

STANDARDIZED SUPERCRITICAL CO₂ EXTRACT OF *ACANTHUS ILICIFOLIUS* (LINN.) LEAVES INHIBITS THE PRO-INFLAMMATORY CYTOKINE TUMOR NECROSIS FACTOR- α IN LIPOPOLYSACCHARIDE-ACTIVATED MURINE RAW 264.7 MACROPHAGE CELLS

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ABSTRACT

Objective: *Acanthus ilicifolius* Linn. (Acanthaceae) is a medicinal mangrove plant used in the treatment of inflammation. Previous phytochemical studies have identified 2-benzoxazolinone (BOA) from the leaves of *A. ilicifolius*. In the present study, we attempted to standardize the supercritical CO₂ leaf extract of *A. ilicifolius* (SCFE-AI) for BOA content and investigate the tumor necrosis factor- α (TNF- α) inhibitory effect of SCFE-AI and BOA on the lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 macrophages. The acute oral toxicity of SCFE-AI and BOA was also established.

Methods: SCFE-AI was standardized for BOA content using high-performance thin-layer chromatography (HPTLC) method. The cytotoxicity of SCFE-AI and BOA was evaluated using MTS colorimetric method. The *in vitro* anti-inflammatory effect of SCFE-AI and BOA on TNF- α production in LPS-activated RAW 264.7 cells was quantified using ELISA method. Acute oral toxicity studies were performed following the Organization for Economic Co-operation and Development test guideline No. 423.

Results: The amount of BOA was found 0.8% w/w of SCFE-AI. The RAW 264.7 cell viability was unaffected by SCFE-AI and BOA treatments within a concentration range <1000 mg/ml after 24 h incubation. SCFE-AI decreased the production of TNF- α in a dose-dependent manner compared to BOA. The LD₅₀ value for SCFE-AI was found to be >2000 mg/kg and ranges from 300 to 2000 mg/kg with BOA.

Conclusion: The HPTLC chromatogram could serve as an analytical tool for authentication and quantification of BOA content. The anti-inflammatory mechanism of *A. ilicifolius* might be through the inhibition of TNF- α production.

Keywords: *Acanthus ilicifolius*, 2-benzoxazolinone, Pro-inflammatory cytokine, Tumor necrosis factor- α , Anti-inflammatory, RAW 264.7 macrophage.

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