Essential Oil from Ceylon Cinnamon (*Cinnamomum ceylanicum.* Blume) Inhibits Oral Bacterial Biofilms

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Ceylon cinnamon (CC) is native to Sri Lanka and commonly available. Although CC essential oils have shown antibacterial effects they have not been tested against biofilms of clinically important oral bacteria such as *Streptococcus mutans, Actinomyces naeslundii* and *Lactobacillus acidophilus.* Our objective was to observe the effects of CC essential oils on biofilms of *S. mutans, A. naeslundii* and *L. acidophilus*. Biofilms were formed *in vitro* using a reactor combined flow system simulating the oral cavity. *S. mutans* and *A. naeslundii* were cultured in Brain Heart Infusion (BHI) broth supplemented with 50 mM sucrose. *L. acidophilus* was cultured in Rogosa and Sharpe agar supplemented with 50 mM glucose. Single-species biofilms of the bacteria mentioned above were formed on Hydroxyapatite Discs placed in the oral biofilm reactor. After 48 hour growth period, the discs were aseptically removed, and treated with following agents for 10 minutes: CC essential oil dissolved in 20% Dimethyl Sulfoxide (DMSO) in 0.2% and 0.4%; Chlorhexidine 0.2%; 20% DMSO as the solvent; respective culture medium as the negative control. Afterwards discs were assessed for viability by XTT assay, CFU units, and imaged using confocal laser scanning microscopy. XTT assay depicted significant reduction of viable bacteria in biofilms treated with 0.4% CC ($p<0.05$) for *L. acidophilus* (0.73±0.023), *A. naeslundii* (0.81± 0.006) and *S. mutans* (0.21±0.015) compared to the negative control. The CFU counts followed the same pattern with a significant reduction in colony forming ability by 0.4% CC ($p<0.05$). Confocal laser scanning microscopy with live/dead staining revealed the highest proportion of dead bacteria in biofilms treated with 0.4% CC. 20% DMSO only did not depicted significant inhibitory effect. In conclusion, 0.4% of CC essential oil in DMSO exerted generalized antimicrobial effects on tested biofilms.

Keywords: *Streptococcus mutans, Actinomyces naeslundii, Lactobacillus acidophilus*, Ceylon cinnamon