

Investigation of *in-vitro* Anti-inflammatory Activity of Hexane Extract of *Costus speciosus* Leaves

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Background: In the current study, non-polar n-hexane fraction of *C. speciosus*, family *Coastaceae*, has been investigated for its anti-inflammatory activity. The previous investigations have proven the biological activity including anti-inflammatory activity of n-hexane fractions of *Costus speciosus* rhizome.

Objectives: To investigate *in vitro* anti-inflammatory activity of hexane extract of *C. speciosus* leaves (HECS)

Methods: A soft extract of *C. speciosus* leaves was obtained after refluxing with hexane. The *in-vitro* anti-inflammatory activity (% inhibition) of HECS was investigated by ova albumin denaturation (OAD) and heat induced human red blood cell membrane stabilization (HRBC) assays against a standard, diclofenac sodium (D. sodium). The activity guided fractionation of HECS was done using a silica column fractionation, with gradual elution of n-hexane, carbon tetrachloride (CCl₄) and ethyl acetate (EtOAc). Fractions were pooled into 7 sub fractions (A-G) using thin layer chromatography (TLC) and determined *in-vitro* activity.

Results: The dose dependent study showed no significant ($p = 0.063$) activity based on OAD-assay at 1.00 mg/mL. However, the HRBC-assay showed a significant ($p = 0.004$) activity, when compared to D. sodium. The maximum activity of 42.32 (± 1.00)% (HECS) and 37.49 (± 1.50)% (D. sodium) was observed in OAD-assay and 50.93 (± 1.53)% (HECS) and 28.74 (± 1.11)% (D. sodium) in HRBC-assay, at a dose of 1.00 mg/mL. The sub fractions, B (100% CCl₄) and G (CCl₄: EtOAc 50:50, CCl₄: EtOAc 25:75 and 100% EtOAc), have shown high *in-vitro* anti-inflammatory inhibition. The % inhibitory activity was found to be, 89.7 (± 6.9)% (B) and 92.0 (± 8.9)% (G) for HRBC-assay and 55.9 (± 4.4)% (B) and 39.4 (± 7.2)% (G) for OAD-assay, respectively.

Conclusions: The anti-inflammatory activity of sub fractions of *C. Speciosus* based on OAD and HRBC assays indicate that HECS could be used as a potential source for developing novel anti-inflammatory agents.

Keywords: Anti-inflammatory activity, hexane extract of *Costus speciosus* leaves, Sub fractions