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Artigo

GC/FID-based authentication of *Baccharis trimera:* a quality control study of products commercialized in Curitiba and metropolitan region (Brazil)

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RESUMO: "Autenticidade de *Baccharis trimera* baseada em CG-DIC: um estudo de controle de qualidade de produtos comercializados em Curitiba e Região Metropolitana (Brasil)". *Baccharis trimera* (carqueja) é uma planta medicinal empregada popularmente para tratamentos de problemas dos sistemas digestório e renal. O presente trabalho tem como objetivo avaliar a qualidade de amostras de carqueja comercializadas em Curitiba e Região Metropolitana (Paraná-Brasil), por meio de análises de cromatografia a gás (CG/DIC) do óleo essencial delas extraído por hidrodestilação usando aparelho de Clevenger. Análises macro e microscópicas foram também realizadas para as amostras, e os resultados demonstraram a ocorrência de contaminação de algumas delas por outras espécies de plantas, fungos e pequenos insetos. Das amostras analisadas, 21 apresentaram perfil cromatográfico semelhante ao encontrado para óleo essencial padrão da espécie, enquanto 7 diferenciaram-se em relação a este perfil. As análises dos cromatogramas obtidos para as amostras de óleo essencial extraídas de ervas comercializadas permitem-nos sugerir o uso de ensaios baseados em CG/DIC como uma ferramenta rápida para garantia da qualidade e monitoramento de aspectos de segurança à saúde deste tipo de erva medicinal.

Unitermos: *Baccharis trimera,* controle de qualidade, óleo essencial, cromatografia gasosa.

ABSTRACT: *Baccharis trimera* (carqueja) is a medicinal plant used for stomach pain, bad digestion, heart bum, kidney problems and constipation. The objective of the present work was a quality study of carqueja commercialized in Curitiba and metropolitan region (Paraná-Brazil) using gas chromatography techniques (GC/FID) for analyses of the essential oil, which was extracted through hydrodistillation using a Clevenger system. Macro and microscopic analyses were also done. Some samples were contaminated by other species of plants, fungi and small insects, some of them could be identified. Among all samples, 21 showed similar chromatographic profile to the standard oil, and 7 had different profile in relation to the standard. The chromatogram analyses showed that most of the analyzed samples had the similar profile as the standard oil of *Baccharis trimera*. GC/FID-based authentication of *Baccharis trimera* may be useful as a rapid tool to ensure quality control and safety monitoring of this kind of herbal pharmaceuticals.

Keywords: *Baccharis trimera,* quality control, essential oil, gas chromatography.

INTRODUCTION

The use of medicinal plants as an alternative therapy is growing, but it is not free of danger to health due to the lack of knowledge of the plant by producers, sellers and buyers. This problem happens because it is not possible to offer standard plants, in the same quantity and regularity (Vilegas et al., 1994).

Botanical misidentification, inappropriate methods of collection and processing with undesirable foreign matters have all contributed to a negative impact of natural plant products. The demand for high quality, safe, effective, and clean natural plant products and their formulations with various substances have been growing significantly in the industrialized world (Duarte; Bardal, 2002; Ribeiro et al., 2005)

Adulteration of medicinal plants is a serious public health problem in Brazil, due to the implications for safety and efficacy of herbal medicine utilization. Quality control is required, both for crude drugs and phytotherapeutic preparations regularity (Vilegas et al., 1994).

Baccharis trimera (Less) DC (Asteraceae), known in Brazil as "carqueja", has been popularly used to treat liver diseases, rheumatism, diabetes, as well as digestive, hepatic and renal disorders (Januário et al., 2004, Mors et al., 2000; Agra et al., 2007). The essential oil of this plant has a lot of therapeutical applications, as digestive, anti-rheumatic attributed to carquejol and carquejile acetate. It is also used in diarrhea, stomach

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and liver problems (Silva Júnior, 1997). The extract of *B. trimera* showed hypoglycemic action in non-diabetic patients and inhibit the *in vivo* growing of *Trypanossoma cruzi*. In dogs, injections of the compound carquejol reduced the blood pressure, the cholesterol level and the respiratory rate, and caused a small elevation of glicemy (Castro; Ferreira, 2000). Studies that were carried out for the aerial part of *B. trimera* also showed other compounds as saponines (Borella et al., 2006), flavonoids (Gené et al., 1996), glycolipids (Mendes et al., 2006), and diterpenes (Torres et al., 2000).

In relation to the quality control of *Baccharis trimera*, although analysis of whole leaves affords an easy identification of authentic and adulterated samples, sometimes for crushed or powdered drugs, morphological evaluations are limited. (Budel et al., 2004). Chromatography analysis could be an alternative for quality control of crude drugs in order to find out fingerprint profiles of samples; however the literature reports only a TLC method for analysis of polar preparations from *Baccharis trimera* (Borella; Fontoura, 2002).

The objective of the present research was the analysis of the quality of *Baccahris trimera* commercialized in Curitiba and Metropolitan region (state of Paraná, Brazil), using gas chromatography/ flame ionization detection (GC/FID) based on its essential oil content, and macroscopical and microscopical experiments.

MATERIAL AND METHODS

Plant material

Authentic samples of aerial parts of *Baccharis trimera* Less were gently provided by Professor Rui Inácio Neiva de Carvalho, PUC, PR, Brazil. Leaves, collected in Pinhais, PR, Brazil, in 2002,

Table 1. Macroscopic and microscopic analyses of the commercial samples of *Baccharis trimera*.

SAMPLES	MACROSCOPIC CHARACTERISTICS			MICROSCOPIC CHARACTERISTICS
	Aspect	Parts of the plant in major amount	Parts of the plant in less amount	
1	non triturated	aerial part	sticks/ flowers	leaves of other species and spider web
2	non triturated	aerial part	sticks/ flowers	not completely decomposed leaves and little rocks
3	non triturated	aerial part/ sticks	flowers	parts of different plants
4	pulverized	sticks	aerial part	incomplete drying
5	pulverized	aerial part/ sticks	non detected	non detected
6	pulverized	aerial part/ sticks	non detected	small alive insects, leaves of other species and non identified materials
7	pulverized	sticks	aerial part / flowers	small alive insects and leaves of other species
8	pulverized	sticks	aerial part / flowers	small rocks and bad drying process
9	non triturated	aerial part / flowers	sticks	steam and leaves of other species, bad drying process
10	pulverized	sticks	aerial part / flowers	insects wings
11	non triturated	sticks	aerial part	leaves and seeds of other species
12	pulverized	sticks	aerial part / flowers	incomplete drying
13	capsule		sticks	small rocks and bad dryness
14	pulverized	sticks	aerial part / flowers	small alive insects
15	non triturated	aerial part	sticks	leaves of other species

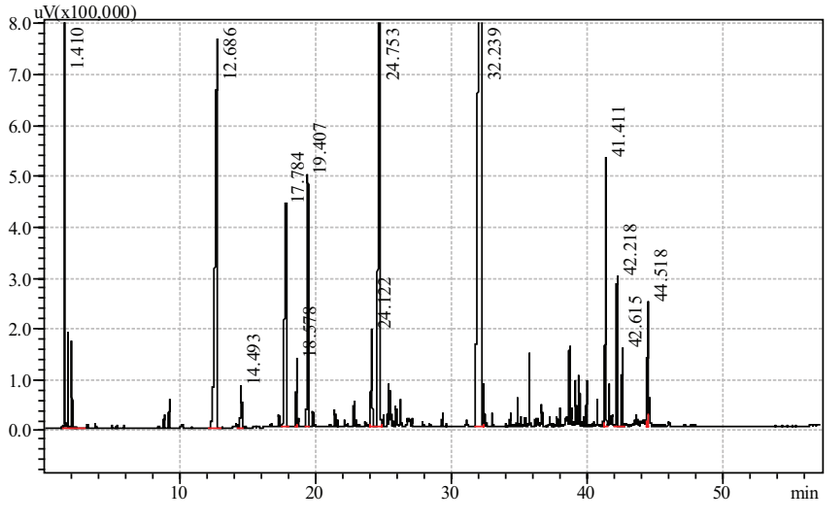

Figure 1. Chromatographic profile of the standard oil of *Baccharis trimera* Less.

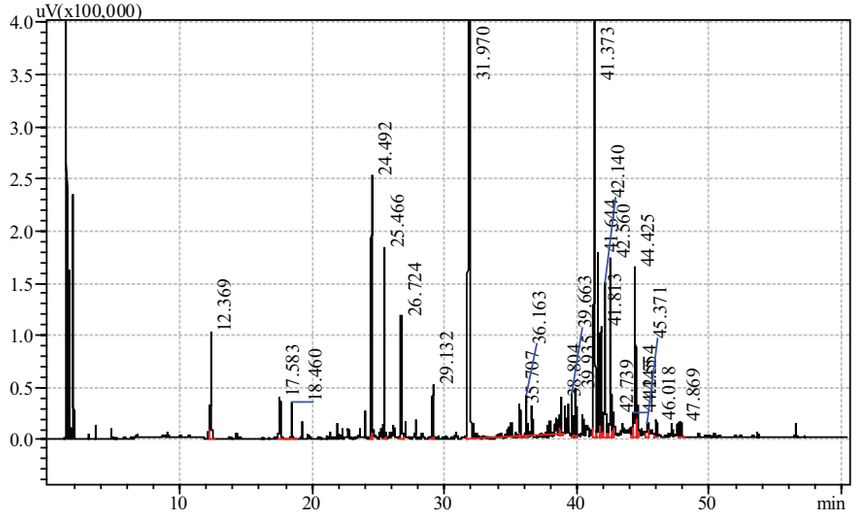

Figure 2. Chromatographic profile similar to the standard obtained to sample 7.

were authenticated by Dr. Renato Goldenberg, from the Department of Botany, Universidade Federal do Paraná, Brazil. A voucher specimen was deposited in the Herbarium of the same university (Voucher number: UPCB 45093).

Several samples of *Baccharis trimera* were acquired in commercial establishments in Curitiba and metropolitan region (PR, Brazil). Two types of products were acquired: boxes containing 10 and 20 little bags of the pulverized vegetal material (sample A) and little bags which were containing parts of the plant, without pulverization (sample B); corresponding to 15 total samples tested.

Dried leaves and commercial samples, when necessary, were powdered and sieved before being submitted to the essential oil extraction.

Study of the presence of non-identified materials

A small portion of each sample was transferred to a Petri plate. The samples were analyzed macroscopically and when the identification of the contaminants was not possible, a magnifying glass and a microscope were used.

Essential oil extraction

The extraction of the essential oil was carried out by hydrodistillation using a Clevenger-type apparatus

Commercial samples: about 20 g of the vegetal material were extracted with 250 mL of water for 7 hours.

Standard oil: The standard oil of *Baccharis*

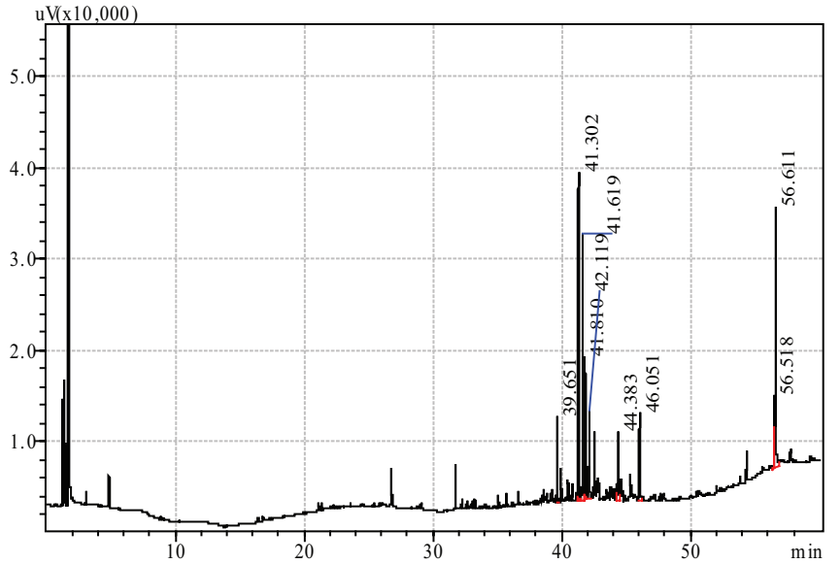

Figure 3. Chromatographic profile different from the standard obtained to sample 12.

trimera Less was extracted by the same procedure as for the commercial samples.

Chromatographic analyses

GC/FID analyses were performed on a Shimadzu 2010 gas chromatograph, using a 30 m x 0.25 mm ID fused-silica open-tubular column, crosslinked and chemically bonded with 95 percent dimethyl and 5 percent diphenyl-polysiloxane (DB-5), 0.25 µM film thickness. The GC operating conditions for the analyses were as follows: Samples were injected using the split mode (split 1:30) with the injector temperature at 200 °C and the detector temperature at 250 °C. The column temperature program was: 50 °C (15 min hold), increased at 5 °C/ min to 100 °C (10 min hold), increased at 5 °C/min to 200 °C (10 min hold); the carrier gas was hydrogen at an average linear velocity of 50 cm/s.

RESULTS AND DISCUSSION

In the macroscopic analyses of the samples, the presence of small sticks was found, as well as some other species of plants. The samples A showed sticks with leaves, and samples B showed less contamination. In the microscopic analyses, many undesirable foreign matters were observed, as pieces of small insects, alive insects, spider web, and some of other non-identified materials (Table 1).

The oil extraction yields corresponded to 0.5 to 1%, in agreement to the theoric values expected to *Baccharis trimera*.

Using chromatographic analyses, it was possible to select the samples in authentic and non-authentic (Table 1), comparing with the chromatographic profile of the standard oil (Figure 1).

The samples 1, 2, 4, 5, 6, 7, 8, 9, 11, 14, and 15 were classified as authentic (Table 1). The similarity of these samples in comparison to the chromatographic profile of the standard oil can be seen in figure 2. The samples 3, 10, 12 and 13 were classified as non-authentic. The differences of these samples in relation to the chromatographic profile of the standard oil were showed in figure 3. The intensity of the peaks in most of cases was different, probably because of the different times of the harvest and the differences of the soil composition (Silva, 2005; Agostini et al., 2005). In addiction to these factors, the presence of a great amount of sticks and other materials can influence in the concentration of the substances found in the essential oil.

The quality of *Baccharis trimera* commercialized in Curitiba and metropolitan region needs to be improved in relation to the presence of non-identified material in the vegetal material used in the elaboration of the products. In relation to the adulteration of the vegetal material, 26.7% of the analyzed samples were not in accordance with the quality standard. We can conclude that more rigidity is needed in the quality control of these kinds of materials in order to provide high quality and uniform products. The GC/FID-based authentication of *B. trimera* can be considered as a rapid screening to further studies of the influence of the seasonal variability on its essential oil.

GC/FID has been shown to be an effective analytical method for chromatographic analysis of the essential oil of *Baccharis trimera*, irrespective of carquejol and carquejile acetate standards. The present work showed first that the GC/FID fingerprint of the essential oil of *B. trimera* can be useful for quality evaluation of commercial samples and can bring

substantial improvement to such products, since it can be used as a tracking system to avoid contaminations during harvesting, processing, and packaging of these products. This method can be used for quality assurance as an important source of information on the authenticity of commercial samples of *B. trimera,* mainly in the case of pulverized drugs and capsules, and it may help as a rapid tool for quality control of the safety and efficacy of this herbal treatment.

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