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Original article

Chemical composition and efficacy in the egg-hatching inhibition of essential oil of *Piper aduncum* against *Haemonchus contortus* from sheep

Gisele Lopes Oliveira[a], *Thallyta Maria Vieira*[b], *Vanessa Francisca Nunes*[c], *Munic de Oliveria Ruas*[c], *Eduardo Robson Duarte*[d], *Davyson de Lima Moreira*[e,*], *Maria Auxiliadora Coelho Kaplan*[f], *Ernane Ronie Martins*[d]

[a]*Programa de Pós-graduação em Biotecnologia Vegetal, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*
[b]*Programa de Pós-graduação em Ciências Agrárias, Universidade Federal de Minas Gerais, Montes Claros, MG, Brazil*
[c]*Ciências Biológicas, Faculdade de Saúde Ibituruna, Montes Claros, MG, Brazil*
[d]*Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Montes Claros, MG, Brazil*
[e]*Departamento de Produtos Naturais, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil*
[f]*Núcleo de Pesquisa de Produtos Naturais, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*

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ABSTRACT

Piper aduncum L., Piperaceae, has been used to treat mainly inflammatory diseases and has shown several biological activities such as insecticidal and larvicidal. The aim of this study was to analyze the chemical composition of essential oil of *P. aduncum* and its efficacy to egg-hatching inhibition of *Haemonchus contortus* from sheep. The essential oil was obtained from leaves and analysed by gas chromatography coupled to flame ionization detector and gas chromatography coupled to mass spectrometry. It was possible to characterize 22 different substances, among them *monoterpenes* (80.6%) and *sesquiterpenes* (13.9%). The major compound was identified as 1,8-cineole (55.8%). Eggs of the nematode were exposed to four concentrations of the essential oil. Levamisole phosphate was used as positive control. The essential oil showed to be effective in inhibiting *H. contortus* hatchability and the LC_{90} was calculated as 8.9 $mg.ml^{-1}$. These results can point out the *P. aduncum* essential oil and its chemical components as potential alternative to control of *H. contortus*.

Introduction

Essential oils have shown interesting biological activity such as antimicrobial, cytostatic, insecticidal, larvicidal and anthelmintic (Franzios et al., 1997; Bakkali et al., 2008; Nerio et al., 2010; Lara Junior et al., 2012; Oliveira et al., 2013a).

Considering the different plant families showing essential oils, special interest in Piperaceae, which has the one of its great features the presence of structures that produce essential oils especially in inflorescences and leaves (Pessini et al., 2003). The family Piperaceae is distributed throughout tropical regions around the world, with the *Piper* being the most

* *Corresponding author*.
E-mail: dmoreira@far.fiocruz.br (D.L. Moreira).
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representative genus (Yuncker, 1972; Souza and Lorenzi, 2005). *Piper aduncum* L., Piperaceae, stands especially by showing biological activity associated with its extracts and essential oil such as insecticide, larvicide, antimicrobial, molluscicidal and leishmanicidal (Orjala et al., 1994; Bernard et al., 1995; Moreira et al., 1998; Torres-Santos et al., 1999; Souto, 2006; Sousa et al., 2008; Lara Junior et al., 2012). This specie is popularly known as pepper jack or false jaborandi and it is considered an opportunistic plant that invades deforested areas, and features rusticity with high resilience to climate change (Lorenzi and Matos, 2002; Sousa et al., 2008). The biological activities attributed to *P. aduncum* and the great chemical diversity of its essential oil lead us to investigate this plant for veterinary applications.

Several helminths infect herds of any age and gender and are a major obstacle for its development (Silva, 2004; Mali and Mehta, 2008). The gastrointestinal nematode *Haemonchus contortus* is the most pathogenic and is responsible for over 80% of the parasite load in small ruminants, causing loss of weight and appetite, significant decrease in the production of milk and meat, submandibular edema, ascites, hypoproteinemia, hipoalbunemia, severe gastritis bleeding, severe anemia and death (Urquhart et al., 1990; Arosemena et al., 1999; Melo, 2005). The treatment of nematode parasites in sheep is most often carried out exclusively with synthetic antihelminthics. This practice has promoted the rapid selection of multiresistant populations of parasites to commercial antihelminthics (Melo and Bevilaqua, 2002; Furtado, 2006).

Thus, the purpose of this study was to the evaluate chemical composition of *P. aduncum* essential oil and its efficacy to egg-hatching inhibition of *H. contortus* from sheep.

Materials and methods

Plant materials, essential oil extraction and analysis

Leaves of *Piper aduncum* L., Piperaceae, were collected in May 2012 in the Gallery Forest of the Angico River, in the rural region of Bocaiúva site, Minas Gerais State, Brazil (S 16° 57,582', W 43° 51,912'). A voucher specimen was deposited at the Herbarium of the Botanical Garden of Rio de Janeiro (RB 501.330). The essential oil was extracted from fresh leaves (150 g) by hydrodistillation in a modified Clevenger-type apparatus, yielding 0.9% (p/v).

The samples were subjected to analysis by gas chromatography coupled to flame ionization detector (HP-Agilent 6890 GC-FID) and by gas chromatography coupled to mass spectrometry (HP Agilent GC 6890 – MS 5973), in the Analytical Platform of Farmanguinhos, Fiocruz, Rio de Janeiro. Initially, the essential oils were diluted in dichloromethane (1 $mg.ml^{-1}$) and analyzed by GC-MS to obtain the mass spectra and to performer chemical characterization. Concomitantly, the diluted samples of essential oils (0.5 $mg.ml^{-1}$) were analyzed by GC-FID for quantification of chemical constituents and to determine the retention indices (RI). Each essential oil component was quantified based only in the individual component's relative peak area in the chromatogram. The substances in the essential oil were identified by comparing their mass spectra with database registration (WILEY7n) and by comparison of RI calculated those records from literature (Adams, 2007). RI were calculated using GC data of a homologous series of saturated aliphatic hydrocarbons within C8 to C20 (Sigma-Aldrich), performed at the same column and the conditions used in the GC analysis for the essential oils, and using the equation proposed by van Den Dool and Kratz (1963).

GC-FID parameters: HP-5ms column (30 m × 0.32 mm × 0.25 µm), temperature programming from 60 to 240°C, with increase of $3°C.min^{-1}$, using the hydrogen and synthetic air to the carrier gas, with a flow rate of 1 $ml.min^{-1}$ and injection volume of 1 µl.

GC-MS parameters: HP-5ms column (30 m × 0.32 mm × 0.25 µm), temperature programming from 60 to 240°C, with increase of $3°C.min^{-1}$, using helium as the carrier gas to the, with a flow rate of 1 $ml.min^{-1}$ and injection volume of 1 µl.

Egg-hatching inhibition

The nematode eggs were obtained from the feces of three castrated male sheep Santa Inés infected with *Haemonchus contortus* only that were raised in pens at the Experimental Farm of the Institute of Agricultural Sciences of the Federal University of Minas Gerais. The average fecal egg count of > 3,000 g^{-1} and the infection was determined using the modified McMaster technique (Gordon and Whitlock, 1939). For the recovery of infective larvae it was used the technique of coproculture in accordance with Ueno and Gonçalves (1998) and larvae of *H. contortus* were confirmed according to the key of Keith (1953). Experimental procedures were carried out in accordance with Experience the Animal Ethical Committee of Minas Gerais Federal University and approved by this committee, under protocol number 042/2008. About 100 g of feces were macerated, homogenized, filtered and washed in sieves with meshes of 106, 53 and 20 mm. The eggs were suspended in supersaturated aqueous sodium chloride solution and subsequent centrifugation according to Bizimenyera et al. (2006). It was standardized at the final concentration of 100 eggs in 240 µl of sterile distilled water. Assess to the activity of the essential oil of *P. aducum* in egg-hatching inhibition (EHI) tests were done with five replicates (Coles et al., 1992). The essential oil was diluted in 1% of tween 80 in sterile distilled water solution. In test tubes it was added 240 µl of the solution with approximately 100 eggs and added equal volume of essential oil solution at the end concentrations of 50.0, 12.0, 6.0 and 3.6 $mg.ml^{-1}$, respectively. Solution of levamisole phosphate (15 $mg.ml^{-1}$) was used as control positive and pure distilled water and 1% tween 80 solutions were used to the negative controls. The cultures were kept at 28°C in biological oxygen demand chamber for 48 h. Subsequently, unembryonated eggs, embryonated eggs, and first-stage larvae (L1) were counted according Coles et al. (1992).

The counting of eggs and L1 was transformed into relative values for the initial number of eggs for replicate. The results were submitted to variance analysis and compared in media tests ($p \leq 0.05$) of the statistical package SAEG 9.1 (2007). Probit regression was employed to analyze the data using this statistical package to determine the concentrations sufficient