Supercritical Fluid Extraction of *Lippia dulcis Trev.*

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Lippia dulcis Trev. is a sweet herb endemic to tropical America. The constituents of this plant were previously investigated and isolated, being the sesquiterpenes (+)-hernandulcin and its epimer (-)-epi-hernandulcin the major compounds. It is known that hernandulcin is the sweet principle of this plant and has a potential application. Although hydrodistillation is commonly applied to essential oil extraction, the high temperature needed may cause thermolabile compounds to degrade. By using organic solvents to obtain the extract, there is the inconvenience of having a post processing step in order to purify it. In the present work, the supercritical extraction process was adopted, using CO_2 as a solvent. Experiments were conducted varying extraction parameters. These extracts were compared to those obtained by hydrodistillation. Preliminary results indicate that supercritical extraction process promotes higher extract yield. Gas chromatography analyses of the extracts were performed to evaluate the composition of both products.

INTRODUCTION

Lippia dulcis Trev. is a medicinal plant from the Verbenaceae family. Its infusion has been reported to treat coughs, bronchitis, colds and urinary retention. It is strongly aromatic, with leaves and flowers of strong sweet taste [1]. Few studies have been conclusive concerning the composition and usage of its essential oil, since it may vary according to the extraction method and cultivation region. The presence of a sweetener compound (hernandulcin) in the extract is mentioned in most of the published researches regarding this vegetable matrix. Its sensory properties were evaluated using a human taste panel. The authors affirmed that hernandulcin was judged to be 1000 times sweeter than sucrose [2].

The constituents of this plant were previously investigated and isolated. Many compounds were identified in this plant extracts, such as camphor, limonene, terpineol, α-pinene, α-copaene, (+)-4β-hydroxy-hernandulcin, (+)-hernandulcin, and so on [3].

The extract composition of *Lippia dulcis* collected in Puerto Rico was evaluated [1]. The authors verified the presence of the major compounds (+)-hernandulcin and its stereoisomer (-)-epi-hernandulcin.

The compound (+)-hernandulcin was isolated from *Lippia dulcis* leaves and flowers extract, obtained by extraction with petroleum ether [4]. The hernandulcin total yield was 0.004% w/w, in a dry basis. The authors also identified its isomer.

A patent of the compound hernandulcin as a non caloric sweetener was deposited [5]. However, it has disadvantages when applied to this purpose, being not very water soluble, and decomposing to ketones 3-methyl-2-cyclohexen-1-one and 6-methyl-5-hepten-2-one when heated [6]. Thus, an extraction method that uses lower temperatures may be advantageous in this case, as it is expected to happen with products obtained by supercritical extraction process.

Earlier works rely on using hydrodistillation or organic solvents to obtain this extract [1,4]. Although hydrodistillation is commonly applied to essential oil extraction, the high temperature needed may cause thermolabile compounds to degrade. By using organic solvents, there is the inconvenience of having a post processing step in order to proceed the extract purification. Carbon dioxide extracts are solvent-free and do not undergo thermal degradation as steam distilled oils do.

In the present work, the supercritical fluid extraction (SFE) process was adopted, using CO_2 as a solvent. Although this process is widely explored to extract natural compounds, there are no reports in literature of its application to obtain *Lippia dulcis Trev.* extract. The main purpose of this research was to investigate the influence of temperature and pressure in the supercritical fluid extraction of *Lippia dulcis*, in order to identify the operating conditions that maximize the global yield. Plants were collected during winter. Results were compared to those obtained by hydrodistillation process, for the same vegetable material. Also, a preliminary qualitative characterization of the products composition, obtained by both processes, was carried out performing gas chromatography analyses.

MATERIAL AND METHODS

Extraction equipments

Hydrodistillation was carried out in a Clevenger apparatus for 7h, using a 1:10 w/w proportion of plant material and solvent (distilled water). Supercritical fluid extraction was conducted in a pilot unit schematically represented in Figure 1. Carbon dioxide (99.9% purity) was supplied to the system through a cylinder (Linde, Brazil) connected to a gas booster (Maxpro Technologies, Germany). The unit was composed by a jacketed extraction vessel (l.8cm internal diameter) pressurized by the gas booster. A jacketed surge tank was placed between the gas booster and the extractor vessel, in order to perform a temperature conditioning of the CO_2 and to avoid eventual pressure overshoots, allowing a better pressure control. The temperature of the surge tank, as well as the extraction vessel was controlled by a thermostatic water bath. In the surge tank, a thermocouple (type J, Consistec) was installed to monitor the CO_2 temperature. The extraction pressure was monitored by a pressure transducer (AEP, Italy). The samples were collected in a separator maintained at 30bar and 35°C. The solvent flux was measured at the separator outlet by a flow meter (Key Instruments, USA).

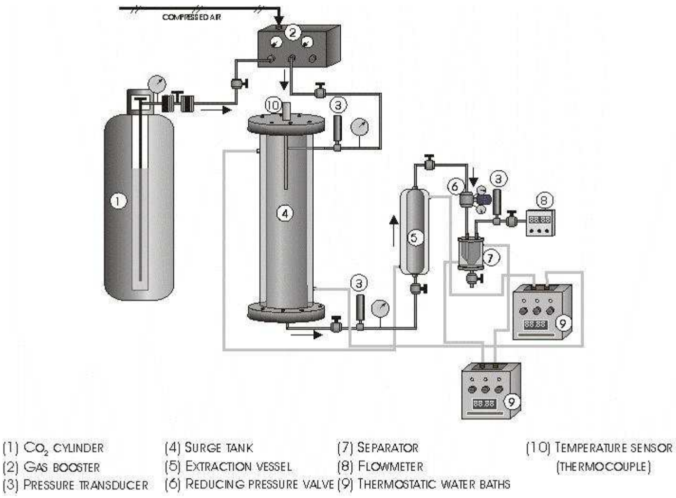

Figure 1 : Supercritical fluid extraction pilot unit.

Extraction procedure

The vegetable matrix used in this research was identified as *Lippia dulcis (Trev.)* Moldenke, from the Verbenaceae family, with the voucher specimen FLOR 36.457 deposited at UFSC, SC, Brazil. The plant was collected, air dried and its leaves and flowers were milled in a knife grinder (Marconi, Brazil). The powdered plant was screened and classified according to its particle size, using a set of sieves with openings varying from 0.044 to 0.295mm. About 96% of the particles were retained in sieves with openings from 0.212 to 0.295mm (Sauter average diameter – d_{32}=0.280mm).

The average CO_2 flow rate was 3.0 L/min (25^{o}C, atmospheric pressure), and the following pressures and extraction temperatures were tested: 100/120/140 bar and 35/40^{o}C, maintaining the other parameters constant. Each extraction experiment was carried out for a period from 100 to 260 min, until there was no more available extract to be collected. Extract samples were collected at time intervals, being the first sample collected always after a static time of 60 min (period of time in which supercritical CO_2 remains in contact with the vegetable matrix). The total yield was determined from the mass of extract obtained in the separator in comparison to the initial mass fed into the extraction vessel.

Extracts composition evaluation

Extracts composition evaluation were carried out performing gas chromatography-mass spectrometry (GC-MS). Exploratory assays used samples from hydrodistillation procedure and the extract obtained from supercritical fluid extraction at 100bar/35^{o}C. The main purpose of this analysis was to identify the main compounds presents in the different extracts. The samples were submitted to a cleanup process before GC-MS analyses, which consisted of eluting it through a silica flash column with pentane and ethylic ether (1:1).

GC-MS analyses were performed on a Varian CP-3800 gas chromatograph coupled with a Saturn 2000 mass spectrometer, using a CP-Sil 8 CB Low Bleed/MS (30m x 0.25mm internal diameter x 0.25µm film thickness) capillary column. The injector temperature was set to 250^{o}C. The oven temperature was adjusted to 50^{o}C for 1min and then programmed to increase to 240^{o}C at a rate of 3^{o}C/min. Helium was used as the carrier gas, at a constant rate of 1ml/min. The software used was Saturn GC/MS workstation 5.1.

RESULTS

Figure 2 presents the supercritical fluid extraction curves obtained under different pressure and temperature conditions. The results are expressed as extraction yield (% w/w).

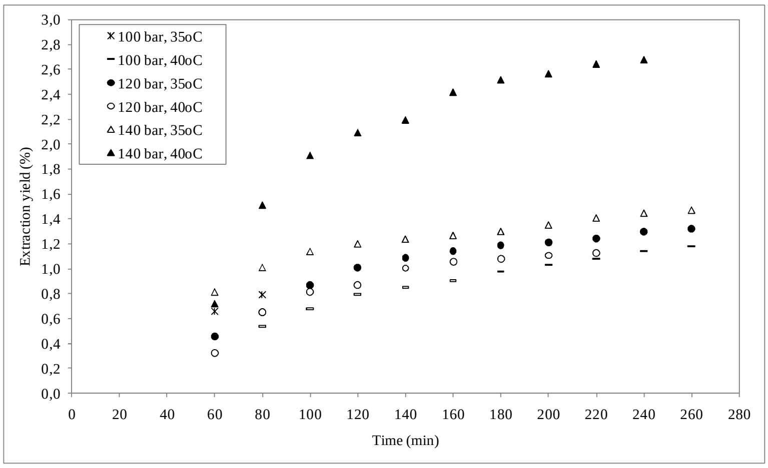

Figure 2: Extraction curves.

The highest extraction yield was achieved under 140 bar and 40°C. However, good results were obtained for 140bar/35°C and 120bar/35°C. Table 1 indicates the CO_2 densities [7] for all process conditions used, as well as the extraction yields obtained.

Table 1: CO_2 parameters and extraction yields obtained.

Pressure (Bar)	Temperature (°C)	CO_2 density (g/cm^3)	Extraction yield (% w/w)
100	35	0.714	0.8
100	40	0.630	1.2
120	35	0.768	1.3
120	40	0.719	1.1
140	35	0.802	1.5
140	40	0.764	2.7

It can be observed that the behavior of the extraction curves do not have always a close relation to CO_2 density, since for the highest CO_2 density the extraction yield was not maximum. In general, by increasing extraction pressure, at a constant temperature, higher global yields are obtained, since CO_2 density values are also increased. However, process selectivity is lowered. This is in accordance to the literature [8].

At 35°C it can be observed a higher extraction yield with the increase in pressure, that is, the larger solubility of the compounds in the supercritical fluid. On the other hand, at 40°C, from 100 to 120bar there is a decrease in the global yield as pressure increased and from 120 to 140 bar a contrary behavior took place.

The raise in temperature at 120bar has caused a decrease in the CO_2 density and, consequently, diminished its solvent power. That is why the extraction yield was lower at 120bar/40°C than at 120bar/35°C. However, at 100 and 140bar the increase in temperature had a positive effect on extraction yield, although CO_2 density was lower. An increase in temperature reduces the supercritical CO_2 density (for a fixed pressure), thus reducing its solvent power; but it increases the vapor pressure of the compounds to be extracted. Therefore, the tendency of these compounds to pass to the fluid phase is increased [8].

Hydrodistillation extraction yield was lower than SFE process, 0.3% of essential oil, in a dry basis. From the qualitative point of view, the volatile oil obtained by hydrodistillation is a clear yellowish oil and the aspect from the product obtained by SFE varies depending on the process parameters applied. It varied from a dark green to an intense yellow color, maybe due to the possible co-extraction of chlorophyll and cuticular waxes, respectively.

It was also noticed a difference in each product aroma, having the hydrodistilled essential oil an intense “burned” smell, probably because of the high temperature maintained for a long time. The extract of the SFE process presented an aroma similar to the original plant material. In fact, SFE process avoids degradation and hydrolysis of thermally sensitive compounds [9].

Figure 3 presents the chromatograms for the essential oil obtained by hydrodistillation (A) and the extract obtained by supercritical fluid extraction (B). The mass spectrum of the hernandulcin peak is shown on (C). Results from (B) and (C) are in accordance to [1].

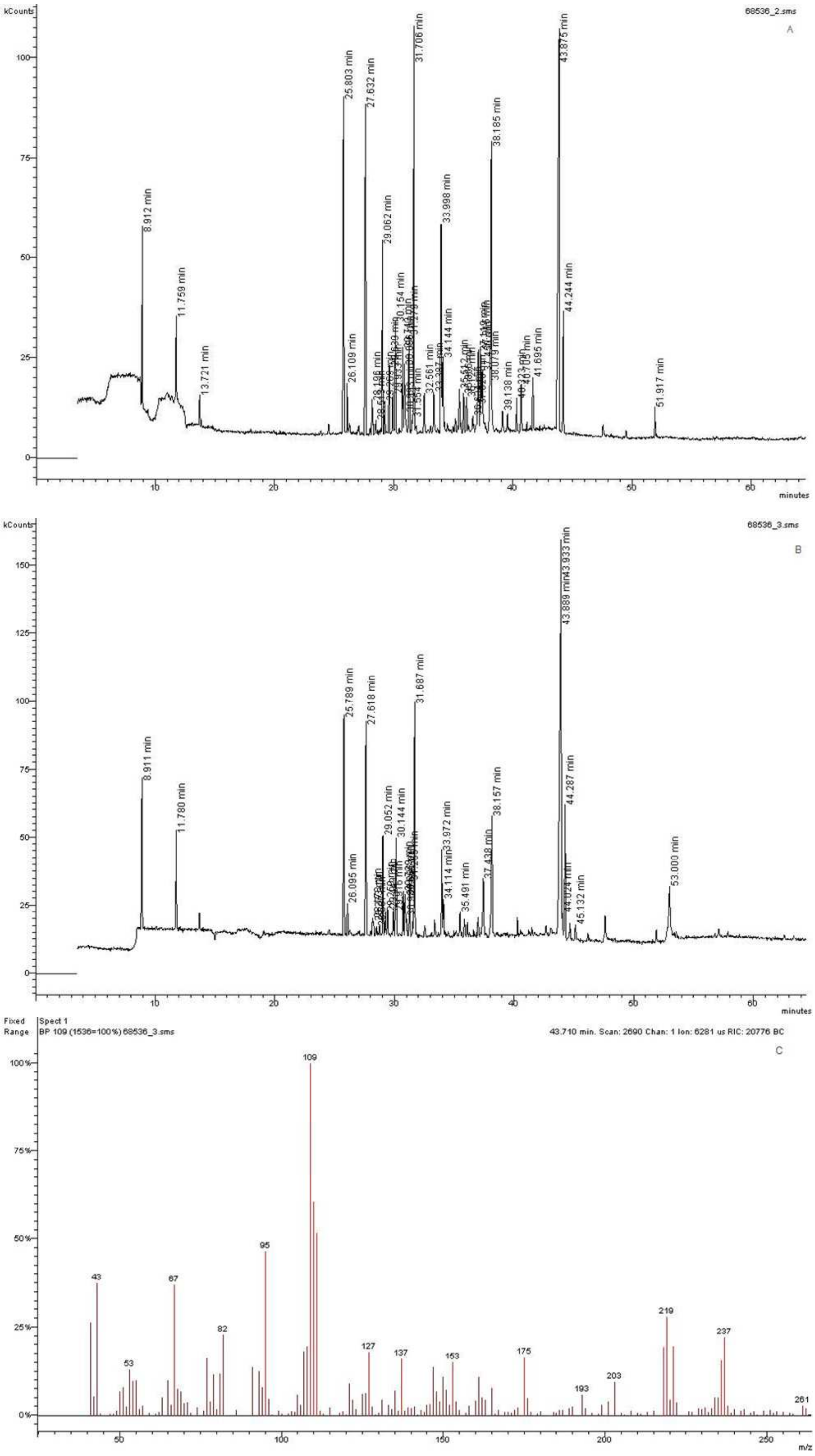

Figure 3: (A) Total ion chromatogram of Lippia dulcis essential oil obtained by hydrodistillation ; (B) Total ion chromatogram of Lippia dulcis extract obtained by SFE and; (C) Mass spectrum of hernandulcin at the maximum peak intensity.

Table 2 presents the major peaks that appeared in the extracts obtained by hydrodistillation and SFE, as a preliminary evaluation of these products. The estimated content of the major compound hernandulcin in the supercritical fluid extraction was 31.580% (sum of 19.215 and 12.365) against 16.328% of the hydrodistilled essential oil. This can be compared to literature results [1], in which *Lippia dulcis* essential oil was obtained by solvent extraction (CH_2Cl_2), affording 36.00% of hernandulcin.

Table 2: Composition of the hydrodistilled essential oil and SFE extract.

Component	Total ion chromatogram			
	Hydrodistillation		Supercritical fluid extraction (100 Bar/35^{o}C)	
	Rt (min)	Area (%)	Rt (min)	Area (%)
3-cyclohexene-1-methanol,6-methyl	8.912	3.221	8.911	3.894
2-cyclohexen-1-one,3-methyl	11.759	1.673	11.780	3.325
Copaene	25.803	7.877	25.789	7.614
Caryophyllene	27.632	7.844	27.618	7.753
Cedrene	29.062	4.241	29.052	3.533
(+)-epi-bicyclosesquiphellandrene	30.154	2.733	30.144	3.484
Naphthalene	31.706	9.315	31.687	7.956
α-bisabolol	38.185	7.499	38.157	4.565
(+)-hernandulcin (identified by comparison with the literature)	43.875	16.328	43.889	19.215
			43.933	12.365
n.i.	44.244	2.913	44.287	5.975
Subtotal (%)		63.644		79.679

n.i. : not identified.

CONCLUSION

Preliminary results indicate that SFE process promotes higher *Lippia dulcis* extract yield, when compared to hydrodistillation method. The best SFE parameters were 140 bar and 40^{o}C, giving a higher global yield. Changes in pressure and temperature in the SFE process had a considerable effect in the extraction yield. Hernandulcin was found to be the major compound of *Lippia dulcis* extracts, for both hydrodistillation and SFE. As an exploratory research, the results found for the composition of *Lippia dulcis* extract obtained by SFE technology seemed to be in accordance with those already published applying other extraction techniques.

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