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Chemical composition and acaricidal activity of the essential oil of *Baccharis dracunculifolia* De Candole (1836) and its constituents nerolidol and limonene on larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae)

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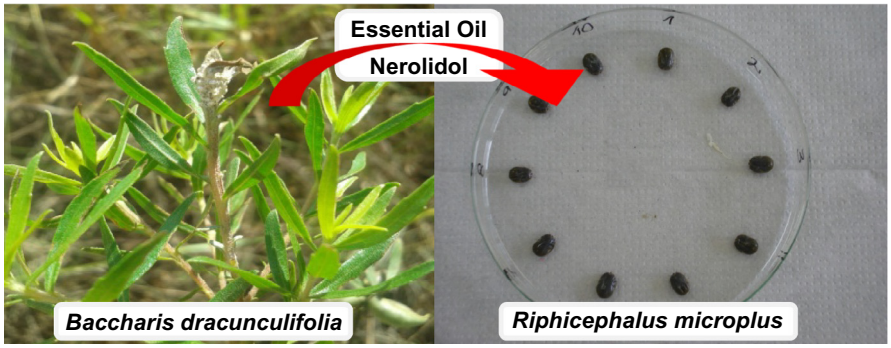
HIGHLIGHTS

- The chemical composition of essential oil (EO) of *B. dracunculifolia* was accessed.
- *B. dracunculifolia* EO comprises mostly nerolidol, germacrene D and limonene.
- The volatile oil is highly active against *R. microplus* larvae and engorged female.
- Nerolidol is the major constituent responsible for acaricidal activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Baccharis dracunculifolia DC (common name "alecrim-do-campo" in Brazil) is a plant with widespread distribution in South America that is the botanical origin of green propolis. The aim of this study was to evaluate the chemical composition and acaricidal activity of the essential oil of *B. dracunculifolia* and its constituents nerolidol and limonene on unengorged larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). The essential oil yield was 0.8% of dry mass and the major constituents were nerolidol (22.3%), germacrene D (7.2%), limonene (6.9%), β-pinene (6.7) and bicyclogermacrene (6.5%). The acaricidal activity of the essential oil and the pure compounds nerolidol and (*R*)-(+)-limonene were assessed in the laboratory through the modified larval packet test (LPT) and the female immersion test (FIT). In the LPT, the essential oil and nerolidol were both active, causing more than 90% mortality at concentrations from 15.0 and 10.0 mg mL^{-1}, respectively, whereas (*R*)-(+)-limonene was not active. In the FIT, the oil and nerolidol caused reduction in the quantity and quality of eggs produced, with control

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percentages of 96.3% and 90.3% at concentrations of 60.0 and 50.0 mg mL^{-1}, respectively. It can be concluded that the essential oil obtained from the aerial parts of *B. dracunculifolia* and its major component nerolidol have high activity on *R. microplus* larvae and engorged females.

1. Introduction

Rhipicephalus microplus (Canestrini, 1888) (Acari: Ixodidae), known popularly as the cattle tick, is the most important parasite species in terms of economic losses caused to cattle breeders in the Neotropical region. Infestation by this ectoparasite can cause reduced weight gain, lower milk production, hide damage, propensity for myiasis and transmission of pathogenic agents, besides increased expenditures for specialized labor, equipment and chemical products for treatment of animals. It is estimated that the losses caused by this parasite amount to some 3.2 billion dollars a year in Brazil (Grisi et al., 2014).

Control of ectoparasites is still mainly carried out through the use of synthetic chemical products. However, the indiscriminate use of these compounds has many drawbacks, among them the development of resistant parasite strains and toxicity to the environment and humans, including through residues in meat and milk (Taylor, 2001). In this context, natural products are in many cases an economically feasible and less toxic alternative. They can be used directly as pesticides or as the leading molecules for synthesis of new compounds (Duke et al., 2010).

Essential oils (EOs) are mixtures of volatile secondary metabolites, obtained mainly by hydrodistillation of various plant parts, such as leaves, roots, fruits, bark and wood. Chemically, they are mainly composed of terpenoids and are produced by specialized organs such as ducts, secretory cavities and glandular trichomes (Benchaar and Greathead, 2011; Kutchan, 2005; Maffei, 2010). The terpenoids or isoprenoids are the main family of natural compounds, represented by over 40,000 substances. They are biosynthesized by the mevalonate metabolic pathway, which is active mainly in cytosol, or by 2-*C*-methyl-*D*-erythritol-4-phosphate, principally in the plastids (Aharoni et al., 2005). The combination of substances contained in EOs can have a synergistic effect through multiple modes of action, therefore reducing the development of resistance (Dayan et al., 2009; Duke et al., 2010; Isman, 2000; Ntalli et al., 2011).

The genus *Baccharis* includes over 500 species, distributed from the United States to Argentina, with predominance in South America (Budel et al., 2005). *Baccharis dracunculifolia* DC (Asteraceae) is a plant native to Brazil, popularly called "alecrim do campo" (Barroso, 1976) and is the biological origin of the product called green propolis (Park et al., 2004). This plant is used in popular medicine as an antipyretic and also as an antiseptic to treat skin injuries (Parreira et al., 2010). Besides these uses, it also has confirmed anti-inflammatory and antinociceptive actions (Santos et al., 2010). Studies have further proved that the essential oil of this plant has antiulcerogenic (Massignani et al., 2009), antimicrobial (Ferronato et al., 2007), antiprotozoan and schistosomicidal actions (Parreira et al., 2010). However, there are no published reports of its potential acaricidal activity.

Therefore, this study aimed to assess the acaricidal activity of the essential oil of *B. dracunculifolia* and its constituents, nerolidol and (*R*)-(+)-limonene (Fig. 1), against *R. microplus* larvae and engorged females.

2. Material and methods

2.1. Plant material

Specimens of *B. dracunculifolia* were collected in August 2011 (winter) on the campus of Viçosa Federal University (UFV), Viçosa, Minas Gerais, Brazil (geographic coordinates: 20°46′05.46″S and 42°51′40.28″W). Voucher specimens were dried and placed in the collection of the VIC Herbarium of the university's Plant Biology Department, under number VIC 35.604 and was identified by Dr. Eric Koiti Okiyama Hattori of Missouri Botanical Garden.

2.2. Essential oil extraction and pure substances used

The fresh aerial parts were weighed and divided into three portions of 100 g each and then subjected to hydrodistillation for 5 hours, using a Clevenger apparatus. The essential oil was collected and the residual water was removed with anhydrous sodium sulphate (Na_2SO_4), after which the oil was filtered and kept under refrigeration (−10 °C) in sealed amber flasks until the moment of analysis.

2.3. Analysis by gas chromatography and gas chromatography combined with mass spectrometry

The essential oil samples were analyzed in a Shimadzu GCMS-QP 5000A gas chromatograph equipped with a Supelco DB-5 capillary column (30 m × 0.25 mm × 0.25 μm) and a selective mass detector, operating with ionization energy of 70 eV. The carrier gas was helium, at a flow of 1.8 mL min^{-1} and the injector temperature was programmed to remain at 230 °C. The oven heating program started at 40 °C for 2 minutes, followed by a gradual increase to 230 °C at a rate of 3.0 °C per minute. This temperature was then maintained for 20 minutes.

The quantification of the constituents was performed with a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector (FID). The same method and column were used with both chromatographs. The compounds were identified by comparing the mass spectra against those in the device's library (Wiley and Nist) and those described in the literature, along with the arithmetic index (Adams, 2007). The pure substances, (*R*)-(+)-limonene and nerolidol, were purchased from Sigma-Aldrich.

2.4. Preparation of the oil samples

For the modified larval packet test (LPT), a stock solution of the EO was prepared at a concentration of 60 mg mL^{-1} (m/v) in 2% Tween and water, while for the nerolidol and (*R*)-(+)-limonene, the stock solutions were at a concentration of 20 mg mL^{-1} using absolute ethanol. The stock solutions were serially diluted to concentrations of 2.5, 5, 10, 15 and 20 mg mL^{-1}. For the Female Immersion Test (FIT), a new stock solution of nerolidol was prepared (60 mg mL^{-1}) using a 1:1 mixture of 2% DMSO and 2% Tween. Then this stock solution was serially diluted to concentrations of 10, 20, 40 and 60 mg mL^{-1}.

2.5. Ticks

The ticks used in this study were of the sensitive Porto Alegre (POA) strain. These ticks are maintained in artificial infestations on cattle at the experimental farm of the Embrapa Dairy Cattle Research Unit (Embrapa Gado de Leite), located in Juiz de Fora, Minas Gerais state, Brazil. The experimental procedure was approved by the animal experimentation ethics committee (AEEC 11/2013) of Embrapa Gado de Leite.