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Chemical composition of essential oil from the bark of *Croton cajucara* Bentham

Fabiano Rosa da Silva[1*], Alberto Wisniewski Junior[2], Valdir Cechinel Filho[3] and Domingos Sávio Nunes[4]

*[1]Laboratório de Química de Fitobiomassa, Departamento de Química, Universidade Federal do Paraná, Cx. Postal 19081, 81531-990, Curitiba, Paraná, Brazil. [2]Departamento de Química, Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brazil. [3]Departamento de Farmácia, Universidade do Vale do Itajaí, Itajaí, Santa Catarina, Brazil. [4]Departamento de Química, Universidade Estadual de Ponta Grossa, Ponta Grossa, Paraná, Brazil.*Author for correspondence. E-mail: frosadasilva@gmail.com*

ABSTRACT. The essential oil from the bark of *Croton cajucara* Bentham, popularly known as sacaca or cajuçara, was obtained by hydrodistillation, yielding 1.28% of the former bark mass, and analyzed by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC-MS). In total, 50 compounds were identified, with oxygenated sesquiterpenes composing the majority of components. The main compounds identified were: cyperene (12.36%), α-guaiene, (11.50%) and *epi*-β-Santalene (8.70%).

Keywords: Sacaca, vapor dragging, CG-MS, cyperene.

Composição química do óleo essencial das cascas de *Croton cajucara* Bentham

RESUMO. O óleo essencial das cascas de *Croton cajucara* Bentham, conhecida popularmente por sacaca ou cajuçara, foi obtido por hidrodestilação com rendimento de 1,28% em relação à massa de material vegetal e analisado por cromatografia gasosa (CG) e cromatografia gasosa acoplada à espectrometria de massas (CG-EM). No total foram identificados 50 compostos, com os sesquiterpenos oxigenados como a maioria dos componentes. Os constituintes majoritários identificados foram o cipereno 12,36%, α-guaieno 11,50% e o epi-β-santeleno 8,70%.

Palavras-chave: Sacaca, arraste com vapor d água, CG-EM, cipereno.

Introduction

The family Euphorbiaceae has more than 8.000 species widely distributed in tropical and temperate regions worldwide. Many of them are known to have medicinal or toxic properties (WILSON et al., 1976; SALATINO et al., 2007). *Croton* is a large genus of this family, which comprises about 1,300 species including trees, shrubs and herbs found in tropical and subtropical regions of northern and southern hemispheres. The species *Croton cajucara* Bentham, popularly known as sacaca or cajuçara, is a medicinal plant commonly distributed in the Amazon region, in the form of a large shrub up to 6 m high (HIRUMA-LIMA et al., 2002a). In folk medicine the leaves and stem bark of this species are used as tea for treating diabetes, controlling high blood cholesterol, liver and gastrointestinal disorders (CAMPOS et al., 2002). A decoction of the leaves is also used against stomach pains, fever, liver problems, jaundice and malaria, whereas the infusion of the leaves mixed with melão-de-são-caetano (*Momordica charantia*), is useful against hepatitis (BRITO et al., 2001; HIRUMA-LIMA et al., 2002a).

The following triterpenes of sacaca stem bark have been isolated: acetyl aleuritolic acid (AAA) and diterpene furanic neo-clerodane *trans*-dehydrocrotonin (DCTN), *trans*-crotonin (TCN), cajucarin A, cajucarin B, cajucarinolide and sacacarine (MACIEL et al., 2000; SALATINO et al., 2007). The main components of the nonpolar solid shells are DCTN, which only occurs in this plant species, and the AAA. The simultaneous occurrence and abundance of these two compounds in a plant material can serve as sufficient proof that it is effectively *C. cajucara* (ITOKAWA et al., 1989).

The biological activities of sacaca and the gastroprotective effect of the bark tea were related to DCTN (HIRUMA-LIMA et al., 1999a), with the CTN (ALMEIDA et al., 2002), as well as the essential oil obtained from the stem bark (HIRUMA-LIMA et al., 1999b, 2000, 2002b). Other studies have shown that the essential oil has anti-nociceptive and anti-inflammatory effects (BIGHETTI et al., 1999). In addition, DCTN e CTN showed hypoglycemic, antispasmodic, antitumor and antiestrogenic activity (MACIEL et al., 2000).

The International Standard Organization (ISO) defines volatile oils as: (a) products extracted from plant parts by steam water distillation, and (b) products obtained by citrus fruits (*Rutaceae*) pericarp expression. These are volatile oily liquids with strong and often pleasant aroma, originated from the secondary metabolism of plants, occupying a major place in the composition of pharmaceutical products, perfumes and cosmetics industries. They are usually synthesized in the leaves, stored in extracellular spaces between the cuticle and the cell wall, and basically constituted by terpenes, synthesized by the mevalonic acid route. Chemically, the vast majority of essential oils are made up of phenylpropanoid derivative or terpenoids, and the latter predominates (CHAAR et al., 2003).

The constituents of essential oils vary from terpenes, simple alcohols, aldehydes, ketones, phenols, esters, ethers, oxides, peroxides, furans, organic acids, lactones, coumarins and sulfur compounds. In the mixture, such compounds are present at different concentrations, there are others at lower levels and some at very low quantities (traces) (SIMÕES; SPITZER, 2003).

The yield and ratio of the oil components vary depending on several factors: geographical origin, climatic conditions, harvest time, genetic modification and extraction process used. The characteristics and compositions are associated with specific environmental factors and growing conditions. Several techniques can be employed in the extraction of essential oils, such as hydrodistillation, steam distillation by drag of water, extraction with organic solvents or CO_2 liquid. Among the factors that interfere with chemical and organoleptic characteristics of essential oils, the extraction process is one of the most important; being the distillation with water steam the most used conventional process (FRIZZO et al., 2001; SILVA et al., 2005; SILVIA et al., 2002).

Among the chromatographic methods available for the characterization of essential oils the gas chromatography coupled to mass spectrometry is the most appropriate method to separate and quantify their constituent substances, due to their selectivity and sensitivity. As these are volatile enough, the sample can be solubilized in solvents such as hexane, before being injected into the equipment without requiring derivatization steps. For separation, generally are used capillary columns suitable for separating the various components of the sample in question (CHAAR et al., 2003; SILVA et al., 2005; SIMÕES; SPITZER, 2003).

The identification of individual compounds from a complex sample of an essential oil can be performed by comparing the retention time of the sample in relation to standards, which may be called the Retention Index (RI), proposed by Kovats in 1958 as a parameter for identification of solutes in chromatograms. The RI of a given solute can be obtained from a chromatogram of a solute mixture with at least two normal alkanes with retention times whose values include the value of the retention time of the solute in question (SKOOG, 2002).

In order to be more independent of variations in the retention time, under different measurement conditions, especially at different heating programs during the analysis, it was introduced Kovats Index (KI), which relates the retention time of the compound to the retention time of a homologous series of hydrocarbons. These indices allow better comparison of data between different laboratories and are calculated by the following formula (CAZES; DEKKER, 2005).

KI is given by the equation

$$KI_x = 100y + 100(z-y)\frac{t_{(r)x} - t_{(r)y}}{t_{(r)z} - t_{(r)y}}$$

y = nº carbons of the pattern to the left;
z = nº carbons of the pattern to the right;
$t_{(r)x}$ = retention time of the compound considered;
$t_{(r)y}$ = retention time of pattern in the left;
$t_{(r)z}$ = retention time of pattern in the right.

This paper reports the identification of the chemical constituents of *C. cajucara* bark essential oils, obtained by hydrodistillation and steam drag, by gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS)

Material and methods

Plant material

Samples of sacaca stem bark were acquired in a medicinal plants street market in Santarém, Pará State, Brazil. The identification of this material was performed qualitatively by thin layer chromatography, using a mixture of 5% methanol in chloroform as eluent on chromatoplates HF-254 of silica gel 60 (Merck®), revealed with a solution of methanol and concentrated sulfuric acid (1:1) followed by heating on plate. For comparison with the botanical extract (chloroform extract), we used DCTN and AAA patterns.

Analytical sample of essential oil

A sample of 132 g composed of dried and ground bark was hydrodistillated in a 2 L flask with 1.3 L

distilled water, in a apparatus built with glass complying with specialized information (Clevenger), thus the obtained yield and oil composition were compared with samples from the same botanical material obtained by steam extraction (STAHL; SCHILD, 1981). After 4h hydrodistillation, the oil obtained was separated using a separating funnel with the aid of ethyl ether. The organic solution was dried with anhydrous sodium sulfate, filtered and the diethyl ether was evaporated on a rotary evaporator at reduced pressure and low temperature.

Prepared sample of essential oil

The essential oil from the sacaca bark was also obtained in large quantities by water steam, in a prototype device built with pieces of aluminum and glass (Figure 1).

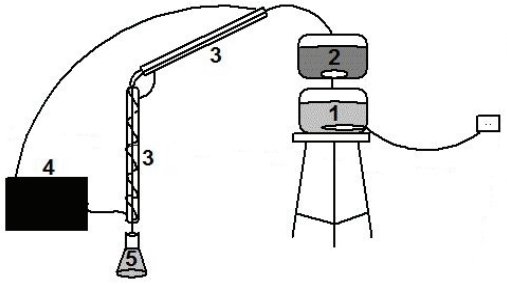

Figure 1. Extractor used to obtain the essential oil sample prepared from the water steam drag extraction of *C. cajucara* bark.

In container 1, distilled water was heated until reach boiling point and generates steam, which is interconnected with the container 2, where 2 kg of ground and dry plant material were used. At the bottom of the container 2, a perforated metal shield was placed to prevent clogging of the link between the two extractors and also to allow steam to be dispersed homogeneously throughout the plant material, preventing preferential pathways. After the steam permeate the plant material, this was conducted for the capacitors shown in 3, which were continuously cooled by means of the container 4, with the condensate recovered in the flask indicated at 5.

After 4h of extraction, the obtained oil was separated from the aqueous phase with the aid of ethyl ether. The ethereal solutions were dried with anhydrous Na_2SO_4, filtered and evaporated at low pressure and temperature.

Analysis of essential oils

The essential oil samples were analyzed by a GC-MS system composed of a gas chromatograph Varian® CP-3800 coupled to a mass spectrometer Saturn®, managed by software Saturn® GC/MS Workstation 5.51. We used a nonpolar capillary column CP-Sil-8-CB-Low-Bleed/MS de 30 m, 0.25 mm thick, film of 0.25 μm and H_2 as a carrier gas with 1 mL $min.^{-1}$ flow. It was experimentally determined a proper warm-up program for better separation of the components: injector temperature 250ºC; oven temperature 60ºC for 3 min. followed by heating 5ºC $min.^{-1}$ to 220ºC, where it remained for 15 min. The identification of compounds was initially performed with data obtained by the analysis GC-ME and by the KI of each component, from the retention time provided by injecting a series of *n*-alkanes (C_{10}-C_{25}) and comparing them with default values provided by Adams (1995).

For the quantification of the components we used the data from new injections with a flame ionization detector (GC-FID), with an identical capillary column, under the same heating conditions, the carrier gas flow etc, previously defined in experiments GC-MS, with the reinjection of the series of *n*-alkanes and a new determination of KI for comparison with those obtained previously (ADAMS, 1995; CAZES; DEKKER, 2005).

Results and discussion

The procedure for the analytical sample achievement resulted in an oil yield of 1.28% p/p in relation to plant material, an oil less dense than water, with a pleasant essence, slightly yellow, sweet-smelling wood.

Since the preparative sample showed a yield of 0.8%, this loss can be attributed to preferential pathways formed during the processing of material extraction, which even minimized could have avoided the contact of all the material with the water steam, unlike the analytical sample obtained by hydrodistillation in Clevenger apparatus, where the entire plant material was in contact with water.

The compounds identified and quantified in the analytical sample of the essential oil are presented in Table 1.

A total of 50 compounds were identified, which represent 71.46% the mass of oil. Of this, the monoterpenes amounted 4.99%, of which only 0.10% is oxygenated monoterpene, with *para*-cymene as the only compound of this class, and 4.89% of non-oxygenated monoterpenes. The oxygenated sesquiterpenes made up the majority of the components with 65.45%, while non-oxygenated, only 1.02%.

Table 1. Chemical composition (%) of essential oil from the bark of *C. Cajucara* Bentham.

Components	%	KI calculated	KI standard*
α-pinene	*t*	940	939
camphene	*t*	956	953
(-)-β-pinene	*t*	982	980
para-cimene	0.10	1027	1026
limonene	*t*	1032	1031
cis-linalool oxide	*t*	1073	1074
trans-linalool oxide	*t*	1089	1088
linalool	2.79	1102	1098
trans-pinocarveol	0.03	1147	1139
cis-verbenol	*t*	1151	1140
camphor	*t*	1153	1143
borneol	1.65	1178	1165
4-terpineol	0.27	1185	1177
para-cimen-8-ol	*t*	1191	1183
α-terpineol	0.15	1200	1189
cis-verbenone	*t*	1214	1204
trans-carveol	*t*	1224	1229
O-methyl-thymol	*t*	1233	1235
carvacrol	*t*	1294	1298
δ-elemeno	*t*	1336	1339
α-cubebene	0.13	1351	1351
(+)-cyclosativene	2.02	1376	1368
α-copaene	4.12	1381	1376
β-patchoulene	*t*	1386	1380
cyperene	12.36	1411	1398
α-*cis*-bergamotene	5.16	1417	1415
α-santalene	2.18	1423	1420
trans-caryophyllene	5.31	1427	1418
β-cedrene	4.19	1433	1418
α-*trans*-bergamotene	0.10	1437	1436
α-guaiene	11.50	1445	1439
aromadendrene	0.85	1449	1439
epi-β-santalene	8.70	1452	1449
(Z)-β-farnesene	0.52	1457	1458
α-humulene	2.45	1463	1454
allo-aromadendrene	3.80	1468	1461
γ-gurjunene	*t*	1481	1473
germacrene D	*t*	1488	1480
α-muurolene	*t*	1504	1499
β-himachalene	*t*	1511	1499
cuparene	*t*	1517	1502
δ-cadinene	*t*	1525	1524
α-calacorene	*t*	1550	1542
(E)-nerolidol	*t*	1564	1564
Spathulenol	*t*	1588	1576
cariofilene oxide	*t*	1593	1581
viridiflorol	*t*	1604	1590
α-cadinol	*t*	1653	1653
cadaline	2.06	1683	1674
α-bisabolol	1.02	1692	1683

t: trace element with a percentage lower than 0.03%; *Default values described by Adams (2005).

The major constituents identified were the cyperene (12.36%), α-guaiene (11.50%) and *epi*-β-santelene (8.70%). It is noteworthy that linalool represented only 2.79% of the essential oil, lower than the values previously observed in the oil from the leaves of this species, which ranged from 13.5 to 64.0% (LEMOS et al., 1999; LOPES et al., 2000).

The GC-MS analysis of the essential oil obtained by water steam showed the same profile in relation to the components of the prepared sample, and even at a larger scale extraction had caused a slight variation in the content of each compound; these values varied less than 1%, especially considering the major components cyperene, α-guaiene, α-copaene, *epi*-β-santelene and α-santalene, where this variation did not exceed 0.5%.

Conclusion

The yield of essential oil obtained by hydrodistillation was 1.28 weight/weight%, whereas the compounds identified represented a total of 71.46% of its mass. Of this total, the sesquiterpenes constituted the majority of the compounds with 66.47%, with cyperene (12.36%) and α-guaiene (11.50%), as the major constituents.

The water steam drag method presented lower yield than the hydrodistillation, however, the composition of both oils showed only small variations, which were restricted to the content of each component, ranging from 0.5% for major components and 1% for the other compounds, demonstrating that with the experimental unit, compared with a standard extraction method, is feasible to obtain larger quantities of essential oil without changing the chemical composition of the material obtained.

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References

ADAMS, R. P. **Identification of essential oil components by gas chromathography/mass spectrometry**. Illinois: Carol Stream, 1995.

ALMEIDA, A. B.; NUNES, D. S.; MIOTO, A.; SPADARI-BRATFISCH, R. C.; SOUZA BRITO, A. R. M. Mechanism of antiulcerogenic activity of semi-synthetic crotonin obtained from *Croton cajucara* Benth. **Revista Brasileira de Farmacognosia**, v. 12, n. 1, p. 105-110, 2002.

BIGHETTI, E. J. B.; HIRUMA-LIMA, C. A.; GRACIOSO, J. S.; SOUZA BRITO, A. R. M. Anti-inflammatory and antinociceptive effects in rodents of the essential oil of *Croton cajucara* Benth. **Journal of Pharmacy and Pharmacology**, v. 51, n. 12, p. 1447-1453, 1999.

BRITO, M. V. H.; SIQUEIRA, R. B. P.; SANTOS, M. T.; CARVALHO, R. A. Revisão crítica do uso da sacaca (*Croton cajucara*, Benth) como fitoterápico. **Revista Paraense de Medicina**, v. 15, n. 2, p. 10-16, 2001.

CAMPOS, A. R.; ALBUQUERQUE, F. A. A.; RAO, V. S. N.; MACIEL, M. A. M.; PINTO, A. C. Investigations on the antinociceptive activity of crude extracts from *Croton cajucara* leaves in mice. **Fitoterapia**, v. 73, n. 2, p. 116-120, 2002.

CAZES, J.; DEKKER, M. **Encyclopedia of chromatography**. New York: CRC Press, 2005.

CHAAR, J. S.; MOUCHREK FILHO, V. E.; SILVA, D. D.; BREVIGLIERI, S. T.; GALHIANE, M. S.; CHIERICE,

G. O. Extraction and quantification of linalool in the essential oil from leaves from *Aniba duckei Kostermans* cultivated in its natural environment. **Anais da Associação Brasileira de Química**, v. 52, n. 3, p. 110-114, 2003.

FRIZZO, C. D.; SERAFINI, L. A.; DELLACASSA, E.; LORENZO, D.; MOYNA, P. Essential oil of *Baccharis uncinella* DC. from Southern Brazil. **Flavour and Fragance Journal**, v. 16, n. 4, p. 286-288, 2001.

HIRUMA-LIMA, C. A.; SPADARI-BRATFISCH, R. C.; GRASSIKASSISSE, D. M.; SOUZA BRITO, A. R. M. Antiulcerogenic mechanisms of dehydrocrotonin, a diterpene lactone obtained from *Croton cajucara* Benth. **Planta Medica**, v. 65, n. 4, p. 325-330, 1999a.

HIRUMA-LIMA, C. A.; GRACIOSO, J. S.; NUNES, D. S.; SOUZA BRITO, A. R. M. Effects of an essential oil from the bark of *Croton cajucara* Benth. on experimental gastric ulcer models in rats and mice. **Journal of Pharmacy and Pharmacology**, v. 51, n. 3, p. 341-346, 1999b.

HIRUMA-LIMA, C. A.; GRACIOSO, J. S.; RODRÍGUEZ, J. C.; HAUN, M.; NUNES, D. S.; SOUZA BRITO, A. R. M. Gastroprotective effect of essential oil from *Croton cajucara* Benth (Euphorbiaceae). **Journal of Ethnopharmacology**, v. 69, n. 3, p. 229-234, 2000.

HIRUMA-LIMA, C. A.; SOUZA-BRITO, A. R. M.; GUIMARÃES, E. M.; SANTOS, C. M.; DI STASI, L. C. Dicotiledonae medicinais na Amazônia e na Mata Atlântica. In: DI STASI, L. C.; HIRUMA-LIMA, C. A. (Ed.). **Plantas medicinais na Amazônia e na Mata Atlântica**. 2. ed. São Paulo: Editora Unesp, 2002a. p. 87-139.

HIRUMA-LIMA, C. A.; GRACIOSO, J. S.; BIGHETTI, E. J. B.; KASSISSE, D. M. G.; NUNES, D. S.; SOUZA BRITO, A. R. M. Effect of essential oil obtained from *Croton cajucara* Benth. on gastric ulcer healing and protective factors of the gastric mucosa. **Phytomedicine**, v. 9, n. 6, p. 523-529, 2002b.

ITOKAWA, H.; ICHIHARA, Y.; KOJIMAA, H.; WATANABE, K.; TAKEYAA, K. Nor-clerodane diterpenes from *Croton cajucara*. **Phytochemistry**, v. 28, n. 6, p. 1667-1669, 1989.

LEMOS, T. L. G.; MACHADO, M. I. L.; MENEZES, J. E. S. A.; SOUSA, C. R. Essential Oil of *Croton cajucara* Benth. **The Journal of Essential Oil Research**, v. 11, n. 4, p. 411-412, 1999.

LOPES, D.; BIZZO, H. R.; SOBRINHO, A. F. S,; PEREIRA, M. V. G. Linalool-rich essential oil from leaves of *Croton cajucara* Beth. **The Journal of Essential Oil Research**, v. 12, n. 6, p. 705-708, 2000.

MACIEL, M. A. M.; PINTO, A. C.; ARRUDA, A. C.; PAMPLONA, S. G. S. R.; VANDERLINDE, F. A.; LAPA, A. J.; ECHEVARRIA, A.; GRYNBERG, N. F.; CÔLUS, I. M. S.; FARIAS, R. A. F.; LUNA COSTA, A. M.; RAO, V. S. N. Ethnopharmacology, phytochemistry and pharmacology: a successful combination in the study of *Croton cajucara*. **Journal of Ethnopharmacology**, v. 70, n. 1, p. 41-55, 2000.

SALATINO, A.; SALATINO, M. L. F.; NEGRI, G. Traditional uses, chemistry and pharmacology of *Croton species* (Euphorbiaceae). **Journal of Brazilian Chemical Society**, v. 18, n. 1, p. 11-33, 2007.

SILVA, F.; SANTOS, R. H. S.; BARBOSA, L. C.; RIBEIRO, R. R.; PASSARINHO, R. V. Basil conservation affected by cropping season, harvest time and storage period. **Pesquisa Agropecuária Brasileira**, v. 40, n. 4, p. 323-328, 2005.

SILVIA, R. S. S.; DEMUNER, A. J.; BARBOSA, L. C. A.; CASALI, V. W. D.; NASCIMENTO, E. A.; PINHEIRO, A. L. Efeito do estresse hídrico sobre características de crescimento e a produção de óleo essencial de *Melaleuca alternifolia* Cheel. **Acta Scientiarum**, v. 24, n. 5, p. 1363-1368, 2002.

SIMÕES, C. M. O.; SPITZER, V. **Farmacognosia da planta ao medicamento**. 5. ed. Porto Alegre: UFRGS Editora, 2003.

SKOOG, D. A. **Princípios de análise instrumental**. 5. ed. Porto Alegre: Bookman, 2002.

STAHL, E.; SCHILD, W. **Pharmazeutische Biologie**. Stuttgart: Gustav Fischer, 1981.

WILSON, S. R.; NEUBERT, L. A.; HUFFMAN, J. C. The chemistry of the euphorbiaceae. A new diterpene from croton californicus. **Journal of the American Chemical Society**, v. 98, n. 12, p. 3669-3674, 1976.

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