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Chemical constituents of *Lippia rigida* Schauer (Verbenaceae)

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1. Subject and source

Lippia L. is considered one of the largest genera of Verbenaceae, and comprises about 100 species distributed in the Neotropics and Africa (O'Leary et al., 2012). Most of them are found in Brazil, Paraguay and Argentina (Salimena, 2002). Recently, a study on species delimitation in *Lippia* section Goniostachyum was reported in the literature (O'Leary et al., 2012), and several species were grouped into only four taxa: *Lippia sericea* Cham., *Lippia grata* Schauer, *Lippia origanoids* Kunth and *Lippia stachyoides* Cham. According to this study, *L. rigida* Schauer is considered synonymy of *Lippia origanoides*. Thus, the study on the chemical composition of *L. rigida* is of chemotaxonomic relevance. *L. rigida* was collected in June 2010 from Mucugê, Bahia state, Brazil, and identified by Professor Maria Lenise Silva Guedes (Departament of Botany, Federal University of Bahia, Brazil). Voucher specimen (1240) is deposited at the Herbarium Alexandre Leal Costa (ALCB, Federal University of Bahia, Brazil).

2. Previous work

No chemical investigation on *L. rigida* is reported so far in the literature.

3. Present study

The dried and powdered leaves (1.6 kg) and trunk (3.1 kg) of *L. rigida* were extracted at room temperature with hexane (3 × 5 L), followed by ethanol (3 × 8 L). Solvent distillation under reduced pressure yielded the respective extracts HEL (13.0 g)

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and EEL (45.0 g) from leaves, and HET (25.1 g) and EET (51.0 g) from trunk. An aliquot (23.0 g) of the ethanol extract from leaves (EEL) was chromatographed over silica gel and provided four fractions after sequential elution with 100% hexane (2.5 L), dichloromethane (3.0 L), EtOAc (2.8 L) and MeOH (1.8 L) and solvent distillation under reduced pressure: EEL-H (0.47 g), EEL-D (3.92 g), EEL-EA (12.11 g) and EEL-M (1.98 g), respectively. Fraction EEL-D (3.02 g) was subjected to column chromatography over silica gel by elution with gradient mixture of dichloromethane/MeOH (10 → 40% v/v), and resulted in nine fractions (EEL-D1–EEL-D9) after TLC analysis. Fraction EEL-D3 (1.61 g) was purified by successive column chromatography over Sephadex LH-20 (100% MeOH) and silica gel (gradient mixture hexane/EtOAc), and yielded compounds **1** (59.0 mg) and **2** (6.0 mg). An aliquot (4.17 g) of EEL-EA was fractionated over Sephadex LH-20 (100% MeOH), and resulted in 14 fractions (EEL-EA1–EEL-EA14). Fraction EEL-EA7 (3.08 g) was submitted to flash column chromatography over silica gel (dichloromethane/EtOAc/MeOH: 89/10/1 → 30/40/30% v/v), and yielded 14 fractions (EEL-EA7-1–EEL-EA7-14) after TLC analysis. Fraction EEL-EA7-5 (360.0 mg) was purified by HPLC (C-18 semipreparative column, MeOH/H_2O (80:20), flux 4.5 mL/min) and yielded compounds **3** (250.0 mg), **4** (42.1 mg) and **5** (7.0 mg).

An aliquot (32.3 g) of the ethanol extract from trunk (EET) was chromatographed over silica gel and provided four fractions after sequential elution with 100% hexane (2.3 L), dichloromethane (3.0 L), EtOAc (2.8 L) and MeOH (1.9 L) and solvent distillation under reduced pressure: EET-H (0.15 g), EET-D (1.77 g), EET-EA (5.74 g) and EET-M (21.18 g), respectively. Fraction EET-EA (5.29 g) was subjected to column chromatography over silica gel by elution with gradient mixture of dichloromethane/EtOAc/MeOH (90/9/1 → 20/50/30% v/v), and resulted in eight fractions (EET-EA1–EET-EA8) after TLC analysis. Fraction EET-EA8 (3.58 g) was purified by successive column chromatography over silica gel (gradient mixture dichloromethane/EtOAc/MeOH), Sephadex LH-20 (100% MeOH) and silica gel (gradient mixture dichloromethane/MeOH), and yielded compounds **6** (60.0 mg).

After the essential oil extraction from the powdered leaves (424.0 g) of *L. rigida* by hydrodistillation, the decoction (2 L) was filtered, and concentrated under reduced pressure. An aliquot (41.6 g) of the aqueous extract (WEL, 63.0 g) was dissolved in 200 mL of MeOH:H_2O (2:3), and submitted to partition with 400 mL of each of the following solvents: CH_2Cl_2, EtOAc and butanol. Solvent distillation under reduced pressure yielded the respective fractions WEL-D (2.01 g), WEL-EA (11.54 g) and WEL-B (8.21 g). Part of the fraction WEL-EA (3.31 g) was chromatographed on flash silica gel by elution with gradient mixture of CH_2Cl_2/MeOH (10 → 80% v/v), and resulted eight fractions (WEL-EA1–WEL-EA8) after TLC analysis. Fraction WEL-EA6 (640.6 mg) was re-fractionated by column chromatography on flash silica gel, eluted with gradient mixture of CH_2Cl_2/MeOH (5 → 80% v/v), and yielded nine fractions (WEL-EA6-1–WEL-EA6-9) after analysis by TLC. Fraction WEL-EA6-2 (30.0 mg) was identified as the pure compound **7**. Fraction WEL-EA6-4 (80.2 mg) was purified by column chromatography on flash silica gel, eluted with CH_2Cl_2/MeOH (5 → 80% v/v), and yielded compounds **8** (32.0 mg) and **9** (9.0 mg).

All isolated compounds were identified on the basis of their spectrometric data (FT-IR, EI-MS, 1D and 2D NMR), and comparison with literature data for the same compounds: sakuranetin (**1**) (Jerz et al., 2005), pinocembrin (**2**) (Yenjai et al., 2009), naringenin (**3**) (Almeida et al., 2005), 7-methoxy aromadrendrin (**4**) (Almeida et al., 2005), genkwanin (**5**), taxifolin (**6**), kaempferol (**7**), quercetin (**8**) and rhamnocitrin (**9**) (Agrawal, 1989), Fig. 1.

4. Chemotaxonomic significance

The chemical investigation of *L. rigida* is being reported for the first time. Although most studies on the chemical composition of *Lippia* species is related to their essential oil, non-volatile compounds from different classes were also identified in this genus. The most frequently reported are flavonoids, iridoids and naphtoquinones (Gomes et al., 2011). According to Gomes et al. (2011), about 56 flavonoids (45 flavone and 11 flavanone derivatives) were isolated from *Lippia* species.

Recently, *L. rigida*, along with twenty-seven congener species, was considered a synonym of *L. origanoides*. Species delimitation was based on a phylogenetic species concept using a modified population aggregation analysis. No chemotaxonomic data were considered (O'Leary et al., 2012). Thus, the study on the chemical composition of *L. rigida* might be useful as a comparative chemotaxonomic tool for establishing the similarity of this species with *L. origanoides*.

Studies on the chemical composition of *L. origanoides* are reported in the literature and most of them report the profile of essential oils. Carvacrol and thymol are the major components in most of the oils (De Morais et al., 1972; Brieskorn and Pöhlmann, 1976; Gallino, 1987; Oliveira et al., 2007; Velasco et al., 2007; Stashenko et al., 2008; Vicuña et al., 2010; Sivira et al., 2011; Borges et al., 2012; Caballero-Gallardo et al., 2012). Chromatographic (GC/FID, GC/MS) and statistical (PCA) analyses of the essential oil from about ten specimens of *L. origanoides* was used to classify this species into three different chemotypes (caryophyllene-rich chemotype A, carvacrol-rich chemotype B, and thymol-rich chemotype C), according to the oil major compounds (Stashenko et al., 2010). Additionally, the extracts from these chemotypes of *L. origanoides* showed different flavonoids profiles by LC and GC analysis. Pinocembrin (**2**), naringenin (**3**), and luteolin were identified in all of the chemotypes but in varied yield, while quercetin (**8**) was present only in two of them (Stashenko et al., 2013).

Herein, we report the isolation of nine know flavonoids from *L. rigida*: sakuranetin (**1**), pinocembrin (**2**), naringenin (**3**), 7-methoxy aromadrendrin (**4**), genkwanin (**5**), taxifolin (**6**), kaempferol (**7**), quercetin (**8**) and rhamnocitrin (**9**). Among them, flavonoids **1**, **4**–**7** and **9** were not isolated so far from *L. origanoides*, and compounds **4**, **5**, and **7** are being reported for the first time in *Lippia* genus. Additionally, compound **4** is new in the family Verbenaceae. Compounds **1**, **2**, **3**, **6** and **8** were produced

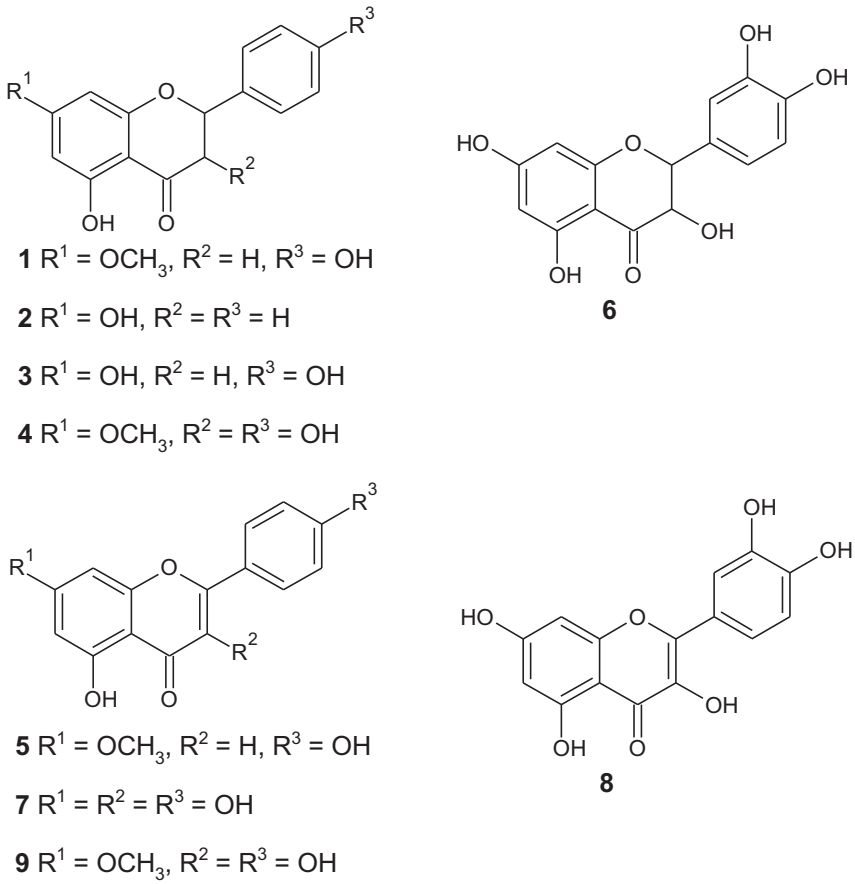

Fig. 1. Chemical structures of the flavonoids isolated from *Lippia rigida*.

by *Lippia graveolens* (Gomes et al., 2011), which is also being considered synonym of *L. origanoides* (O'Leary et al., 2012). However, the establishment of these flavonoids as chemotaxonomic markers of the *L. origanoides* group requires a more comprehensive study.

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