CARACTERIZAÇÃO DE COMPOSTOS VOLÁTEIS E SEMIVOLÁTEIS DOS ÓLEOS DOS FRUTOS DE *Solanum lycocarpum* POR CROMATOGRAFIA GASOSA-ESPECTROMETRIA DE MASSAS

CHARACTERISATION OF VOLATILE AND SEMI-VOLATILE COMPOUNDS OF OILS FROM *Solanum lycocarpum* FRUITS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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RESUMO

Os óleos foram obtidos a partir de frutos verdes e maduros de *Solanum lycocarpum*. Neste estudo, os compostos voláteis e semivoláteis dos óleos foram caracterizados por cromatografia gasosa-espectrometria de massa (GC-MS). O óleo dos frutos verdes de *S. lycocarpum* exibiu principalmente ácidos graxos, ésteres, hidrocarbonetos e esteróis. No entanto, o óleo dos frutos maduros apresentou como principais compostos ésteres e esteróis. Em OUF-7, o composto predominante foi ácido octadecanoico (73,37%), em ORF-2, o octadecanoato de octadecila (59,30%) e em ORF-3, o hexadecanosato de hexadecila. O sitosterol, identificado em OUF-2 e OUF-6, é o principal esteroide encontrado em várias espécies de *Solanum* podendo ter importância quimiotaxonômica para este gênero. Esta é a primeira vez que, ao nosso melhor conhecimento, caracterizaram-se os compostos voláteis e semivoláteis de óleos dos frutos de *S. lycocarpum*.

Palavras-chave: *Solanum lycocarpum*, compostos voláteis, compostos semi-voláteis, frutos, GC-MS.

ABSTRACT

Oils were obtained from unripe and ripe fruits of *Solanum lycocarpum*. In this study, the volatile and semi-volatile compounds of oils were characterized by gas chromatography-mass spectrometry (GC-MS). Oil of unripe fruits (OUF) of *S. lycocarpum* exhibited principally fatty acids, esters, hydrocarbons and sterols. However, oil of ripe fruits (ORF) presented the major compounds esters and sterols. In OUF-7, the predominant compound was octadecanoic acid (73.37%), in ORF-2, octadecanoic acid octadecyl ester (59.30%) and in ORF-3, hexadecanoic acid hexadecyl ester (97.98%). Sitosterol, identified in OUF-2 and OUF-6, is predominant sterol found in various species of *Solanum* can have chemotaxonomy importance for this genus. This is the first time, to the best of our knowledge, that volatile and semi-volatile compounds of oils from *S. lycocarpum* fruits have been characterized.

Keywords: *Solanum lycocarpum*, volatile compounds, semi-volatile compounds, fruits, GC-MS.

INTRODUCTION

The Solanaceae family comprises about 150 genera and 3000 species, distributed predominantly in tropical and subtropical regions of South America, and several species of the *Solanum* genus are cultivated for food, such as eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*), gilo (*Solanum gilo*) and potato (*Solanum tuberosum*) (Souza and Lorenzi, 2005).

The species *Solanum lycocarpum* A. St. Hil., popularly known as the 'fruit of the wolf' or 'apple wolf', is distributed in a savannah stretching across more than 1.2 million square miles (Brazilian Cerrado) (Vieira *et al.*, 2003). The ripe fruits are prepared in jellies, jams or pasta or consumed '*in natura*' (Clerici and Carvalho-Silva, 2011). In traditional medicine, this species is widely used as a sedative, in the treatment of epilepsy, asthma, diabetes and obesity, and for the reduction of cholesterol levels and abdominal and renal pain (Munari *et al.*, 2012).

Several classes of secondary metabolites with interesting biological activities, such as anti-inflammatory, antioxidant, anti-tumour, cytotoxic, genotoxic, antibacterial, allelopathic and larvicidal (Chiavegatto *et al.*, 2017; Costa *et al.*, 2015; Morais *et al.*, 2013, 2015, 2017; Pereira *et al.*, 2014; Silva *et al.*, 2015) have been identified in this species. Phytochemical investigations demonstrated that the solasonine and solamargine (glycoalkaloids) are the two predominant compounds in *S. lycocarpum* (Miranda *et al.*, 2013). Recent studies also reported the presence of caffeic and chlorogenic acids in ripe fruits (Morais *et al.*, 2015), apigenin and kaempferol in the leaves (Costa *et al.*, 2015) and fatty acids in ripe and unripe fruits (Silva *et al.*, 2015).

Aiming to contribute to the phytochemical study of this species, the present work showed the characterization of volatile and semi-volatile compounds of oils from the fruits of *Solanum lycocarpum* by Gas Chromatography-Mass Spectrometry (GC-MS).

MATERIALS AND METHODS

General

Silica gel 230-400 mesh from Sorbline (Darmstadt, Brazil) was used for column chromatography, and silica gel 60G F₂₅₄ Sorbline (Darmstadt, Brazil) was used for thin-layer

chromatography. All PA solvents used were purchased from Vetec (Brazil).

Plant material

The fruits of *S. lycocarpum* A. St. Hil. were collected in São Sebastião do Oeste, Minas Gerais, Brazil, in August 2011. The species was identified by Dr. Alexandre Salino and a voucher specimen (BHCB 159397) was deposited at the Instituto de Ciências Biológicas Herbarium, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Extraction and fractionation

Samples of the dried and powdered unripe (170.01 g) and ripe (250.58 g) fruit were subjected to oil extraction using a Soxhlet extractor with petroleum ether as the solvent (700 mL, 6 h). The extracted oils were concentrated in a rotary evaporator at 50 °C under reduced pressure to produce 26.95 g of oil of unripe fruit (OUF) and 29.09 g of oil of ripe fruit (ORF) (Silva *et al.*, 2015).

The oil of unripe fruit, OUF (1.0 g), was chromatographed over silica gel, employing gradient elution of hexane/dichloromethane/methanol with increasing polarities. Fourteen groups of fractions were obtained; G-2 (403 mg) and G-3 (99 mg) were chromatographed on silica gel column, with hexane/dichloromethane, as eluents in mixtures of increasing polarity, obtained **OUF-1** (15 mg), **OUF-2** (20 mg), **OUF-3** (9 mg) and **OUF-4** (12 mg). G-4 (360 mg) was fractionated over silica gel column (eluents: hexane and dichloromethane, with increasing polarities), obtained nine subgroups. Subgroup 8 (275 mg) was submitted to a preparative-layer chromatography (dichloromethane/methanol 5:0.2), leading to 15.1 mg of **OUF-5**, 8.2 mg of **OUF-6** and 35 mg of **OUF-7**.

The oil of ripe fruit, ORF, (1.0 g) was fractionated on silica gel column (eluents: hexane, dichloromethane and methanol, with increasing polarities) and eleven group of fractions were obtained. G-5 (205 mg), G-8 (128 mg) and G-9 (75 mg) were chromatographed over silica gel, obtained **ORF-1** (13 mg), **ORF-2** (8 mg) and **ORF-3** (10 mg), respectively.

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) of the samples was performed on a

Shimadzu model QP5050A instrument equipped with a DB-5 column (30 m x 0.25 mm, 0.25 μ m). The initial temperature of the column was 80 °C for 1 min, which was then increased at a rate of 7 °C/min to 300 °C and held for 5 min; the injector temperature was kept at 250 °C (split 1:20), and the detector temperature was kept at 260 °C. Helium (He) was used as the carrier gas, with a linear flow-rate of 39.3 mL/min (115.4 Kpa). For each analysis, 1 μ L of the sample was injected into the GC. The scan range was from 50 to 600 *m/z* at a scan rate of 2 scans/s. The solvent delay was 2.5 min. The compounds were identified by a mass spectral database search by using the *National Institute of Standards and Technology* (NIST) 2.0 database, followed by matching of MS data and expressed as relative percentages of each compound, calculated by internal normalization of the chromatographic peak area. All volatile compounds showing mass spectra with match factors $\geq$ 95% were put on a *positive list* of tentatively identified metabolites.

RESULTS

Oil of unripe fruits of *S. lycocarpum* exhibited especially fatty acids, esters, hydrocarbons and sterols. Oil of ripe fruits showed as the major compounds esters and sterols. In OUF-3, the main compounds identified were hydrocarbons; in OUF-6, sterols; in OUF-7, fatty acids; and in ORF-3, esters (**Figure 1**). Three fractions presented predominant compounds representing more than 50% of the samples. In OUF-7, the majority compound was octadecanoic acid with 73.37% (fatty acid); in ORF-2 was octadecanoic acid octadecyl ester with 59.30% (ester); and in ORF-3 it was hexadecanoic acid hexadecyl ester with 97.98% (ester) (**Figure 2**).

Other compounds also found in the fractions in large percentages were: hexadecane (31.31%) and docosene (28.01%) in OUF-3; hexadecanoic acid methyl ester (31.30%) in OUF-5; sitosterol (45.27%) in OUF-6 and hexadecanoic acid octadecyl ester (36.29%) in ORF-1. However, several compounds have also been identified in more than one of the fractions of *S. lycocarpum* fruits (**Table 1**), such as the fatty acid, octadecanoic acid (OUF-1, OUF-5 and OUF-7), esters, hexadecanoic acid methyl ester (OUF-1 and OUF-5), hexadecanoic acid octadecyl ester (OUF-4 and ORF-1) and hexadecanoic acid hexadecyl ester (OUF-4, ORF-1 and ORF-3), and sterol substances,

sitosterol (OUF-2 and OUF-6), stigmasta-3,5-dien-7-one (OUF-1, ORF-1 and ORF-2) and cholesta-4,6-dien-3-ol (OUF-1 and ORF-2).

DISCUSSION

Solanum genus exhibits a wide variety of secondary metabolites as alkaloids, flavonoids, cinnamic acids, hydrocarbons, sterols, esters and fatty acids. Several compounds identified in the fractions of oils from *S. lycocarpum* have been reported in other *Solanum* species.

Sitosterol, stigmasterol and campesterol are commonly present in various species of *Solanum*, such as *S. atriplicifolium*, *S. chenopodioides*, *S. riparium*, *S. pseudocapsicum*, *S. diflorum*, *S. argentinum*, *S. sisymbriifolium*, *S. palinacanthum*, *S. elaeagnifolium* and *S. juvenile*. Sitosterol was also described in *S. palitans*, *S. incisum* and *S. sublobatum* (**Figure 2**), being more abundant sterol found in these species (46.6%-87.9%) (Zigadlo, 1994), showing chemotaxonomy significance for the *Solanum* genus.

Stigmasta-3,5-dien-7-one and cholesta-4,6-dien-3-ol were identified in *S. nigrum* (Fatima, 2016). Hexadecanoic acid methyl ester, octadecanoic acid methyl ester, 9-octadecenoic acid methyl ester and 9,12-octadecadienoic acid methyl ester have been previously found in fruits of *S. lycocarpum* (Silva *et al.*, 2015) and octadecanoic acid in fruits of *S. sessiliflorum* (Marx *et al.*, 1998).

The hydrocarbons heptacosane and docosane and hexadecanoic acid ethyl ester were described in extracts of *S. habrochaites* (Santos, 2015). Hexadecane, eicosane and phytol have been reported in *S. nigrum* (Riaz *et al.*, 2013). Phytol, hexadecanoic acid ethyl ester and pentadecanal were identified in *S. macranthum* (Essien *et al.*, 2012). The other compounds were not found in *Solanum* species.

CONCLUSION

The volatile and semi-volatile compounds of oils from *S. lycocarpum* fruits were characterized in this study. Sitosterol, stigmasterol, campesterol, stigmasta-3,5-dien-7-one, cholesta-4,6-dien-3-ol, heptacosane, docosane, hexadecanoic acid ethyl ester, hexadecane, eicosane, phytol and pentadecanal have been reported previously in *Solanum* species. The other compounds were not found in *Solanum* species, being described in *Solanum*

genus for the first time, to the best of our knowledge, contributing of phytochemical study of this species.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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Figure 1. Total ions chromatograms obtained by GC/MS. A) OUF-6, B) OUF-7, C) ORF-2 and D) ORF-3. Major compounds identified in the fractions: 1) Sitosterol, 2) Octadecanoic acid, 3) Octadecanoic acid octadecyl ester and 4) Hexadecanoic acid hexadecyl ester.

Sitosterol (45.27%, OUF-6)

Octadecanoic acid (73.37%, OUF-7)

Octadecanoic acid octadecyl ester (59.30%, ORF-2)

Hexadecanoic acid hexadecyl ester (97.98%, ORF-3)

Figure 2. Structures of major compounds characterised in the fractions by Gas Chromatography-Mass Spectrometry (GC-MS)

Table 1. Major compounds of oils from *S. lycocarpum* fruits.

Fraction	Majoritary compounds	%
OUF-1	Octadecanoic acid	19.10
	Hexadecanoic acid eicosyl ester	17.51
	Stigmasta-3,5-dien-7-one	15.92
	Cholesta-4,6-dien-3-ol	12.34
	Hexadecanoic acid methyl ester	9.24
OUF-2	9,12-octadecadienoic acid methyl ester	22.98
	Heptacosane	17.80
	Sitosterol	10.73
	Octanoic acid	10.08
	2,4-decadienal	8.95
	Nonadecane	6.36
	Tetracosane	6.26
OUF-3	Hexadecane	31.31
	Docosene	28.01
	Eicosane	20.20
	5-octadecene	18.09
OUF-4	Hexadecanoic acid octadecyl ester	20.95
	Cicloartanol	20.59
	Hexadecanoic acid hexadecyl ester	17.38
	1,22-docosanodiol	8.99
	Stigmasta-3,5-diene	8.76
	11-hexadecenoic acid	5.74
OUF-5	Hexadecanoic acid methyl ester	31.30
	Octadecanoic acid	22.11
	Eicosane	21.55
	Octadecanoic acid methyl ester	12.02
	Hexadecanoic acid ethyl ester	7.30
OUF-6	Sitosterol	45.27
	Stigmasterol	20.70
	Campesterol	18.44
	9-octadecenoic acid methyl ester	9.03
OUF-7	Octadecanoic acid	73.37
	Hexanoic acid	18.40
ORF-1	Hexadecanoic acid octadecyl ester	36.29
	Stigmasta-3,5-dien-7-one	19.88
	Hexadecanoic acid hexadecyl ester	11.19
	Phytol	10.60
	4-methyl-stigmasta-4,22-dien-3-ol	4.70
	Pentadecanal	4.13
ORF-2	Octadecanoic acid octadecyl ester	59.30
	Stigmasta-3,5-dien-7-one	13.75
	Cholesta-4,6-dien-3-ol	12.44
ORF-3	Hexadecanoic acid hexadecyl ester	97.98

OUF: oil unripe fruit; ORF: oil ripe fruit. * Concentration in percentage, calculated relative to the normalised areas of the peaks.