

Enhancing Antimicrobial Activity of *Moringa oleifera* Leaf through Biofermentation with Mixed Lactic Acid Bacteria

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Structured Abstract

Background: Antibiotic resistance has become a major global health issue due to the reduced effectiveness of conventional antibiotics and the slow development of new drugs. As an alternative, *Moringa oleifera* have gained attention for their therapeutic potential, as its leaves contain bioactive compounds such as flavonoids, phenolics, and alkaloids with known antibacterial properties, although their efficacy can be influenced by the processing method used. This study aims to evaluate the antibacterial activity of *M. oleifera* leaf (MOL) extract fermented with various lactic acid bacteria (LAB) species against *Escherichia coli* and *Staphylococcus aureus*, and to compare the effects of single, double, and triple LAB combinations to determine whether mixed species fermentation enhances antimicrobial potency.

Methods: Biofermented MOL extract derivatives were prepared using different combinations of LAB. These included single species (D1–D3), double species combinations (D4–D6), and a triple species combination (D7) involving *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* Shirota. The antibacterial activity of each fermented extract was evaluated against *E. coli* and *S. aureus* using the agar well diffusion method at a concentration of 500 mg/ml.

Results: All fermented MOL extract derivatives exhibited antibacterial activity, with *S. aureus* showing greater susceptibility than *E. coli*. Among the samples, the triple LAB fermentation (D7) demonstrated the highest inhibitory effect against *E. coli*, with an inhibition zone of 17.67 mm. In contrast, the most effective treatment against *S. aureus* was the single LAB strain D3 (*L. casei* Shirota), which produced an inhibition zone of 19.00 mm. These findings indicate that LAB fermentation enhances the antimicrobial activity of MOL, particularly when multiple LAB species are combined, likely due to synergistic effects.

Conclusion: This study successfully developed fermented MOL extracts using single, double, and triple LAB combinations, and confirmed their antibacterial activity against *E. coli* and *S. aureus*. *S. aureus* was more susceptible, while D7 (triple LAB) showed the strongest effect on *E. coli*, and D3 (*L. casei* Shirota) was most effective against *S. aureus*. These results suggest that LAB fermentation enhances antimicrobial activity, with both mixed and single species showing selective advantages. Future work should include HPLC analysis, MIC and MBC determination, and broader pathogen testing.

Keywords: *M. oleifera* leaf extract, Biofermentation, Lactic Acid Bacteria, Antibacterial activity

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