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SHORT COMMUNICATION

Chemical composition of essential oils of shells, juice and seeds of *Passiflora ligularis* Juss from Ecuador

Iván Andrés Chóez Guaranda[1*], Diana Alejandra Herrera Hurtado[1], Migdalia Miranda Martínez[1], Patricia Isabel Manzano Santana[1,2]

[1]Escuela Superior Politécnica del Litoral (ESPOL), Centro de Investigaciones Biotecnológicas del Ecuador (CIBE), Guayaquil, Ecuador,
[2]Escuela Superior Politécnica del Litoral (ESPOL), Facultad de Ciencias Naturales y Matemáticas (FCNM), Guayaquil, Ecuador

ABSTRAT

The objective of this research was to analyze the chemical composition of essential oil of shell, juice and seeds of *Passiflora ligularis* Juss or granadilla from Ecuador. The essential oils were obtained by microwave assisted hydrodistillation (MWHD) extraction method using Clevenger apparatus and were analyzed by gas chromatography mass spectrometry (GC-MS). Fifty compounds were identified altogether. The majority compounds were the squalene (34.92%), pentadecanal (15.28%) and ionol (19.16%) in shells, juice and seeds respectively. However, aromatic compounds, sesquiterpenes, alcohols, aldehydes and hydrocarbons were detected as minority compounds with potential utility in food and pharma industry. These results have not been reported and allow adding value to this kind of agricultural waste.

Keywords: Essential oil; GC-MS; Granadilla; MWHD; *Passiflora ligularis* Juss

INTRODUCTION

Passiflora ligularis Juss is a species of passion flowers known in America by the name of Granadilla (Lindley et al., 1830), is a climbing plant belonging to the family Passifloracea and it is native from Peru (Curtis et al., 1830). Studies have argued that seed extracts of some *Passiflora* species possess antifungal activity, antioxidant activity (Shanmugam et al., 2013) and antibacterial activity (Patil, 2010). In fact, an active substance called passicol obtained from shell extracts of *Passiflora mollissima* and *Passiflora caerulea* was reported by (Birner et al., 1973). Even, aqueous extract of *Passiflora ligularis* fruit can decrease the blood glucose and reduce the oxidative stress by removing free radicals in diabetes (Palanirajan et al., 2014)

Currently there are no studies about the chemical composition of essential oil of granadilla. However it is known that the essential oil of the flower of this fruit is used as an insecticide but is not suitable for human consumption due to toxicity thereof (Cabrera, 2006). For this reason, the objective of this study was to characterize by GC-MS the essential oil of shells, juice and seeds of ecuadorian *Passiflora ligularis* Juss in order to provide added value to this fruit waste and improve the production chain.

MATERIALS AND METHODS

Plant material

Fresh plant material was collected on a food market in the city of Guayaquil. The plant material was washed and went through by different separation processes to obtain shells, juice and seeds.

Microwave assisted hydrodistillation (MWHD)

The procedure used for the extraction of essential oils of shells, juice and seeds of *Passiflora ligularis* Juss was adapted from the method described by (Rodríguez et al., 2012). Microwave assisted hydrodistillation system with Clevenger apparatus was used for extraction. Fresh plant material with water was distilled at 100°C and atmospheric pressure for four consecutive cycles of 10 minutes. The essential oils were separated with diethyl ether, were dried with anhydrous sodium sulfate, were

***Corresponding author:**
Iván Andrés Chóez Guaranda,Escuela Superior Politécnica del Litoral (ESPOL), Centro de Investigaciones Biotecnológicas del Ecuador (CIBE), Km 30.5 Vía Perimetral, Guayaquil, Ecuador. E-mail: iachoez@espol.edu.ec, ivanchoezg@outlook.com

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weighed and stored in amber vials before the GC-MS analysis. This process was performed in triplicate for each sample.

Gas chromatography mass spectrometry (GC-MS)

The analysis of essential oils were performed by a gas chromatography mass spectrometry equipment Agilent Technologies (7890A GC system and 5975C inert XL MSD with triple axis detector). A capillary column HP-5MS (30 m × 0.25 mm) with phenyl dimethylpolysiloxane was used as stationary phase (0.25 micron film thickness) and helium as the carrier gas (1 mL/min). The injection of 0.5 μL of sample diluted in diethyl ether was done at a temperature of 250°C with splitless mode, the detector temperature was 280°C, the oven temperature was maintained at 50°C for 0.5 minutes, then increased to 250°C at 4°C/min. Compounds were identified by comparison of their mass spectra and mass reference of Nist 2011 MS Library.

Statistical analysis

The results of yield of each sample were subjected to analysis of variance and Tukey test for comparison of average values between samples with 5% significance, using InfoStat statistical software, version 2008.

RESULTS AND DISCUSSION

Table 1 shows the yield percents of essential oils of shells, juice and seeds of granadilla. These results have not been referenced, seed yield (0.0328 %) proved to be the highest in comparison with juice (0.00048 %) and shell (0.00047 %).

The chromatograms of Fig. 1 shows the chromatographic profile of essential oil of shells (a), juice (b) and seeds (c) of granadilla.

Table 2 lists the components identified in the essential oil of granadilla's shells. 22 chemical compounds were determined: 1 aromatic compound, 3 fatty alcohols, 1 fatty aldehyde, 2 esterified fatty acids and 15 hydrocarbons. Hydrocarbons, fatty alcohols and fatty aldehydes are components of waxes (Pond et al., 2005), which are substances deposit in the cell wall of the fruit to prevent excessive water loss (Zeraik et al., 2010).

Table 1. Percent of yield of essential oils of shells, juice and seeds of *Passiflora ligularis* Juss

Sample	% of yield*
Shells	0.00047±0.00036 B
Juice	0.00048±0.00021 B
Seeds	0.0328±0.016 A

*Average values (n=3) ± standart deviation. Different letters indicate significant differences between samples according to Tukey test (p>0.05)

As a characteristic flavor component in shells only was found the ionol (10.58%). Nonetheless, the major component was squalene (34.92 %), lipid compound with potential use in the cosmetic area as a humectant and in the pharmaceutical industry as immunological adjuvant in vaccines (Smith, 2000).

Table 3 shows the components identified in the essential oil of granadilla's juice. 20 chemical compounds were determined: 4 aromatic compounds, 1 sesquiterpene, 3 hydrocarbons, 5 aldehydes, 1 alcohol, 4 esterified fatty acids and 2 fatty acids in the free state.

The pentadecanal was the majority component (15.28%), followed by ionol (12.93%) and nerolidol (7.75%). The

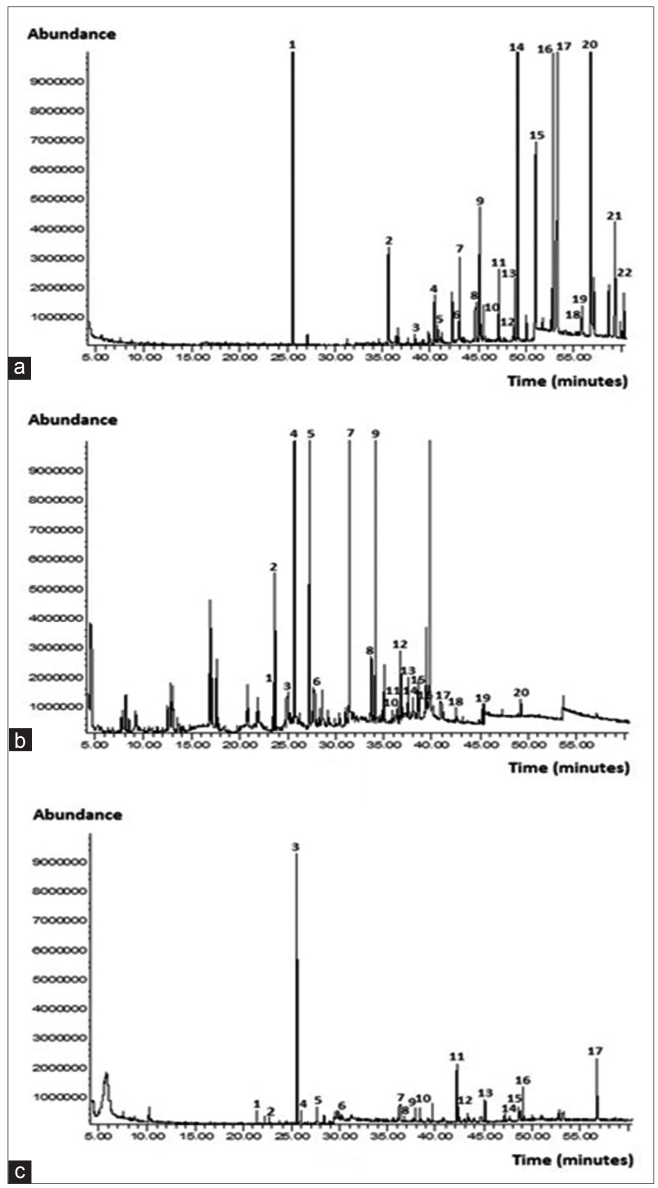

Fig 1. Chromatographic profiles of essential oil from shells (a), juice (b) and seeds (c) of *Passiflora ligularis* Juss obtained by GC-MS.

latter compound is characterized as a sesquiterpene present in essential oils of various plants, is approved by the Food and Drug Administration of the United States as a food flavoring agent. It also has antitumor activity (Wattenberg, 1991), antimalarial activity (Lopes et al., 1999), antileishmanial activity (Arruda, 2005) and has been evaluated as a penetration enhancer in the skin for transdermal administering of therapeutic drugs (Cornwell et al., 1994; Yamane et al., 1995).

Table 2. Chemical composition of essential oil of shells of *Passiflora ligularis* Juss

Peak	Retention time (min)	Compound	Peak area %
1	25.62	Ionol	10.58
2	36.53	Octadecanal	0.30
3	38.37	Hexadecanoic acid ethyl ester	0.18
4	40.42	1-Octadecanol	0.99
5	40.80	Heneicosane	0.31
6	42.91	Octadecanoico acid ethyl ester	0.16
7	42.99	1-Octadecene	1.82
8	44.71	9-Tricosene	0.63
9	45.13	Tricosane	2.29
10	47.07	9-Nonadecene	0.49
11	47.17	Docosane	1.36
12	48.63	Z- 12- Pentacosene	0.24
13	48.78	1- Heneicosanol	1.15
14	49.14	Hexadecane	6.21
15	51.05	n-Hexacosane	4.02
16	52.83	1-Docosanol	6.05
17	53.23	Heptacosane	11.41
18	55.80	1- Nonadecene	0.37
19	55.92	Eicosane	0.96
20	56.87	Squalene	34.92
21	59.28	Nonacosane	1.14
22	59.32	Tetracosane	0.45

Table 3. Chemical composition of essential oil of juice of *Passiflora ligularis* Juss

Peak	Retention time (min)	Compound	Peak area %
1	23.49	Dihydro-β-ionone	0.78
2	23.71	Dihydro-β-ionol	2.47
3	24.95	Trans-β-ionone	0.82
4	25.77	Ionol	12.93
5	27.26	Nerolidol	7.75
6	27.75	Octanoic acid hexyl ester	0.99
7	31.45	Pentadecanal	15.28
8	33.68	10,12-Hexadecadienal	1.06
9	34.13	Hexadecanal	5.19
10	35.93	9,12 Octadecadienoic acid	0.30
11	36.42	9,12 Octadecadien-1-ol	0.33
12	36.68	Tetradecanal	1.17
13	36.88	Hexadecanoic acid methyl ester	0.74
14	38.08	9,12 Hexadecadienoic methyl ester	0.36
15	38.54	9,12,15-Octadecatrienoic acid	0.72
16	38.63	Cis-9-Hexadecenal	0.77
17	40.94	Heneicosane	0.39
18	42.48	Ethyl 9,12,15- octadecatrienoate	0.24
19	45.26	Eicosane	0.34
20	49.26	Heptadecane	0.33

Table 4 lists the components identified in the essential oil of granadilla's seeds. 17 chemical compounds were determined: 1 aromatic compound, 5 sesquiterpenes, 4 hydrocarbons, 6 esterified fatty acids and 1 fatty acid in the free state.

Despite the presence of fatty acids in seeds as linoleic acid ethyl ester (4.15%), stand out the presence of ionol (19.16%) as most abundant compound; and the sesquiterpenes: α-cubebene (0.95%), trans-caryophyllene (0.57%), cis-calamenene (0.77%), caryophyllene oxide (1.78%) and cadalene (0.39 %) but with low area percents.

The trans-caryophyllene is used as a preservative in foods, drugs and cosmetics. Furthermore, it has been proven its bioactivity in vitro against dermatophytes because it has antifungal properties, this biological activity it has been compared with ciclopiroxolamine and sulconazole, compounds commonly used in the treatment of onychomycosis (Yang et al., 1999).

Is of importance to mention that ionol was identified in the three parts of the fruit, but in juice were found three other derivatives of this compound: Dihydro-β-ionone (0.78%), dihydro-β-ionol (2.47%) and trans-β-ionone (0.82%). These results agree with those reported by (Winterhalter, 1990) and (Hederich et al., 1991); who note that compounds are important in the aromas of fruits, besides being precursors of edulans (C13-norisoprenoids), important aroma components of *Passiflora edulis* and *Passiflora* in general. A

Table 4. Chemical composition of essential oil of seeds of *Passiflora ligularis* Juss

Peak	Retention time (min)	Compound	Peak area %
1	21.36	alpha-Cubebene	0.95
2	22.74	trans-Caryophyllene	0.57
3	25.59	Ionol	19.16
4	25.92	cis-Calamenene	0.77
5	27.67	Caryophyllene oxide	1.78
6	30.26	Cadalene	0.39
7	36.25	9,12-Hexadecadienoic methyl ester	1.47
8	36.71	Hexadecanoic acid methyl ester	0.40
9	37.91	9,12-Octadecadienoic acid	0.98
10	38.35	Hexadecanoic acid ethyl ester	1.12
11	42.16	Linoleic acid ethyl ester	4.15
12	42.30	Ethyl oleate	1.37
13	45.11	Eicosane	1.75
14	48.54	Methyl 9-cis, 11-trans-octadecadienoate	1.18
15	48.76	9-Nonadecene	1.01
16	49.11	Pentacosane	2.63
17	56.78	Squalene	7.69

compound present just in the waste of the fruit was the ethyl palmitate (hexadecanoic acid ethyl ester). Indeed, the seeds had the greater area percent of the compound (1.12%).

CONCLUSIONS

The best yield was found in essential oil of seeds (0.0328%), this is due to the majority presence of sesquiterpenes and fatty acids unlike the juice (0.00048%) and shells (0.00047%). 50 compounds were identified altogether, 22 correspond to shells, 15 to juice and 13 to seeds. The majority compounds were squalene (34.92%), pentadecanal (15.28%) and ionol (19.16%) in shells, juice and seeds respectively. However, aromatic compounds, alcohols, aldehydes and hydrocarbons were found as minor components with potential utility in the food and pharmaceutical industries.

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Author contributions

I. A. C. G. and M. M. M. designed, conducted the work and wrote the paper, D. A. H. H. made the experimental work and P. I. M. S. made a major contribution to the review paper.

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