South African Journal of Botany 108 (2017) 163–174

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

In vitro and *In vivo* wound healing studies of methanolic fraction of *Centella asiatica* extract

H.A. Azis [a], M. Taher [a,*], A.S. Ahmed [a], W.M.A.W. Sulaiman [b], D. Susanti [c], S.R. Chowdhury [d], Z.A. Zakaria [e,*]

[a] Department of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200, Pahang, Malaysia
[b] Department of Basic Medicinal Sciences, Faculty of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200, Pahang, Malaysia
[c] Department of Chemistry, Faculty of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200, Pahang, Malaysia
[d] Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Centre, 56000 Kuala Lumpur, Malaysia
[e] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 29 March 2016
Received in revised form 5 August 2016
Accepted 13 October 2016
Available online xxxx

Edited by V Steenkamp

Keywords:
Centella asiatica
Methanol fraction
Asiaticoside
Scratch assay
Circular excision wound

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.

1. Introduction

Wounds are an unavoidable and inescapable part of our life. Various types of wounds may require different types of treatments. Fortunately, the human body is equipped with a complex self-healing mechanism. Even so, under conditions such as microbial infection, diabetic condition and poor blood circulation, the management of wounds become complicated and sometimes costly (Thakur et al., 2011). In order to overcome these problems, various products have appeared in the market to heal wounds in the shortest time possible and to increase patient compliance by minimizing pain, discomfort and scarring. However, it is important to recognize that wound care should always support the natural healing process to avoid any unwanted complications as well as to ensure a smooth healing process.

* Corresponding authors.
E-mail addresses: mtaher@iium.edu.my, tahermuhammad@gmail.com (M. Taher), zaz@upm.edu.my, dr_zaz@yahoo.com (Z.A. Zakaria).

http://dx.doi.org/10.1016/j.sajb.2016.10.022
0254-6299/

The present study would like to introduce *Centella asiatica* extract as a traditional herb that has wound healing properties to support the body's natural healing process. *C. asiatica* (L.) Urban is commonly known as Asiatic pennywort and is known locally as *pegaga* (Fig. 1). This herb has been consumed as medicine since ancient times especially in the Ayurverdic system of India and in folk medicine in China and Madagascar. Although in Malaysia, *C. asiatica* is also used by traditional healers in herbal remedies, its popularity is more confined as a vegetable rather than a medicinal plant (Jabatan Perhutanan Semenanjung Malaysia, n.d.). The World Health Organization (WHO) has documented *C. asiatica* as one of the most important medicinal plants to be conserved and cultivated (Jabatan Perhutanan Semenanjung Malaysia, n.d.)

Previous studies on *C. asiatica* extracts have reported its potential as an antioxidant, antimicrobial agent, agent of collagen synthesis and even as a wound healer (Taemchuay et al., 2009; Hashim et al., 2011; Idrus et al., 2012). Most studies report asiaticoside as the active constituent producing the said effect. Asiaticoside is a triterpenoid compound that is found as a saponin glycoside due to the attachment of sugar molecules to a triterpene unit. The sugar molecules are glucose–glucose–rhamnose (Fig. 2).

In various wound healing models, topical application (0.2%–0.4%), injection (1 mg) or ingestion (40 μg/disk) of asiaticoside has been shown to increase hydroxyproline content, improve tensile strength, increase collagen synthesis and remodeling of the collagen matrix, promote epithelialization, stimulate glycosaminoglycan synthesis and elevate antioxidant levels (Shukla et al., 1999a; Shukla et al., 1999b; Somboonwong et al., 2012). This study aimed to validate the pharmacological activity of asiaticoside by using extracts with standardized asiaticoside content.

In the present study, the wound healing potential of *C. asiatica* extract was determined using *in vitro* scratch assay and *in vivo* circular wound excision model. The basic wound healing process involves proliferation, migration and functioning of fibroblasts and keratinocytes. Therefore, for the *in vitro* study, human dermal fibroblast (HDF) and human keratinocyte (HaCaT) cell lines were deemed suitable for use. The *in vivo* study conducted afterwards confirmed the *in vitro* results, considering some compounds that show promising activity *in vitro* may be metabolized into inactive metabolites *in vivo* (Saad and Said, 2011). However, this does not indicate that the *in vivo* test is more significant compared to the *in vitro* test. The latter is more suitable for quick, inexpensive screening tests. While both tests have different roles in research, they actually complement each other (Saad and Said, 2011). According to Fronza et al. (2009), scratch assays used in *in vitro* studies provide first insights on the positive influence of plant preparations in the formation of new tissue.

Although studies on the effectiveness of traditional herbs or plants are quite common, they may be a very helpful step in the commercialization of traditional herbs. Accordingly, it is hoped that this study would promote *C. asiatica* as an agent to overcome poor wound healing among patients.

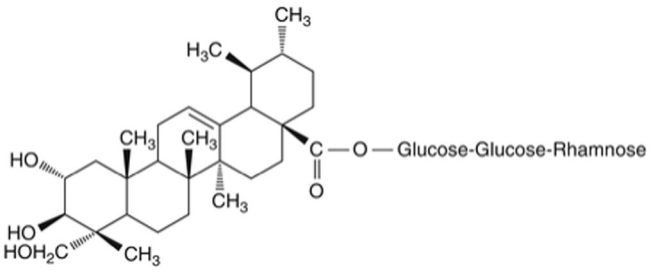

Fig. 2. Asiaticoside structure.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and solvents used in this study were of reagent or analytical grade. Only selected chemicals were of HPLC grade. Hexane and methanol were purchased from Merck (Germany), ethanol was obtained from R&M Chemicals (UK) while dichloromethane and ethyl acetate were purchased from Fisher Scientific (UK). HPLC grade methanol and acetonitrile were purchased from Fisher Scientific (UK) and Acros Organics (Belgium), respectively. Dulbecco's modified Eagle medium (Sigma, USA), fetal bovine serum (JR Scientific Co., US), penicillin streptomycin (Gibco, USA), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) (Calbiochem, Germany) and platelet-derived growth factor-BB (Calbiochem, Germany) were purchased for *in vitro* study. Solcoseryl jelly 10% (INVIDA, Singapore), aqueous cream (Pharmaniaga, Malaysia), ketamine as HCl (100 mg/mL) and xylazine as HCl (100 mg/mL) (Ilium, Australia), normal saline solution (Opticare, Malaysia), 37% formaldehyde (Merck, Germany), absolute ethyl alcohol (Fisher Scientific, UK), toluene (Fisher Scientific, UK), xylene (Fisher Scientific, UK), Masson's trichrome staining kit (R&M Chemicals, UK), hematoxylin (Harris Formula) and eosin (Surgipath® Leica Microsystem, USA) were purchased for *in vivo* study.

2.2. Raw material

Whole part of *C. asiatica* (L.) Urban var. *Nyonya* was collected at Kampung Pandan 2, Kuantan Pahang, Malaysia. The plant was identified by Dr. Shamsul Khamis, Coordinator of Biodiversity Unit, Institute of

Fig. 1. *Centella asiatica*.