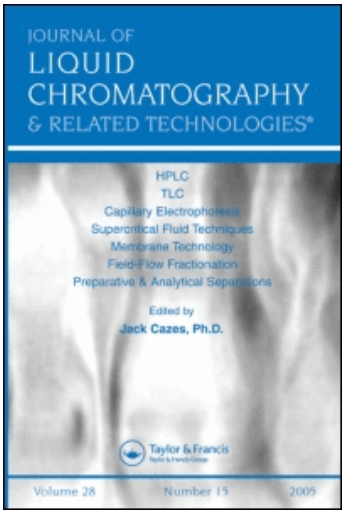

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Differentiation of South Brazilian *Baccharis* Species by TLC

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Differentiation of South Brazilian *Baccharis* Species by TLC

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Abstract: The *Baccharis* species, known in Brazil as "carquejas" are used for digestive disorders in folk medicine. Phytochemical studies reported the presence of phenolic and terpenoid compounds as the main constituents and the literature also describes anti-inflammatory, antioxidant, and antimicrobial activities for the *Baccharis* species. Due to difficult botanical characterization, the development of chemical differentiation of these plants is important. In this paper, we present the chemical characterization of five native *Baccharis* species to Southern Brazil by TLC, using just one mobile phase and two colors reagents.

Keywords: *Baccharis*, TLC, Phenolic compounds, BaII compound, Control quality

INTRODUCTION

The genus *Baccharis* (Asteraceae) is exclusive to America, encompassing nearly 400 species of which approximately 120 grow in Brazil. Around 70 species in the State of Rio Grande do Sul[1–3] are described. Popularly

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known as "carquejas", *Baccharis* species aerial parts are used in traditional medicine, mainly for digestive disorders.[4] Previous studies reported the presence of phenolic compounds as flavonoids and phenolic acids,[5,6] diterpenes, and saponins.[7–9] Their pharmacological and biological properties are described as anti-inflammatory, analgesic, antioxidant, and antibacterial for the *Baccharis* species.[4,9–11]

Although their use is traditional, the botanical differentiation among these species is complex, the correct nomenclature, the synonyms of the members, and especially botany morphology is controversial. It is very difficult for the correct identification of the three winged species, such *B. trimera*, *B. cylindrica*, and *B. usterii*[3,4,12] in the absence of flowers. On the other hand, *B. articulata* is considered to be a very close taxonomically related species to *B. gaudichaudiana*, both two-winged species.[8]

Considering that, recently, we reported the isolation and identification of BaII compound (4′-O-β-D-glucopyranosyl-3′,5′-dimethoxybenzyl-caffeate) from *B. articulata*, and this compound showed antioxidant properties comparable to Trolox®,[6] BaII could be used as a good chemical marker for this species.

Altogether, considering that these species are used as medicinal plants, often without correct identification, the development of fast and easy TLC methodology may contribute significantly to the differentiation between these plants and to their control quality as raw materials.

Herein, we report a chemical characterization by thin-layer chromatography (TLC) of *B. articulata, B. cylindrica, B. spicata*, *B. trimera*, and *B. usterii*, native species to the State of Rio Grande do Sul, Brazil.

EXPERIMENTAL

Plant Material

Aerial parts of *B. articulata* (Lam.) Person (BA), *B. cylindrica* (Less.) DC (BC), *B. spicata* (Lam.) Baillon (BS), *B. trimera* (Less.) DC (BT), and *B. usterii* Heering (BU) were collected in Porto Alegre, State of Rio Grande do Sul, Brazil. Herbarium specimens are on deposit in the Herbarium of the Botany Department of Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. Each plant material was air dried and powdered separately.

Reference Substance

Pure BaII compound (4′-O-β-D-glucopyranosyl-3′,5′-dimethoxybenzyl-caffeate) was previously isolated from *B. articulata*.[6]

Preparation of Plant Extracts

The aqueous extracts from *Baccharis* species were prepared, separately, by decoction of the plant material (1g, 2 × 100 mL) during 30 min. Part of each aqueous extract was evaporated under reduced pressure. The other part of each aqueous extract was partitioned, separately, using *n*-BuOH of which the solvent was evaporated under reduced pressure. Yields of each extract, i.e., of the *Baccharis* species (aqueous extract/*n*-BuOH fraction, mg): *B. articulata* (200/178), *B. cylindrica* (253/117), *B. spicata* (230/104), *B. trimera* (290/200), and *B. usterii* (270/90) were as given here. Fifteen μL of each sample, dissolved in MeOH (10 mg/mL), was applied to a TLC plate as a band, at the starting line.

Thin-Layer Chromatographic Analysis

Phytochemical profiles of *Baccharis* species were established by thin-layer chromatography (TLC) on silica gel plates (Merck F_{254}, 20 × 20 cm) using as mobile phase chloroform-ethanol-acetic acid ($CHCl_3$:EtOH:HOAc, 60:40:6, v/v). The mobile phase was allowed to migrate 15 cm from the starting line. Detections were performed using, separately, two colours of reagents: anisaldehyde-H_2SO_4, heating to 100°C, and diphenylboryloxy-ethylamine 1% in methanol, followed by PEG 400 (5%, w/v) (Natural Reagent).[13] Spots were observed under visible light and long-wave UV light after being sprayed by the coloured reagents. Figures 1 and 2 are photographed chromatographs in long-wave UV light. Figure 3 is a photographed chromatograph in visible light.

RESULTS AND DISCUSSION

Considering the large use of *Baccharis* species in folk medicine, and the morphological similarity of some native species from Southern Brazil, an analytical method to differentiate these species was developed. The extracts of plants were prepared in order to obtain polar compounds, considering their popular uses,[4] So, aqueous extracts and a *n*-BuOH fraction were used in our TLC analysis. A quick and economical method that had been performed to obtain the differentiation of these *Baccharis* species through their phytochemical profiles is described.

Figure 1 presents the chromatographic profile of aqueous extracts from the *Baccharis* species using silicagel 60 plates GF_{254}, $CHCl_3$:EtOH:HOAc (60:40:6, v/v) as mobile phase, anisaldehyde-H_2SO_4, heating to 100°C as colour reagent, and visualisation under UV light, 356 nm. It is possible to verify that some extracts have important characteristics. *B. articulata* has a major blue spot (Rf 0.6) not detectable elsewhere and identified as BaII

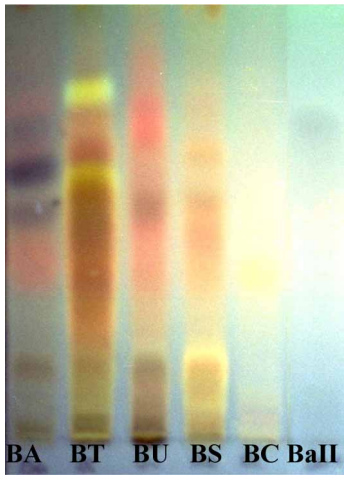

Figure 1. TLC profile of aqueous extract from *Baccharis* species. Chromatographic system: Si gel GF_{254}, $CHCl_3$:EtOH:HOAc (60:40:6, v/v), anisaldehyde-H_2SO_4 then heating (100°C)/UV_{356}. For sample codes see Experimental section.

compound. Additionally, it was also possible to differentiate *B. trimera* aqueous extract that shows two yellow spots (Rf 0.6 and 0.8) and *B. usterii* with two major spots (one red and another one brown), at Rf 0.7 and 0.5, respectively. *B. spicata* has one yellow spot at Rf 0.2. *B. cylindrica* extract did not show any characteristic using this chromatographic condition.

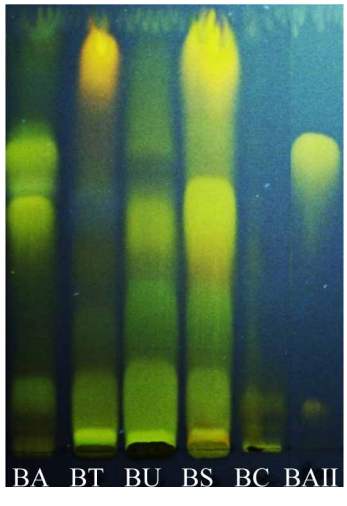

Figure 2. TLC profile of aqueous extract from *Baccharis* species. Chromatographic system: Si gel GF_{254}, $CHCl_3$:EtOH:HOAc (60:40:6, v/v), natural reagent/UV_{356}. For sample codes see Experimental section.

Figure 2 shows the chromatographic profile of *Baccharis* species using the same chromatographic conditions of Figure 1, except the natural reagent as colour reagent was used. Using this reagent, it is possible to observe the profile of the phenolic compounds present in the aqueous extracts. First, it is clear to observe that *B. cylindrica* aqueous extract does not present phenolic compounds. *B. articulata, B. spicata, B. trimera*, and *B. usterii* extracts can be differentiated through their chromatographic profile.

Figure 3 presents the pattern of *n*-BuOH fractions from *Baccharis* species using silicagel plates GF_{254}, $CHCl_3$:EtOH:HOAc (60:40:6, v/v) as mobile phase and anisaldehyde-H_2SO_4 then heating (100°C) as colour reagent. A predominance of polar compounds with terpenoid colour characteristic was observed at all studied species. *B. articulata* is the only species presenting the BaII compound (Rf. 0.6). Another important characteristic is that the *B. usterii* chromatographic profile showed three major violet spots undetectable in the other species. In relation to the other *Baccharis* species, *B. cylindrica* has an important quantity of terpenoid compounds.

Through the use of one mobile phase, and changing only the colour reagent, it was possible to differentiate the five South Brazilian *Baccharis* species studied herein. It is possible to observe qualitative and quantitative differences on the chemical profile of aqueous extract and a *n*-BuOH fraction from these species. Although the botanical differentiation among

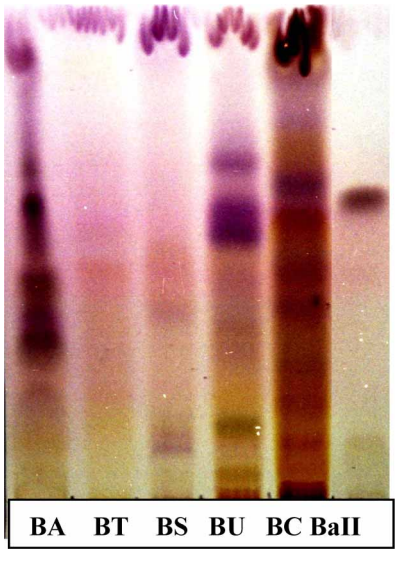

Figure 3. TLC profile of *n*-BuOH fraction from *Baccharis* species. Chromatographic system: Si gel GF_{254}, $CHCl_3$:EtOH:HOAc (60:40:6, v/v), anisaldehyde-H_2SO_4 then heating (100°C). For sample codes see Experimental section.

these plants is complex due to morphological similarity, their chemical profiles can be easily differentiated by the chromatographic conditions presented herein.

Accordingly, terpenoids and phenolic compounds can be used as quality markers in the identification of the species studied herein. Especially, the BaII compound could be used as a quality marker in the identification of *B. articulata*.

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