Antibacterial activity of leaf essential oils of *Citrus crenatifolia* and *Citrus reticulata*: a comparative study

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Abstract

Plant essential oils (EOs) are smart alternatives to synthetic antibiotics. *Citrus crenatifolia* (CC, Heen naran), a native plant to Sri Lanka and *Citrus reticulata* (CR, Mandarin) are two species of genus *Citrus* in the family Rutaceae. Both species are widely used in traditional medicine in Sri Lanka. This study aimed to determine the antibacterial activity of pure and combined formulae of EOs of CC and CR (1:1 ratio) against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* bacterial species. Hydrodistillation was used to collect CC and CR leaf EOs. Kirby-Bauer disc diffusion method was used to assess antimicrobial susceptibility to EOs. Amoxicillin (0.01mg/mL for *E. coli* and *S. aureus*, 0.1mg/mL for *B. cereus*) was used as a positive control in the experimental setup. Diameters of inhibition zones were measured after 24-hour incubation at 37ºC. Descriptive and inferential statistical analyzes were performed using R studio version 4.1.1. Least significance test determined the differences at $p<0.05$. The CC EO showed significantly strong antibacterial activity against *B. cereus* compared to amoxicillin, CR and CC+CR and against *S. aureus* compared to amoxicillin. This is suggestive that a synergistic effect drawn by the presence of high concentrations of antibacterial compounds in CC EO. In contrast, CR EO showed significant and moderately strong growth inhibition of *S. aureus* and *E. coli* compared to that of *B. cereus*. Low CR effectiveness in *B. cereus* could be due to weaker CR penetration, cell wall thickening and reduced hydrophobicity. The CC+CR combination also showed significant, moderate growth inhibition of all three bacterial strains. *B. cereus* and *E. coli* showed significantly higher inhibition on CC+CR, however, *S. aureus* showed least susceptibility. In conclusion, both CC and CR EOs demonstrated potent antibacterial effects against the three tested bacterial species whereas CC+CR combination showed additive effect.

Keywords: *Antibacterial activity, Citrus crenatifolia, Citrus plants, Essential oil, Kirby-Bauer disc diffusion*