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Screening and characterization of potential antibacterial activity in *Alpinia calcarata* (Heen araththa)

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Bacterial infections continue to be a major public health threat worldwide, leading to substantial morbidity and mortality. Therefore, it is crucial to investigate potential antibacterial sources capable of combating antibacterial resistance. Plant-based antibacterials render a potential alternative approach to addressing this paramount concern. *Alpinia calcar*ata (Heen Araththa) is an important Sri Lankan medicinal plant in the family Zingiberaceae. Therefore, this study aims to evaluate the antibacterial potential of *A. calcarata* rhizome and leaf extracts. In this study, methanol, ethyl acetate and hexane crude extracts were tested against *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC BAA-1706) using the agar-well diffusion method. Ciprofloxacin (0.02 mg/mL) and 10% DMSO were used as positive and negative control respectively. The tests were conducted in triplicates. Preliminary phytochemical screening and thin-layer chromatography (TLC) studies were conducted. Further, potentially active Ethyl acetate Rhizome Extract (ERE) and Hexane Rhizome Extract (HRE) were characterized using Gas chromatography/mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FT-IR) and UV-Visible spectroscopy (UV-VIS). The bioactive compounds in HRE and ERE were determined using the TLC contact bioautography method with some modifications. A one-way ANOVA test was used to test the antibacterial properties among three treatments using SPSS software. The findings demonstrated that the rhizome extract exhibited significantly higher antibacterial activity compared to the leaf extract. Also, a significant antibacterial action ($p < 0.05$) against both Gram-positive and Gram-negative bacteria, with notably higher activity against gram-positive bacteria was observed with *A. calcarata* extracts. HRE was the most effective and showed a significant inhibition (14.33 ± 1.52 mm) against *B. cereus* and moderate inhibition for *S. aureus* (9.66 ± 0 mm). ERE also showed similar potent antibacterial activity compared to the HRE against *B. cereus* (13.33 ± 1.52 mm) and *S. aureus* (8.33 ± 0.41 mm). Both extracts exhibited less than 8 mm inhibition zones with *E. coli* while neither HRE nor ERE inhibited *P. aeruginosa* and *K. pneumoniae* growth. Phytochemical analysis and TLC studies of both HRE and ERE of *A. calcarata* confirmed the presence of triterpenoids. GC-MS analysis of the HRE and ERE confirmed the presence of terpenoids and their derivatives. FT-IR spectrum data revealed the presence of carboxylic acid functionality in the HRE and alcohol functionality in the ERE. Based on the UV-Visible spectrum data, the λ_{max} of the ERE was 249 cm^{-1} indicating the presence of unsaturated nonconjugated terpene hydrocarbons. Bioautography results revealed an inhibition zone was observed only for the HRE, and no such inhibition activity was observed for the ERE against *B. cereus*. This study has the potential to develop antibacterial agents against *B. cereus* using different concentrations of HRE to optimize the activity. Further isolation and characterization of HRE will lead to the development of precursor molecules for new effective antibacterials.

Keywords: Antibacterial potential, *Bacillus cereus*, Rhizome extracts, Terpenoids, Hexane extracts.

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