

Enhancing Anti-*Escherichia coli* Activity of a Functional Beverage Formulation Containing Kefir, *Moringa Oleifera*, and Tualang honey Through Response Surface Methodology Optimization

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Structured Abstract

Background: Kefir, *Moringa oleifera*, and Tualang honey are recognised as functional foods that contribute to health benefits. These ingredients are rich in bioactive compounds that have anti-*Escherichia coli* properties. However, blending kefir, *M. oleifera*, and Tualang honey (KMOT) into functional beverage formulation would synergistically improve anti-*E. coli* activities remain unexplored. Therefore, this study aimed to identify the best ratio between kefir, *M. oleifera*, and Tualang honey to enhance anti-*E. coli* of a functional beverage using Response Surface Methodology (RSM).

Methods: The minimum and maximum quantities for kefir, *M. oleifera*, and Tualang honey were set at 30-50 mL, 1-3 g, and 20-30 g, respectively. Anti-*E. coli* activity of 15 KMOT formulations designed by RSM was assessed through disc diffusion assay (DDA), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). This method involved measuring the diameter of the inhibition zone produced by KMOT against *E. coli*.

Results: The highest diameter of the inhibition zone observed was 1.73 mm (F12), while the highest percentage inhibition of MIC was 90.89% (F6). Kefir milk and Tualang honey were identified as the main contributing factors to determine the anti-*E. coli* properties. RSM recommended 44.31 mL of kefir milk, 1 g of *M. oleifera*, and 39.24 g of Tualang honey. The closest practical formulation (40 mL of kefir milk, 1 g of *M. oleifera*, and 40 g of Tualang honey) was validated, with predicted values for the diameter of inhibition zone and percentage inhibition of MIC at 1.47 mm and 90.17%, respectively. However, the experimental values obtained were slightly lower at 1.43 mm and 89.94%, respectively.

Conclusion: In conclusion, this study identified the optimal ratio between kefir, *M. oleifera*, and Tualang honey to enhance anti-*E. coli* properties of KMOT, thereby creating a functional beverage with protective benefits against bacterial pathogens.

Keywords: *Escherichia coli*, Kefir, *Moringa oleifera*, Response Surface Methodology, Tualang honey

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