SUPERCRITICAL EXTRACTION OF ESSENTIAL OIL FROM *Cordia curassavica* (JACQ.) ROEMER AND SCHULTES

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In this work, the results of a preliminary experimental study to obtain extracts from leaves of *Cordia curassavica* using CO_2 supercritical as solvent is presented. The influence of operational conditions (pressure and temperature) was studied by response surface method with three central points. The operating pressure in the extraction was varied from 78 to 341 bar and the temperature was varied from 26 to 54 ºC. The yields varied from 0.11 to 5.46 % (dry basis), depending on the experimental condition. The linear terms of pressure and temperature, the quadratic term of pressure, and the interaction pressure/temperature significantly ($p<0.05$) affected the yield. The extract chemical composition was analyzed by GC-MS. Results were compared with Soxhlet extraction and distillation method.

Keywords: SFE, *Cordia curassavica*, Volatile oil

INTRODUCTION

In the last few decades, there has been a growing interest in plant extracts due to the wide spectrum of metabolites present in it. Brazil has a large agricultural resource and it is one of the major plant producers in the world. *Cordia Curassavica* (Jacq.) Roemer and Schultes, a plant known in Brazil as “erva-baleeira”, is a brunched perennial tree with 1-4 m of height [1]. In this tree, the brunches are resinous and the leaves are aromatic [1],[2]. It is cultivated in the tropical regions of America, mainly in Central America and Caribbean Islands [3]. In Brazil, it is native in Rio de Janeiro, Bahia, and São Paulo [4].

The infusion of the leaves of this plant is popularly used in the treatment of cold, flu, pneumonia, cough, headache, and parasitic diseases [2],[5]. The dichloromethane extracts from *C. curassavica* are very effective against the phytopathogenic fungi *Cladosporium cucumerinum* [3],[6], the *Candida albicans* yeast [3],[7], and the eggs of the *Aedis egypt* fly, the yellow fever vector [3]. In cordia extracts are found some phenolic compounds [8] and some terpenic quinones [9]. In *Cordia curassavica* leaves were identified three main components: b -terpinene, a -pinene e sabinene [10],[11]. From the roots were isolated two meroterpenoids naftoquinones named cordiaquinones J and K, in addition to the known cordiaquinones A and B [3].

The conventional methods to produce essential oils and oil resins, such as steam distillation and organic solvent extraction can cause some degradation in thermolabile compounds and leave some amount of toxic solvents in the solute. Since one of the quality requirements of the extracts used in food or pharmaceutical industry is its purity, the supercritical fluid extraction (SFE) can be a technically viable way to produce them.

There is no available data related to the production of extracts from *C. curassavica* using SFE. Thus, the main goal of this work was to verify the influence of extraction pressure and temperature on the global yield and in the extract composition of the SFE process. The global yield is defined as the maximum amount of extract that can be obtained at a given extraction pressure and temperature. This parameter is used in the mathematical models used in the design of SFE units [12] and in the purification process.

MATERIALS AND METHODS

Raw Material Preparation and Characterization

The *Cordia curassavica* used in this work was cultivated in the "Centro de Pesquisas Químicas, Biológicas e Agronômicas" – UNICAMP (Campinas, Brazil). The leaves were dried in a tray drier at 40°C and comminuted in a hammer mill. The particles were packed in a polypropylene bag under vacuum, and stored in a freezer at – 10 °C (Brastemp, Brazil). The humidity of the particles was measured using the xylol distillation method [13]. The particle size distribution was determined using a vibratory sieve shaker (Produtest, model 3580, São Paulo, Brazil) and sieves of 48, 60, 80, and 100 mesh. The particles from 60 to 100 mesh were used in the extraction experiments. The Sauter mean diameter (d32) was calculated to represent the mean particle size.

Extraction Procedures: SFE, Hydrodistillation, and Soxhlet Extraction

The SFE experiments were carried out in a Speed_SFE unit (Applied Separations, model 7071, Allentown, USA). The Thar design 5 mL extraction column (Pittsburgh, USA, with internal volume of 6 mL) was used in these experiments. The particles (3.37±0.01 g) were packed into the column, resulting in a fixed bed with density of 540±2 kg/m^3. The static period of CO_2 (99.98% of purity, White Martins, Campinas, Brazil) in contact with the particles was 5 minutes. The CO_2 mass flow rate was 4.2±0.4 g of CO_2 / min. The extract was collected in an ethylene glycol bath at – 10°C to decrease the lost of more volatile compounds. The extraction was finished when there was no noticeable extract leaving the system (30-45 minutes). The extract mass was determined by weighting the collection flask (Sartorius, model A200S, ±0.0001 g, Goettingen, Germany) at the beginning and end of the experiment.

The Soxhlet extraction used hexane (200 mL) (Merck, HPLC grade, lot 41123118, São Paulo, Brazil) as solvent. The *C. curassavica* particles (8.00 g) were loosely packed inside a tube prepared with Whatman nº 42 filter paper. The top and the bottom part of the tube were closed with cotton plugs. This cartridge was placed in the Soxhlet device and the system was kept under reflux for 3 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, Germany), in a thermostatic bath at 40 ºC.

The hydrodistillation was accomplished in a Clevenger distillation system. In a 2000 mL glass flask was added the particles (28.89±0.01 g) and distilled water (1200 mL). The system was leaved under reflux during 2.5 hours. The essential oil was collected from the top part of the water and placed in a dark glass flask.

Experimental Design

The effect of pressure and temperature on the global extraction yield was studied using a response surface methodology. The experimental design matrix used is presented in Table 1. The results of the experiments were analyzed using the software STATISTICA 5.0.

Table 1. Matrix of the experiments experimental design

Level	-1,41	-1	0	+1	+1,41
Pressure (bar)	78*	100	200	300	341
Temperature (°C)	26	30	40	50	54

* The value of experimental design procedure of 59 bar could not be investigated due to experimental limitations

Chemical Composition 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, Japan) equipped with a capillary column of fused silica (DB-5; 30 m x 0.25 mm x 0.25 μm, J&W Scientific, USA). 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 split ratio was 1:30. The temperature programming was 60-240 (3°C/min). The injector and detector temperatures were 240 and 230ºC, respectively. The identification of the chemical constituents was based on: (i) GC-MS mass spectrum data bank (Wiley 139 Library); (ii) mass spectra present in literature [14]; and (iii) retention index [15].

The quantification of the substances was done by GC-FID (Shimadzu, GC 17A, Kyoto, Japan) equipped with a capillary column DB-5 (30 m x 0.25 mm x 0.25 μm, J&W Scientific, USA) and split injector. The temperature programming was 50°C (5 min) – 280°C (5 min), 5°C/min. The injector and detector temperatures were 240 and 280ºC, respectively. The quantitative analysis used the external standard method [16]. The quantification was done using a standard of β-caryophyllene (Sigma, Lot: 38H2503, 98.6% of purity) and α-humulene (Sigma, Lot: 97H2505, 98.8 % of purity).

RESULTS AND DISCUSSION

Material Characterization

The particle humidity was 9.3 % wt. The Sauter mean diameter of the particles (from 60-100 mesh) was 2.15×10^{-4} m.

Supercritical Extraction

Table 2 presents the yield obtained (mass of extract/mass of raw material in dry basis x 100) in the experiments. The maximum yield (5.46%) was obtained at 300 bar / 50 °C and the minimum (0.11%) at 78 bar / 40 °C. The repeatability of the experiments was tested in the central point of the experimental design. The standard deviation of the yield was 0.02 %.

The response surface of the global yield as a function of temperature and pressure can be visualized in Figure 1. The Pareto chart of effects (Figure 2) shows the significance of the linear terms of pressure and temperature, the quadratic term of pressure, and the interaction pressure/temperature term.

Effect of solvent pressure

A comparison of the global yield of extract from *C. curassavica* predicted by the empiric model and the experimental data is presented in Figure 3. The empirical model predict one decrease of the global yield with pressure for high pressure values but the isothermal experimental data presented in Table 2 (run 1 and 2 for 30 °C, run 2 and 4 for 50 °C, run 5,7 and 9-11 for 40°C) show an increase of the global yield with pressure.

A set of experiments was carried out to determine the global yield isotherm (54°C) of *C. curassavica* for the pressure range of 78 to 341 bar. The results are presented in Figure 4.

The global yield tends to reach a plateau for high pressures

Table 2. Influence of temperature and pressure on the total yield of *C. curassavica* SFE

Run	Pressure (bar)	Temperature (°C)	Total yield (% dry basis)
1	300	30	2.867
2	300	50	5.461
3	100	30	1.603
4	100	50	0.469
5	341	40	3.355
6	200	54	3.902
7	78	40	0.108
8	200	26	2.349
9	200	40	3.357
10	200	40	3.347
11	200	40	3.321

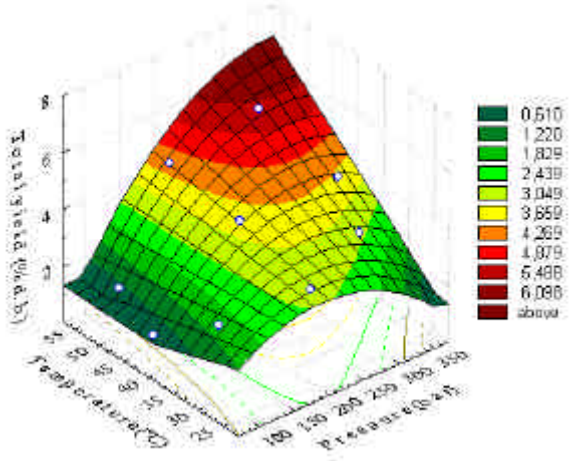

Figure 1 – Response Surface of total yield as a function of pressure and temperature.

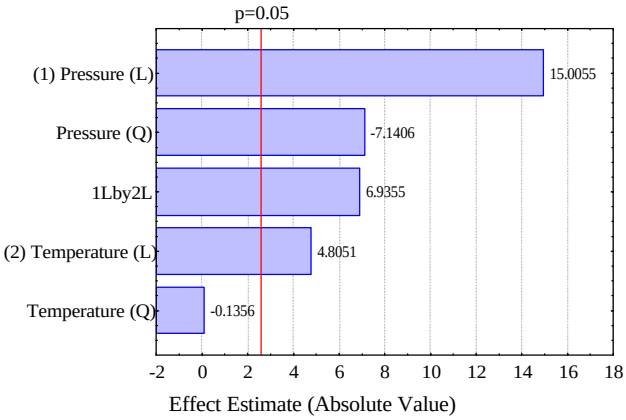

Figure 2. Pareto chart of effects.

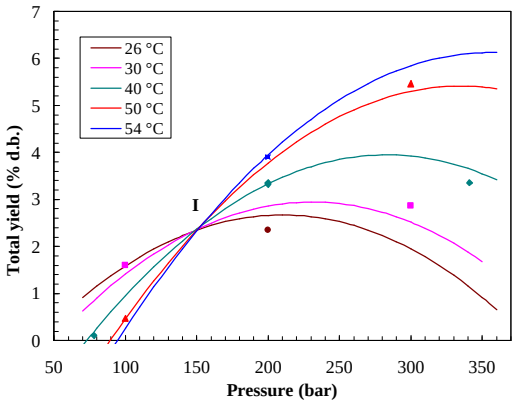

Figure 3 – Predicted total yield vs. pressure at various temperatures

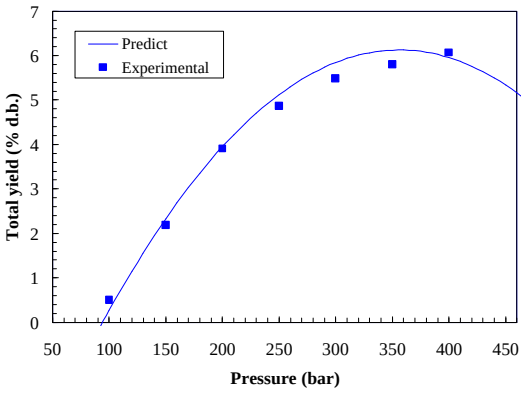

Figure 4 – Predicted and experimental total yield vs. pressure at 54 °C

Effect of solvent temperature

The global yield decrease with temperature for pressure lower than 150 bar. At these

pressures, the CO_2 density decreases considerably with temperature, thus, decreases the CO_2 solvation power and, consequently, the global yield decreases. The joint point in the isotherms (point I in Figure 3) can be considered as the solubility inversion point [17]. For pressures larger than 150 bar, the global yield increases with temperature being the increase of vapor pressure more important than the density decrease [18]. Gomez et al. [1] also observed the decrease of the extraction yield with temperature for pressure lower than 150 bar.

The hexane Soxhlet and hydrodistillation yields were 8.12 and 0.50% (dry basis), respectively. Although the high yield obtained with organic solvent, the extract was darker and with a solid appearance. On the other hand, the hydrodistilled oil was lighter and less viscous.

Chemical composition of the extracts

The major compounds presented in the extracts were caryophyllene, alo-aromadendrene, b-sesquiphellandrene, a-humulene, b-atlantone, Germacrene-D. The Caryophyllene and a-humulene were quantified and the results are presented in Table 3. In this table the yield values are expressed as the amount of solute extracted divided by the amount of raw material used in the extraction (in dry basis) multiplied by 100. The soxhlet extraction produces the largest amount of total extract but the SFE at 300 bar and 50°C produced the largest amount of both caryophillene and α-humulene.

Table 3. Percentage composition of the extracts

Substance	SFE		Soxhlet extraction	Hydrodistillation
	200 bar/54°C	300 bar/50°C		
Caryophyllene	0.075	0.228	0.144	0.017
a-humulene	0.085	0.225	0.160	0.031
Total Yield	3.90	5.46	8.12	0.50

CONCLUSIONS

The influence of the extraction temperature and pressure on the SFE global yield of *Cordia curassavica* extract was studied. The global yield decrease with temperature for pressures lower than 150 bar. For pressures larger than that, the global yield increase with temperature. This behavior is similar to the influence of temperature on the oil solubility. The best operational condition was 300 bar and 50°C. At this condition was found the maximum yield of extract as well as the maximum yield of caryophyllene and a-humulene.

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