyentes volátiles de la fruta madura se aislaron por destilación-extracción simultáneas utilizando éter dietílico como disolvente y se analizaron por cromatografía de gases con detector de llama de hidrógeno y cromatografía de gases acoplada a espectrometría de masas. Se identificaron 77 compuestos volátiles agrupados en: 45 terpenos, 18 ésteres, siete ácidos, cuatro aldehídos, dos fenoles y un alcohol, de los cuales 66 se encontraron por primera vez en la grosella. La concentración total de compuestos volátiles fue de 109 mg/kg de fruta fresca. De acuerdo con las clases de compuestos, los terpenos (100,1 mg/kg) y los ácidos (6,7 mg/kg) dominaron el perfil cuantitativo en las frutas, mientras que los terpenos y los ésteres lo hicieron cualitativamente. En el grupo de los terpenos se identificaron varios monoterpenos y sesquiterpenos, siendo los más representativos el epi- α -muurolol (32,9 mg/kg) y el α -cadinol (22,1 mg/kg). El ácido hexadecanoico (3,8 mg/kg) fue el compuesto mayoritario en los ácidos.

ABSTRACT. *Phyllanthus acidus* (L.) Skeels is a small tropical tree that grows up to 12 m tall, which bears pale-yellow waxy fruits in abundance on the branches, which are edible and tastes sour; it is one of the few members of the Euphorbiaceae family that has edible fruit. The tart flesh is used as a flavoring or made into jelly. Volatile constituents from the ripe fruit were isolated by simultaneous distillation-solvent extraction with diethyl ether and analyzed by gas chromatography with flame ionization detector and gas chromatography-mass spectrometry. Seventy-seven volatiles were identified: 45 terpenes, 18 esters, seven acids, four aldehydes, two phenols, and one alcohol, of which 66 were found for the first time in grosella. The total concentration of volatiles was ca 109 mg per kg of fresh fruit. According to the class of compounds, terpenes (100.1 mg/kg) and acids (6.7 mg/kg) dominated the quantitative profile in the fruits, whereas qualitatively terpenes and esters were the most representative. In the terpene group, many monoterpenes and sesquiterpenes were identified, with the major representatives being epi- α -muurolol (32.9 mg/kg) and α -cadinol (22.1 mg/kg). Hexadecanoic acid (3.8 mg/kg) was the major compound among the acids.

INTRODUCTION

Phyllanthus acidus (L.) Skeels is a small tropical tree with ovate pointed leaves that grows up to 12 m tall, commonly named grosella (in Cuba) or Tahitian gooseberry (in USA). Probably native of Brazil, but introduced into tropical Asia at an early date and now widespread. Grosella bears fruits in abundance on the branches. It has reddish flowers and pale-yellow waxy fruits, 1.5 to 2.5 cm thick and is 6 to 8 ridged, in the center is a hard stone containing 4 to 6 seeds. The yellow fruit of the

grosella fruit is edible and tastes sour; it is one of the few members of the Euphorbiaceae family that has edible fruit. The tart flesh is used as a flavoring or made into jelly.¹ Despite its characteristic unique aroma, there was no report on its aroma constituents until now, and only it was reported the composition of the fermented fruit.²

This study reports the isolation, characterization and quantification of the major volatile compounds associated with grosella fruit.

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MATERIALS AND METHODS**Fruits**

Fresh ripe fruits (1 kg) were picked from the same bushes grown in Güira de Melena, Havana province. Identification of the fruit was done by Dr Víctor Fuentes (Instituto de Investigaciones de Fruticultura Tropical, Havana). The isolation of volatile compounds was concluded within 24 h after harvest. Fruit pulp was prepared in a commercial blender for analysis.

Isolation of volatile compounds

The simultaneous distillation-extraction method was used.³⁻⁵ After addition of internal standard (methyl nonanoate, 1 mg), pulp (200 g) was blended with distilled water (600 mL) and simultaneously distilled and extracted for 60 min in a Likens-Nickerson microapparatus with 25 mL of diethyl ether P.A. (previously redistilled and checked as to purity). The volatile concentrate was dried over anhydrous sulfate and concentrated to 0.6 mL with a Vigreux column and then, to 0.1 mL with a gentle nitrogen stream. The concentrated extract possessed the typical aroma, indistinguishable from that associated with ripe grosella.

Gas chromatography (GC-FID)

A Konik 4000A GC equipped with a 30 m X 0.25 mm X 0.25 mm HP-5MS fused-silica capillary column and a flame ionization detector (FID) was used. Injector and detector temperatures were both 250 °C. Oven temperature was held at 50 °C for 2 min and then raised to 250 °C at 4 °C/min and held for 10 min. Carrier gas (hydrogen) flow rate was 1 mL/min. Injector and detector temperatures were 250 °C. Linear retention indices (LRI) were calculated against those of n-paraffins. Quantitative data were obtained from the electronic integration of the GC peak areas (EZChrom V. 6.7 software) with the use of the internal standard method, neglecting FID response factors.

Gas chromatography-mass spectrometry (GC-MS)

Analysis was performed on a Hewlett-Packard series 6890N gas chromatograph equipped with a 5973 mass-selective detector. The chromatographic conditions were the same as those described for GC (FID). Carrier gas (helium) flow rate was 1 mL/min. The detector operated in impact electron mode (70 eV) at 230 °C. Detection was performed in the scan mode between 30 and 400 Da. Constituents were identified by comparison of their mass spectra with those in NBS, NIST or authors' FLAVORLIB data base and confirmed in many compounds by their relative retention indices. Mass spectra from the literature were also compared.^{6,7}

RESULTS AND DISCUSSION

The volatile compounds of grosella fruit were obtained by simultaneous distillation-solvent extraction and analyzed by GC-FID and GC-MS. The concentrated extracts showed aroma notes resembling the flavor of fresh fruit, described as sour.

Quantification was based on GC-FID peak integration data, so accuracy is potentially limited by a number of factors, including coelution of two or more components and differences in FID response factors among compounds. In total *ca.* 109 mg of volatile compounds per kilogram of fruit was obtained (Table 1).

Overall, 77 volatile compounds were identified: 45 terpenes, 18 esters, seven acids, four aldehydes, two phenols, and one alcohol (Table 1). Sixty-six of them are reported for the first time in this fruit.

Table 1. Volatile compounds quantified in grosella fruit.

Compound	LRI	Concentration (mg/kg)
hexanal	802	< 0.1
(E)-2-hexenal	855	< 0.1
(Z)-3-hexenol	859	0.2
α -thujene ¹	929	< 0.1
myrcene ¹	991	< 0.1
butyl butyrate ¹	995	< 0.1
ethyl hexanoate ¹	998	< 0.1
(Z)-3-hexenyl acetate	1005	< 0.1
<i>p</i> -cymene ¹	1026	0.1
limonene ¹	1029	3.6
β -phellandrene ¹	1031	< 0.1
<i>trans</i> -linalool oxide (furanoid) ¹	1073	< 0.1
terpinolene ¹	1089	< 0.1
<i>p</i> -cymenene ¹	1091	< 0.1
methyl benzoate	1092	< 0.1
ethyl heptanoate ¹	1097	< 0.1
nonanal ¹	1102	0.2
methyl octanoate ¹	1127	0.1
camphor	1146	< 0.1
hexyl isobutyrate ¹	1153	< 0.1
α -terpineol	1189	< 0.1
ethyl octanoate	1195	0.6
methyl chavicol ¹	1196	< 0.1
decanal ¹	1202	< 0.1
<i>trans</i> -carveol ¹	1217	< 0.1
<i>cis</i> -carveol ¹	1230	< 0.1
carvone ¹	1243	0.1
piperitone ¹	1253	< 0.1
nonanoic acid ¹	1280	0.1
isobornyl acetate ¹	1286	< 0.1
ethyl nonanoate	1288	0.1
thymol ¹	1290	< 0.1
benzyl butyrate ¹	1345	0.1
α -terpinyl acetate ¹	1349	< 0.1
cyclosativene ¹	1371	< 0.1
α -copaene ¹	1378	5.2
β -bourbonene ¹	1387	0.6
β -cubebene ¹	1388	0.1
benzyl isopentanoate ¹	1392	< 0.1
ethyl decanoate ¹	1396	< 0.1
α -gurjunene ¹	1410	< 0.1
β -caryophyllene ¹	1419	0.9
β -copaene ¹	1432	0.3
aromadendrene ¹	1440	0.1
α -humulene ¹	1455	0.2
allo-aromadendrene ¹	1461	0.1
9-epi-(E)-caryophyllene ¹	1467	2.3
γ -muurolene ¹	1480	0.7
α -amorphene ¹	1485	< 0.1
germacrene D ¹	1486	5.5
β -selinene ¹	1491	0.2
γ -amorphene ¹	1497	1.2
α -muurolene ¹	1501	5.5
δ -amorphene ¹	1511	0.4
γ -cadinene ¹	1514	1.0
δ -cadinene ¹	1523	6.8
<i>trans</i> -cadina-1(2),4-diene ¹	1535	0.3
α -cadinene ¹	1540	0.5
α -calacorene ¹	1546	2.9
β -calacorene ¹	1566	0.8
dodecanoic acid ¹	1567	< 0.1
ledol ¹	1570	0.5
caryophyllenyl alcohol ¹	1572	0.2
epi- α -muurolol ¹	1642	32.9
α -cadinol	1654	22.1
cadalene ¹	1678	5.0
tetradecanoic acid ¹	1767	0.8
ethyl tetradecanoate ¹	1793	< 0.1
isopropyl tetradecanoate ¹	1830	0.2
pentadecanoic acid ¹	1864	0.2

(To be continued on following page.)

Table 1. (Continued.)

Compound	LRI	Concentration (mg/kg)
benzyl salicylate ¹	1866	0.2
hexadecanoic acid ¹	1966	3.8
ethyl hexadecanoate	1992	0.1
methyl octadecanoate ¹	2123	< 0.1
oleic acid ¹	2141	1.3
octadecanoic acid ¹	2166	0.5
ethyl octadecanoate ¹	2196	< 0.1

LRI Lineal retention index in HP-5MS.

¹ Reported for the first time in this fruit.

According to the class of compounds, terpenes (100.1 mg/kg) and acids (6.7 mg/kg) dominated the quantitative profile in the fruit. In the terpene group, many monoterpenes and sesquiterpenes were identified, with the major representatives being epi- α -muurolol (32.9 mg/kg) and α -cadinol (22.1 mg/kg). Among the acids, hexadecanoic acid (3.8 mg/kg) was the major compound.

Comparing the results of the present work with the reported composition of fermented Colombian grosella,² some qualitative and quantitative differences were found, which could be attributed to a different cultivar, ripeness of the fruit or the isolation method (in the first report liquid-liquid extraction was used).

CONCLUSIONS

Seventy-seven compounds were identified in the present study, 66 of them reported for the first time as volatile constituents of grosella fruit. Among the identified compounds, terpenes and acids were quantitatively the most important groups, whereas qualitatively terpenes and esters were the most representative.

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