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In vitro and In vivo wound healing studies of methanolic fraction of Centella asiatica extract (Article)

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Abstract

Ethnopharmacological relevance Asiaticoside is claimed as a bioactive compound capable of **wound healing**. **In** order to ensure that the pharmacological activity of the **extract** is traceable and measurable, the present study attempted to evaluate the bioactivity of rich fractionated **extract** of asiaticoside. Aim of the study The current study evaluates the **wound healing** efficacy via **in vitro** scratch assay and **in vivo** circular **wound** excision model. Materials and methods The ethanol **extract** was fractionated into seven fractions via vacuum liquid chromatography. The compound of interest **in** the fractions was qualitatively identified using thin layer chromatography and the positive **fraction** containing asiaticoside was further quantified using reverse-phase HPLC. The asiaticoside-rich **fraction** was subjected to (i) colorimetric MTT (methylthiazoltetrazolium) cytotoxicity assay following incubation with human dermal fibroblast (HDF) and human dermal keratinocyte (HaCaT); (ii) **in vitro** 12-well plate scratch assay (using HDF and HaCaT cells) and (iii) topically apply (40%, 10% and 2.5%, w/w) on **in vivo** circular **wound** excision of rabbits. Data on **wound** contraction, epithelisation period, hydroxyproline content and histophatological analysis was collected from **in vivo** study. Results The results showed that the methanol **fraction** of the **extract** contained about 2.4% asiaticoside. Based on the results of colorimetric MTT (methylthiazoltetrazolium) cytotoxicity assay, both HDF and HaCaT showed significant stimulation upon application of the **methanolic fraction** of **extract** at concentrations of 100 µg/mL and 0.19 µg/mL. The methanol **fraction** showed almost no toxicity effect at the concentrations tested since their IC_{50} could not be determined **in** concentrations ranging from 100 µg/mL to 0.19 µg/mL. Since all the concentrations tested allowed for more than 90% cell viability, the concentrations chosen for the scratch assay were randomly chosen and designated as highest (100 µg/mL), medium (6 µg/mL) and lowest (0.2 µg/mL) concentrations. **In** the scratch assay, methanol **fraction** of **extract** with concentration of 0.2 µg/mL and 100 µg/mL showed significant effect on HDF and HaCaT compared to the positive control ($p < 0.05$). **In vivo**, it was shown that the methanol **fraction** of the **extract** induced collagen synthesis. Histopathology data also concluded that dose-dependent effect of the tested **extract** as a **wound** healer was present. Conclusions Taken together, recent findings suggest that methanol **fraction** of C. **asiatica** demonstrated remarkable polyvalent activity, and thus has potential as an effective **wound** healer. **In** conclusion, the claim of the presence of **wound healing** properties **in** C. **asiatica** had been well supported based on the results obtained **in** this study. © 2016 South African Association of Botanists

Author keywords

Asiaticoside; **Centella asiatica**; Circular excision **wound**; Methanol **fraction**; Scratch assay

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