Traditional Sweetener to Functional Food: Improving the Bioactive Profile of Kithul Treacle through *Tabernaemontana divaricata* Nectar-Microbiome Fermentation

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Floral nectar harbours unique microbial communities and natural antimicrobial properties. Whereas kithul treacle, a traditional Sri Lankan sweetener with physicochemical properties similar to bee honey, has its natural food preservative potential largely understudied. This study investigated the biotechnological application of *Tabernaemontana divaricata* floral nectar microbiome to enhance the food preservative capabilities and bioactive profile of kithul treacle through microbial fermentation. To achieve this, bacteria from *T. divaricata* nectar were isolated and screened for sugar fermentation and antagonistic properties to prepare a compatible consortium that could ferment fructose, sucrose, and glucose. Selected strains were phenotypically characterized using Gram staining and molecularly characterized through 16S rRNA gene sequencing, BLAST analysis and phylogenetic mapping. The consortium was used to ferment kithul treacle for 21 days at room temperature. Fermented treacle was evaluated using a PEN3.5 E-Nose system for volatile organic compound (VOC) profiling and functional enhancements were evaluated using anti-microbial activity assays (disc diffusion) and anti-inflammatory assays (albumin denaturation). All steps except the initial culturing involved duplicates, while the anti-inflammatory assay involved quadruplicates. Initial culturing yielded 14 isolates, of which eight fermented all three sugars. The antagonistic assay identified a compatible three-strain consortium of Gram-negative bacilli, consisting of *Atlantibacter hermannii* (PZ138294), *Pantoea* sp. (PZ142719), and *Klebsiella pneumoniae* (PZ143343). Phylogenetic analysis placed them into distinct, genus-level clades supported by high bootstrap values ranging from 86% to 93%. The PCA biplot of E-Nose sensor readings showed a distinct shift in fermented treacle's VOC profile, closely aligning it with that of bee honey. One-way ANOVA and Tukey's HSD *post hoc* tests of inhibition zone diameters of the disc diffusion assay showed greater antimicrobial activity of fermented treacle (0.15 cm) than the control (0.05 cm) against *Candida parapsilosis*, with a statistically significant effect ($p < 0.05$). Its activity was comparable to bee honey (0.13 cm; $p > 0.05$). Furthermore, the anti-inflammatory assay revealed that the fermented treacle had an inhibition percentage of 87.57% at 100% concentration, exceeding the control (82.62%) and almost equalling bee honey (88.08%) in anti-inflammatory activity. In conclusion, enhanced anti-inflammatory and antimicrobial activities, together with altered VOC profiles, indicate that nectar-derived microbial fermentation improves the functional properties of kithul treacle.

Keywords: bee honey, floral nectar, food preservative, kithul treacle, *Tabernaemontana divaricata*

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