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Short communication

Inhibitory activities of essential oils against *Babesia canis*

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Abstract

The *in vitro* anti-*Babesia canis* activities of nine essential oils were investigated. Among the tested essential oils *Achillea millefolium*, *Eugenia caryophyllus* and *Citrus grandis* were the most active (IC₅₀ values of 51.0, 60.3 and 61.3 µg/mL, respectively). The oils from *Abies sibirica*, *Rosmarinus officinalis*, *Eucalyptus globulus*, *Cinnamomum zeylanicum*, *Mentha piperita* and *Pinus sylvestris* were less active (IC₅₀ values of 134.3, 237.3, 239.3, 367.9, 837.5 and 907.3 µg/mL, respectively). The results support the concept that some essential oil constituents may be useful in the clinical management of babesiosis.

Key words: *Babesia canis*, essential oil, natural product

Introduction

Babesiosis is a tick-transmitted disease caused by protozoans of the genus *Babesia* which has been recognised as an emerging infectious disease of dogs (Adaszek and Winiarczyk 2008). Medicinal plants are an important natural source of biologically active compounds, and have been shown to possess antimicrobial

and antiparasitic properties (Murnigsih et al. 2005, Santoro et al. 2007, AbouLaila et al. 2010a,b, Vawazola et al. 2018, Guz et al. 2019). With the aim of discovering new natural products against *Babesia canis*, and developing active essential oils (EOs) for application, nine plants were selected for anti-babesial investigation in our work. It is the first time that these EOs were tested on *Babesia* species.

Table 1. Inhibitory concentration (IC₅₀) of examined essential oils.

Essential oils	IC ₅₀ (µg/mL)	95% Confidence intervals
<i>Achillea millefolium</i>	51.0	18.1 – 71.3
<i>Eugenia caryophyllus</i> bud	60.3	15.9 – 62.2
<i>Citrus grandis</i> peel	61.3	15.5 – 61.1
<i>Abies sibirica</i>	134.3	7.4 – 29.3
<i>Rosmarinus officinalis</i> leaf	237.3	4.0 – 15.7
<i>Eucalyptus globulus</i> leaf	239.3	4.8 – 18.8
<i>Cinnamomum zeylanicum</i> bark	367.9	2.5 – 10.0
<i>Mentha piperita</i>	837.5	1.1 – 4.3
<i>Pinus sylvestris</i>	907.3	1.0 – 4.0

Materials and Methods

Essential oils

The antibabesial activities of 9 essential oils: 1 – from *Mentha piperita*, 2 – *Achillea millefolium* oil, 3 – *Abies sibirica* oil, 4 – *Rosmarinus officinalis* leaf oil, 5 – *Citrus grandis* peel oil, 6 – *Eugenia caryophyllus* bud oil, 7 – *Cinnamomum zeylanicum* bark oil, 8 – *Pinus sylvestris* oil, and 9 – *Eucalyptus globulus* leaf oil, were studied (1, 4, 5, 6, 7, 8, 9 – from PPHU KEJ Sp. z o.o., Wieliczka, Poland; 2 – from Alteya Organics, Bulgaria; 3 – from ETJA S.C., Elbląg, Poland).

In vitro test for anti-babesial activity

The anti-babesial assay was performed against *B. canis* in vitro according to the reported method by Rickmann et al. (1978) cited by Vawazola et al. (2018). The essential oil was prepared in a solution of 100% methanol/dimethyl sulfoxide at 800 µg/mL. The final concentrations of the extracts were 80.0, 8.0, 0.8, 0.08 and 0.008 µg/mL. These dilutions were performed in three replicates in aseptic conditions. Microplates thus impregnated were placed in an oven at 37°C until completely dry.

Heparinised blood from a normal dog was washed three times with Vega y Martinez (VYM) solution (CaCl₂·2H₂O – 16.0 mg, KCl – 400.0 mg, KH₂PO₄ – 1415.4 mg, MgSO₄·7H₂O – 154.0 mg, Na₂HPO₄·7H₂O – 1450.0 mg, NaCl – 7077.0 mg, and dextrose – 20.5 mg, in 1L of H₂O containing 0.25 mM of adenine and 0.5 mM of guanosine) supplemented with penicillin (100 IU/mL), streptomycin (100 µg/mL), and amphotericin B (0.25 µg/mL), and the blood was then washed twice with RPMI 1640. After washing, erythrocytes were resuspended to a final cell volume of 6% in a culture medium consisting of 60% RPMI 1640 and 40% normal dog serum. The erythrocyte suspension was mixed with parasitised erythrocytes to obtain 0.7% parasitaemia at the start of the incubation. Fifty microlitres of this mixture were distributed in each

well of the previously impregnated microplates.

The plate was incubated at 37°C under a humidified atmosphere of 5% CO₂. After leaving the plate in the incubator for 72 h, a Giemsa stained thin smeared specimen was prepared. The percentage of parasitaemia of *B. canis* was determined by counting the number of parasitised cells in 1000 erythrocytes. In this study, PBS with DMSO 0.1% was used as a negative control. All experiments were performed in triplicate in two independent assays. The concentration of essential oils that inhibit 50% of the parasites (IC₅₀) was estimated by interpolation from dose-response regressions plotted by Excel 2016.

Results and Discussion

Table 1 shows the IC₅₀ values of 9 essential oils effective against *B. canis*. Among the essential oils examined, the most active was *A. millefolium* (IC₅₀ = 51.0), followed by *E. caryophyllus* (IC₅₀ = 60.3) and *C. grandis* (IC₅₀ = 61.3). The oils of *A. sibirica*, *R. officinalis* and *E. globulus* also caused growth inhibition, but at higher concentrations (IC₅₀ = 134.3, 237.3 and 239.3, respectively). On the other hand, very weak inhibition of *B. canis* growth was noticed as a result of treatment at the tested concentrations with the essential oils of *M. piperita*, *C. zeylanicum*, and *P. sylvestris*.

The need to identify more effective and less toxic compounds exhibiting anti-babesial activity has motivated research on natural products isolated from plant species. Many plant extracts (Guz et al. 2019) and phytochemicals isolated from plants (AbouLaila et al. 2010a,b) have been tested for their anti-babesial activity. Yarrow was found to display strong inhibition of *Babesia gibsoni* (Murnigsih et al. 2005) and *B. canis* (Guz et al. 2019) activity. Moreover, yarrow essential oil at high concentrations may have some activity against *Trypanosoma cruzi* (Santoro et al. 2007). Nerolidol (a sesquiterpene compound) found in several plants' essential oils i.e. from *A. millefolium* (Judzentiene and Mockute 2010) and *C. grandis*

(Susandarini et al. 2016) has shown anti-babesial activity (AbouLaila et al. 2010a).

In conclusion, the present findings suggest that *A. millefolium*, *E. caryophyllus* and *C. grandis* possess strong activity against *B. canis* and some of the essential oils' constituents might be potential therapeutic agents useful in the clinical management of babesiosis. Additional studies, including gas chromatography-mass spectrometry analysis of the essential oils, and *in vitro* and *in vivo* experiments, are essential.

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