SUPERCRITICAL FLUID EXTRACTION OF RESIDUAL ITAÚBA WOOD (*MEZILAURUS ITAUBA*)

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ABSTRACT – Itauba (*Mezilaurus itauba*) is an Amazonic tree from the Lauracae family and its wood has significant economic importance. Its extensive use generates large amounts of residue, which frequently causes environmental damage. We studied the supercritical extraction (SFE) of residual *M. Itauba* for the production of natural extracts with high aggregated value. The extraction yield and composition were compared to other techniques (soxhlet using n-hexano and hydrodistillation) and to the use of ethanol as a co-solvent during the SFE. The SFE extracts were obtained using CO_2 as solvent with pressures from 80 to 220bar, temperatures from 38 to 52^0C and 3mL/min flow. The highest output obtained using SFE was 1.04% at 220bar and 45^oC. On extractions using ethanol as a co-solvent (2 to 8% concentration), the maximum yield was of 1.56%. Soxhlet and hydrodistillation showed yields of 5.47 and 0.04%. Important composites were identified in the extracts and of highlight α-Cadinol, T-muurolol and δ-Cadinene.

1. INTRODUCTION

The unordered world population growth and technological advancements over the last decades created a greater dependency on renewable sources of raw materials, which makes up a relevant problem for the environment and economy. Wood is an important source of organic material, which stimulates the whole use of its constituents. *Mezilaurus itauba* is a tree belonging to the botanical family Lauraceae (Lorenzi, 1998). Lauraceae has a special relevancy among the other families due to its economic importance, as, beyond the use of its wood, its species are aromatic, being frequently used as raw material in the industry. The antimicrobial properties of the methanolic and dichloromethanic extracts from *Mezilaurus itauba* were researched by Rodrigues *et al*. (2008). *Mezilaurus itauba* wood is generally employed in hydraulic constructions, bridges, laminations, high grade furniture, nautical constructions, etc. The forestry sector is a strong consumer of natural resources with renewable potential. However, it also produces a large amount of residues. It is estimated that around 40 and 60% of the volume from logs is properly made use of, which means that from each 10 trees, half of these are wasted (Mady, 2000). The main wood components with commercial value are: cellulose, lignin, hemicellulose, extractives and nonextractives (pectins and inorganic compounds). Extractives are substances formed from alteration or the carbohydrates produced during photosynthesis and may correspond to more than 20% of the dry weight in tropical trees and are constituted of a number

of organics compounds. These substances are responsible for several characteristics from the wood, such as: scent, resistance to decay, avor and abrasive properties. Its composition may vary between different tree species, segments of the tree, age, preceding region, etc. (Gonzaga, 2006). The economic retrieval of many of these materials is still a challenge. Some composites may be obtained using vapor or organic solvents, but these approaches present some disadvantages that limit their utility. For example, the high operation costs associated to organic solvents. Hence, the solid residues from the oil extraction (solid-liquid extraction and pressing), essential oil (hydrodistillation) or supercritical extraction industries may be considered a promising source of raw material. Thus, this study aims to assess the extraction yield using CO_2 in supercritical phase ($scCO_2$) at a range of temperature and pressure settings and compare the results with extraction by hydrodistillation and soxhlet using n-hexano.

2. MATERIALS AND METHODS

Residues from *Mezilaurus itauba* wood from the state of Pará (Brazil) were provided by Madeireira Flor do Sol (Santa Catarina, Brazil) - a lumberyard. Drying was performed in an oven for three days at 40^{o}C. The raw material was then ground with a coffee grinder (MDR 301, Cadence, Brazil) and classified in mesh -25+30 to form a fixed bed of particles. Microphotography with scanning electron microscope (model JEOL JSM-6390LV) was applied to determine the geometry and average size of the particles. The average measured size of the particles was of 0.38mm. Figure 1 shows the microphotography taken of the dried ground wood particle. The real density was computed using the pycnometry with Helium gas and was of 0.3635 kg/m^3. Meanwhile, the apparent density was obtained for the wood mass used to occupy the extraction cell volume and its value was 378.6 kg/m^3. The particle porosity was estimated to be 0.74.

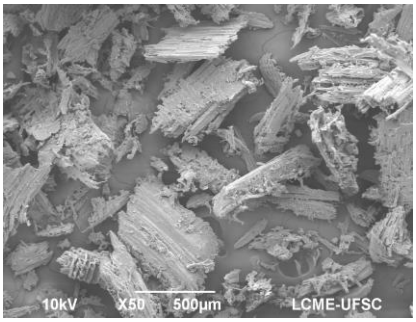

Figure 1 - Microphotograph of the *Mezilaurus itauba* particles.

2.1 Supercritical Fluid Extraction (SFE)

Extractions with supercritical CO_2 were performed in a pilot unit (JASCO). It is composed of three high pressure pumps: B1 (CO_2 pump), B2 (co-solvent pump), B3 (eluent pump), heating stove (3), lined UV-Vis detector (4), back-pressure automated valve (5), controlled heating system (6) (to mitigated the cooling effect caused by CO_2 expansion, the Joule-Thompson effect)

and collection separator (7). An extraction cell with 0.01L of internal volume was used with a 10µm extractor exit filter to avoid contamination of solid particles within the line.

2.2 Supercritical Extraction Procedure

Carbon dioxide (99.9% purity) was supplied to the system through a cylinder (Linde, Brazil). The experiments were conducted in a dynamic mode. After reaching the desired temperature and pressure, CO_2 is released to the extraction cell (E), coming in direct contact with the material sample. The extract is then continuously retrieved under pre-determined flow and times. The extraction time was established as 120 minutes for all experiments. The CO_2 flow was of 3mL/min and also kept constant during the whole extraction. When ethanol was used as co-solvent (in 2, 5 and 8%), the extracts after 120 minutes were taken to the heating stove for 24 hours at 40^{o}C. The total yield was defined as the mass of extract obtained at the end of the experiment over the initial mass of solid material (3,80±2 g of wood) fed into the extraction cell. All experiments were performed in triplicate.

2.3 Experimental Procedure for Hydrodistillation and Soxhlet Extraction

Hydrodistillation was carried out on a Clevenger apparatus using a 1:10 (w/w) proportion of dried and sieved *M. itauba* to solvent (distilled water), according to similar experiments performed by Galhiane and Silva (2006). The separated oil was collected in an amber ask and weighted immediately after collection. The extraction experiment lasted 180 minutes, after which there was no increase in the yield of natural extract. Soxhlet extraction was performed using n-hexane and a solvent-to-solid ratio of 30:1 (v/w). The total extracting time was 180 minutes. n-hexane was removed from the extracts using a rotary evaporator with vacuum control.

2.4 Composition of the Extracts

The composition of the extracts was evaluated by gas chromatography coupled with mass spectrometry (GCMS, Shimadzu GCMS-GC2010-QP2010 plus + AOC 5000) equipped with a RTX 5 column (30 m of length x 0,25mm of diameter x 0,25 µm of film thickness). Helium (He) was the utilized carrier gas at a constant flow of 1 mL/min. The injection temperature remained at 250^{o}C during the process, while the stove temperature was kept at 60^{o}C for one minute and, then, increased up to 240^{o}C at a 3^{o}C/min rate. The mass spectrometer (MS) was set to the following temperatures: ion trap temperature (250^{o}C) and interface temperature (280^{o}C). GCMS Solutions, from Shimadzu, was the managing software utilized and the compound identification libraries were NIST08s and Willey.

3. RESULTS

3.1 *Mezilaurus Itauba* Extraction Yield

In the hydrodistillation process, 0.04% of the essential oil was obtained (percentage yield expressed as the mass of essential oil recovered in relation to the initial mass of sieved and dried *M. itauba* loaded into the system). The hydrodistilled essential oil presented an intense burned smell, a consequence of the high temperature maintained for a long period of time (100°C for 180 min). The extraction using soxhlet presented 5.47% yield (w/w). Table 1 shows the temperature and pressure settings, for a 5 level factorial planning, for the supercritical extraction experiments, as well as the extraction yields (x_o) and the solvent (CO_2) density at each setting. The highest extraction yield is obtained with the conditions of 22 MPa and 45°C where 1.04% of the material was extracted as solute. Conversely, the worst outcome, 0.42%, was found with 8 MPa and the same temperature (45°C), which may be explained due to the lowest tested CO_2 density (241.05 kg/m^3) found at this condition. The lower density reduces the solubility of the oil in the solvent, and was translated into lower extraction yield on the performed experiments, a phenomenon attested by the results shown in Table 1.

Table 1 - Supercritical fluid extraction of *Mezilaurus Itauba*, yield for different experimental settings of temperature and pressure

Experiment	Temperature [°C]	Pressure [MPa]	CO_2 density (ρ_{CO_2}) [kg/m^3]	Yield (x_0) [%]
1	40	10	628,61	0,58
2	40	20	839,81	0,91
3	50	10	384,33	0,52
4	50	20	784,29	0,95
5	45	8	241,05	0,42
6	45	22	832,36	1,04
7	38	15	794,55	0,99
8	52	15	681,71	0,68
9	45	15	741,97	0,78
10	45	15	741,97	0,79

In general, increasing the pressure at a constant temperature leads to a greater yield, as the CO_2 density is higher, thus improving the capability of carbon dioxide to diffuse into the plant matrix and solubilizing the compounds. Analyzing experiments 5, 6 and 7 from Table 1, all at the same temperature, 45°C, the increase in pressure caused a greater solute output. However, in some cases the extraction curves did not correspond to the solvent density. As an example, the highest density (ρCO_2= 839, 31kg/m^3), obtained with 20 MPa pressure and 40°C, lead to a suboptimal yield, 0.91%. This effect had been observed by other works on supercritical extraction (Galvão, 2004; Donelian *et al*., 2009). There is no definitive justification for this result, it could be caused by the heterogeneity found on the wood, which may lead to samples from different parts of the tree with less essential oil to be used in the experiment with higher pressure. Another possible reason for this outcome is the fact that, at these conditions, increasing the temperature leads to a higher vapor pressure of the compounds being extracted, leading them to be converted to the fluid phase and hindering the extraction (Mukhopadhyay, 2000). Alternatively, analyzing the temperature effect at isobaric conditions, at 10 MPa (experiments 1 and 3) and 15 MPa (experiments 7, 8 and 9), the increase in temperature lead to a decrease in yield. Once again, this is caused by the greater solubility found when the solvent has higher density. However, for experiments 2 and 4, performed at 20 MPa, the yield was inversely proportional to the temperature. This phenomenon is justified by an increase in vapor pressure of

the extract at higher pressures, in addition to the greater diffusivity and lower solvent viscosity at 50°C when compared to 40°C (Choi, 2000). In order to evaluate the influence of the process parameters, pressure and temperature, on the yield of *M. itauba* extracts obtained by supercritical fluid extraction, the results were statistically analyzed and the data obtained are shown in Table 2.

Table 2 - Supercritical extraction yield of *Mezilaurus itauba* ANOVA

Effect	SS	Degrees of freedom	MS	F_{action}	p Value
Intercept	0,00225	1	0,00225	40,2026	0,09958
P	0,33848	1	0,33848	6039,82	0,0081
T	0,025789	1	0,025789	460,176	0,02965
P×T	0,00225	1	0,00225	40,2026	0,09958

According to the results shown in Table 2, it is verified that pressure and temperature have significant effect over the extraction output, this is confirmed by the statistical results with a pValue lower than 0.05. Figure 3 shows the response surface generated by a quadratic model regression, describing the yield behavior as a function of pressure and temperature.

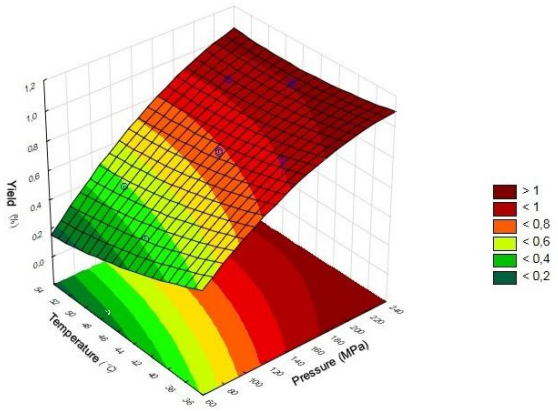

Figure 3 - Surface graph of the *Mezilaurus Itauba* supercritical extraction yield in function of the temperature and pressure (T and P).

Analyzing Figure 3, there is an indication that for pressures lower than 22 MPa there is a tendency to have higher extraction outputs with lower temperatures. Conversely, at pressures higher than 22 MPa there is an increase of the yield with higher temperatures. Choi (2000) and Leal *et al*. (2003) also observed in their works such effect of the temperature on the yield on high and low pressures. On the extractions using ethanol as a co-solvent with 2, 5 and 8% volume concentrations in relation to CO_2, the observed yields were 1.05, 1.21 and 1.56%, respectively. These yields were superior to the ones when using pure CO_2 (which had 1.04% maximum). Nonetheless, at 2% concentration, the output using the co-solvent was not significantly different. At higher concentrations, a greater difference was found on the output caused by the co-solvent managing to break the solute from the solid matrix and substitute them at the active sites of the matrix (Hollender, 1997). It also accelerates the extraction process of compounds with elevated polarity. However, the addition of the co-solvent decreases the selectivity and, as it is in liquid state at atmospheric pressure, it is collected alongside the desired solute compounds, requiring another step of separation between them. The yield obtained with hydrodistillation (0.04%) was

inferior to all other experiment results in this work. Since this process requires the solvent (water) to boil, which causes the formation of steam and drags the most volatile compounds in the sample, for an extended period (3 hours) there may be loss and elimination of some of the compounds, consequently leading to a lower yield. Moreover, during hydrodistillation, the obtained product is essential oil, while, in other processes, like soxhlet and supercritical fluid extraction, the solvents and extraction conditions allow solutes with more complex compositions and higher yields. The hydrodistillation output found in this work was similar to the one shown in Seabra *et al*. (2012), where the bark of *Pinus pinaster Ait*. had 0.014% yield. The extraction of this same tree via soxhlet using ethanol as a co-solvent obtained 6.58% yield.

3.2 Extract Composition

The composition of the *Mezilaurus itauba* extracts and essential oil was studied through gas chromatography/mass spectrometry (GCMS) analysis. For the supercritical fluid extraction, the extracts from the experiments with highest yield were used in this analysis (22 MPa, 45°C and 150 MPa, 38°C) and the one with the lowest output (8 MPa, 45°C). Table 3 presents the compounds identified in the extraction products (hydrodistillation and supercritical fluid extraction).

Table 3 - Composition of the *Mezilaurus itauba* extracts

Compounds	Chromatogram data GC/MS							
	Hidrodistillation		**SFE (22 MPa 45 °C)**		**SFE (15 MPa 38 °C)**		**SFE (8 MPa 45 °C)**	
	Rt(min)	Relative area (%)	Rt(min)	Relative area (%)	Rt(min)	Relative area (%)	Rt(min)	Relative area (%)
β-Caryophyllene	-	-	29.22	6.33	29.24	4.61	-	-
Germacrene-D	-	-	31.78	8.19	31.79	5.71	-	-
β-Cubebene	-	-	32.23	7.95	-	-	-	-
δ-Cadinene	33.67	12.70	33.52	10.16	33.53	9.03	33.54	26.15
Muurolol-T	38.22	5.22	38.10	16.41	38.12	16.54	38.12	24.69
α-Cadinol	38.68	4.16	38.58	25.53	38.59	25.26	38.59	15.50
Torreyol	-	-	-	-	38.28	6.78	38.28	6.34
Naphthalene	31.80	15.1	-	-	-	-	31.62	7.08
Muurolene	-	-	-	-	-	-	32.60	4.51
Cadinene	-	-	-	-	-	-	33.13	9.28
Epicubenol	37.68	2.91	-	-	-	-	37.59	6.47
α-Copaene	27.46	7.47	-	-	-	-	-	-
Caryophyllene	29.31	5.03	-	-	-	-	-	-
trans-α-bergamotene	30.00	3.54	-	-	-	-	-	-
α-Amorphene	31.89	2.47	-	-	-	-	-	-
Selinene	32.12	2.62	-	-	-	-	-	-
Valencene	32.47	4.79	-	-	-	-	-	-
α -Muurolene	32.72	7.71	-	-	-	-	-	-
Cis-α-Bisabolene	34.36	2.59	-	-	-	-	-	-
Caryophyllene Oxid	35.96	5.69	-	-	-	-	-	-
β-Bisabolene	33.05	4.80	-	-	-	-	-	-

It can be observed by these results that the extracts from *M. itauba* obtained by CO_2 supercritical extraction are comprised of a mixture of chemical compounds, with high value relative areas (%), where the majority of these extracts are sesquiterpenes. It is also noticeable that the extracts are similar to the majority compounds, as in all analysis a greater chromatograph area (%) was found for α-Cadinol, T-muurolol e δ-Cadinene. Among the experiments with higher pressure (and consequently higher CO_2 density), difference was found in two compounds, β-Cubebene and Torreyol. However, on the experiment with low pressure, a broader range of compounds were observed, even though a similar tendency was found on the chromatograph peaks. Chang *et al*. (2008) researched the composition of the oil from Calocedrus macrolepis var. formosana and concluded that T-muurolol and α-Cadinol are strong inhibitors of Rhizoctonia solani and Fusarium oxysporum growth, two fungi species. Feitosa *et al*. (2007) identified the constituents from the leaves of Mezilaurus mahuba which were in its majority hydrocarbons, non-oxygenated sesquiterpenes, β-Caryophyllene, Germacrene-D and δ-Cadinene. When the dry twigs were analyzed, δ-Cadinene was the main component found. Sesquiterpene β-Caryophyllene is notable for its anti-carcinogenic and anti-inammatory properties (Zheng *et al*., 1992), while the caryophyllene oxide (found in the hydrodistilled extract) possesses an anti-bacterial effect (Limberger *et al*., 2004). In the extracts obtained by hydrodistillation, a wider range of compounds were found than when CO_2 supercritical extraction was performed, even though sesquiterpenes were still common in both extracts. The major compounds according to the chromatogram area (%) were Naphthalene, δ-Cadinene and α-Muurolene. Naphthalene is an aromatic hydrocarbon and has known properties against insects (moths, termites, among others). α-copaene is commonly found in twigs, leaves and tree stems, as observed by Alcântara *et al*. (2010). The greater amount of different compounds found when the supercritical extraction was carried out with CO_2 of lower density or by hydrodistillation is explained by the fact that at high pressures heavier compounds, not distinguishable with GCMS, were obtained. Thus, a purer oil, but with lower yield, is obtained in such condition. extraction was carried out with CO_2 of lower density or by hydrodistillation is explained by the fact that at high pressures heavier compounds, not distinguishable with GCMS, were obtained. Thus, a purer oil, but with lower yield, is obtained in such condition.

4. CONCLUSION

The extraction of *M. itauba* with $scCO_2$ at 22 MPa and 45^{o}C gave the best yield (1.04%), which was higher than that of steam distillation (0.04%), though not as good as when utilizing ethanol as a co-solvent (1.05, 1.21, 1.56%) or soxhlet (5.47%). However, supercritical extracts showed a better purity with a lower amount of undesirable substances, such as resins. In the supercritical fluid extraction process both the pressure and the temperature had a considerable effect on the extraction yield. Regarding the composition of the extracts obtained by hydrodistillation, supercritical fluid extraction and soxhlet, it was found that they were mainly characterized by the presence of sesquiterpene compounds in all cases. The extract composition when larger pressures were applied had a higher concentration of α-Cadinol, the main component of the extract. However, regardless of the operating conditions, the compounds present in greatest

quantities in all of the *M. itauba* with $scCO_2$ were the same: α-Cadinol, T-muurolol and δ-Cadineno.

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