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WOUND HEALING PROPERTIES OF *SWIETENIA MAHAGONI* SEED EXTRACTED USING $SCCO_2$: AN IN VITRO STUDY

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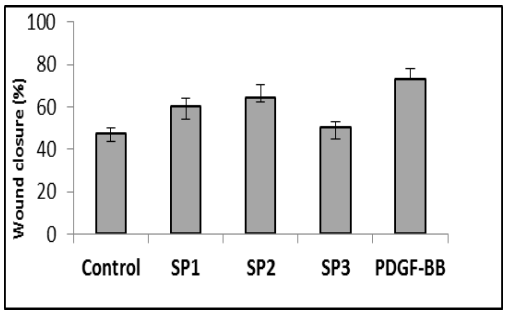
Graphical abstract



Abstract

Swietenia mahagoni seed has been traditionally used as a wound healing agent. The aim of this study was to investigate the in vitro healing properties of *Swietenia mahagoni* seed extract. A supercritical carbon dioxide ($SCCO_2$) extract of *Swietenia mahagoni* seeds was tested regarding its ability to stimulate the growth of human skin fibroblasts (HSF 1184) in vitro using the colorimetric methylthiazol tetrazolium (MTT) assay and scratch assay in a selected range of extract concentrations. The results show that the *Swietenia mahagoni* seed extract at a concentration of 0.01 mg/mL was able to stimulate the growth of human skin fibroblasts. The scratch assay showed that all of the extracts led to a significantly higher percentage of wound closure compared to the control. The wound healing capabilities of *Swietenia mahagoni* seed extract may be due to its fibroblast stimulation activity, which may promote repair of the wounded dermis. Thus, it may serve as a good herbal component to incorporate into products designed to promote wound healing.

Keywords: Wound healing, *Swietenia mahagoni*, supercritical carbon dioxide

Abstrak

Biji Swietenia mahagoni telah digunakan secara tradisional sebagai agen penyembuhan luka. Tujuan kajian ini adalah untuk menyiasat sifat penyembuhan in vitro dari ekstrak biji Swietenia mahagoni. Pengekstrakan superkritikal karbon dioksida biji Swietenia mahagoni diuji mengenai keupayaannya untuk merangsang pertumbuhan fibroblas kulit manusia (HSF 1184) secara in vitro menggunakan *assimetric methylthiazol tetrazolium* (MTT) colorimetric dalam pelbagai kepekatan ekstrak yang dipilih. Hasilnya menunjukkan bahawa ekstrak biji Swietenia mahagoni pada kepekatan 0.01 mg / mL mampu merangsang pertumbuhan sel fibroblas kulit manusia. Ujian awal menunjukkan bahawa semua ekstrak membawa kepada peratusan penutupan luka yang lebih tinggi berbanding dengan kawalan. Keupayaan penyembuhan luka dari ekstrak biji Swietenia mahagoni mungkin disebabkan oleh aktiviti rangsangan fibroblast, yang boleh menggalakkan perawatan dermis yang cedera. Oleh itu, ia mungkin boleh menjadi komponen herba yang baik untuk dimasukkan ke dalam produk yang direka untuk mempromosikan penyembuhan luka.

Kata kunci: Penyembuhan luka, Swietenia mahagoni, karbon dioksida superkritikal

1.0 INTRODUCTION

The manufacture and clinical evaluation of herbal remedies and/or their constituents have made it possible to transform traditional medicine into a modern industry capable of making a significant contribution to the delivery of healthcare [1]. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe, clinically effective, better tolerated by patients, less expensive and globally competitive [2]. In most of the developing world, plants or herbal products have long played important roles in the treatment of wounds, intestinal problems, coughs and sneezes, general torpor, etc. For treating wounds, the choice of herbal products varies between regions and cultures [3]. Wound healing is a complex multifactorial process that results in the contraction and closure of the wound and restoration of a functional barrier. The repair of injured tissues occurs as a sequence of events, including inflammation, proliferation and migration of different cell types [4]

Swietenia mahagoni (Linn.) Jacq. grows mainly in tropical areas of Asia, such as India, Malaysia, Indonesia and southern mainland China. Its seeds have been applied as folk medicine for the treatment of hypertension, diabetes and malaria, while the decoction of its bark has been used as a febrifuge [5]. The therapeutic effects associated with the seeds are mainly caused by their biologically active ingredients, including fatty acids and tetranortriterpenoids [6]. There are reports of *S. mahagoni* seeds having anti-inflammatory, antimutagenic and antitumour activities [7]. Plant extracts have been shown to possess antibacterial [8] and antifungal activities. Limnoid, obtained from *S. mahagoni*, has antifungal and antidiabetic activity [9]. The seeds of *S. mahagoni* are a good agricultural product and are potentially rich in fat (64.9%) [10].

The aim of this study was to investigate the potential healing activity of *S. mahagoni* seed using in vitro models, i.e. the MTT assay and the scratch assay.

2.0 METHODOLOGY

2.1 Extraction Preparation (Supercritical Carbon Dioxide Extraction)

The ground sample (5 g) was placed into an extractor vessel. The extracts were collected into a glass vial placed in the separator at ambient temperature and pressure. The flow rate of CO_2 was 2 mL/min. The investigated values of pressure and temperature were varied from 20 to 30 MPa and 40 to 60°C, respectively. The extraction processes were designated SP1 (pressure 20 MPa and temperature 40°C), SP2 (pressure 25 MPa and temperature 50°C) and SP3 (pressure 30 MPa and temperature 60°C). After each extraction, the obtained extract was placed into a glass vial, sealed and stored at 4°C to prevent degradation.

2.2 Cell Proliferation and Viability Assay

A previously described method was used [11]. Human skin fibroblasts (HSF 1184) proliferation in the presence of the crude extracts was evaluated using the methylthiazol tetrazolium (MTT) colorimetric assay. All cells were cultured in minimum essential medium (MEM) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin (PS) in a humidified 5% CO_2 incubator. The cells were seeded at a density of 2 x 10^5 cells/well in 96-well plates excluding the first row and incubated for 24 h prior to treatment. The test samples were prepared by dissolving the extract in MEM to yield the final concentration of the crude extracts of *S. mahagoni* (10, 1, 0.1, 0.01, 0.001 and 0.0001 mg/mL). The medium was replaced after 24 hours with 200 µL of MEM containing 10% FBS and 1% PS and serial dilutions of the plant extracts. After incubation, the cells were washed with phosphate buffered saline (PBS), then 20 µL of freshly prepared MTT solution (5 mg/mL) was added to each well and cells were incubated at 37°C for 5 hours. The MTT solution was then removed and replaced with 200 µL of DMSO to allow dissolution of the purple MTT formazan crystals. The absorbance was measured at 540 nm using an ELISA plate reader.

2.3 Scratch Assay

The wound closure of human skin fibroblasts was assessed using the scratch assay, which measures the expansion of a cell population on a surface, as previously described [12] with slight modifications. Cells were seeded into 12-well plates at a density of 3 x 10^5 cells/well and cultured in MEM supplemented with 10% FBS and 1% PS until confluent cell monolayers were achieved. Then, a linear scratch was generated in the monolayer with a sterile 200 µL plastic pipette tip. Any cellular debris was removed by washing the cells with PBS. MEM (control), platelet-derived growth factor (positive control) or the crude extract (0.01 mg/mL) was added to the cells and incubated for 24 h at 37°C. Two representative images from each well of the scratched areas for each condition were taken at 0, 12 and 24 h to measure scratch closure. The data were analysed with NIH ImageJ software [11]. Scratch closure was determined as the difference in scratch width at 0 hours and 24 h.

2.4 Statistical Analysis

Data are expressed as the mean ± SEM. Results were submitted to the one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, using SPSS 16.0 software. Values of $p < 0.05$ were considered statistically significant.

3.0 RESULTS AND DISCUSSION

3.1 Percent Yield of the Extract

The purpose of standardised extraction procedures for crude drugs (medicinal plant parts) is to obtain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent, known as menstrum. These products contain a complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans [13]. Carbon dioxide (CO_2) is the most common choice of supercritical fluid, because of its advantages of being non-toxic, non-flammable and cost-effective; moreover, it can be easily removed from the extract following decompression [13, 14]. *S. mahagoni* (L) Jacq seeds extract have excellent free radical scavenging and xanthine oxidase inhibitory activity [15]. The seed extract has also been reported to have medicinal value for the treatment of hypertension, diabetes, and malaria [16]. It has also been reported to have medicinal value for the treatment of cancer, amoebiasis, cough, chest pain and intestinal parasitism. The biologically active ingredients, i.e. tetranortriterpenoids and fatty acids, are considered to be responsible for these therapeutic effects [6]. The yields of the SC-CO_2 extract of *S. mahagoni* seed for SP1 (pressure 20 MPa and temperature 40°C), SP2 (pressure 25 MPa and temperature 50°C) and SP3 (pressure 30 MPa and temperature 60°C) were found to be 15.52%, 10.52% and 20.68%, respectively.

3.2 Influence of SP1, SP2 and SP3 on Cell Viability in HSF 1184 Fibroblast Culture

In vitro cytotoxicity test is based on the idea that toxic chemicals affect basic functions of cells which are common to all cells, and that toxicity can be measured by assessing cellular damage. Early toxicity can help identify whether a compound can be further utilised for evaluating biological activity [17]. The cell proliferation and viability of human skin fibroblasts was evaluated by the MTT assay with different concentrations of the extracts. The MTT assay was quantified as sample absorbance/control absorbance versus concentration; sample absorbance/control absorbance values higher than 1 can promote the proliferation of human skin fibroblasts [18]. As shown in Figures 1, 2 and 3, the SP1, SP2 and SP3 at 0.001-1 mg/mL, 0.001-1 mg/mL and 0.01-0.1 mg/mL, respectively, induced cell survivability of more than 1. The data obtained in this study indicate better viability of human skin fibroblasts at the lowest concentration of SP1 at 0.001 mg/mL (1.156), SP2 at 0.001 mg/mL (1.496), and SP3 at 0.01 mg/mL (1.025). SP3 promoted the viability of fibroblasts at a higher concentration compared to SP1 and SP2. The lack of cytotoxic effects on fibroblasts suggest that *S mahagoni* seeds can be classified as non-toxic and can be used safely for external application and wound dressing. However, direct application in a mammalian system is recommended in order to prevent cytotoxicity. On the basis of these results, all of the extracts showed the highest cell viability at 0.01 mg/mL; this concentration was used in subsequent experiments.

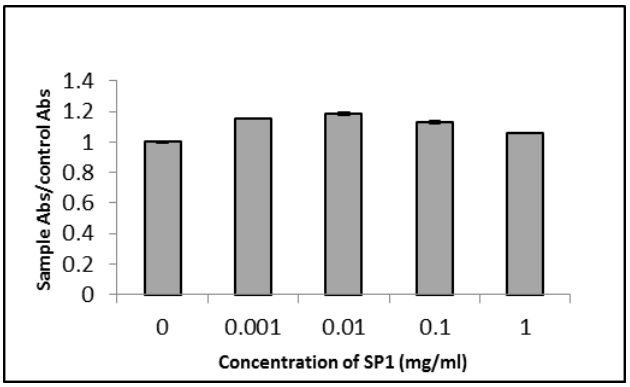


Figure 1 Effect of concentration SP1 extract on fibroblast growth as determined by MTT assay

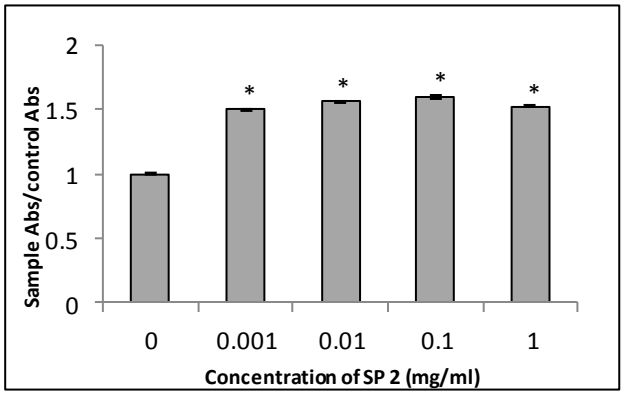


Figure 2 Effect of concentration SP2 extract on fibroblast growth as determined by MTT assay

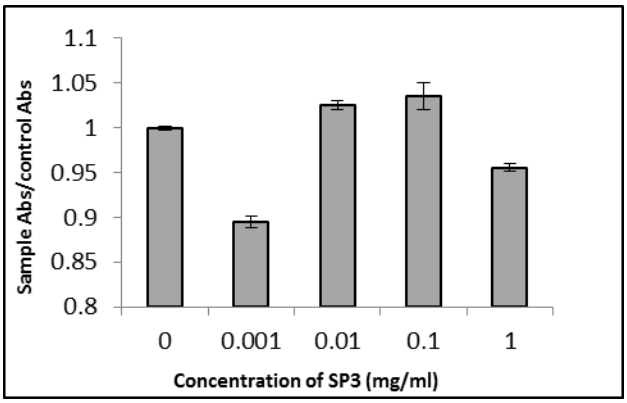


Figure 3 Effect of concentration SP3 extract on fibroblast growth as determined by MTT assay

3.3 Effect of SP1, SP2 and SP3 in the in Vitro Scratch Assay

The scratch assay is commonly used to study cell migration in vitro. This method involves the creation of an artificial gap, i.e. a "scratch", on a confluent cell monolayer by making a scratch with a pipette tip [4]. Confluent monolayers of human skin fibroblasts were scratch wounded as described in the methods and then allowed to migrate for 24 h at 37°C in the presence or absence of *S. mahagoni* seed extracts at specific concentrations. Platelet derived growth factor was used as a positive control as it has been documented to promote scratch healing by fibroblasts [11]. Cell proliferation and migration was studied from 0 to 24 h, as shown in Figures 4 and 5. The negative control (complete MEM) only promote about 47.252% wound closure while the positive control strongly promoted scratch closure (72.861%). The extracts also promoted scratch closure: SP2 (64.468%), SP1 (60.172%) and SP3 (50.355%). SP2 exhibited the most promising effects in the scratch assay at a concentration of 0.01 mg/mL. SP2 increased the population of fibroblasts in the scratched area due to the migration of cells and/or by the proliferation of migrated cells.

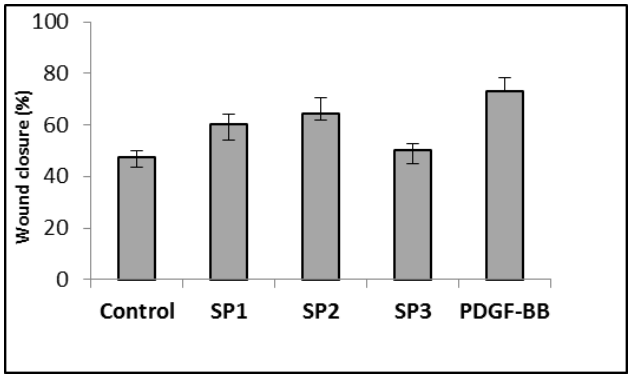


Figure 4 Percentage of wound closure of fibroblast confluent monolayers. The scratched fibroblast were treated with MEM as negative control, platelet derived growth factor (PDGF-BB) as positive control and *S. mahagoni* extract (SP1, SP2 and SP3) at 0.01 mg/mL

Generally, the scratch assay represents the second phase of wound healing characterised by either the proliferation or migration of fibroblasts. Consequently, *S. mahagoni* seeds can be used as a wound healing remedy to promote the closure of a wound by increasing cell numbers at doses relevant to the scratch assay. Although the scratch assay cannot replace in vivo studies as final evidence of the effectiveness of these extracts in wound healing, this finding provides the first evidence that these extracts have the potential to repair the injured dermis. Several previous studies using plant extracts for fibroblast growth and wound healing, among others is [12] which showed that *Callendula officinalis* extract stimulated proliferation and migration of fibroblast at low concentration 10 µg/ml enhanced cell number 64,35%. [3] reported that ethanol extracts of *Bridelia ferruginosa* Benth (1-30 µg/ml) and *Parkia biglobosa* jacq (15-30 µg/ml) influenced the proliferation of dermal fibroblast significantly ($p < 0,05$). Furthermore, [19] report that ethanol extracts of *Eupatorium laevigatum*, *Kalanchoe tubiflora* and *Xanthium cavanillesii* showed a moderate wound healing effect as 30,14%; 46,22% and 41,17% respectively. The platelet derivate growth factor (PDGF-BB) was used as positive control at a 2 ng/ml concentration and had an average of stimulating effect of 64,84%.

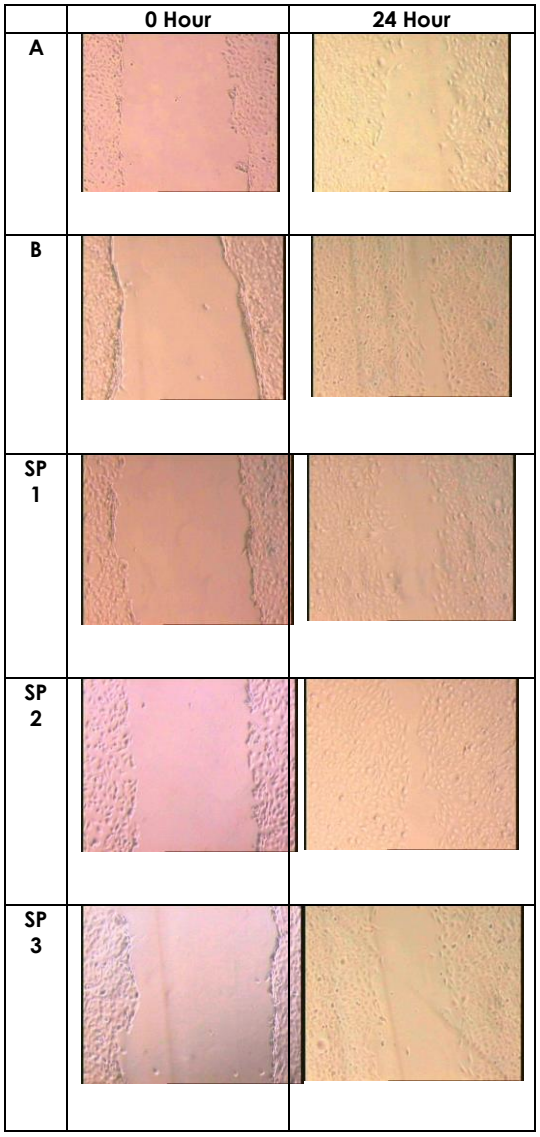


Figure 5 Wounded human skin fibroblast treated with MEM (A=Negative control), PDGF-BB (B=Positive control) and *S. mahagoni* seed extract (SP1, SP2 and SP3) at 0 and 24 hour

4.0 CONCLUSION

The present study reports that these extracts of *Swietenia mahagoni* seeds can moderately stimulate fibroblast growth and may enhance skin tissue repair.

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