SUPERCRITICAL FLUID EXTRACTION OF WOODS AND ROOTS FROM THE BRAZILIAN TROPICAL FOREST REGION

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Abstract

Extensive studies have been carried out in order to extract, identify and quantify pharmaceutical active ingredients in the Amazonas flora (e.g. Preciosa (*Aniba Canellia H.B.K MEZ*), Sacutiaba *(sideroxylon obtusifolium (Roem.&Schult)* , Mangoe branco *(Mangifera indica)*, Casca-doce (*Glycoxylum inophyllum*) and Pequia (*Aspidosperma australe*). Different extraction techniques have been determined: steam distillation, Soxhlet extraction and Supercritical Fluid Extraction (SFE). All extracts were analyzed using gas chromatography GC and the chromatograms for the different extraction methods compared qualitatively. Low volatile and high volatiles compounds were specified. Some wood barks extracts were analyzed by GC-MS in order to identify substances of particular interest.

1 INTRODUCTION

The Amazonas region in Brazil hides many plant species of pharmaceutical importance. Lot of work has been carried out and is still being carried out in order to extract, identify and quantify these extracts/active ingredients in the flora. Different extraction techniques have been used and this leads to the problem of comparing different extraction results as each has a particular group of compounds they preferentially extract. It is therefore necessary to do a screening and a comparison of analysis results between various extraction techniques. This work should give an idea of the total extractable compounds and a comparison between three chosen extraction methods[1]. Certain wood barks from the Amazon region were subjected to extraction by steam distillation, Soxhlet extraction and supercritical fluid extraction, with more emphasis on the latter technique. The extracted wood barks were –among others- *Mangoe Branco, Sacutiaba*, *Casca Doce*, *Preciosa*, and *Pequisa.* The extracts were analyzed using gas chromatography and the chromatograms for the different methods compared qualitatively. Similarities in retention times and the presence or absence of low volatile (LVC, late eluting) or high volatile (HVC, early eluting) compounds were compared.

2 MATERIALS AND METHODS

2.1 Steam Distillation

For steam distillation a Karlsruher Apparatus was used. 5 g of the milled wood was weighed and introduced into about 1–1.5 liters of water in a round bottom flask. The mixture was boiled for about 4 hrs. The essential oils flow with the water vapor through the pentane and are retained while the water vapor condenses and flows back to the round bottom flask. The pentane with the essential oils is drawn off and the pentane allowed to evaporate.

2.2 Soxhlet Extraction

The extraction apparatus consists of a heating device, a solvent reservoir and an extract flask, an extraction chamber containing a porous paper extraction thimble and a condenser. 10 g of the sample was placed in the thimble and covered with glass wool. It was then placed into the

extraction chamber. The extract flask was weighed and about 200 ml of hexane was poured into it. After about 6 hrs, the hexane and extract in the extract flask was vaporized using a rotavapor unit. 10 ml of hexane was added to the extract in order to prepare it for the analysis.

2.3 Supercritical Fluid Extraction (SFE), CO_2 as supercritical solvent

The apparatus used was a Spe-ed SFE unit (Applied Separations, Allentown, PA). CO_2 is condensed and pressurized up to the operating pressure by means of a pneumatic pump. The samples (4.0 g) were filled into the 10 mL column, and glass wool was placed at both ends of the solid bed. First, the CO_2 valve and the pressurized air valves are opened, and the condenser is started. After the system becomes stable with the operating pressure (30.0 MPa), and operating temperature (40°C), and the equilibrium time of 15 min is reached, the micro-regulating valve is set to maintain a CO_2 flow rate from 2.7 to 4.5 g/min (solvent ratio equivalent to 0.675–1.125 min^{-1}, respectively) and the experiment is started. The extraction time was 4 h. For experiments with modifier a HPLC pump was used to pump 10 % ethanol to the extraction vessel at a flow rate of 0.5 ml/min. The flow rate of the CO_2 was maintained at 5.4 g/min. All other procedures were as explained above. Samples are collected and kept in a cold trap weighed and preserved in a refrigerator at 4°C until being analyzed.

2.4 Analysis

The used analysis method for extract comparison was GC-FID[2].The extracts were analyzed using a Hewlett Packard (Avondale, PA) HP 5890A GC, equipped with a cold on column capillary inlet system, which was maintained at 0°C. A 30 m, 0.25 mm I.D, 0.10 µm FD DB-5-MS (J & W Scientific, Folsom, CA) fused silica capillary column was used for the separation. Grade 5.0 Nitrogen (Linde, Germany) was used as the carrier gas with a flow rate of 2 ml/min. The temperature program contained a 1 min initial temperature of 70°C, followed by a ramp of 10°C/min to a final temperature of 300°C, which was maintained for 20 min. For all the extracts, 1 µL was injected for each analysis. Peaks eluting in the time interval from 0 - 22 min were considered as **H**igh **V**olatile **C**ompounds (HVC), peaks eluting after 22 min were considered as **L**ow **V**olatile **C**ompounds (LVC). In the figures the chromatograms of Soxhlet extraction (chromatogram A), SFE (chromatogram B) and of steam distillation (chromatogram C) of different plant species are shown.

3 RESULTS AND DISCUSSION

Table 1 concludes the extraction yield obtained with the three extraction methods studied.

Table 1 : Extraction yields (%DW) by using different extraction methods

Wood	Extraction yield (%)		
	Soxhlet	**Steam Distillation**	**SFE**
Mangoe Branco	1.85	0.06	0.18 (NM) 14.8 (WM)
Sacutiaba	1.14	0.03	0.32
Casca Doce	0.45	0.11	3.1/ 0.95*
Preciosa	2.68	2.35	8.15 (NM) 3.37 (WM)
Pequia	0.46	0.07	0.75
Barba Timao	1.75	0.03	0.46
Kina	1.09	0.15	0.48
Eanaipe	0.27	0.06	1.64 3.87
Pau d'onco	0.57	0.06	0.45
Pau Darco	1.03	0.16	1.0
Aroeipa	2.15	0.08	2.0

NM – no modifier; WM – with modifier; * – first/second trial

Soxhlet extraction gave higher yields for most of the samples, when compared to steam distillation and SFE. By adding of a modifier or liquid solvent can be manipulated the selectivity of the extraction process. Two reasons are responsible: the affinity between solvent and extractible substances and increasing of elution processes in the extraction vessel. However, the SFE extracts show in all the chromatograms less peaks than the other extraction techniques. Latter confirm the SFE process is a clean extracts producing extraction technique.

3.1 Mangoe Branco(Mangifera indica)

The Soxhlet extraction chromatogram showed HVC and LVC, which are characteristic for this technique. The SFE chromatogram showed one specific HVC. Steam distillation appeared to extract preferably HVC. Essential oils should be the likely compounds present. Similarities in retention times (14 and 18min for steam distillation and Soxhlet extraction, 18min and 21min for all three) were observed inferring that all the processes extracted identical or closely related compounds, however, with varying concentrations.

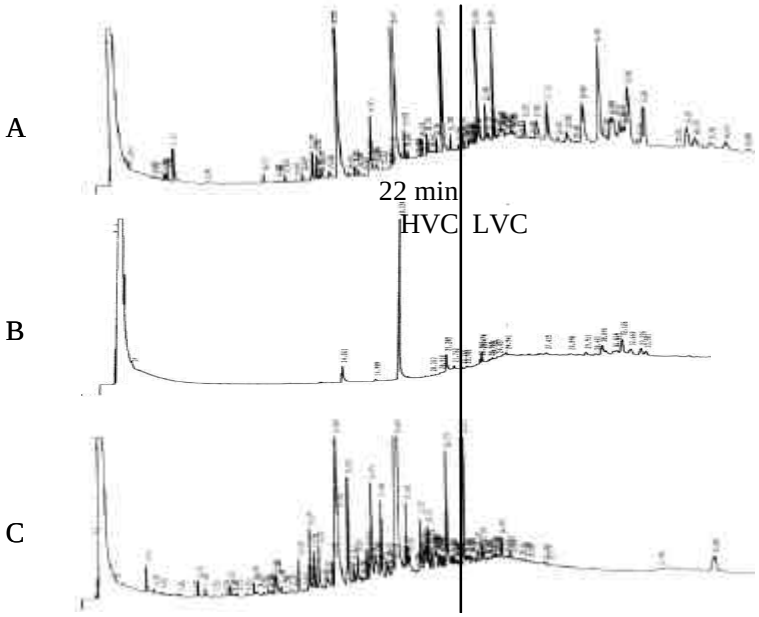

Fig. 1 : *Mangoe Branco* (A Soxhlet 9.26mg/mL, B SFE 1.44mg/mL, C Steam 3.2mg/mL)

3.2 **Sacutiaba** ***(sideroxylon obtusifolium,Roem.&Schult)***

LVC in the chromatograms of SFE and Soxhlet extraction were of interest, with more late peaks being present in the chromatogram of the latter. This suggests that the Soxhlet method appears to extract more substances than the SFE. This trend was also observed for the HVC, though in very low concentrations. The steam distillation chromatogram showed only HVC in the range of 16 and 22min with concentrations higher than those for the other two processes. This occurred, notwithstanding the higher sample concentrations used in the GC analysis for the Soxhlet and SFE techniques. The SFE extract of Saca Tudo was analyzed by GC-MS. It was possible to identify the substances

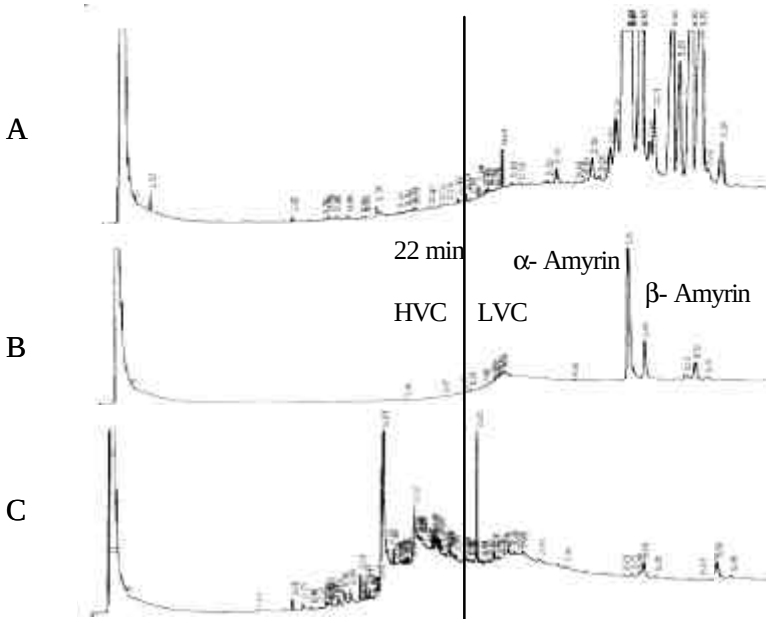

Fig 2 : *Saca Tudo* (A Soxhlet 5.67mg/mL, B SFE 0.96mg/mL, C Steam Distillation 0.7mg/mL)

α-Amyrin and β-Amyrin with retention times of 34.12 and 35.14 min respectively. As seen in Fig.2 SFE is able to extract these substances with high selectivity These substances have similar properties in some respects to mianserin (antidepressant) and might process a sedative action [3].

3.3 Casca-doce (Glycoxylum inophyllum)

The Soxhlet method extracted a few HVC at retention times of 16,18 and 22min. Eluting peaks were also observed at 33 and 34min. Similar early eluting peaks were observed for steam distillation. Judging by the concentration of sample analyzed by GC, we can infer that the steam distillation extracted larger amounts of early eluting extracts. Obviously, SFE produced a different extract spectrum as the other techniques in the HVC range. The latter observation was not the case for the peaks occuring after 22 min run time and could imply that the different techniques prefe-rentially extract identical substances in higher amounts. An early eluting peak (18min.) was noticed in the chromatogram for the Soxhlet and steam distillation, but missing in that for the SFE. Identical compounds were observed at 22 and 33min for all three processes.

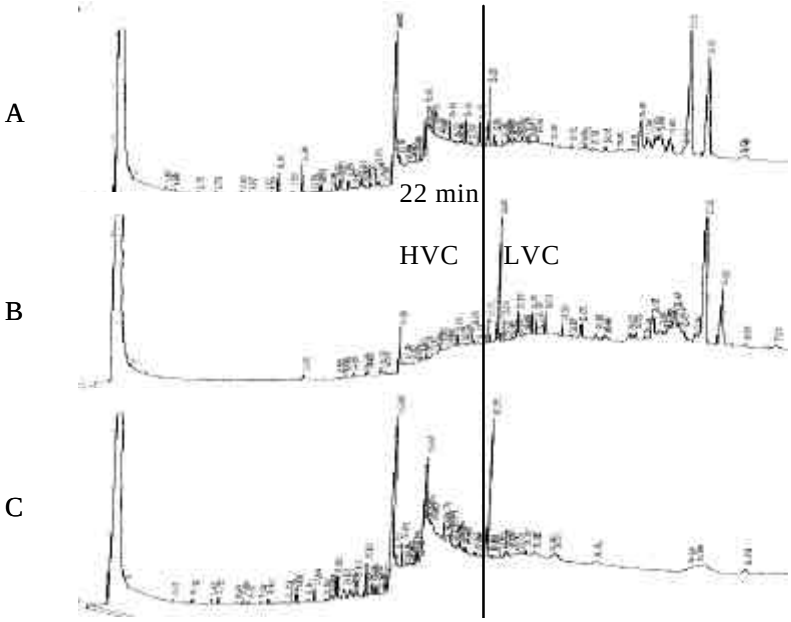

Fig. 3 : *Casca-doce* (A Soxhlet 4.46mg/mL,B SFE 7.74mg/mL, C Steam 1.14mg/mL)

3.4 Preciosa (Anniba Canellila)

Preciosa is commonly known as rosewood and is an evergreen tree with a reddish bark and yellow flowers. The tree is usually felled for its essential oils. The latter has been extracted by steam distillation from wood chippings and produces a colorless to pale yellow liquid. It has a floral spicy scent. Traditionally, rose-wood oil is used in Brazil for acne, colds, coughs, dermatitis, fevers, frigidity, headaches, infections, nausea, nervous tension, skin care and wounds and in

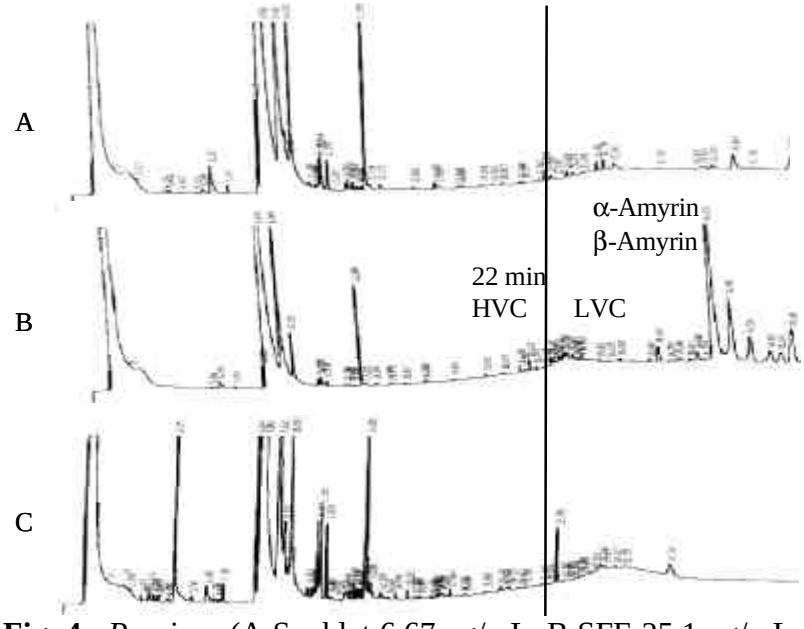

Fig. 4 : *Preciosa* (A Soxhlet 6.67mg/mL, B SFE 25.1mg/mL, C Steam Distillation 11.7mg/mL)

Venezuela for arthritis, catarrh, edema, leucorrhoea, the nerves and Venereal diseases. Essential oils and lemonoids are the important phytochemicals present, they have the following properties and actions: analgesic, anticonvulsant, antidepressant, antimicrobial, antiseptic, aphrodisiac, bactericidal, cephalic, deodorant, stimulant and tonic [4].
Peaks of interest were those of HVC for steam distillation and Soxhlet extraction. The latter did not extract LVC as expected. The SFE chromatogram showed presence of HVC and LVC, the moderately volatile compounds were not significant. Identical peaks were seen from the chromatogram of the Soxhlet and SFE techniques at a retention time of about 9 min. By GC-MS α-Amyrin and β-Amyrin (again with retention times of 34.12 and 35.14 min, respectively) were identified in the SFE extract.

3.5 Pequia (*Aspidosperma australe*)

The Soxhlet extraction chromatogram showed early and late eluting peaks (14-35min.), which are characteristic for this technique. With a retention time of 16.8 and 22.2 min were observed peaks in the chromatograms for all the extraction processes. The chromatogram from the SFE shows late eluting peaks form 16 to 33 min. The extraction methods Soxhlet and SFE appear to extract the same or narrow related substances, particularly in the region between 30 and 33 min retention time. Steam distillation chromatograms shows early eluting peaks, the most in the region between 16 and 18 min. retention time. Identified substances in the apisto SFE-extracts were: linoleic acid, stearic acid, palmitic acid, pentacosan, terpene, alkanes and alkenes.

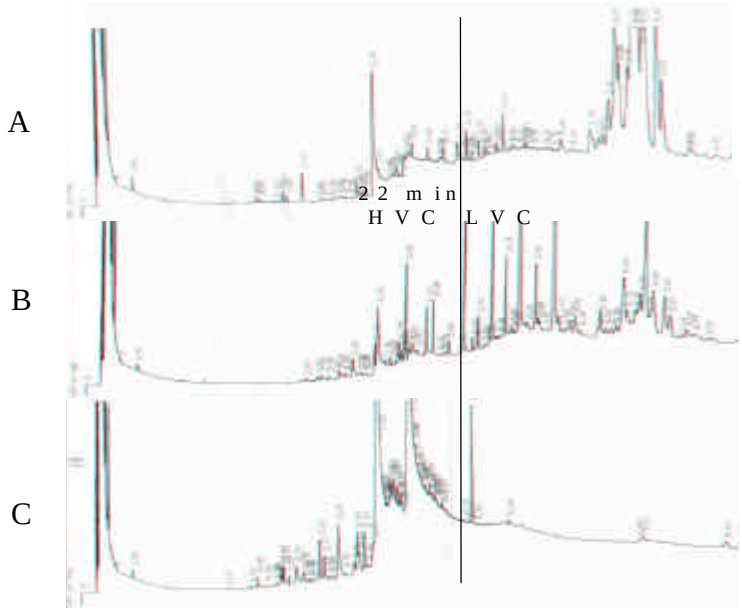

Fig. 5 : *Apisto* (A Soxhlet 4.6mg/mL, B SFE 2.0mg/mL , C: Steam 1.9mg/mL)

4. CONCLUSIONS

From the results obtained in this project, one can infer that the three processes extracted materials according to the solvent affinity of the extracting fluid :

The steam distillation extracted mainly high volatile compounds, suspected to be essential oils and proved to be better than the other two techniques with respect to extraction of this particular group of compounds.
The Soxhlet and SFE processes extracted almost all compounds with the exception of some of the wood samples. Some of these extracted substances could be identical or closely related substances as seen in the chromatograms. These compounds could range from essential oils to fatty acids to resins and waxes.

However, it was observed that the Soxhlet extraction with hexane gave a higher extract yield (%) than the other two methods.
The SFE in general gave a higher yield than that for steam distillation. One reason could be the high solvent ratio (150 – 480gCO_2/g_{solid}) used for the process. The combination of a modifier (polar) with supercritical carbon dioxide was observed to increase the yield of the extracts, however this depends on the type of wood, as one sample gave a contradicting result.

5. REFERENCES

[1] **Miller, R. B**., Structure of Wood, Balsa Wood Structure Information, www.zimsweb.com/balsa/information/balsainfo.htm
[2] **Brunner, G.** Gas Extraction. An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes, Springer-Verlag: New York**, 1994**
[3] Raintree; Rosewood **www.rain-tree.com/rosewood.htm**
[4] **Subarnas, A.et al.:** J. Pharmacol. 45(6). **1993**