EXTRACTION OF VOLATILE OIL FROM *Piper aduncum L.* LEAVES WITH SUPERCRITICAL CARBON DOIXIDE

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Piper aduncum L. is a plant of the Piperaceae family, usually found in tropical regions. The leaves of *Piper aduncum* L. are used in folk medicine to treat stomachaches, trachoma and other diseases, and also as an insect repellent. Another possible application of the extract from *Piper aduncum* L., found in the literature, is the treatment of Lehismaniasis, a common disease in tropical countries. This treatment can be done through the use of DMC (2´-6´-Dihidroxy-4´-Methoxychalcone), a substance found in the *P. aduncum* extract. In this work, the extraction of the volatile oil from the leaves of *Piper aduncum* L. grown in an experimental farm in Brazil (Botucatu, SP) was performed, using supercritical carbon dioxide as solvent, at different conditions of pressure and temperature. The process global yield was determined, in order to define the operational condition in which the highest relative amount of oil can be extracted from the raw material, to increase the process viability. The chemical composition of the *Piper aduncum* L volatile oil was analyzed by gas chromatography and mass spectrometry (GC-MS) in order to identify the major; this information was compared to literature data to verify if the SFE was capable of producing an extract useful for the chemical, pharmaceutical and/or food industry.

Keywords: SFE; global yield; *Piper aduncum*; chemical composition

INTRODUCTION

Piper aduncum L. is a native plant from tropical regions such as South and Central America, Asia and Pacific islands. This plant is popularly used in medicine bathes, and also in the treatment against trachoma and vaginitis, and stomachaches [1]. In Brazil, *Piper aduncum* L. is known as "pimenta-longa", "jagurandi" or "aperta-ruão". This specie is native from both Amazon and Atlantic forests in Brazil. There is another specie, *Piper hispidinervum*, known as wild "pimenta-longa", which contains about 80% of safrole in its essential oil. *Piper hispidinervum* is found only few regions of the Amazon and may not be adaptable to non-Amazonian climates [2]. Safrole is a compound of great interest for chemical and pharmaceutical industries that use it in the production of cosmetics and insecticides. In the specie *Piper aduncum* L. the amount of safrole in the essential oil was found to be about 15% [2].

Until the sixties, safrole used to be obtained from *Ocotea pretiosa* Mezz, a native plant from the Atlantic forest. The excessive exploration of this forest made the Brazilian Institute for Environment (IBAMA) forbid the exportation of *Ocotea pretiosa* Mezz, so the

offer of safrole sources in the international market was reduced. The natural reserves of *Ocotea pretiosa* Mezz became almost depleted in Brazil. In this context, the extraction of oil from *Piper aduncum* L. may be a viable alternative for Brazil to supply the internal and international market of safrole.

Another possible application of the extracts from *Piper aduncum* L. is for the treatment of lehismaniosis, an infectious disease that normally attacks people in undeveloped countries. Torres-Santos et al. [3] had isolated the substance 2´-6´-Dihydroxi-4´-Methoxychalcone from the extract of *Piper aduncum* L. This compound showed to be efficient against the agents that cause lehismaniosis.

The oil from *Piper aduncum* L. can be obtained by steam distillation [2], but there is not, in literature, any description of supercritical fluid extraction (SFE) of oil from the leaves of this plant.

MATERIALS AND METHODS

Piper aduncum L. was cultivated under controlled conditions at the Experimental Farm of Lageado (Plant Production Department, Agronomy Science College/UNESP, Botucatu, SP, Brazil). The leaves, already dried, were triturated in a laboratory mill (Marconi Equipamentos para Laboratórios, Wiley MA 340, Piracicaba, Brazil). The particle mean diameter was measured after separation with a system of vibratory sieves (WS Tyler, Mesh 12, 16, 24, 32, 48, Wheeling, IL). 138.49 grams of *Piper aduncum* L. milled leaves were put on the top of the sieve with the lowest Mesh. The system was shook by 20 minutes, and after that the content remaining on each sieve was weighed. The mean geometrical particle diameter was calculated by the method recommended by ASAE Standard [4] and its value was of 0.52 mm.

The oil was extracted from the triturated leaves of *Piper aduncum* L. by three different methods: hydrodistillation, Soxhlet and SFE.

Hydrodistillation

The hydrodistillation was done using a Clevenger distillation system: In 2 L round bottom glass flask was added 38.61 grams of triturated *Piper aduncum* L. leaves and one liter of distillated water. The mixture was left under reflux for 2.5 hours. The oil was collected, centrifuged and stored under refrigeration.

Soxhlet Extraction

The Soxhlet extraction used ethanol (150 mL) (LabSynth, 99.5%, lote 58428, São Paulo, Brazil) as solvent. The *Piper aduncum* particles (10.03 g) were loosely packed inside a tube prepared with Whatman nº 44 filter paper. The extremities of this tube were filled with cotton. This cartridge was placed in the Soxhlet device and the system was kept under reflux for 1.25 hours. After cooling, the solvent was separated from the extract in a rotovap system (Laborota, model 4001, Viertrieb, Germany), with vacuum control (Heidolph Instruments GMBH, Viertrieb, Germany) with the thermostatic bath (Heidolph Instruments WB, Viertrieb, Germany) at 50 ºC. The remaining extract was weighted in order to determine the yield of the process.

Supercritical Fluid Extraction (SFE)

Figure 1 illustrates the scheme of the SFE equipment used in this work. This equipment was built at the Technische Universität Hamburg-Harburg (TUHH), as a part of

a cooperation project (SuperNat) which involves TUHH and some Brazilian research groups that work on SFE of natural products, such as LASEFI-DEA/FEA-UNICAMP.

The pressure regulator, inlet, outlet and micrometric valves, and the extraction column were kept under a heating bath, to achieve the required temperature of the SFE process. Before reaching the pump, the CO_2 was cooled under 0°C by a refrigeration bath, in order to be at the liquid state to be pumped into the line.

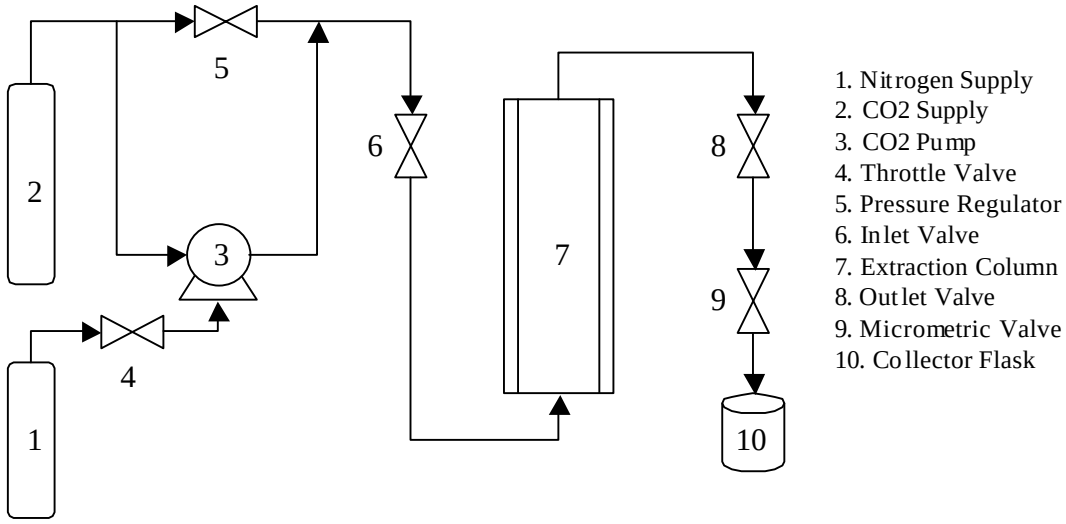

Figure 1: Experimental setup used in the supercritical fluid extraction.

In each experiment, a sample of triturated *Piper aduncum* L. leaves was weighted and introduced inside a stainless steel 100 ml extraction column. The mass of each sample was about 7 grams, so the remaining space in the column was filled with glass spheres. Pieces of glass wool were put on the extremities of the column, and between the glass spheres and the *Piper aduncum* L. sample. Figure 2 shows a scheme of the extraction bed.

Figure 2: SFE Column

The solute-free solvent should contact the glass spheres before contact the particle bed; otherwise some adsorption of the solute may occur on the spheres surface.

The experimental procedure was as follows: Initially, all the valves were closed, except the pressure regulator. After the extraction column formed as shown in Figure 2 was

assembled, the SFE process began, with the following steps:

- The CO_2 (99.0%, Gama, S.S ONU 1013, Campinas, Brazil) supply was opened, so the line became pressurized at the pressure of the CO_2 cylinder (about 60 bar), until the inlet valve;
- The nitrogen supply and the throttle valve were opened, in order to pump CO_2 into the line;
- The pressure regulator was closed, until the pressure reached the required value in the line before the inlet valve;
- The inlet valve was opened, in order to pressurize the extraction column;
- After the system reached the required pressure, a static period of 5 minutes was allowed;
- After the static period, the outlet valve was opened, and so was the micrometric valve, which is responsible to regulate the CO_2 flow rate. The extract began to be collected in the flask;
- After one hour of extraction, the inlet valve was closed and the micrometric valve was completely opened, so the extraction column was depressurized;
- The line after the column was opened, and ethyl acetate was injected into the line, to remove the remaining extract and collect it in another flask;
- After the ethyl acetate was removed from the flask, the extract from both flasks were weighted, in order to calculate the extraction yield;
- The extracts were stored under refrigeration to be analyzed by GC-MS.

In order to establish the operational conditions which provide the higher extraction yields an factorial experimental design was performed with two factors (pressure and temperature), two levels for each factor and a central point. The pressure levels were 100 and 300 bar, and the temperatures, 30 and 40°C. All conditions were tested in duplicate.

Analysis of the Extracts

The chemical composition of the extracts (essential oil and part of the oleoresin) was analyzed by GC-MS (Shimadzu, model QP-5000, Kyoto, Japan) equipped with a capillary column of fused silica DB-5 (30 m × 0.25 mm × 0.25 μm, J&W Scientific, Folsom, CA). The electron impact technique (70 eV) was used. The carrier gas was helium (White Martins, 99.9% purity) (1.7 mL/min). The extracts (0.005 grams) were diluted in ethyl acetate (1mL-solvent, analytical grade, LabSynth, lot 55893, São Paulo, Brazil); 1 μL of sample was injected and the sample split ratio was 1:30. The temperature programming was 60-240 ^{o}C (3°C/min). The injector and detector temperatures were 240 and 230ºC, respectively. The identification of the chemical constituents was based on their retention index [5].

The extracts were also analyzed by thin layer chromatography (TLC) on a silica plate (60-PF254, Merck 20×20 cm, 0.25 mm of height, lot 940378601, Germany), using a solution 80:20 of hexane (Merck, analytical grade, lot HX0290-44, Germany) and ethyl acetate (Merck, analytical grade, lot K225488323, Germany), and the plate was revealed

with iodine (LabSynth, analytical grade, lot 57738, São Paulo, Brazil).

RESULTS AND DISCUSSION

The hydrodistillation process resulted in yield of 0.25% (wet basis), in 2 hours of process. This extract was light yellow. The SFE extract was dark yellow. The yields (wet basis) obtained in the SFE, at different conditions of pressure and temperature, are presented in Table 1. The Soxhlet extraction produced a dark green solid like extract with yield of approximately 10%.

Table 1 - SFE Yields for *Piper aduncum* L. Oil

Pressure (bar)	Temperature (°C)	Yield (kg oil/kg sample x 100)
100	30	1.35 ± 0.33
100	40	1.69 ± 0.23
300	30	1.44 ± 0.01
300	40	1.84 ± 0.02
200	35	1.54 ± 0.05

A statistical analysis of the results (ANOVA) was done, to evaluate the effects of pressure and temperature of extraction in the yield. The results of the ANOVA had shown that the temperature had a significant effect on the extraction yield ($p = 0.0198$). On the other side, pressure did not have a significant effect on the yield ($p = 0.3525$).

Figure 3 shows the GC-MS analysis of the extracts obtained by hydrodistillation (sample dilution: 5 mg/mL), SFE at 300 bar and 40°C (sample dilution: 15 mg/mL), and by ethanol Soxhlet (sample dilution: 15 mg/mL). The TLC plate obtained for the extracts from *Piper aduncum* L. leaves is presented in Figure 4. One can observe in both figures that the extract obtained by hydrodistillation presents the smaller number of compounds. Table 2 shows the major compounds identified in the extracts. The percentages shown in Table 2 refer to the area of the peaks detected by the GC.

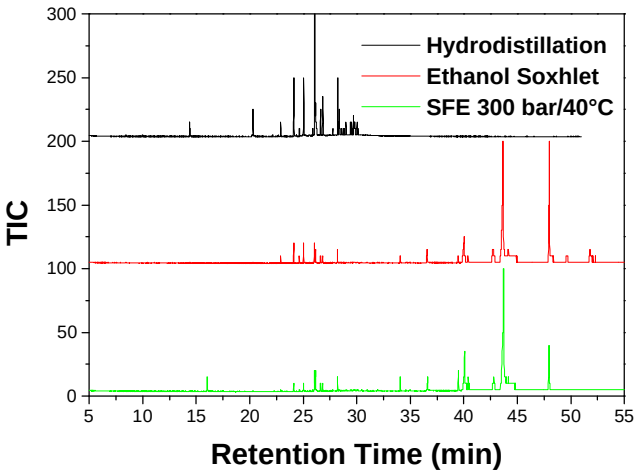

Figure 3 – GC-MS chromatograms of *Piper aduncum* extract. The concentration of the hydrodistilled extract was 5 mg/mL and for the others 15 mg/mL.

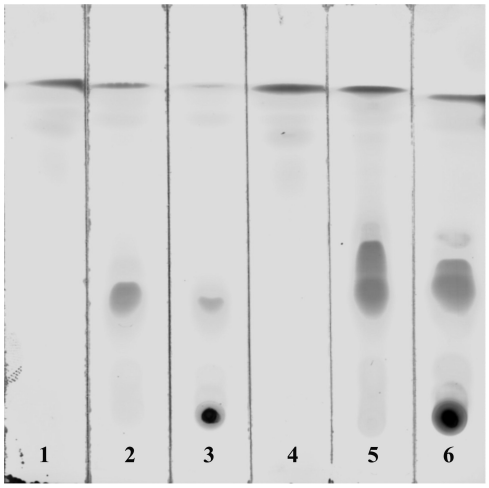

Figure 4 – TLC of *P. aduncum* extracts. 1 and 4 - Hydrodistillation 5 mg/ml, 2 – SFE 300bar/40°C at 5 mg/mL, 3 – Soxhlet at 5 mg/mL, 5 – SFE 300 bar/40°C at 15 mg/mL, 6 – Soxhlet at 15 mg/mL.

Table 2. Major Compounds Identified in *Piper aduncum* L. Oil

Compound	Hydrodistillation (%)	SFE (%)	Compound	HD (%)	SFE (%)
Safrole	1.64	0.27	δ-cadinene	1.73	0.72
Caryophyllene	3.33	2.18	Spathulenol	3.15	1.79
α-humulene	3.88	2.22	Caryophyllene Oxide	1.67	0.63
Asaricin	6.74	2.76	Epi-α-cadinol	1.17	0.36
γ-cadinene	1.49	1.18			

CONCLUSIONS

The results obtained using the different extraction methods had shown, as expected, that SFE process provides better yield than hydrodistillation. The Soxhlet method provided the highest extraction yield, but many compounds of high molecular weight were extracted together with the oil. Among these compounds, there are probably waxes, and other substances that may not be desirable.

The comparison of the GC-MS chromatograms with the TLC shows that many compounds obtained by Soxhlet and by SFE were not present in the oil obtained by hydrodistillation. Unfortunately, there was not an accessible library where these compounds could be identified. However, the chromatograms indicate that these non-identified compounds represent the major part of the oil obtained by Soxhlet and by SFE. The TLC indicates that these compounds are probably more hydrophobic than the ones obtained by hydrodistillation.

Although safrole was detected in the extracts, the amount obtained was very small to consider *Piper aduncum* L. as a potential source of these compounds to the industry.

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