UNIVERSITI TEKNOLOGI MARA

EVALUATION OF SYNERGISTIC ANTI-INFLAMMATORY EFFECTS OF *VITEX TRIFOLIA* LEAVES HYDROALCOHOLIC EXTRACT WITH SELECTED NON-STEROIDAL ANTI-INFLAMMATORY DRUGS, IN SILICO AND IN VITRO

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MSc

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Inflammation is the immune system response to different types of stimuli and it is related to the pathogenesis of several chronic diseases. Conventional drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) possess life-threatening side effects after prolonged use. *Vitex trifolia* is a shrub from the family Verbenaceae, which can possess potential anti-inflammatory effects and hence treat inflammation-related diseases in several Asian countries, including Malaysia. However, its synergistic effect with NSAIDs is yet to be investigated. This study aims to investigate the synergistic anti-inflammatory effect of *V. trifolia* leaves hydroalcoholic extract (VT) with NSAIDs such as diclofenac (DICL), ibuprofen (IBU), and indomethacin (IND) using different *in silico* and *in vitro* models after the phytochemical screening of the extract. VT was prepared using ultrasonic-assisted maceration, and the extract was qualitatively analysed using validated HPTLC methods. *In silico* studies were performed to investigate the synergistic effect of identified active compounds of *V. trifolia* and NSAIDs using Network target-based Identification of Multicomponent Synergy (NIMS) and pathway enrichment analysis. Then, i*n vitro* studies were conducted on the synergistic effects of VT with DICL, IBU, and IND against RAW 264.7 cells induced with H_2O_2 and LPS. The cell viability and ROS production were evaluated in the H_2O_2-induced RAW 264.7 cells, whereas the levels of IL-1β, IL-6, TNF-α, and COX were measured in the LPS-induced RAW 264.7 cells. The qualitative analysis indicated the presence of alkaloids, flavonoids, phenols, phytosterols, and terpenoids in the leaves extract. The HPTLC analysis further confirmed the presence of phenols and alkaloids in the extract with total alkaloids content of 21.13 ± 2.89 mg atropine equivalent/g dried extract and total phenolic content of 136.94 ± 4.02 mg gallic acid equivalent/g dried extract. *In silico* studies were performed on 21 active compounds of *V. trifolia* and DICL, IBU, and IND in 63 compound-NSAID pairs. The results of NIMS analysis showed that 57 out of 63 compound-NSAID pairs had synergy scores ranging from 0.083 to 1. Additionally, the pathways enrichment analysis revealed that compound-NSAID pairs' targets were enriched in 255 inflammatory-related pathways. For the MTT cell viability assay, VT (25 and 50 µg/mL) showed protective effects and significantly ($p<0.05$) increased the cell viability compared to that of RAW264.7 cells treated with 300 µM H_2O_2 alone, NSAIDs, however, increased the H_2O_2-induced cytotoxicity. Hence, there were no synergistic protective effects of VT and NSAIDs on H_2O_2-induced cytotoxicity. VT (25-100 µg/mL) significantly ($p<0.05$) reduced the levels of TNF-α, IL-6, and COX compared to the RAW 264.7 cells treated with LPS (1 µg/ml) alone. The inhibitory effects of DICL (100 µg/ml) and IND (100 µg/ml) were also recorded on TNF-α, IL-6, and COX production. However, IBU (100 µg/ml) only inhibited the production of TNF-α. Moreover, no significant ($p<0.05$) inhibitory effect of VT and NSAIDs was detected on IL-1β. The synergistic effect was only observed with VT+DICL combination on LPS-induced IL-6 production with coefficient of drug interaction (CDI) values lesser than 1. However, no synergistic effect was detected on IL-1β, TNF-α, and COX with any other VT+NSAID combination. This study concluded that VT can potentially exhibit anti-inflammatory properties through its inhibitory effects on inflammatory cytokines production and COX activity attributed to the presence of various secondary metabolites. Further molecular investigations on the isolated compounds of the plant and *in vivo* studies are suggested for future works.

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