OLEORESIN EXTRACTION OF *Polygala cyparissias* USING SFE AND ORGANIC SOLVENT METHODS

WEINHOLD, T.S.; TRIDAPALLI, C. W.; HENSE, H. FERREIRA, S.R.S.*

LATESC/EQA/CTC – UFSC, CP 476 - 88040-900, Florianópolis, SC – BRAZIL
* Corresponding author: sandra@enq.ufsc.br; FAX: 55 48 331.9687

Supercritical fluid extraction (SFE) is a reliable technology and its application has been increased in the last decades. It is an alternative extraction process, compared to the conventional ones, especially due to the focus on product quality and process selectivity.

A SFE unit with a fixed bed extractor was used to evaluate the oleoresin extraction using supercritical CO_2. The high pressure unit allow the control of the operational conditions temperature and pressure, and as a consequence, the solvent density, in order to fix the CO_2 ability in the solubilization.

The aim of this investigation was the extraction of the oleoresin from *Polygala cyparissias*, a bush commonly find in dunes in the Brazilian coast. The therapeutic activity related to the *Polygala cyparissias* extract is attributed mainly to the presence of xanthones and triterpenoids. The composition profile of the extracts, identified using gas chromatography - mass spectrometry indicate the presence of squalene, α-amyrin, friedelin, β-sitosterol, among others, for the supercritical fluid extract, while the literature indicate the presence of xanthones such as 1,3-dihydroxy-7-methoxyxanthone, in the conventional extract. The extracts are potentially used against allergies, asthma and inflammations and as diuretic.

Therefore, the objective of this study was to evaluate the efficiency of the SFE process, compared to conventional solvent extractions, to obtain the *Polygala cyparissias* oleoresin. The process conditions were 150 and 200bar, 20 and 40°C, with solvent density varying from 781.27 to 937.48 kg CO_2/m^3 and a solvent flow rate from 1.5 to 4.0 g CO_2/min. The SFE yield was up to 0.35 % w/w (for 840.4 kg CO_2/m^3) and, for the conventional extractions were 0.44; 0.48; 0.59 and 4.09 % w/w for n-hexane, dichloromethane, ethyl acetate and buthanol.

Key-words: SFE, *Polygala cyparissias*, xanthones, triterpenoids, composition profile.

INTRODUCTION

Medicinal plants and its extracts represented for several centuries the base for therapeutic. Although, only from the beginning of last century, contributions from pharmacology, biochemistry, and organic chemistry became imperative. Then, studies involving medicinal plants have been increased in the last few years concerned to the characterization of new components with therapeutic activity. Also, Brazil is the greatest worldwide reservoir, due to the availability and diversity of its natural resource, which remains basically scientifically unexplored.

One of the main limitations of the phytotherapy is the use of natural products as source for medicaments, especially due to the process complexity and the presence of biologic mixtures, difficult to characterize. Although, innovative techniques and new

engineering processes have been overcoming this limitation, which indicates a promising future for the phytotherapy [1].

The supercritical fluid extraction (SFE) has became an alternative technique for the extraction of natural products due to the use of low temperature process and to obtain solvent free products. Also, it allows to obtain extracts more representative of the original material, than the conventional ones.

Therefore, the objective of this investigation was the determination of the composition profile of *Poligala cyparissias* extracts, commonly called as "avenca-da-praia" (*beach maidenhair*), a bush easily found in the dune regions of the Brazilian coast. This study was also concern to the comparison between SFE and organic solvent extracts, in order to evaluate the process efficiency.

The "avenca-da-praia" was first taxonomically identified in 1992 as *Polygala cyparissias ST. Hill & Moq.*, and the initial studies were related to the conventional extracts [2].

The popular medicine has been indicating the use of *Poligala cyparissias*, specially its root, as topic anesthetic. The organic solvent extracts from aerial parts are indicated for allergies, asthma and inflammations, among others. The importance of the "avenca-da-praia" as potential phytotherapic is related to the presence of the methyl salicylate in the root and the xanthones composition in the plant extract [2, 3].

I - MATERIAL E MÉTODOS

The plant material used in this investigation was collect from the dunes in Florianópolis, SC, Brazil. The plant was then dried at room temperature, grounded using a domestic coffee grinder and classified using sieves. The particle size selected for the SFE was +20 –32 mesh.

A high pressure unit operating in a dynamic mode and with a fixed bed extractor was used to obtain the supercritical fluid extracts of "avenca-da-praia" (LATESC – UFSC – Brazil). 40g of the grounded material were used to prepare the fixed bed.

The extractions were performed using supercritical carbon dioxide (SC-CO_2) from White Martins, at 150 and 200 bar and at 20 and 40^{o}C, which indicate solvent density varying from 781.27 to 937.48 kgCO_2/m^3 [4], obtained using the pump (Model 3200 P/F, Thermo Separation Products, Fremont, CA, USA) where the operational pressure was adjusted.

The fixed bed of grounded particles was formed inside the extractor column. And the process temperature was controlled by a thermostatic water bath (Model MQBTZ99-20, Microquímica, Florianópolis, SC, Brazil). Samples were collected at predetermined time intervals up to 5 hour extraction after 6 hours static time (initial solvent/solid contact). Overall extraction curves were obtained with the experimental data of mass of extract against mass of CO_2.

Organic solvent extraction of "avenca-da-praia" was developed using methanol for exhaustive extraction (12 L/5 days) for 1.452 kg of raw material, to obtain the crude extract (CE). The CE was then fractionated using hexane, dichloromethane, ethyl acetate and buthanol, solvents with a polarities ranging from non-polar to polar.

Extract analysis

The chemical profile of the "avenca-da-praia" extracts, obtained using SC-CO_2 were developed in the *Instituto de Pesquisas Tecnológicas* (Laboratório de Análise de

Combustíveis – FURB, SC – Brazil), using gas chromatography-mass espectrometry analysis – GC/MS (GC Varian CP-3800, MS Saturn 2000), for the components identification, using a database Saturn GC/MS Workstation 5.51.

The composition of the SC-CO_2 (at different operational conditions) and conventional extracts were evaluated by gas chromatography (Shimadzu, Model CGS-14A, Kyoto, Japan) equipped with a FID detector. The column temperature started at 40ºC, up to 305ºC at 8ºC/min. The detector temperature was 320ºC and the injector temperature was 280ºC with 1µl sample injection.

II - RESULTS AND DISCUSSION

Overall extraction curves (OEC) for the "avenca-da-praia" oleoresin extraction, obtained at 40°C and 150 and 200 bar for different solvent flow rate, are shown in **Figure 1**. The experiments at 200 bar were performed at 3.35; 1.49 and 2.82 gCO_2/min. and the extraction at 150 bar was developed with solvent flow rate set at 3.21 gCO_2/min.

The process behavior show in **Figure 1** for 40°C indicate an increase in the extraction rate with the pressure, due to the increase in the solvent density and therefore the solute solubility in the solvent phase.

The flow rate effect, also shown in **Figure 1**, indicate an increase in the extraction rate with the solvent flow rate. This behavior is not expected, specially if we consider that low flow rate indicate larger contact time between solid and solvent phases. Although, we may evaluate this behavior according to the different mass transfer effects. At low flow rate, the intra-particle diffusion may be overlapping the convective effect, decreasing the extraction rate [5].

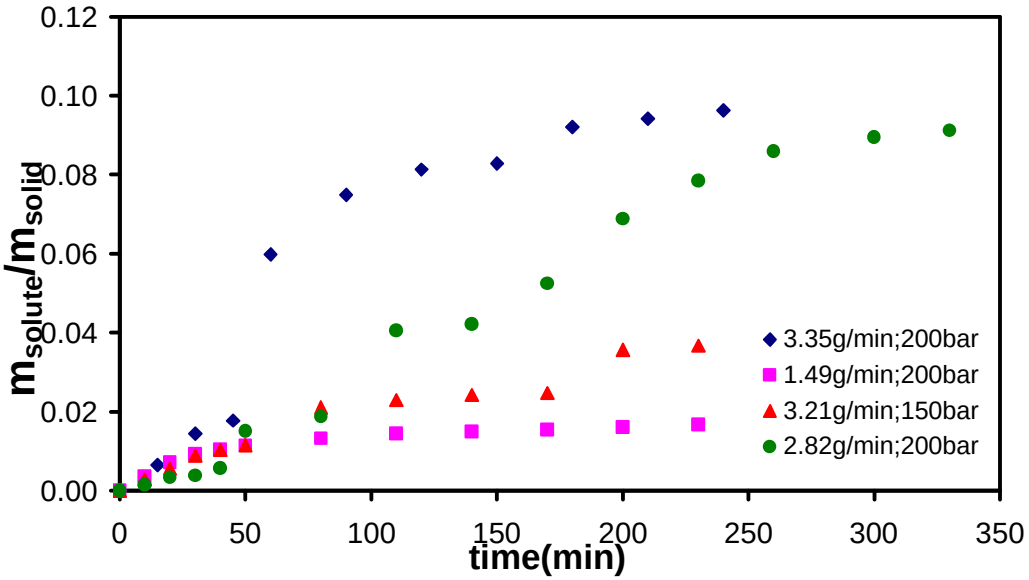

Figure 1. Overall Extraction curves for "avenca-da-praia" at 40ºC.

To evaluate the efficiency of the supercritical fluid extraction to obtain "avenca-da-praia" oleoresin, the SFE process was compared to the conventional processes and the results for the extraction yield are compared in **Table 1**. The results are presented for SFE at different operational conditions and for organic solvent extractions using

hexane (500mL), dichloromethane (400mL), ethyl acetate (400 mL) and buthanol (100 mL), for the 98.54g of CE. The highest yield for SEF was obtained at 200 bar and 40°C (0.35% w/w). This result are compared, in terms of order of magnitude, with extractions using hexane, dichloromethane and ethyl acetate. Solvent extraction with buthanol indicate much higher process yield than the SFE, probably due to the polarity of the solvent and to the presence of methanol, added to this fraction to improve the yield.

The temperature effect in the SFE yield, evaluated for 150 bar, indicate an increase in the extraction rate with temperature, from 20 to 40°C. This behavior may be explained by the solute vapor pressure, that increases with temperature, promoting an enhancement of the solute concentration in the solvent phase. The studied range of temperature and pressure indicate, from the extraction results, the proximity from the cross-over region. Also, the density effect in the process yield agrees with the croos-over region, i.e., at 150 bar the yield increased with decrease in density and at 40°C the yield increased with density (from 781.27 to 840.40 kg/m^3).

Table 1. Extraction yield: Comparison between SFE and conventional process

Organic solvent extractions		SFE	
Solvent	Yield (%)	Conditions (bar / °C / ρ_{CO2})	Yield (%)
Hexane	0.44	200 / 40 / 840,40	0.35
Dichloromethane	0.48	150 / 40 / 781,27	0.09
Ethyl Acetate	0.59	150 / 20 / 904,51	0.01
Methanol/BuOH	4.09		

Although the yields obtained using organic solvent extractions were higher if compared with the SFE results, to evaluate the process efficiency is important to analyze the solute composition profile. Besides the conventional extraction yield, this processes present disadvantages such as high organic solvent volumes and time of extraction, if compared with SFE.

Chromatographic analysis were performed for “avenca-da-praia” extracts obtained using organic solvent and supercritical CO_2. The results for the hexane extract and for the SFE at 200 bar and 40°C are shown in **Figures 2** and **3**, respectively.

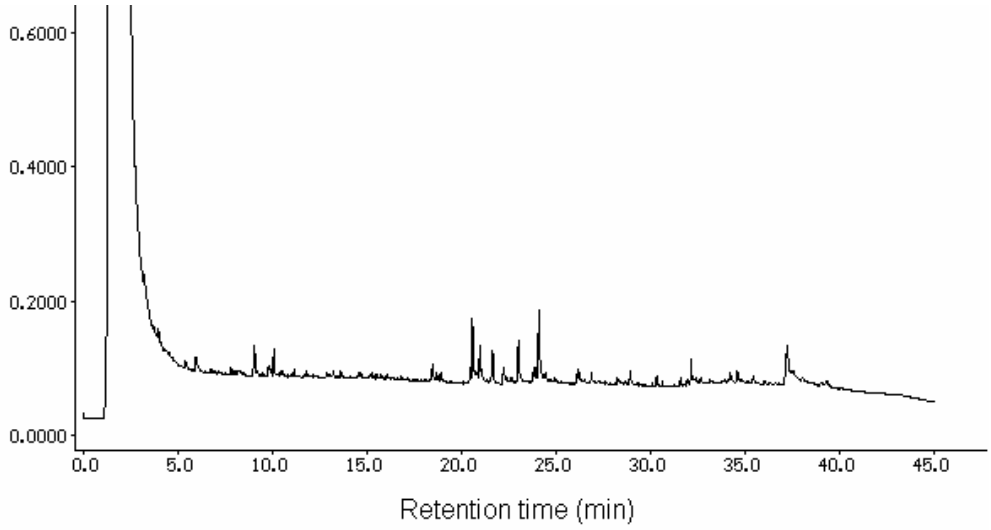

Figure 2. Gas chromatogram from conventional extract – hexane fraction

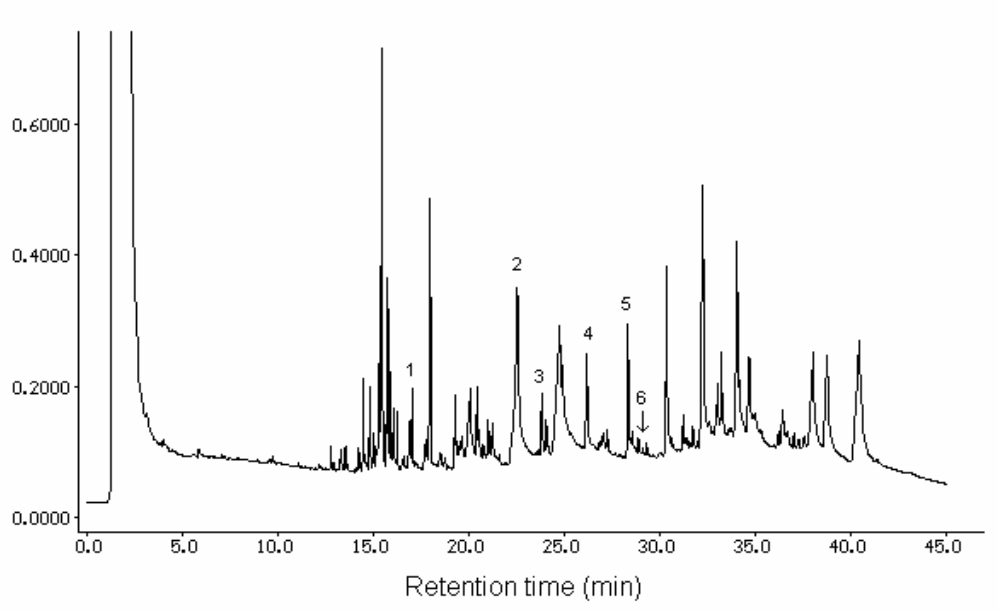

Figure 3. Gas chromatogram from SFE at 200 bar and 40ºC

According to the results shown in **Figures 2** and **3** we observe that the CO_2 extract present the richest composition profile, with a large number of separated components (pick number) (**Figure 3**), compared to the hexane fraction (**Figure 2**). This behavior proof the process differences in terms of product quality. Also, to evaluate the CO_2 efficiency, related to target components, the extract was analyzed by GC-MS in order to identify the components present in the extract. The results are shown in **Table 2** with the pick numbers listed in **Figure 3**, the identified components and the respective molecular weight.

Table 2. Identified components for "Avenca-da-praia" extract.

Peak number	Component	Molecular weight
1	N-butyl-Benzenosulfonamide	213
2	γ-sitosterol	414
3	Dibenz(a,c)acridine	279
4	Cholest-4-ene-3,6-dione	398
5	Friedelin	426
6	Decosane	310

Besides the components listed in **Table 2**, the GC-MS analysis also identified the presence of squalene and α-amyrin (triterpenes like friedelin), stigmasterol, a diterpene, and also β-sitosterol and dehydromevalonic lactone. From the identified components, the α-amyrin is a triterpenic saponine present predominantly in the families Sapindaceae, Hippocastanaceae and Polygalaceae, among others. The saponines are indicated as expectorant and diuretic, important therapeutic activities [1].

CONCLUSION

The extraction of *Polygala cyparissias* oleoresin indicate the CO_2 is as efficient as hexane, ethyl acetate and diclormethane in terms of process yield. For SFE the pressure effect indicate an increase in the extraction rate due to the increase in the solvent density, but the temperature effect indicate the importance of the solute vapor pressure in the extraction rate, which increases with temperature although the solvent density decreases. The composition profile of the extracts shown the significant difference between extracts (CO_2 and hexane) and also the presence of an important group of components, the saponines, represented by the α-amyrin, not detected in the hexane fraction.

REFERENCES

[1] SIMÕES, C. M. O.; SCHENKEL, E. P.; GOSMANN, G.; MELLO, J. C. P.; MENTZ, A. L.; PETROVICK, P. R.; Famacognosia: da planta ao medicamento. 2 ed. rev. – Porto Alegre/Florianópolis: Ed. Universidade/UFRGS/ Ed. UFSC, p. 21, 2000.

[2] PINHEIRO, T.N.; CECHINEL, V.F.; SANTOS, A.R.S.; CALIXTO,J.B.; MONACHE, F.D.; PIZZOLATTI,M.G.; YUNES,R.A.; Three xanthones from *Polygala cyparissias*. Phytochemistry. Vol.48 (4), 1998, p.725-728,.

[3] PINHEIRO, T. R.; Isolamento, identificação e avaliação do potencial farmacológico dos constituintes de *Polygala cyparissias ST. Hill & Moq*, Dissertação (Mestrado em Química), Centro de Ciências Físicas e Matemáticas da Universidade Federal de Santa Catarina, Florianópolis, 1996.

[4] ANGUS, S.; ARMSTRONG, B.; REUCK, K. M.; International Thermodynamic Tables of the Fluid State. v.3. Carbon dioxide. Pergamon Press, 1976.

[5] RODRIGUES, M.V.; MEIRELES, M.A.A.; MARQUES, M.O.M. Determination of the solubility of clove essential oil in SC-CO_2: a standardization of the dynamic method. In: Proceedings of the 5th International Symposium on Supercritical Fluids. Westin, Atlanta, USA, 2000. CD-Room.