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In vitro wound healing and cytotoxic activity of the gel and whole-leaf materials from selected aloe species

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ABSTRACT

Ethnopharmacological relevance: *Aloe vera* is one of the most important medicinal plants in the world with applications in the cosmetic industry and also in the tonic or health drink product market. Different parts of *Aloe ferox* and *Aloe marlothii* are used as traditional medicines for different applications. Although wound healing has been shown for certain aloe gel materials (*e.g. A. vera*) previously, there are conflicting reports on this medicinal application of aloe leaf gel materials.

Aim of the study: The present study aimed at determining the wound healing properties of the gel and whole-leaf materials of *Aloe vera*, *Aloe ferox* and *Aloe marlothii,* as well as their cytotoxic effects on normal human keratinocyte cells (HaCaT).

Materials and methods: Nuclear magnetic resonance spectroscopy was used to chemically fingerprint the aloe gel and whole-leaf materials by identifying characteristic marker molecules of aloe gel and whole-leaf materials. An MTT assay was performed to determine the cytotoxicity of the various aloe whole-leaf and gel materials on HaCaT cells. Wound healing and *in vitro* cell migration were investigated with HaCaT cells by means of the CytoSelect™ assay kit.

Results: The *in vitro* wound healing assay suggested that all the aloe gel and whole-leaf materials examined, exhibited faster wound healing activity than the untreated control group. After 48 h, all the aloe gel and whole-leaf materials almost completely caused full wound closure, displaying 98.07% (*A. marlothii* whole-leaf), 98.00% (*A. vera* gel), 97.20% (*A. marlothii* gel), 96.00% (*A. vera* whole-leaf), 94.00% (*A. ferox* gel) and 81.30% (*A. ferox* whole-leaf) wound closure, respectively. It was noteworthy that the gel materials of all the three aloe species exhibited significantly faster ($p < 0.05$) wound healing actions when compared to their respective whole-leaf materials at 32 h.

Conclusion: The gel and whole-leaf materials of *A. vera*, *A. ferox* and *A. marlothii* have shown the ability to heal wounds at a faster rate and to a larger extent than untreated keratinocytes. The MTT assay results suggested that the gel and whole-leaf materials of all the selected Aloe species showed negligible toxicity towards the HaCaT cells.

1. Introduction

A wound can be described as a laceration or break of the skin surface caused by thermal or physical injury (Hashemi et al., 2015). Wound healing is a dynamic and multi-faceted process that can be divided into four phases, namely hemostasis, inflammation, proliferation (granulation and contraction) and re-modeling (maturation) (Orsted et al., 2004). Each phase of the wound healing process is characterized by the migration of specific cell types into the wound to interact with the environment and other cells (Topman et al., 2013).

The use of medicinal plants for the treatment of various skin conditions has been popular for decades. Some of these natural medicines are believed to possess considerable therapeutic potential and should therefore be investigated for use in the advancement of products in the treatment of skin burns and wounds (Serafini et al., 2014).

The medicinal properties of aloe plants, especially *Aloe vera* (*Aloe barbadensis* Miller) are well-known worldwide. It has been reported that *A. vera* possesses various therapeutic properties, specifically in promoting wound, burn, and frost-bite healing. Additionally, this

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species has showed anti-inflammatory, anti-fungal, hypoglycemic and gastro protective properties (Choi and Chung, 2003). Several studies have shown the vulnerary activity of systemic or topically administered aloe gel material, but other studies indicated that it caused a delay in wound healing or no effect at all (Hamman, 2008).

Maenthaisong et al. (2007) concluded from a comprehensive literature survey that *A. vera* containing dosage forms may efficiently shorten the duration of wound healing of first and second degree burns. *A. vera* gel was found to increase the rate of epithelialization and thereby could contribute to successful wound healing. On the other hand, contradictory findings were documented by Schmidt and Greenspoon (1991), which included delayed wound healing after *A. vera* containing gel was applied on obstetric and gynecologic patients with wound complications.

An *in vitro* study by Topman et al. (2013) showed that *A. vera* had no effects on the migration kinematics of cultured fibroblasts after infliction of localized mechanical damage. However, due to the complexity of the wound healing process it was thought that *A. vera* may affect *in vivo* wound healing *via* alternative pathways.

Some of these contradictory results may be explained by the variation in chemical composition between plants from different regions as well as differing isolation techniques used for extracting the compounds (Hamman, 2008). Even though the wound healing properties of *A. vera* has been proven clinically and experimentally, additional studies are needed for confirmation (Hashemi et al., 2015).

Aloe ferox is another important aloe species that is often used for its curative properties (Coopoosamy and Naidoo, 2013). An investigation into the wound healing effect of *A. ferox* whole-leaf juice on incisional wounds in a rat model showed progression of wound closure as well as facilitation of the healing process. Treatment with *A. ferox* whole-leaf juice indicated an increase in the wound healing rate and a shortened time period for full or partial epithelialization (Jia et al., 2008).

Although anecdotal evidence exists for use of *Aloe marlothii* leave materials for wound treatment, no scientific evidence regarding the vulnerary activity of this aloe species could be found. Consequently, the aim of this study was to determine the *in vitro* wound healing and cytotoxic properties of the gel and whole-leaf materials of *A. ferox*, *A. marlothii* and *A. vera* on normal human keratinocyte cells (HaCaT).

2. Materials and methods

2.1. Materials

Methylthiazol tetrazolium [3-(4, 5-dimethylthiazol-2-yl)−2, 5-diphenyltetrazolium bromide] (MTT) was procured from Life Technologies (USA). Dimethyl sulfoxide (DMSO), phosphate buffer solution (PBS) and 0.4% trypan blue solution were obtained from Sigma-Aldrich (USA). CytoSelect™ wound healing and cell migration kits were obtained from Cell Biolabs, Inc., USA.

The dehydrated gel and whole-leaf materials (DaltonMax 700®) of *Aloe barbadensis* Miller (*Aloe vera* (L.) Burm.f.) were obtained from Improve USA, Inc. (DeSoto, Tx, USA, certified by the International Aloe Science Council)). Leaves of *Aloe ferox* Mill. were obtained from Organic Aloe (Pty) Ltd. (Albertinia, South Africa). *Aloe marlothii* A.Berger leaves were collected in the North-West Province of South Africa from natural populations near Koster (S25°47.100'; E026°46.725'). A specimen voucher (collection number: PUC 1151) was deposited in the North-West University's Herbarium. The names of the selected aloe species have been checked on www.theplantlist.org (date of access: 24/08/2016).

Human immortalized keratinocyte (HaCaT) is an immortalized non-tumorigenic human keratinocyte cell line, which was originally derived from normal human trunk skin and these cells are able to stratify. For this study, the cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) (Lonza, Switzerland), supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS) (Biochrom, Merck, Germany) and 1% penicillin/streptomycin (Lonza, Switzerland) at 37 °C in a 5% CO_2 humidified environment. PBS solution, free of calcium and magnesium (Lonza, Switzerland) was used in washing of cell monolayers.

2.2. Methods

2.2.1. Preparation of A. ferox and A. marlothii leaf materials

The traditional hand-filleting method was used to obtain the gel and whole-leaf materials from the *A. ferox* and *A. marlothii* leaves as previously published (Fox et al., 2015; Lebitsa et al., 2012; Ramachandra and Rao, 2008). In short, the base, tapering point and margins of the leaves were removed, followed by the removal of the rind from both the top and bottom side of the leaves. The remaining gel fillet was rinsed with water and liquidized in a kitchen blender, either alone or in combination with pieces of the green rind to obtain the gel and whole-leaf material, respectively. Thereafter; the aloe leaf materials were lyophilized with a freeze dryer (VirTis, UK) and the obtained dried powder passed through a sieve (150 – 180 µm).

2.2.2. Chemical characterization of aloe leaf materials

Nuclear magnetic resonance (^{1}H NMR) fingerprinting was performed on the selected aloe leaf materials as previously described to identify the presence of marker molecules (*i.e.* aloverose, glucose, malic acid and isocitric acid) that can confirm the origin of the aloe gel and whole-leaf materials (Fox et al., 2015). In short, solutions containing approximately 30 mg of the aloe gel and whole leaf materials were prepared with D_2O (1.5 ml) and subsequently filtered through cotton wool. Thereafter, a small quantity of 3-(trimethylsilyl) propionic acid-D4 sodium salt was added and the H^1-NMR spectra recorded with an Avance III 600 Hz NMR spectrometer (Bruker, Rheinstetten, Germany).

2.2.3. Passaging of human immortalized keratinocytes (HaCaT) cell line

HaCaT was cultured at 37 •C in a 5% CO2 humidified environment and maintained in Dulbecco's Modified Eagle's medium (DMEM) (Lonza, South Africa), with 10% (v/v) fetal bovine serum and 0.1% mixture of streptomycin and penicillin (Sigma, USA). The monolayer of cells were washed twice with PBS and then treated with trypsin-EDTA solution for 5–10 min at 37 C to remove the cells from the substratum. Cell pellets were harvested at 1000 rpm centrifugation for 5 min, then the supernatant was discarded and the cell pellet was re-suspended into the fresh medium. The culture medium was changed every two to three days and the concentration of cells were determined by utilizing trypan dye exclusion Mazumder et al. (2015).

2.2.4. In vitro cytotoxic assay

The cytotoxicity of the selected aloe gel and whole-leaf materials was performed by means of an MTT assay. The MTT solution (2 mg/ml) was prepared in a serum free DMEM medium. Solutions of all the selected aloe leaf powder materials were prepared in PBS at a concentration of 5 mg/ml and PBS was used as the control. All the samples were filtered using 0.45 µm filters. A total of $2{\times}10^4$ HaCaT cells were seeded into 96-well plates, incubated for 24 h and allowed to grow as a monolayer. The cells were then treated with different concentrations (0.40, 0.66 and 1.30 mg/ml) of gel and whole-leaf materials of aloe species and PBS. After 24 h, the test solutions were removed and discarded from the 96 well plates and 50 µl of the MTT was added to the well plates. The plate was then incubated at 5% CO_2, 37 °C, for 1.5 h. Thereafter, the MTT solution was discarded and 200 µl of DMSO was added to each well and mixed gently Dwivedi et al. (2015). The absorbance of each well was subsequently recorded at 560 nm utilizing a VERSA max microplate reader (Lab system Multiskan RC, USA). The absorbance gives an indication of the viable cells because they reduce the yellow MTT to purple formazan. The

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