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HSCCC separation and enantiomeric distribution of key volatile constituents of *Piper claussenianum* (Miq.) C. DC. (Piperaceae)

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ABSTRACT

High Speed Countercurrent Chromatography (HSCCC) technique was used for the preparative isolation of the major leishmanicidal compounds from the essential oils of *Piper claussenianum* species in Brazil. The essential oils from inflorescences of *P. claussenianum* were analyzed by GC-FID and GC-MS. The enantiomeric ratio of the major constituents of the *P. claussenianum* essential oils were determined using a Rt-DEXsm chiral capillary column by GC-FID analysis. It was found an enantiomeric excess of (+)-(*E*)-nerolidol in the leaves, and (+)-linalool and (+)-(*E*)-nerolidol in the inflorescences essential oil. The major volatile terpenes alcohols were isolated in preparative scale from inflorescences: linalool (320.0 mg) and nerolidol (95.0 mg) in high purity level. The HSCCC, a support-free liquid–liquid partition chromatographic technique, proved to be an effective and useful method for fast isolation and purification of hydrophobic and similarly structured bioactive components from essential oils of *Piper* species.

1. Introduction

The Piperaceae family is composed by more than 4500 species widely distributed in the tropical and subtropical regions (Gutierrez et al., 2016). The species of the genus *Piper* are the most studied from chemical (Parmar et al., 1997) and pharmacological point-of-views (Marques and Kaplan, 2015; Brú and Guzman, 2016; Brambilla et al., 2017). In the last report, Marques et al. (2010) showed *Piper claussenianum* species as a valuable natural source of terpenes with biological and economic relevance such as linalool and nerolidol.

The isolation of pure bioactive compounds from natural sources on a preparative or semi-preparative scale is frequently necessary for further pharmacological investigations (Carini et al., 2015). Essential oils (EO) from plants are complex hydrophobic liquids containing a wide variety of volatile flavoring compounds. Terpenes and phenylpropanoids are the most common volatile components present in the EO composition. Many of terpene volatile compounds contain asymmetric C-atom(s) forming possible chiral variant isomers compounds, such as linalool (König and Hochmuth, 2004) and (*E*)-nerolidol (Juchelka et al., 1996), (Fig. 1). The enantiomer compounds are indiscernible by their general chemical/physical properties and usually can not be separated by GC conventional columns (Bardarov and Veltcheva, 2011). However, in many cases, optical isomers differ drastically by their biological activity resulting in different therapeutic effects. The information about their ratios in essential oils is important characteristic for their quality assessment, and can be used for confirmation of their origin, as well as for elucidation of the mechanisms of their biological formation and quality control (Singh and Sharma, 2015).

Since the essential oils are mainly composed by nonpolar metabolites such as terpenes and phenylpronapoids, the preparative separation of these volatile components is a challenge due their structural similarity, strongly hydrophobic properties and poor stability (Yanagida et al., 2007; Wu et al., 2008; Wybraniec et al., 2009).

The High Speed Countercurrent Chromatography (HSCCC), a support-free liquid–liquid partition chromatographic technique, represents a universal preparative chromatographic method that permits both normal and reversed-phase operation which eliminates irreversible adsorption of the sample onto the solid support (Feger et al., 2006; Berthod et al., 2009; Friesen and Pauli, 2009; Koichi et al., 2010). The general basis of the target compounds separation by HSCCC is the fast, continuous mixing and demixing operations of immiscible biphasic solvent systems in strong and rapidly alternating centrifugal force fields (Berthod et al., 2009).

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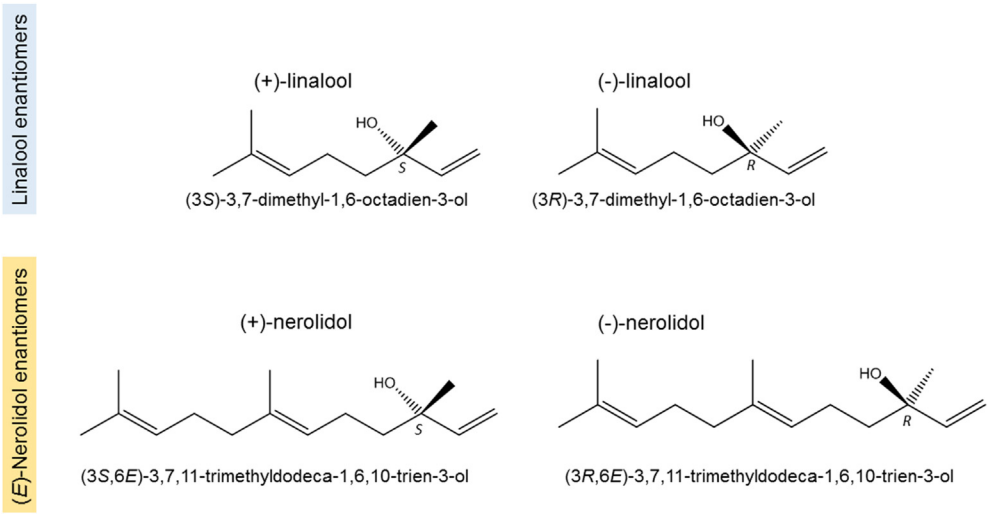

Fig. 1. (+,−)-Linalool and (+,−)-(*E*)-Nerolidol images are not superimposable and so they are enantiomers.

During the past few decades, HSCCC has been applied to both analytical and preparative separations of a wide variety of organic and/or inorganic compounds and is useful for the investigation of complex mixtures of natural compounds as plant extracts (natural library) (Woźniak and Garrard, 2014; Luan et al., 2016) and essential oils (Marques and Kaplan, 2013). The aim of this work was to perform the chemical characterization and the preparative isolation of the major bioactive volatile compounds from the inflorescences of *P. claussenianum* EO for their use in complementary pharmacological investigations.

2. Experimental

2.1. Solvents and reagents

All of the organic solvents used for HSCCC were of analytical grade.

2.2. Essential oil extraction

Leaves (300 g) and inflorescences (300 g) of *Piper claussenianum* were collected in Castelo, ES in January 2014. The botanical vouchers were identified by Dr. Elsie Franklin Guimarães and kept at the Herbarium (HB) of the Rio de Janeiro Botanical Garden (JBRJ), registered under number RB 489043. The fresh plant material was submitted separately to hydrodistillation for 2 h in a modified Clevenger-type apparatus. The obtained essential oils (EO) were dried over anhydrous sodium sulphate, yielding 1.0% (w/w) in leaf EO and 0.9% (w/w) in inflorescences EO samples.

2.3. Preparation of the two-phase solvent system and sample solutions

For HSCCC, we tested four different solvent systems composed by Hexane/Acetonitrile (1:1); Hexane/Methanol (1:1); Hexane/Acetonitrile/Ethyl acetate (1:1:0.4) and Hexane/Acetonitrile/Methanol (1:1:0.5). The sample solutions were prepared by diluting the essential oil in a mixed solution of the lower phase and upper phase (1:1, v/v) of the used solvent system for HSCCC separation. The tubes were vigorously shaken and the two phases allowed to settle. Equal volumes of upper and lower phases were separated before the TLC and GC-FID analyzes. The distribution of the inflorescences EO components into the phases was first estimated by thin-layer chromatography (TLC, silica gel 60 F254 nm) with Hexane/Ethyl acetate (3:2) as the eluting solvent system. The separation of the compounds was observed under a UV lamp at 254 nm and by spraying a sulphuric acid/methanol reagent (1:1, v/v), followed by heating to assist the visual estimation of the relative distribution of the compounds in each phase. The Distribution coefficients (K_D) were calculated by GC-FID analyses. According to the best distribution results found on CG-FID distribution profiles, we selected a two-phase solvent system composed of Hexane/ACN (1:1, v/v).

2.4. Determination of distribution coefficient (K_D) for the major terpene compounds by GC - FID

One drop (approximately 5 mg) of the inflorescences essential oil from *P. claussenianum* was added to the mixture of equal volumes of the upper phase and the lower phase from the two-phase solvent system in each flask (Section 2.3) and analyzed separately by GC-FID using the conditions described in Section 2.6. The solvent systems were composed of 1 mL of both phases in each flask. The distribution coefficient (K_D) was calculated as K_D = SP (peak area of compound)/MP (peak area of compound), where SP and MP represent the GC-FID peak areas of *P. claussenianum* EO components in the Stationary and Mobile phases, respectively. The target metabolites (K_D) are shown in the Table 1.

2.5. Apparatus and separation procedure

A CCC (model HSCCC No. 403, PC Inc., Potomac, MD, USA), consisting of a PTFE 80-mL coil, an HPLC pump (model M-45, Waters, USA), a low-pressure injection valve (Rheodyne 5020, USA), and a PTFE 5-mL sample loop were used. This system was coupled to a fraction collector (model L-7650, Merck, Darmstadt, Germany) programmed to collect at 1-min intervals. Appropriate volumes of the solvents Hexane/ACN (1:1, v/v) were vigorously hand-mixed in a separatory funnel, transferred to a flask, and degassed (ultrasonic bath) for 30 min. HSCCC running was performed initially in two different elution modes: head-to-tail and tail-to-head. First, an isocratic elution was conducted in a tail-to-head manner, with the acetonitrile (lower phase) as the stationary phase (normal elution mode). The same isocratic elution was performed using the reversed phase (head-to-tail mode). The coil was entirely filled with the stationary phase of the solvent system with no rotation.

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