

Insecticidal activity *in vitro* of the essential oil of *Pogostemon cablin* against *Ctenocephalides felis felis*

Atividade inseticida *in vitro* do óleo essencial de *Pogostemon cablin* frente *Ctenocephalides felis felis*

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Abstract

The aim of this study was to evaluate the *in vitro* insecticidal activity of the essential oil (EO) of *P. cablin* against immature and adult stages of *C. felis*. The chemical composition of *P. cablin* EO was determined by gas chromatography (GC). To evaluate the insecticidal activity, the egg, larva, pupa, and adult stages of *C. felis* were exposed to different concentrations of *P. cablin* EO diluted in acetone and impregnated in filter paper in a concentration range of 25 to 200 $\mu\text{g}\cdot\text{cm}^{-2}$. After exposure, the material was incubated in climatized chambers and mortality assessment was performed after 24 h for the larval and adult stages, 72 h for the egg stage and 15 days for the pupal stage. After GC analysis, the major constituents of the EO of *P. cablin* were found to be patchoulol (27.4%), α -bulnesene (19.7%) and α -guayene (15.0%). The mortality percentage was 100% at the concentration of 200 $\mu\text{g}\cdot\text{cm}^{-2}$ for all stages. The LC_{50} values were 46.1, 49.2, 60.6 and 62.7 $\mu\text{g}\cdot\text{cm}^{-2}$, respectively for the pupal, egg, larva and adult stages. Based on the results obtained, we can conclude that the EO of *P. cablin* contained patchoulol, α -bulnesene, and α -guayene as major constituents and had insecticidal activity against the immature and adult stages of *C. felis*.

Keywords: flea, volatile oil, sesquiterpene alcohol, patchouli.

Resumo

O objetivo deste estudo foi avaliar a atividade inseticida *in vitro* do óleo essencial (OE) de *Pogostemon cablin* frente estágios imaturos e adultos de *Ctenocephalides felis felis*. A composição química do OE de *P. cablin* foi determinada por cromatografia gasosa (CG). Para a avaliação da atividade inseticida os estágios de ovo, larva, pupa e adultos de *C. felis* foram expostos a diferentes concentrações do OE de *P. cablin* diluídos em acetona e impregnados em papel filtro com a faixa de concentração de 25 a 200 $\mu\text{g}\cdot\text{cm}^{-2}$. Após a exposição, o material foi incubado em câmaras climatizadas e a avaliação da mortalidade foi realizada após 24 h para os estágios de larvas e adultos, 72 h para o estágio de ovo e 15 dias para o estágio de pupa. Após análise cromatográfica, os constituintes majoritários para o OE de *P. cablin* foram: patchoulol (27,4%), α -bulneseno (19,7%) e α -guaieno (15%). Foi obtido 100% de mortalidade na concentração de 200 $\mu\text{g}\cdot\text{cm}^{-2}$ para todos os estágios. As CL_{50} foram de 46,1; 49,2; 60,6 e 62,7 $\mu\text{g}\cdot\text{cm}^{-2}$, respectivamente para os estágios de pupa, ovo, larva e adultos. Com base nos resultados obtidos é possível concluir que o OE de *P. cablin* apresentou o patchoulol, α -bulneseno, e α -guaieno como constituintes majoritários e atividade inseticida frente aos estágios imaturos e adultos de *C. felis*.

Palavras-chave: pulga, óleo volátil, sesquiterpeno álcool, patchouli.

Fleas of the subspecies *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) are parasitic insects of major importance to the health of dogs and cats worldwide. Fleas are responsible for transmitting pathogenic agents, causing blood spoliation, and leading to the development of allergic dermatitis. Added to this is the emergence of flea populations resistant to some ectoparasiticides. Therefore,



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research to identify molecules and technologies for the of these insects is relevant (Lappin et al., 2020; Rust, 2020).

Currently, there is a growing search for alternative eco-friendly to control these insects that are less toxic to the host and the environment. In this context, products from the secondary metabolism of plants, such as EOs, are promising due to their repellent and insecticidal activity against a wide variety of insects of importance to agriculture and one health (Pavela & Benelli, 2016). Some EOs have already shown promise in *in vitro* assays against fleas (Conceição et al., 2020; Freitas et al., 2021). The EO of *Pogostemon cablin* (Lamiaceae), popularly known as patchouli, has already demonstrated toxicity against some groups of insects (Costa et al., 2013; Santos et al., 2022), but there are no published studies on its activity against fleas. Therefore, the present study aimed to demonstrate the insecticidal activity of the EO of *P. cablin* against the immature and adult stages of *C. felis*.

The different stages of this flea came from the experimental colony present in "Laboratório de Quimioterapia Experimental em Parasitologia Veterinária do Departamento de Parasitologia animal do Instituto de Veterinária da Universidade Federal Rural do Rio de Janeiro".

The essential oil from the leaves of *P. cablin* was purchased from Via Aroma Indústria de Aromatizadores de Ambientes Ltda (Porto Alegre, Brazil). Gas chromatography (GC) for the determination of EO constituents was performed using a flame ionization detector (FID) and a split/splitless injector. The substances were separated in an HP- fused silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 m, Agilent J & W), according to the method proposed by Adams (2007).

For the development of *in vitro* assays, the EO of *P. cablin* was diluted in 99.5% pure acetone and then impregnated in Whatman #1 filter paper discs with an area of 23.76 cm² for the immature stages and strips of 10 cm² for adult fleas. The tests were performed in a concentration range of 25 - 200 µg.cm⁻². The entire test was performed in sextuplicate, along with a placebo group (acetone), negative control (without impregnation) and positive control (fipronil for larva, pupa, and adult stages and pyriproxyfen for the egg stage, both at the concentration of 8 µg.cm⁻²).

The methodology for *in vitro* mortality assays was adapted from Conceição et al. (2020). To determine the EO activity against adult fleas, five pairs of fleas with age of 14 days were selected for each repetition. For the evaluation of insecticidal activity against immature stages, 10 eggs aged less than 24 hours, 10 larvae aged five days and 10 pupae aged 10 days were selected for each repetition.

After the test, the material was incubated in acclimatized chambers at a temperature of 27±1 °C and relative humidity of 75±10%. The evaluation periods for adult fleas and larvae were 24 and 48 hours, for eggs 72 hours and for pupae 15 days after incubation. The motility criterion used for adult fleas and larvae was movement, where any minimal movement presented by the insect characterized it as alive. In turn, eggs from which larvae did not hatch were considered unviable, while for pupae, those that did not develop into the adult stage were considered dead.

After the evaluations, the data were tabulated and the percentage was calculated using the formula described by Abbott (1925). Also, the LC₅₀ values for each of the stages were calculated using Probit analysis with the R software (RStudio, 2020) with the ecotoxicology package with 95% confidence interval (p<0.05).

The GC result showed that the EO of *P. cablin* contained patchoulol (27.4%), α-bulnesene (19.7%) and α-guayene (15.0%) as major constituents. Other chemical compounds found were seychelene (7.5%), α-patchoulene (5.4%), β-caryophyllene (3.7%), γ-patchoulene (3.7%), β-guayene (3.6%), β-patchoulene (3.6%), spathulenol (1.9%), β-elemene (1.1%), pogostone (1.1%), α-gurjunene (0.7%), nor-patchoulol (0.6%) and pogostol (0.5%), while 4.6% of the compounds were not identified. The results obtained in this study are similar to those described by Costa et al. (2013), where the sesquiterpene alcohol patchoulol (C₁₅H₂₆O) was the major constituent of the EO of *P. cablin* and was probably responsible for its insecticidal activity.

Regarding the insecticidal activity, 100% mortality was observed against all stages at the concentration of 200 µg.cm⁻². The LC₅₀ values were 46.1, 49.2, 60.6 and 62.7 µg.cm⁻², respectively, for the pupal, egg, larva, and adult stages (Table 1). This is the first study that has evaluated the activity of the EO of *P. cablin* against fleas, demonstrating its insecticidal activity. The susceptibility to this EO was similar to that described by Conceição et al. (2020) for the EOs of *Thymus vulgaris*

Table 1. Mortality (%) and median lethal concentration (LC₅₀) of *Pogostemon cablin* essential oil against immature and adult stages of *Ctenocephalides felis*.

| Concentration | Eggs | Larvae | Pupae | Adults |
|------------------------|-------|--------|-------|--------|
| µg.cm ⁻² | 72 h | 24 h | 15 d | 24 h |
| Neg. Cont. | 6.7 | 0 | 3.3 | 0 |
| Placebo | 3.3 | 0 | 3.3 | 0 |
| Pos. Cont. | 100.0 | 100.0 | 100.0 | 100.0 |
| 25 | 23.3 | 30.0 | 28.7 | 1.7 |
| 50 | 65.0 | 48.3 | 64.4 | 15.0 |
| 100 | 79.7 | 81.7 | 86.4 | 61.7 |
| 150 | 94.4 | 96.7 | 91.5 | 88.3 |
| 200 | 100.0 | 100.0 | 100.0 | 100.0 |
| LC₅₀ | 49.2 | 62.7 | 46.1 | 60.6 |
| Minimum | 35.7 | 47.4 | 18.3 | 48.7 |
| Maximum | 62.8 | 79.1 | 72.5 | 73.2 |
| Slope | 2.2 | 2.7 | 2.9 | 4.0 |
| R² | 0.859 | 0.861 | 0.868 | 0.961 |
| p-value | 0.432 | 0.366 | 0.173 | 0.684 |

Lethal concentration was calculated using Probit analysis with the RStudio Team software (RStudio, 2020). LC = lethal concentration; h = hours; d = days; Neg. Cont. = negative control (filter paper without impregnation); Placebo (filter paper impregnated with pure acetone); Pos. Cont. = positive control (filter paper impregnated with 8 µg.cm⁻² of fipronil for larvae and adults and pyriproxyfen for eggs and pupae).

and *Origanum vulgare* and two other plants of the Lamiaceae family, but with thymol and carvacrol as major constituents. According to the LC₅₀ values, there was no difference in potency of this EO in relation to the stages. Therefore, it seemed to be effective in the same concentration range for all stages. This EO has also been shown to be toxic to other insects. (Lima Santos et al., 2019; Santos et al., 2022).

Based on the results obtained in this study, we can conclude that the EO of *P. cablin* has insecticidal activity against the immature and adult stages of the flea *C. felis*. Moreover, this EO and its major constituents have potential for the control of other arthropods of importance in veterinary medicine. For this reason, new *in vitro* studies with patchoulol and the development of formulations for further *in vivo* tests for target species are needed to confirm this hypothesis.

Ethics statement

The flea colony has been approved at the Animal Use Ethics Committee of the Veterinary Institute of the Federal Rural University of Rio de Janeiro with protocol number 4313110419.

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Conflict of interests

MBST, ABRF, DRC, IRLJ, YPC, FBS, KC - No conflict of interest

Authors' contributions

MBST, ABRF, DRC, IRLJ - Development of methodology; preparation and writing the initial draft. DRC - Application of statistical study data, Review and Editing manuscript. YPC and KC - Review and Editing manuscript.

FBS - Acquisition of the financial support for the project leading to this publication.

Availability of complementary results

None

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