รายงานฉบับสมบูรณ์

โครงการวิจัยประเภททุนทั่วไป

ชื่อโครงการ

Investigation of wound healing potential of *Wedelia trilobata* (L.) leaves

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านวิจัยความเป็นเลิศระบบนำส่งยา คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์

สนับสนุนทุนวิจัยโดย: มหาวิทยาลัยสงขลานครินทร์ ประจำปีงบประมาณ 2554

Abstract

Wedelia trilobata (L.) Hitchc (Asteraceae) has been used in traditional medicine in the Caribbean and Central America for stubborn wounds, sores, swelling, arthritic painful joints. Despite the use of this plant in wound healing, there is a scarcity of scientific data to support its therapeutic application. The aim of the study was to investigate the wound healing potential of leaves of *W. trilobata* (L.) and to isolate the phytoconstituent responsible for the said activity. An ethanolic extract of *W. trilobata* leaves was subjected to column chromatography. Hexane, ethyl acetate (WEA) and chloroform:methanol (50:50) (WCM) fractions were obtained. The fractions were tested using relevant *in vitro* wound healing assays. Antioxidant activity was measured by the DPPH assay. The fibroblast proliferation, oxidative stress using hydrogen peroxide, an *in vitro* scratch assay, and increasing collagen content was determined using fibroblast L929. Minimum inhibitory concentrations (MICs) were determined against *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*. WEA (3 μg/mL) promoted fibroblast L929 survivability up to more than 90% before and more than 85% after hydrogen peroxide induced oxidative stress. WEA (3μg/mL) induced a 70% migration rate in the in vitro scratch assay and the collagen content was increased to 261 μg/mL compared to the control (57.5 μg/mL). WCM exhibited a scavenging activity for DPPH with an IC50 value of 179.5 μg/mL comparable to BHT (139.3 μg/mL). WEA was active against Gram positive bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis* with MIC values of 62.5 and 31.25 μg/mL, respectively. The WEA displayed antibacterial and fibroblast stimulatory activities while WCM exhibited antioxidant to indicate its potential wound healing properties. WEA fraction obtained from ethanolic extract of *Wedelia trilobata* (L.) leaves was further separated into five fractions (WEA1-A, B, C, D, and E). Out of the five fractions only the fraction (WEA1-B) containing ent-kaura-9(11), 16-dien-19-oic acid showed promising antibacterial activity with MIC value of 15.62 μg/mL against *S. aureus* and 7.81 μg/mL against *S. epidermidis*. WEA1-B was purified and identified as grandiflorenic acid (ent-kaura-9(11), 16-dien-19-oic acid). It was then further assessed for its possible activity on fibroblasts by measuring their percentage cell viability, collagen content, oxidative stress induced by hydrogen peroxide, LDH activity after an oxidative stress induced by hydrogen peroxide, and an *in vitro* scratch assay. Grandiflorenic acid (2.5-0.08 μg/mL) produced an increase in the percentage viability of mouse fibroblast L929 cells from 97-117% and protection of the fibroblast

L929 cells against oxidative stress induced by hydrogen peroxide (94-80%). The grandiflorenic acid (2.5 μg/mL) increased collagen content of fibroblast L929 to 95.2 μg/mL as compared to the control (23.8 μg/mL). Cells treated with hydrogen peroxide exhibited 71% of LDH release whereas cells treated grandiflorenic acid (2.5 and 1.25 μg/mL) and then treated with hydrogen peroxide showed less percent release of LDH (25 and 28%) indicating protection of cell membrane integrity. The grandiflorenic acid (2.5 μg/mL) induced a 98.9% migration rate in the *in vitro* scratch assay on day 1, while control showed 57.9% migration rate on day 1. The grandiflorenic acid was also assessed for its possible activity on nitric oxide, TNF-α and IL-1β determination using Raw 264.7 cells. There was no increased level of cytokines (IL-1β and TNF-α) when treated with RAW 264.7 cells which are inflammatory mediators.

The WCM fraction was further separated in fractions (M1-M9).The M9 fraction from the WCM fraction of *W.trilobata* contains two flavonoids which showed an antioxidant activity thereby contributing to wound healing activity of leaves of *W. trilobata.* Further separation of compounds and its structural elucidation is under process.

The ethanolic extracts of leaves of *W. trilobata* afforded two compounds, one terpenoid, ent-kaura-9(11),16-dien-19-oic acid also called grandiflorenic acid and two flavonoids. Grandiflorenic acid exhibited antibacterial, fibroblast and keratinocytes activity while flavonoids showed appreciable antioxidant activity. Results of the present study have clearly demonstrated that the leaves of *W. trilobata* possess wound healing potential which supports its traditional in various folk medicines.