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Optimisation of Functional Beverage Containing *Moringa oleifera*, Tualang Honey and *Kefir* Milk to Increase Antioxidant Properties Using Response Surface Method

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Structured Abstract

Background: *Moringa oleifera*, Tualang honey, and *kefir* milk are well-known functional food ingredients with strong antioxidant properties. However, the synergistic effects of the blends remain unknown. This study aims to identify the best ratio for mixing *M. oleifera*, Tualang honey, and *kefir* milk (MTK) as a functional beverage to improve their antioxidant properties using the response surface method (RSM). This study utilizes Ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1picrylhydrazyl (DPPH) assays to examine the antioxidant activity of the MTK formulation.

Methods: The list of MTK formulations was first designed using Design of Experiment (DOE), employing RSM based Box Behnken Design (BBD). The factors were then mixed according to the list of formulations. The formulations were then kept in a freeze drier for 2 weeks before further assessments. The antioxidant properties of the MTK formulations were then assessed by FRAP and DPPH assays. The optimal formulation was further determined by employing RSM.

Results: This study found that formulation 1 exhibits the highest percentage inhibition in both FRAP (91.47%) and DPPH (93.21%) assays. The quadratic model deployed by RSM has shown synergistic effects between the ingredients with R^2 values of 0.8144 for FRAP and 0.8044 for DPPH. *M. oleifera* plays a major factor in yielding the highest antioxidant activity in MTK due to its consistency ratio, which may affect the formulation's bioactivity. The optimal ratio of MTK deployed by RSM further validates formulation 1 as the most optimal formulation by comparing the predicted values with the experimental values.

Conclusion: In conclusion, the optimal formulation that yields the highest antioxidant activity from MTK was seen in formulation 1 which consists of 1 g of *M. oleifera*, 30 g of Tualang honey and 30 (w/v) of *kefir* milk. Out of all the factors, *M. oleifera* plays the major role in providing high antioxidant activity.

Keywords: *Moringa oleifera*, tualang honey, *kefir* milk, response surface methodology, antioxidant

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