

¹H NMR-based metabolomics of *Clinacanthus nutans* leaves extracts in correlation with their anti-neuroinflammation towards LPS-induced BV2 cells

ABSTRACT

The metabolomics approach successfully explained the possible neuroprotective effect of *Clinacanthus nutans* (Burm. f.) Lindau (CN) leaf extracts. Forty-four metabolites were putatively identified via proton Nuclear Magnetic Resonance (¹H NMR and J-resolved NMR) metabolic profiling of CN leaf extracts in three types of solvents, namely water, 50% ethanol, and ethanol. Metabolite fingerprinting has efficaciously differentiated aqueous between the other two extracts. The variable importance of projection (VIP) showed that 30 metabolites were responsible for the discrimination of the extracts by component 1 in the Partial Least Square (PLS) score plot. The lipopolysaccharides (LPS)-induced murine microglial of the BV2 cell line successfully exhibited aqueous CN as the closest extract related to the nitrite oxide (NO) inhibitory activity via PLS biplot, with an IC₅₀ value of 336.2 ± 4.7 µg/mL through Griess assay. The cytotoxicity assay also indicated that all CN extracts were non-toxic. Schaftoside, acetate, propionate, alanine, and clinacoside C were identified as the most potential biomarkers in the anti-inflammatory assay. Hence, the aqueous CN extract could be further investigated, particularly relating to the anti-neuroinflammation study.

Keyword: ¹H NMR; *Clinacanthus nutans*; Anti-neuroinflammation; BV2 cells