

Colloquium on Applied Sciences 2024

19-21 January 2024, Faculty of Applied Sciences, UiTM Shah Alam, Malaysia

Elucidation of antibacterial of *Moringa oleifera* leaf and *Ganoderma lucidum* extract

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Structured Abstract

Background: The growing prevalence of multi-resistant bacterial strains is a global public health concern, necessitating the search for alternative infection treatments. Rapid genetic changes in bacteria can render standard antibiotics ineffective within five years. To combat resistance, researchers are exploring novel therapeutic alternatives, including medicinal plants with antibacterial qualities. *Moringa oleifera* and *Ganoderma lucidum* are well-documented for their exceptional nutritional and medicinal properties, such as antibacterial, antioxidant, anticancer, and anti-inflammatory effects.

Methods: This research was aimed to determine the antibacterial activity of *M. oleifera* extract (MOE) and *G. lucidum* extract (GLE) that has been prepared via bio fermentation method using *Bacillus subtilis* and ethanolic method in various concentrations (500, 250, 125, 62.5, 31.25 and 15.63 mg/mL) against *Staphylococcus aureus*, *B. subtilis* and *Escherichia coli* using the agar well diffusion assay. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of MOE and GLE was also conducted using the broth microdilution assay.

Results: The agar well diffusion assay results revealed that *E. coli* showed the highest sensitivity to biofermented MOE and GLE with inhibition zones ranging from 12.3 - 23.7 mm. While inhibition zones around *S. aureus* and *B. subtilis* was observed at 0 and 16.7 mm, respectively. Both MIC values of the biofermented and ethanolic MOE and GLE against *E. coli* is higher at 62.5 mg/mL in comparison to *S. aureus* and *B. subtilis* at 31.25 mg/mL, which suggests that this extract is less efficient against the Gram-negative bacterium *E. coli*. Based on MIC/ MBC ratio, ethanolic MOE and GLE was determined as bactericidal. From the observation of MIC assay, both biofermented and ethanolic MOE and GLE showed antibacterial activity when direct contact with tested bacteria occurred.

Conclusion: In conclusion, the findings of this study indicated that there is considerable the antimicrobial effectiveness of polyherbal MLE and GLE was determined using agar well diffusion assay. For the recommendation, specific identification of potential phytochemical compounds that are responsible for the antimicrobial activity in *M. oleifera* and *G. lucidum*. Lastly, filtration *Bacillus subtilis* biofermentation extract before freeze-drying to reduce the *Bacillus subtilis* cells in the extract.

Keywords: *Moringa oleifera*, *Ganoderma lucidum*, Biofermentation, Agar well diffusion assay

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