

Short Communication

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Extraction with supercritical fluid and comparison of chemical composition from adults and young leaves of *Zanthoxylum tingoassuiba*

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Abstract: Plants differ in metabolism during their life cycle. In species used as phytotherapies, these changes determine the quality and effectiveness of the product. The aim of this study to evaluate the differences of chemical extracts obtained with supercritical CO₂ from young and adult leaves of *Zanthoxylum tingoassuiba* St.-Hil., Rutaceae, a species used in the folk medicine in Brazil. The chemical composition of the extracts was elucidated by spectroscopic analysis and comparison with literature data. The results showed difference in the composition of the leaves from *Z. tingoassuiba* and allowed the determination of parameters for the extraction of α -bisabolol and furanocoumarins in this vegetal matrix.

Introduction

Natural products have been the source of most of the active ingredients of medicines (Harvey, 2008). Although in the past 15 years the pharmaceutical industry has significantly reduced the research, natural products are still an important source for the development of new drugs. Survey conducted between the years 1981 to 2007 showed that nearly half of the drugs approved in the U.S. since 1994 was obtained directly or indirectly from natural products. Thirteen new drugs derived from natural products have been approved in the period from 2005 to 2007 (Harvey, 2008; Li & Vederas, 2009).

Taking into account the expense of purified and isolated natural products, the use of herbs is expanding throughout the world (Breevoort, 1998). However, the use of herbs for therapeutic applications demands the assurance of quality, safety and efficacy of these products. The plants show differences in metabolism during their life cycle (Phillipson, 2001). According to this, the harvest season is an important variable, since the secondary metabolites or active ingredients, vary with seasonal and circadian consequent change in the safety and efficacy of the product (Reis et al, 2007).

Previously study of fruits and stem from *Zanthoxylum tingoassuiba* A. St.-Hil., Rutaceae, resulted in the isolation of fifteen substances: two alkaloids:

norchelerythrine and arnottianamide; one lignin: sesamin; four terpenoids: citronellyl acetate, lupeol, α -bisabolol and spatulenol; five coumarins: xanthotoxin, isopimpinelin, *O*-prenylumbelliferone, imperatorin and aurapten; one protoalkaloid: methyl *N*-methylantranilate and two steroids: stigmasterol and β -sitosterol (Silva et al., 2008). Continuing the chemical characterization of *Z. tingoassuiba*, leaves at different stages of maturity were analyzed because they showed clear morphological differences in the field.

The young leaves of *Z. tingoassuiba* are smaller, lighter and brighter than adult ones (Santana et al., 2006a). Concerning to this, this study aimed to assess the correlation between differences in morphology and chemical composition of extracts from leaves, by supercritical carbon dioxide (CO₂) extraction.

Natural products are often obtained from medicinal plants by separation techniques and conventional phytochemical extraction, using organic solvents, column chromatography with silica or HPLC. However, these methods are time consuming; possess multiple steps, adsorption losses of the sample, besides polluting waste solvents (Peng et al., 2006). One alternative is the supercritical fluid extraction (SFE) which consists of an analytical technique, using as an organic solvent inert fluid in supercritical state, such as CO₂, light alkanes, gas noble and olefins (Lanças, 2002).

The CO₂, in particular shows high selectivity, efficiency, under conditions that allow the minimization of chemical and thermal treatments used in conventional phytochemical, a smaller amount of material, avoiding the degradation of components of matrix studied, in addition to its treatment environmentally responsible. (Santana et al., 2006b; Lang & Wai, 2001).

Material and Methods

The specimen of *Zanthoxylum tingoassuiba* St.-Hil., Rutaceae, was collected in the city of Feira de Santana-BA, identified by Prof. Lenise Maria Guedes da Silva and a voucher specimen was deposited in the Herbarium Alexandre Leal Costa of the Institute of Biology, Federal University of Bahia, under the number 67,894.

Leaves of *Z. tingoassuiba* were separated according to the degree of maturation in young and adult, as evidenced by morphological differences. After drying and grinding, the two groups of leaves were submitted to supercritical CO₂ at pilot plant SFE 500 SEPAREX. This unit consists of a cylinder containing CO₂ (A), a pump (P) for the transport of CO₂, an external module for the application of co-solvent (C), a Extractor cylinder (E) of 400 mL and three tabs (S1, S2, S3) of 16 mL each (Figure 1).

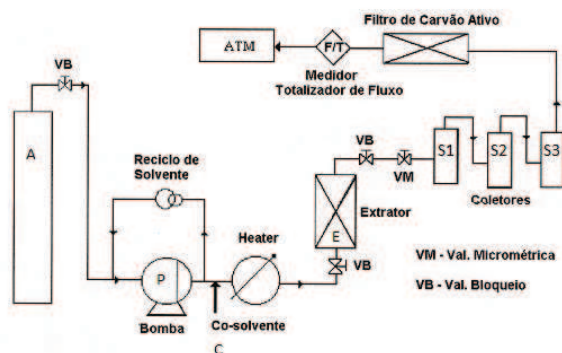


Figure 1. Schematic of pilot plant for supercritical fluid extraction SFE 500 SEPAREX containing: CO₂ cylinder (A), a pump (P) for the transport of CO₂, an external module for co-solvent (C), a extractor cylinder (E) and three tabs (S1, S2, S3).

Five samples were extracted from 30 g for each group of leaves, keeping the conditions of pressure and temperature for 45 min for each extraction. The extractor vessel (E) maintained at 10×10^7 pascal and 313 K, while the tabs had pressure in decreasing order of 1.38×10^7 , 0.69×10^7 and 0.55×10^7 pascal, occurring variation only on the used co-solvent. On the first condition no co-solvent was used, whereas in the second and third, 5% methanol and isopropanol, respectively, were added to CO₂.

NMR analyses were performed on a Varian spectrometer (Gemini 300 model), operating at 300 to 75 MHz for ¹H and ¹³C with reference to tetramethylsilane as internal standard. GC analyses were performed on a gas chromatograph (GC) Perkin Elmer coupled to a mass spectrometer (Model Clarus 500) equipped with a capillary column (30 m x 0.25 mm x 0.1 μm). The injector temperature was maintained at 250 °C. The sample (1.0 μL) dissolved to 2 ppm in CH₂Cl₂, was injected using helium as carrier gas at the linear velocity of 1 mL.min⁻¹. The oven temperature was initially kept at 60 °C for 5 min, then at 7 °C.min⁻¹ was raised up to 210 °C and remained constant for 34.5 min.

Results and Discussion

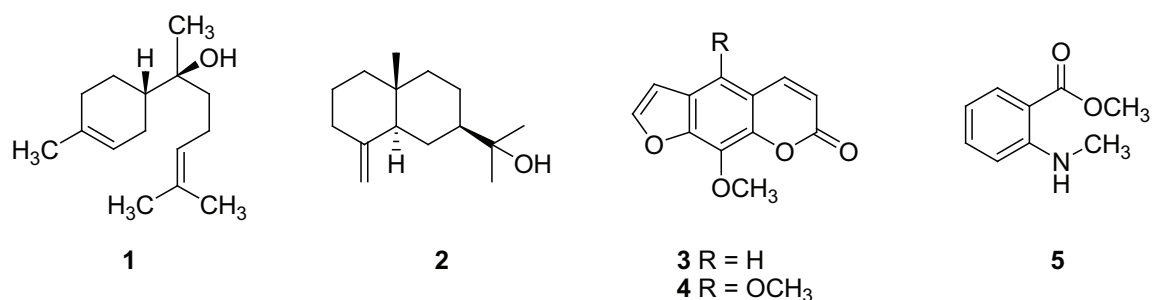
The extracts initially analyzed by (NMR) ¹H (300 MHz) and ¹³C (75 MHz), showed differences in chemical compositions of extracts obtained from young leaves and those from mature leaves. The interpretation of NMR spectra of the samples and the comparison with the spectra of substances isolated by conventional phytochemical techniques (Silva, 2006) and literature data (Stevenson, 2003; Schwartz, 1979) allowed characterizing mixtures derived samples, as sesquiterpenes (1 and 2), furanocoumarins (3 and 4) and a protoalkaloid (5) extracted from young and adults leaves of *Z. tingoassuiba* by SFE.

The sesquiterpenes α-bisabolol (1) and β-eudesmol (2) were identified as major components of young leaves extracts, whereas samples from mature leaves contained a mixture of furanocoumarins xanthotoxin (3), isopimpinelin (4) and α-bisabolol (1), as well.

The second stage of research consisted of analysis of samples by gas chromatography coupled to mass spectrometry (GC/MS) and comparison of Kovat's retention indexes with those described by Adams (1995), confirming the results obtained by NMR (Figure 2). The high sensitivity of this method allowed identifying substances that were not detected by NMR analysis, such as the sesquiterpene β-eudesmol (2) and protoalkaloid methyl *N*-methylantranilate (5), both found in extracts of mature leaves. Compounds 1, 3, 4 and 5 were previously isolated in fruit and stems of *Z. tingoassuiba* (Silva, 2008). Spectroscopic data of the substances identified:

α-bisabolol (1): ¹³C RMN (CDCl₃, 75 MHz), δ ppm: 43.3 (C1), 26.1 (C2), 120.8 (C3), 133.8 (C4), 31.1 (C5), 24.0 (C6), 74.4 (C7), 39.3 (C8), 22.3 (C9), 124.6 (C10), 131.7 (C11), 25.7 (C12), 17.6 (C13), 23.9 (C14), 23.3 (C15). GC-MS. *m/z* (%): 204 (10), 161 (8), 121 (23), 119 (48), 109 (78), 95 (39), 93 (62), 79 (30), 69 (100); KI: 1686.

β-eudesmol (2): due to low concentrations in mixtures this



component was only identified by GC-MS technique. *m/z* (%): 222 (2), 204 (5), 189 (8), 164 (12), 149 (30), 135 (9), 122 (10), 108 (15), 93 (21), 81 (27), 67 (20), 59 (100), 41 (28). KI: 1651.

xanthotoxin (3): ¹H RMN (CDCl₃, 300 MHz): δ 6.30 (1H, *d*, *J*=9.6 Hz, H-3), 7.78 (1H, *d*, *J*=9.6 Hz, H-4), 7.36 (1H, *s*, H-5), 7.63 (1H, *d*, *J*=2.7 Hz, H-2'), 6.82 (1H, *d*, *J*=2.1 Hz, H-3'), 4.31 (3H, *s*, 8-OCH₃); ¹³C RMN (CDCl₃, 75 MHz): δ 160.3 (C2), 147.7 (C7), 145.0 (C2'), 144.3 (C4), 139.3 (C8a), 132.5 (C8), 126.0 (C6), 116.3 (C4a), 114.4 (C3), 112.8 (C5), 106.6 (C3'), 61.5 (8-OCH₃); GC-MS. *m/z* (%): 216 (100), 201 (29), 188 (11), 173 (69), 145 (24), 143 (1), 89 (51), 63 (39), 44 (25).

isopimpinelin (4): ¹H RMN (CDCl₃, 300 MHz): δ 6.38 (1H, *d*, *J*=9.6 Hz, H-3), 8.14 (1H, *d*, *J*=9.9 Hz, H-4), 4.18 (3H, *s*, 5-OCH₃), 7.70 (1H, *d*, *J*=2.1 Hz, H-2'), 7.01 (1H, *d*, *J*=2.4 Hz, H-3'), 4.17 (3H, *s*, 8-OCH₃); ¹³C RMN (CDCl₃, 75 MHz): δ 160.3 (C2), 147.4 (C7), 145.0 (C-2'), 144.3 (C4), 139.3 (C8a), 132.5 (C-8), 126.0 (C6), 116.3 (C4a), 114.4 (C3), 146.5 (C5), 106.6 (C3'), 61.1 (8-OCH₃); GC-MS *m/z* (%): 246 (81), 231 (100), 188 (31), 175(30%), 160 (25) 147 (18), 132 (10), 104 (17).

methyl *N*-methylantranilate (5): due to its low concentration in the mixture was only possible to identify it using the technique of GC-MS. *m/z* (%): 165 (94), 150 (1) 134 (16), 132 (51), 105 (100), 104 (98), 77 (81); KI: 1406.

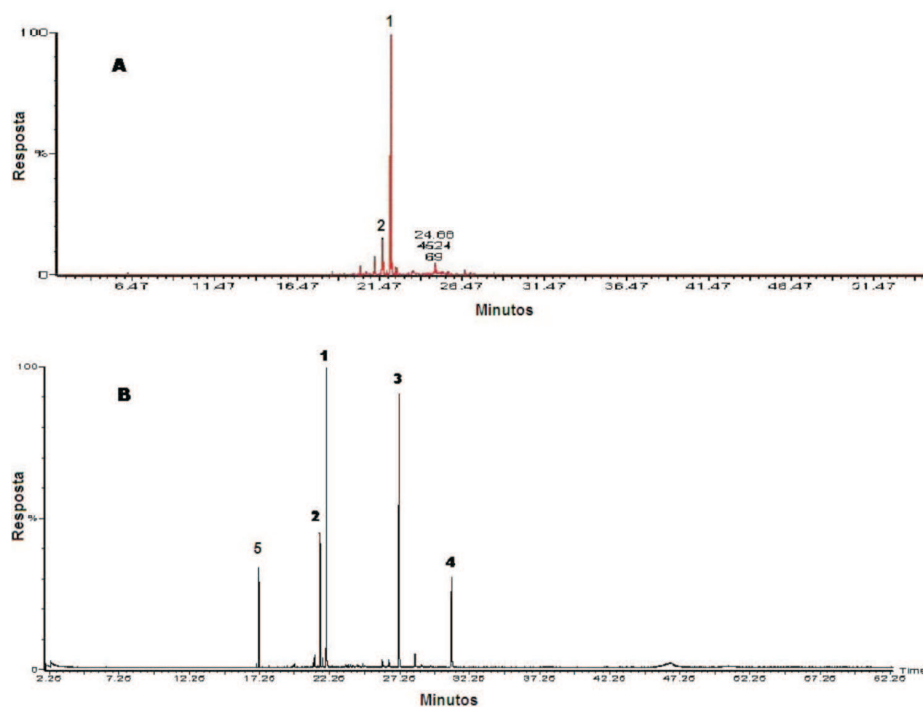


Figure 2. Comparative chromatograms of extracts: A. young leaves and B. mature leaves, allowed to observe the differences to quantity and composition between young and adult leaves of *Zanthoxylum tingoassuiba*.

This study found a significant difference in chemical composition from those obtained from young and mature leaves of *Zanthoxylum tingoassuiba* by supercritical fluid extraction, demonstrating once again the importance of the collection period and characteristics of the plant for medical use. Besides, it was possible to determine parameters for the selective extraction of substances such as α -bisabolol and furanocoumarins of plant using supercritical CO₂.

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References

- Adams RP 1995. *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream, Illinois: Allured Publishing Corporation.
- Brevoort P 1998. The booming US botanical market: a new overview. *HerbalGram* 44: 33-46.
- Harvey AL 2008. Natural products in drug discovery. *Drug Discov Today* 13: 894-901.
- Lanças FM 2002. Extração com fluido supercrítico: quo vadis? *Revista Analytica* 2: 30-37.
- Lang Q, Wai CM 2001. Supercritical fluid extraction in herbal and natural product studies - a practical review. *Talanta* 53: 771-782.
- Li JW-H, Vederas JC 2009. Drug discovery and natural products: End of an era or an endless frontier? *Science* 325: 161-165.
- Peng J, Fan G, Chai Y, Wu Y 2006. Efficient new method for extraction and isolation of three flavonoids from *Patrinia villosa* Juss. by supercritical fluid extraction and high-speed counter-current chromatography. *J Chromatogr A* 1102: 44-50.
- Phillipson JD 2001. Phytochemistry and medicinal plants. *Phytochemistry* 56: 237-243.
- Reis MS, Mariot A, Steenbock W 2007. In Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR (org.). *Farmacognosia: da planta ao medicamento*. Porto Alegre: Editora da UFRGS. p.45-74.
- Santana LLB, Silva CV, Velozo ES 2006a. Estudo fitoquímico das folhas de *Zanthoxylum tingoassuiba* St Hil. *XIX Simpósio de Plantas Mediciniais do Brasil*. Salvador, Brasil.
- Santana LLB, Cardoso LA, Druzian JI, Souza VF, Costa TAC, Nóbrega DA, Hohlemweger SV, Velozo ES 2006b. Selectivity in the extraction of 2-quinolones alkaloids with supercritical CO₂. *Braz J Chem Eng* 23: 525-553.
- Schwartz MA, Swanson GC 1979. Stereospecific syntheses of the diastereomeric(+)- α -bisabolol. A caveat on the assignment of stereochemistry to natural α -bisabolol. *J Org Chem* 44: 6.
- Silva CV 2006. *Alcalóides benzofenantridínicos e outros metabólitos do caule e frutos de Zanthoxylum tingoassuiba* A. St.-Hil. Salvador, 123p. Dissertação de Mestrado, Programa de Pós-graduação em Química, Universidade Federal da Bahia.
- Silva CV, Detoni CB, Velozo ES, Guedes MLS 2008. Alcalóides e outros metabólitos do caule e frutos de *Zanthoxylum tingoassuiba* A. St.-Hil. *Quim Nova* 31: 2052-2055.
- Stevenson PC, Simmonds MSJ, Yule MA, Veitch NC, Kite GC, Irwin D, Legg M 2003. Insect antifeedant furanocoumarines from *Tetradium danielli*. *Phytochemistry* 63: 41-46.

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