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Optimization of Antioxidant Activity and Antimicrobial Effects of Bentong Ginger (*Zingiber Officinale*) against *Streptococcus Mutans*

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Abstract
The bioactive compounds in ginger (*Zingiber officinale*), especially from the Malaysian Bentong variety, are known for their potent antioxidant and antimicrobial properties. However, optimal processing conditions for maximizing these benefits remain unclear. This study aimed to optimize drying and extraction methods to enhance the antioxidant and antimicrobial properties of Bentong ginger against *Streptococcus mutans*, a key oral pathogen. Three drying methods (sun-drying, oven-drying at 60°C, and 100°C) and ethanol concentrations (50%, 70%, and 100%) were systematically evaluated, along with solid-to-liquid ratios (1:2, 1:5, and 1:10 g/mL). Sun-dried ginger extracted with 100% ethanol at a 1:10 ratio exhibited the highest total phenolic content (1238.82 mg GAE/g), the second highest TFC (3849.75 ± 336.21 mg QE/g), and strong antioxidant activity (IC_{50} = 45.31 µg/mL). Antimicrobial assessment revealed a minimum inhibitory concentration (MIC) of 400 mg/mL against S. mutans, with bacteriostatic effects. These findings establish optimal processing parameters and underscore Bentong ginger's potential as a natural antioxidant and antimicrobial agent for functional foods and oral healthcare products.
Keywords: Bentong ginger; Antioxidant activity; Antimicrobial effect; *Streptococcus mutans*

Introduction

Ginger (*Zingiber officinale)* is a widely used medicinal plant and spice, valued for its bioactive compounds such as gingerols, shogaols, and phenolic compounds, which contribute to its antioxidant, anti-inflammatory, and antimicrobial properties (Ma et al., 2021). Among ginger varieties, Malaysian Bentong Ginger stands out for its superior quality and high bioactive compound concentration, earning it the title "King of Ginger" (Mustafa & Chin, 2023). Antioxidants in ginger combat oxidative stress, linked to chronic diseases like cancer and cardiovascular disorders (Ozkur et al., 2022). However, their preservation depends critically on drying and extraction conditions.

Despite ginger's benefits, inconsistencies exist regarding optimal processing methods. Some studies advocate for lower drying temperatures (60°C) to retain phenolic content (Muthukumar et al., 2022), while others suggest higher temperatures (100°C) enhance bioactivity (Cherrat et al., 2019). Additionally, limited research has quantitatively assessed Bentong Ginger's antimicrobial potential against *S. mutans* using standardized assays such as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). This study addresses these gaps by systematically evaluating the effects of drying methods (sun-drying, oven-drying at 60°C and 100°C), ethanol concentrations (50%, 70%, 100%), and solid-to-liquid ratios (1:2, 1:5, 1:10 g/mL) on ginger's bioactive properties.

The findings of this research hold significant implications for the food, nutraceutical, and pharmaceutical industries. By identifying optimal processing conditions, this study aims to enhance the preservation of ginger's bioactive compounds, supporting its use in functional foods and oral healthcare products. Furthermore, the antimicrobial evaluation against *S. mutans* could pave the way for natural alternatives to synthetic preservatives and therapeutics, aligning with global sustainability goals (SDG 3 and SDG 12). This comprehensive approach bridges the gap between process optimization and practical applications, positioning Bentong Ginger as a multifunctional natural agent with both health-promoting and antimicrobial benefits.

Materials and methods

The study began with the collection and preparation of fresh Bentong ginger, which was cleaned and sliced into uniform pieces. The ginger slices were dried using three methods: sun-drying, oven-drying at 60°C, and oven-drying at 100°C. Once dried, the ginger was ground into a fine powder and stored for further analysis.

The extraction of bioactive compounds was performed using maceration with varying ethanol concentrations (50%, 70%, and 100%) and solid-to-liquid ratios (1:2, 1:5, and 1:10 g/mL). The mixtures were shaken for 24 hours, filtered, and concentrated using a rotary evaporator. The extracts were freeze-dried to obtain crude powdered extracts, and their yields were calculated.

The total phenolic content (TPC) was quantified using the Folin-Ciocalteu method, with absorbance measured at 760 nm. The total flavonoid content (TFC) was determined via the aluminum chloride method at 415 nm. Antioxidant activity was assessed using the DPPH radical scavenging assay, measuring absorbance at 517 nm. All analyses were conducted in triplicate.

An orthogonal experimental design (L9 array) was employed to evaluate the effects of drying methods, ethanol concentrations, and solid-to-liquid ratios on TPC, TFC, and DPPH activity. The optimal extract, selected based on these results, was further tested for antimicrobial activity against *Streptococcus mutans* (ATCC 25175). The minimum inhibitory concentration (MIC) was determined using a broth microdilution method with resazurin staining, while the minimum bactericidal concentration (MBC) was assessed by plating on BHI agar. Statistical analysis was performed using one-way ANOVA and Dunnett's test to validate the results.

This systematic approach ensured a comprehensive evaluation of Bentong ginger's antioxidant and antimicrobial properties under optimized conditions.

Results and discussion

The optimization of extraction conditions revealed significant impacts on the bioactive properties of Bentong ginger. Sun-drying coupled with 100% ethanol extraction at a 1:10 solid-to-liquid ratio yielded superior results, demonstrating the highest total phenolic content (1238.82 ± 10.34 mg GAE/g) and flavonoid levels (3849.75 ± 336.21 mg QE/g). These findings align with previous reports on the advantages of sun-drying for phenolic preservation (Mustafa et al., 2019). The extract's potent antioxidant capacity (IC50 = 45.31 µg/mL in DPPH assay) further supports the effectiveness of this gentle drying method in maintaining heat-sensitive compounds (Mustafa & Chin, 2023).

Figure 1 illustrates the resazurin-based MIC assay, where 400 mg/mL of the optimized extract completely inhibited *S. mutans* growth (blue wells), comparable to chlorhexidine (positive control). Figure 2 quantifies this dose-dependent inhibition, with 400 mg/mL achieving >90% suppression (*****p* < 0.0001). However, this MIC was higher than values reported for other ginger extracts (256–800 µg/mL) (Hasan et al., 2015; Babaeckhou & Ghane, 2021; Mohammad et al., 2023). This discrepancy may stem from experimental limitations, such as testing only the supernatant, which potentially excluded pellet-bound antimicrobial compounds like lipophilic gingerols during extraction.

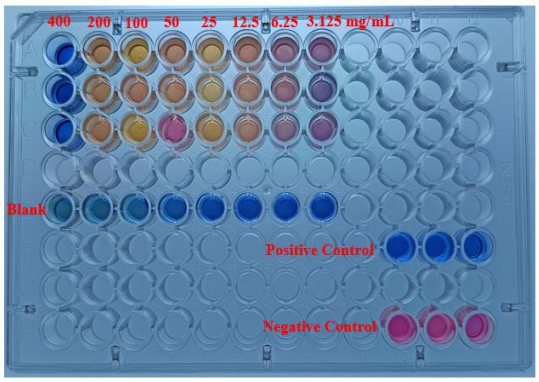

Figure 1 Visualization of *S. mutans* Growth Inhibition by Ginger Extract Using Resazurin-Based MIC Assay

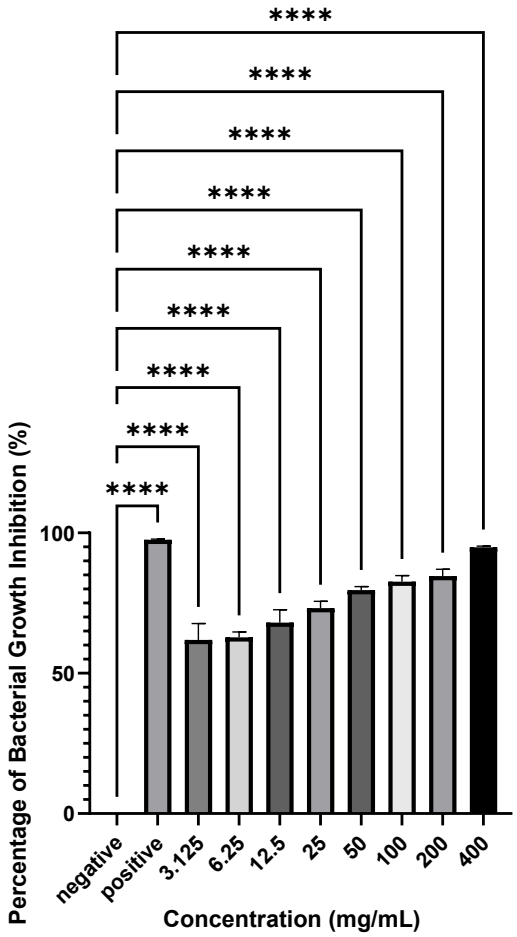

Figure 2 Bar Chart of Percentage of *S. mutans* Growth Inhibition against Concentration of Ginger Extract **(*p < 0.05, **p < 0.01, *p < 0.001, ****p < 0.0001; one-way ANOVA with Dunnett's test)

Figure 3 shows *S. mutans* colonies regrowing on BHI agar after exposure to 400 mg/mL extract, confirming bacteriostatic rather than bactericidal effects. This contrasts with studies demonstrating bactericidal activity of gingerols against *E. coli* (Hughes et al., 2021), likely due to incomplete solubilization of bioactive compounds in the aqueous phase. Future work should optimize extraction protocols to enhance bioavailability, such as testing pellet fractions or incorporating emulsifiers.

Figure 3 *S. mutans* Colony Growth on BHI Agar Following Exposure to 400 mg/mL Ginger Extract

The correlation between high antioxidant activity and antimicrobial efficacy suggests that polyphenols and gingerols play synergistic roles in disrupting bacterial membranes (Silva et al., 2016). While the extract's bacteriostatic nature limits its therapeutic use, its antioxidant-rich profile supports applications in preventive oral care, such as anti-plaque formulations. Further research should investigate the use of combinatorial therapies in conjunction with conventional antimicrobials to enhance clinical relevance.

Conclusion

This study demonstrates that sun-drying combined with 100% ethanol extraction optimally preserves the bioactive compounds of Bentong ginger, yielding exceptional phenolic content (1238.82 mg GAE/g), flavonoid levels (3849.75 mg QE/g), and antioxidant activity (IC50 = 45.31 µg/mL). The optimized extract showed significant bacteriostatic activity against *Streptococcus mutans* (MIC = 400 mg/mL). While demonstrating growth inhibition rather than bactericidal effects, these results position Bentong ginger extract as particularly valuable for preventive oral care applications. Future research should focus on extraction optimization to enhance bioavailability and investigate synergistic combinations with conventional antimicrobials. These findings substantiate the potential of Bentong ginger as a natural therapeutic agent in oral healthcare.

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