SUPERCRITICAL FLUID EXTRACTION OF PHYSALINS FROM *Physalis angulata*

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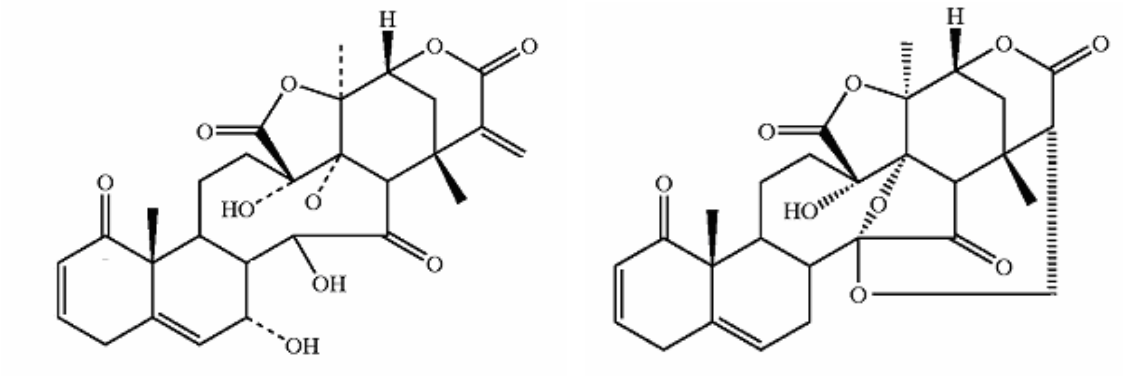
The extraction of physalins from the aerial parts and the fruits of *P. angulata* using supercritical carbon dioxide as solvent was tested. 30 g of the dried raw material were packed in a 300 mL extraction column partially filled with glass beads in the CO_2 entrance. The extraction was conducted at two pressures (100 and 300 bar) and at two temperatures (40 and 60 °C). A CO_2 mass flowrate of $5.7x10^{-5}$ kg/s was employed and the total extraction time was 4 hours. The physalins A and B were detected by reverse phase HPLC using a C8 column. The extraction yield for the aerial parts was comprehended from 0.16% (100 bar and 60°C) to 1.62% (300 bar and 60°C), while for the fruits it varied from 0.13% (100 bar and 60°C) to 1.58% (300 bar and 60°C). Physalin A could only be identified in the aerial part extract obtained at 300 bar and 40°C, which also presented the largest concentration of physalin B. The low physalin A and B fractions in the extracts can be attributed to their small concentration in the raw material (from 30 to 500 ppm [1]).

INTRODUCTION

Physalis angulata is a 1-2 m height very bushy shrub from the *Solanaceae* family. In Brazil, it is native in the North and Northeastern regions, being known as Camapú, Juá, Saco de Bode, Bucho de Rã, and Mata-Fome. In the folk medicine, its fruits, leaves and roots are used in the treatment of diabetes, rheumatism, hepatitis, and asthma. The main substances responsible for therapeutical activities of *P. angulata* extracts are the physalins. There are nineteen known physalin types (A to S), and A and B are the main forms found in *P. angulata*. Figure 1 shows the structural form of these compounds. Physalins are ergostane-type steroidal derivatives and the B form has very high anti-inflammatory activity [2].

The conventional method to obtain physalins from the aerial parts and fruits is the organic solvent extraction. In this work, the technical feasibility to extract physalins from *Physalis angula* using supercritical carbon dioxide as solvent was studied.



Physalin A Physalin B

Figure 1 – Chemical structure of physalin A and B.

MATERIAL AND METHODS

Physalis angulata

The raw material was obtained from a farmer in São Paulo State, Brazil. The aerial parts of the plants were dried in a tray drier for 30 hours at 40-45 °C. The resulting material was comminuted in a knife mill, packed into plastic bags and storage at room temperature until use. The lyophilized fruits were acquired from the same producer.

Supercritical Fluid Extraction

Figure 2 presents a schematic representation of the supercritical extraction system used in this work (AUTIC, Campinas, Brazil).

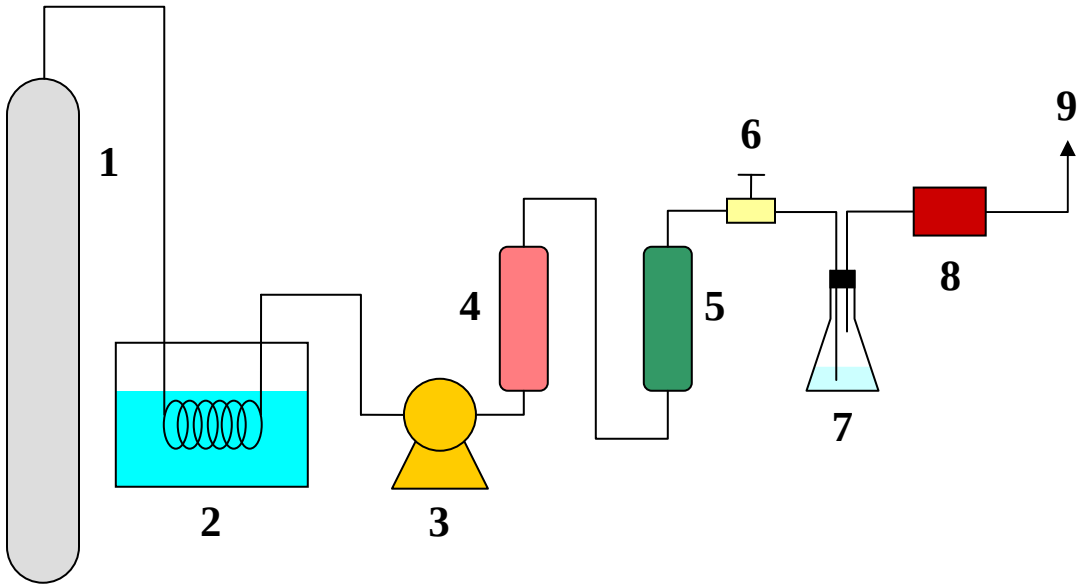


Figure 2 – Schematic representation of the supercritical fluid extraction unit. 1 – CO_2 cylinder; 2 – Cooler; 3 – Pump; 4 – CO_2 Reservoir; 5 – Extraction Column; 6 – Micrometer valve; 7 – Extract Collector; 8 – CO_2 Flowmeter; 9 – Environment.

During the experiments, 30 g of raw material were packed into a 500 mL extraction column. It was used glass wool plugs at the column entrance and exit. The remaining free space of the extraction column was filled with glass beads (CO_2 entrance). The CO_2 mass flowrate was $5.7x10^{-5}$ kg/s and the extraction time was set in 4 hours. The operational conditions used were pressure of 100 and 300 bar and temperatures of 40 and 60 °C. The extract was collected in a filtering flask partially filled with absolute ethanol, to enhance the collection efficiency. After extraction, the tubing after the extraction column was rinsed with ethanol to recuperate the extract deposited on it after the micrometer valve. The rinsed solution was recovered at filtering flask. The Ethanol was evaporated under vacuum in a rotovap system. Then, the mass of extract was determined. The extraction yield was defined as the mass of extract divided by the mass of raw material used in the experiment multiplied by 100.

Physalins Analysis

The extracts were analyzed by HPLC using a HP1100 system with a UV-VIS (G1314A) detector (230 nm). The separation was carried out in a Supelcosil LC-8 column (250 x 4.6 mm) with 5 μm particles, in a gradient mode of water in acetonitrile. A mixture of Physalin A and B was used as standard. Figure 3 presents the standard chromatogram. There is a good resolution of Physalin A and B. Some impurities of the standard can be observed. This standard was used to identify the physalins in the supercritical extracts. All samples were prepared using methanol as solvent.

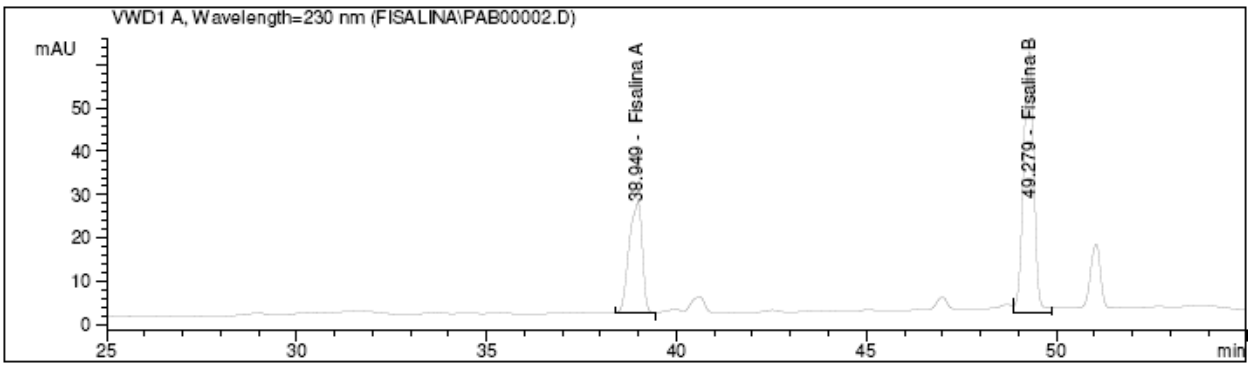


Figure 3 – Chromatogram of physalin standard.

The relative amounts of physalin A and B present in the extracts were estimated using the following equation:

$$CRF = \left(\frac{CP / AP}{CA / AA} \right) \quad (1)$$

where CRF is the physalin relative concentration, CP is the physalin A and B standard concentration (0.53 mg/g), AP is the physalin A or B peak area in the standard solution, CA is the sample concentration, AA is the physalin A or B peak area in the sample solution.

RESULTS AND DISCUSSION

Table 1 presents the extraction yields obtained in the experiments. The extracts colors changed considerably with the raw material and operational conditions. For instance, the extract from fruits obtained at 100 bar and 60 °C was whitish while the aerial part extract obtained at 300 bar and 60 °C was greenish.

Table 1 – Extract yields obtained in the supercritical fluid extraction.

Raw Material	Sample	Pressure (bar)	Temperature (ºC)	Extraction Yield (%)
Aerial Parts	1	100	40	0.70
	2	100	60	0.16
	3	300	40	1.15
	4	300	60	1.62
Fruits	5	100	40	1.23
	6	100	60	0.13
	7	300	40	0.84
	8	300	60	1.58

For both raw materials, an increase in the extraction temperature, for low extraction pressure, resulted in a yield decrease. At this pressure, the influence of the temperature in the solvent density was stronger than the increase of the extract vapor pressure. On the other hand, an increase in the extraction temperature for extraction pressure of 300 bar led to an increase of the extraction yield, confirming the larger effect of temperature in the vapor pressure than in the density for higher pressures. The yields obtained for each raw material was comparable.

Table 2 shows the HPLC analysis of the obtained extracts. The physalin A was observed only in the extract from the aerial parts acquired at 300 bar and 40 °C. This condition also presented the highest physalin B relative concentration.

Table 2 – HPLC analysis of the supercritical fluid extracts.

Sample	Sample Concentration (mg/g)	CRF of Physalin A	CRF of Physalin B
1	13.36	nd	Nd
2	11.97	nd	Nd
3	13.25	0.00110	0.00401
4	15.49	nd	Nd
5	23.54	nd	0.00005
6	11.87	nd	0.00127
7	15.88	nd	Nd
8	18.46	nd	Nd

The extraction results were interesting since the aerial parts of *P. angulata* probably have a lower cost than the fruits since it is commercialized as an exotic fruit in Brazil. Figure 4 shows the chromatograms obtained with sample 3. The top chromatogram shows the analysis of the extract and the bottom one the analysis of the extract spiked with the physalin standard.

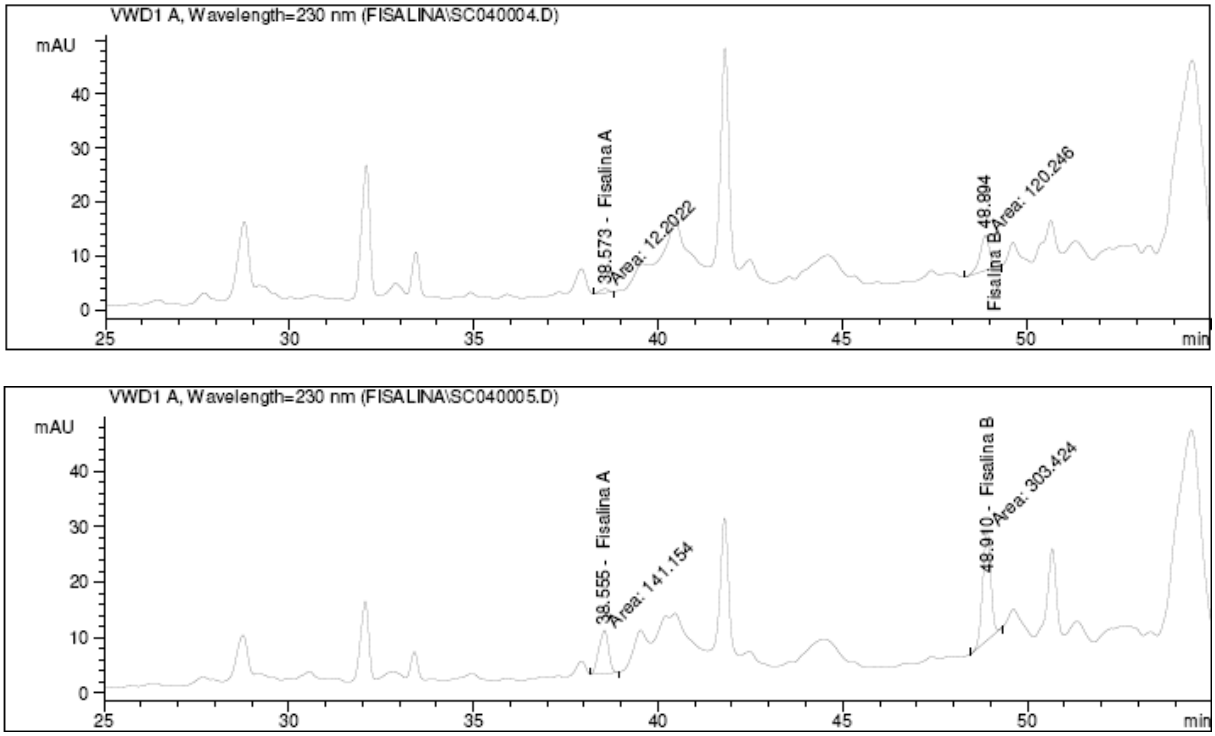


Figure 4 – HPLC analysis of the supercritical extract from the aerial part of *P. angulata* obtained at 300 bar and 40 ºC. The top figure represents the analysis of the extract and the bottom one the extract spiked with physalins standard.

One can observe in Figure 4 that the extract contains only a small fraction of physalin A and B. The concentration of physalins in *P. angulata* is low, ranging from 30 to 500 ppm [1]. In spite of that, this extract presents a huge *in vitro* anti-inflammatory effect (result not shown).

CONCLUSION

Supercritical fluid extraction was a technically viable method to extract physalins from *P. angulata*. The highest amount of physalins was obtained from the aerial parts of the plant extracted at 300 bar and 40 ºC.

ACKNOWLEDMENTS

The authors would like to acknowledge FAPESP for the financial support to this work and Prof. Dr. Theo Guenter Kieckbusch from the School of Chemical Engineering, State University of Campinas, for the use of the supercritical fluid extraction unit.

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