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#### **ACCEPTED MANUSCRIPT**

# In vivo wound-healing activity of Euphorbia characias subsp. wulfenii: Isolation and quantification of quercetin glycosides as bioactive compounds

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#### **Abstract**

#### Ethnopharmacological relevance

The latex and the aerial parts of *Euphorbia characias* L. (Euphorbiaceae) have been used as medicinal plant to treat wounds and warts in traditional medicine.

#### Aim of the study

The effect of the plant extract was tested in vivo and in vitro with experimental models to find scientific evidence for traditional use in wound healing. Potentially active wound-healer compounds were isolated from the active fraction using fractionation procedures under the guidance of biological assay and the possible role of the compounds in the wound healing process was also determined.

#### Material and methods

N-hexane, ethyl acetate, and methanol extracts were successively prepared from the aerial parts of *E. characias* subsp. *wulfenii*. The extracts were tested with linear incision, circular excision wound models and the hydroxyproline assay method to assess the wound-healing activity. The inhibition of the increase in capillary permeability induced by acetic acid, an acute inflammation model, was used to assay the anti-inflammatory activity. Different chromatographic separation techniques on sephadex and silica gel columns, and bioassay guided assay techniques have been used to isolate the active compounds of the plant. Moreover, hyaluronidase, collagenase and elastase enzymes inhibitory effect of active principle were investigated *in vitro* to find out the mechanism of action.

#### Results

The methanol (MeOH-ex) extract of the aerial parts of *E. characias* subsp. *wulfenii* showed significant wound healing activity (linear incision wound model: 43.04%; circular excision wound model 65.24%) and anti-inflammatory activity (34.74%). The methanol extract was separated into its fractions by column chromatography for isolation of efficient compounds. Biological activity of the fractions were assessed and further isolation and purification processes have been carried out in the active fraction. Isolation studies were carried out from the MeOH-ex fraction to obtain active constituents and their structures were elucidated to be quercetin-3-*O*-rhamnoside (quercitrin), quercetin-3-*O*-galactoside (hyperoside), and quercetin-3-*O*-arabinoside

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