CELLS MIGRATION POTENTIAL OF QUERCUS INFECTORIA AQUEOUS EXTRACT EVIDENCED IN HUMAN SKIN FIBROBLAST SCRATCH ASSAY METHOD

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ABSTRACT

Quercus infectoria (manjakani) galls is one of the common medicinal herbs which is widely used in traditional remedies. It contains several bioactive compounds which are proven to treat many diseases through its pharmacological activities such as astrigent, antibacterial, antifungal, larvicidal or antilarvicidal and many more. However, to date, the mechanism of pharmacological activity relevant to wound healing properties of Q. infectoria galls is not well established. Hence, this study was designed to investigate the effect of Q. infectoria to cell migration potential by scratch assay method using human skin fibroblast (HSF1184) cell culture. HSF1184 cells were exposed to different concentrations of Q. infectoria aqueous extract and their effects on cell growth, potential toxicity and ability to promote cell migration were evaluated. Platelet Derived Growth Factor (PDGF) exposed cells were used as positive control and non-exposed cells were used as negative control. No cytotoxicity (IC\textsubscript{50}) was observed when cells were exposed to the extracts between 0.0001μg/ml to 100 μg/ml. The results also showed that the extracts at concentration between 0.001μg/ml to 1.0 μg/ml significantly enhanced growth of HSF1184 relative to negative control. This concentration range of the extract was shown to significantly improve the cell migration rate two times faster after 24 hours exposure in the scratch assay test comparable to the positive control, as compared to the negative control culture. This result suggested that the faster cell migration rate could have been contributed by the presence of unknown compounds with similar activities to the PDGF. This finding supports the traditional application of Q. infectoria in wound healing. The lead compound in the extract that promote active cell migration could be further isolated and subjected to more intensive in vivo wound healing studies.

1.0 Introduction

Quercus infectoria or widely known as manjakani is a small tree native to Greece, Asia Minor and Iran [1], which is also popular as oak tree. The galls arise on young branches of this tree as a result of attack by the gall-wasp Adleriagallae-tinctoria. In Asian countries, Quercus infectoria galls has been used for centuries as traditional medicine for treating inflammatory diseases [2, 3]. Many traditional skincare products and ‘jamu’ incorporate the powder of Q. infectoria to function as external skin wound healing and internal wound healing, respectively. The potential of Q.Infectoria in medical and cosmeceutical areas have tempted the researchers to study and investigate further details about its usage and application. The main constituents found in the galls of Q. Infectoria are tannin (50-70%) and small amount of free gallic acid and ellagic acid [4, 5, 6]. Tannin, which is derived from phenolic compounds, has been reported to have antioxidant activity and has the ability to be antimicrobial [7], antibacterial [8] and the antifungal agent [9].
Recently, we reported that the aqueous extracts of *Q. infectoria* have antioxidant capacity of approximately 94.55% with inhibition zone of 15.0±0.00, 13.0±0.14, 17.0±0.14, and 14.5±0.21 for *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*, respectively, in disc diffusion assay [10]. The main bioactive compounds were found to be 51.14 mg/g gallic acid and 1332.88 mg/g tannic acid [10]. Such unique combination of medicinal properties of *Q. infectoria* offer great potential for its usage clinically, especially in wound management.

Cells migration is one of many *in vitro* tests used to evaluate the capability of tissue formation during embryonic development, wound healing process and immune responses. It will demonstrate the orchestrated movement of cells in particular directions to specific locations, mimicking *in vivo* wound healing to certain extent. Therefore, in this study, the aqueous extract of *Q. infectoria* galls were evaluated for its capability to promote cell migration using scratch assay method. The finding could scientifically support the traditional claims of *Q. infectoria*’s wound healing properties and open its new application in modern medication especially related to skin.

## 2.0 Material and Method

### 2.1 Materials

Phosphate buffer saline (PBS), Minimum Essential Medium (MEM), 3-4,5-dimethylthiazol-2-yldiphenyl tetrazolium bromide (MTT) were purchased from Sigma Aldrich (M) Sdn Bhd.

#### 2.1.1 Plant material

The galls of *Q. Infectoria* were purchased from a herbal shop in Kota Tinggi, Johor Bahru, Malaysia. The galls were crushed to fine powder and washed under tap water to remove the undesired particles. After that, all the samples were dried in the oven at 50°C.

### 2.2 Method

#### 2.2.1 Extraction preparation

To prepare the extract, 5 g of powdered galls were weight and placed in the timble while 150 ml of distilled water was placed at the bottom of the apparatus. The extraction process was done for 6 hours using soxhlet extractor. Lastly, the extract was put in rotary evaporator at 40°C to totally remove the solvent (water). The extract was kept in a cold dry place prior to further experiment.

#### 2.2.2 Serial Dilution of Extract

The test materials of the extract were prepared by dissolving the extract in MEM to achieve the final concentration of the crude aqueous extracts of *Q. infectoria* (100, 10, 1 0.1, 0.01, 0.001, 0.0001 and 0.00001 µg/ml).

## 2.3 Experimental work

### 2.3.1 Cell Proliferation and Cytotoxicity Assay

Cell proliferation and cytotoxicity assay was performed according to the method described by Wang et al. [11]. The effect of crude aqueous extracts of *Q. infectoria* on human skin fibroblast (HSF1184) cells proliferation activity was evaluated by MTT colorimetric assay. All cells were cultured in Minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin (PS) under 5% CO2 in humidified incubator. The cells were seeded at a density of 2x10⁵ cells/well in 96-well plate (excluding the first row) and incubated for 24hr prior to treatment. The medium was replaced after 24 hours with 200 µL of test samples (MEM containing 10% FBS and 1% PS and plant extracts). After incubation the cells were washed with phosphate buffered saline (PBS). 20 µL freshly prepared MTT solution (5 mg/ml) was added into each well and cells were incubated at 37°C for 5 hours. The MTT solution was then removed and replaced with 200 µL DMSO to allow dissolution of the purple MTT formazan crystal. The absorbance was measured at 540 nm using ELISA plate reader.

### 2.3.2 Scratch Assay

The migration rate of human skin fibroblast was assessed using scratch wound assay which measure the expansion of a cell population on surfaces as described by Fonza et al. [12] with slight modification. The cells were seeded into 12-well tissue culture dishes at a concentration of 3x10⁵ cells/ml and cultured in MEM supplemented with 10% FBS and 1% PS until a confluent cell monolayer is achieved. Then a linear scratch was generated in the monolayer with a sterile 200µL plastic pipette tip. Any cellular debris was removed by washing the cells with PBS. The MEM (negative control), PDGF (positive control) and the various concentration of *Q. infectoria* aqueous extracts (0.001, 0.01, 0.1 and 1µg/ml) were added to the cells and incubated for 24 hour at 37°C. Two representative images of each well of the scratched areas from each treatment were photographed at 0 and 24 hours to measure the cell migration rate. The data was analyzed with NIH Image J software [13]. The percentage of cell migration was determined using the following equation:

\[
\text{Cell migration} (%) = \frac{W_{t=0h} - W_{t=24h}}{W_{t=0h}} \times 100
\]
W_{w0h} = Width of wound at 0 hours (pixel)
W_{24h} = Width of wound at 24 hours (pixels)

3.0 Result and Discussion

3.1 Cell Proliferation and Cytotoxicity Evaluation

The proliferation of human skin fibroblast was evaluated by MTT assay with different concentration of *Q. infectoria* aqueous extract. The MTT assay was performed as percentage of relative cell viability (%) versus various concentration of *Q. infectoria* extracts (µg/ml) and the extracts with higher percentage of relative cell viability than negative control was considered to possess growth stimulant activity while lower value than negative control indicates a toxic effect [11, 13], while value below 50% will be regarded as cytotoxic concentration with 50% inhibition effect (IC50). As shown in Figure 1, the extract concentration between 0.001 to 1 µg/ml gave higher value than control, indication of promoting cell proliferation. All the tested concentrations between 0.00001 to 100µg/ml also did not indicate cytotoxic effect, with no IC50 value. The result showed that *Q. infectoria* has an extremely low toxicity on human skin fibroblast at concentration of 0.00001 to 0.0001 µg/ml and from 10 to 100 µg/ml. This mild effect could have been contributed by the minute (diluted) concentration of growth promoting compound in the extracts while at higher concentrations (10 to 100 µg/ml), the toxic compound in the extracts has superseded the growth promoting compound in the same extracts. Both cytotoxic and growth promoting compounds could co-exist in the extracts since they are only crude extract of *Q. infectoria*. However, at the concentration range of 0.001 to 1 µg/ml, the concentration of growth promoting compounds could be enough to promote cell proliferation while at the same time suppress the activity of cytotoxic compounds in the same extracts. The low cytotoxic effects on fibroblast suggest that *Q. infectoria* could be classified as non-toxic substance and can be used safely for external application such as wound dressing. On the basis of the obtained results, the extract at concentration between 0.001 to 1µg/ml was chosen for the next cell migration evaluation in scratch assay.

![Fig.1 Effects of various concentrations of *Q. Infectoria*aqueous extract on human skin fibroblast cell growth](image1)

![Fig.2 Effects of various concentrations of *Q. Infectoria*aqueous extract on cells migration. The scratched fibroblasts were treated with complete MEM medium (negative control), PDGF (positive control) and *Q. infectoria* extract at the concentrations from 0.001 to 1 µg/ml.](image2)
3.2 Scratch Assay

Confluent monolayers of human skin fibroblasts were scratched as described in the method section and then allowed to re-epithelialize for 24 hours at 37°C in the presence or absence of *Q. infectoria* extracts of various concentrations. PDGF was used as positive control, which had been documented to promote scratch wound healing in fibroblasts [13] while complete MEM was used as negative control. From the graph in Figure 2, the negative control, which is MEM, only promotes about 29% of cell migration while positive control (PDGF) enables higher cell migration rate, which was around 74%. The test samples of aqueous extracts achieved migration rates up to around 78%. The extract at concentrations between 0.001 to 1 μg/ml showed significantly higher percentage of cells migration rate compared to the negative control (p>0.05). Figure 3 shows representative images of cells migration during the 24 hours exposure of scratched fibroblast monolayer with 1 μg/ml of *Q. infectoria* aqueous extract.

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![Cell migration during 24 hours exposure of scratched fibroblast monolayer with concentration of 1μg/ml of *Q. infectoria* aqueous extracts. a) Complete MEM, b) PDGF and c) 1μg/ml *Q. infectoria* aqueous extract.](image7)

Generally, the scratch assay indicates the second phase of wound healing characterized either by proliferation or migration of fibroblasts [14, 15]. *Q. infectoria* aqueous extracts, which can be used to promote cell migration, also successfully increased the cell number at relevant concentrations as demonstrated in the scratch assay, which could be due to its effect in enhancing proliferative activity. Although the scratch assay cannot replace *in vivo* studies as final confirmation for its effectiveness in wound healing, this finding confirmed the capability of *Q. infectoria* aqueous extract to facilitate dermal repair.

4.0 Conclusion

The present study showed that aqueous extracts of *Q. infectoria* can stimulate fibroblast growth and could play a role in tissue repair. In conclusion, the increased skin fibroblast proliferation and migration in presence of aqueous extracts of *Q. infectoria* reported here support the potential usage of *Q. infectoria* in the topical treatment of wounds and has validated its traditional claim on wound healing activity.

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