Preliminary investigation of *in vitro* evaluation of cinnamon *(Cinnamomum zeylanicum)* bark oil potentiated intramammary infusion against *Prototheca zopfii*

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Introduction and Objectives: *Prototheca zopfii* is a pathogenic microalga that cause bovine mastitis and canine protothecosis. It exhibits intrinsic resistance against conventional antibiotics and antifungals. *In vitro* studies reveal essential oils exhibit inhibitory activity against *Prototheca*. This study aimed to assess the efficacy of intramammary infusion supplemented with cinnamon bark oil against *P. zopfii*.

Methods: Five *P. zopfii* isolates were used in the study. Commercial grade organic cinnamon bark oil was characterised using Fourier Transform Infrared Spectroscopy (FTIR). Intramammary infusion (IMI) for dry cows was used as the carrier. IMI was assessed using FTIR. Cinnamon bark oil was incorporated manually at 2.5–50 µL/mL with IMI. Potentiated IMI was mixed with *P. zopfii* suspension in nutrient broth and incubated at 37 °C. Cell viability was determined by streaking on Potato Dextrose Agar (PDA) at 0-, 24-, 48-, and 72-hours and culture plates were incubated for 24 hours. Cytological variations were observed at same time intervals.

Results: Inhibitory effect of cinnamon bark oil was dose and time-dependent on all *Prototheca* isolates. Distinct reduction in cell viability was observed when exposed to 5-10 µL/mL of oil over 0-24 hours of exposure. Complete inhibition of viability was achieved within 72 hours of exposure across all tested concentrations. Cytological alterations revealed *Prototheca* cells exposed to cinnamon oil showed progressive loss of viability along concentration gradient. FTIR analysis of cinnamon bark oil showed presence of C=O bonds with absorbance band at 1675.51 cm^{-1} which indicates possible presence of trans-cinnamaldehyde as the main bioactive compound. FTIR spectra of potentiated IMI revealed characteristic peaks for N–H, C=O stretching, and C–H vibrations, along with carboxylate groups and benzathine-derived absorption bands, which support effective interaction with hydrophobic components of cinnamon bark oil, enhancing its stability in lipid-based formulations.

Conclusions: Commercial organic cinnamon bark oil rich in trans-cinnamaldehyde and other bioactive constituents, exhibited strong dose- and time-dependent inhibition against *Prototheca zopfii* when incorporated into a commercial IMI. A minimum concentration of 10 µL/mL effectively inhibited all strains, with complete inactivation at 72 hours. Microscopy and FTIR analysis confirmed membrane disruption in *P. zopfii* and formulation stability of the potentiated IMI.

***Keywords**: Antimicrobial activity, bovine mastitis, cinnamaldehyde, cinnamon bark essential oil, Prototheca zopfii,*

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