

Ceylon Cinnamon Bark Oil Inhibits *In Vitro* Growth of *Prototheca zopfii* Isolated from Four Dogs Diagnosed with Disseminated Protothecosis

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Prototheca zopfii is a ubiquitous, achlorophyllous, pathogenic microalga that causes disseminated protothecosis in dogs, a fatal disease with limited treatments due to its resistance to conventional antibiotics and most antifungals. Following a recent cluster of fatal canine cases in Sri Lanka, this study aimed to determine the efficacy of selected essential oils available in Sri Lanka, including Ceylon Cinnamon bark and leaf oil (*Cinnamomum zeylanicum*), Clove (*Syzygium aromaticum*), Citronella (*Cymbopogon nardus*), Nutmeg (*Myristica fragrans*), and Black pepper (*Piper nigrum*) against *Prototheca* isolates. Fecal samples from infected dogs reported to the Veterinary Teaching Hospital, Peradeniya, Sri Lanka, were cultured on Potato Dextrose agar (PDA) supplemented with chloramphenicol for 24-48 hours and characterized by PCR using genus-specific and species-specific primers. The minimum inhibitory concentrations (MICs) of essential oils were determined using microdilution in 96-well microtiter plates with two-fold dilution series. The cytological changes of *Prototheca* cells in each concentration of essential oils were also observed by light microscopy under 40× magnification using wet mount slides and Gram-stained smears on albumin-coated slides. All experiments were triplicated. Four *Prototheca* isolates were recovered, and all were confirmed as *P. zopfii* by PCR. Among the tested essential oils, only cinnamon bark and leaf oil exhibited an anti-algal effect with corresponding MICs of 1.25 µL/ mL and 5 µL/ mL, respectively. Thus, the highest anti-algal efficacy of cinnamon bark oil was likely due to its high content of trans-cinnamaldehyde. Cytological alterations observed in *Prototheca* exposed to Cinnamon oils suggest that sequential loss of viability along the concentration gradient is most likely to result from lysis of the outer capsule of the organisms. These findings indicate that Ceylon cinnamon oil can be used as a promising alternative therapeutic for canine protothecosis.

Keywords: *Prototheca zopfii*, canine protothecosis, Ceylon cinnamon oil, essential oils

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