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AN *in vitro* STUDY OF WOUND HEALING ACTIVITY OF *Ficus deltoidea* LEAF EXTRACT

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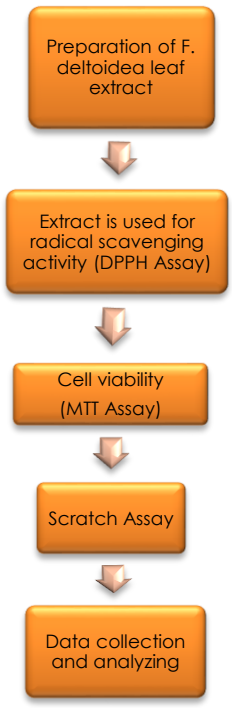
Graphical abstract



Abstract

Ficus deltoidea (Mas Cotek) is one of the popular herbs that has been used traditionally to alleviate illnesses. The present study aimed to investigate the *in vitro* wound healing activity of *F. deltoidea* leaf extract on skin cell. Cell proliferative and migration assay were done on the Human Skin Fibroblast cell (HSF 1184) which were treated with different concentrations of *F. deltoidea* leaf extract. The data of wound closure were collected at time intervals of 0, 6, 12 and 24 hours and analyzed using ImageJ™ software. MTT assay revealed that the *F. deltoidea* leaf extract could induce cell proliferation at a dose dependent manner. *F. deltoidea* leaf extract significantly accelerated the wound closure process in comparison to cells treated with ascorbic acid and untreated cells in scratch assay. Following 24 hours of incubation, cells treated with 50 µg/mL of *F. deltoidea* leaf extract showed remarkable proliferative and wound closure effect with 143.67% and 5.96%, respectively as compared to other concentrations. Collectively, these findings suggested that *F. deltoidea* leaf extract possesses wound healing potential and may be useful for the development of efficient wound healing drug.

Keywords: *Ficus deltoidea*, scratch assay, fibroblast, cell proliferative, wound healing

Abstrak

F. deltoidea (Mas Cotek) merupakan salah satu herba popular yang digunakan secara traditional bagi menyembuhkan penyakit. Kajian ini bertujuan mengkaji aktiviti penyembuhan luka secara '*in vitro*' oleh ekstrak daun *F. deltoidea* ke atas sel kulit. Ujian sel proliferatif dan asai migrasi dilakukan ke atas Sel Kulit Fibroblast Manusia (HSF 1184) yang telah dirawat dengan ekstrak daun *F. deltoidea* pada kepekatan yang berbeza. Data penutupan luka telah dikumpul pada selang masa 0, 6, 12 dan 24 jam dan dianalisis menggunakan perisian ImageJ™. Asai MTT menunjukkan ekstrak daun *F. deltoidea* mampu menggalakkan kesan proliferatif ke atas sel bergantung kepada dos. Ekstrak daun *F. deltoidea* menunjukkan keupayaan untuk mempercepatkan proses penutupan luka dibandingkan dengan sel dirawat dengan asid askorbik serta sel tidak dirawat pada asai calar. Selepas 24 jam tempoh pengeraman, sel dirawat dengan 50 µg/mL ekstrak daun *F. deltoidea* menunjukkan kesan proliferatif yang signifikan sebanyak 143.67% dan kesan penutupan luka sehingga 5.96% berbanding kepekatan lain. Secara keseluruhan, penemuan ini menunjukkan ekstrak daun *F. deltoidea* mempunyai potensi menyembuh luka dan mungkin berguna dalam pembangunan ubatan penyembuhan luka yang efektif.

Kata kunci: *Ficus deltoidea*, asai calar, fibroblast, sel proliferatif, penyembuhan luka

1.0 INTRODUCTION

Ficus deltoidea belongs to Moraceae family and also known as Mas Cotek. Traditionally this plant was used to alleviate and heal illnesses such as wounds, sores, as an anti-diabetic drug and after-birth tonic [1, 2].

A number of studies have demonstrated that this plant is beneficial to human health [3]. It is believed that *F. deltoidea* has high antioxidant potential that assisted in scavenging free radicals [4]. A study by Hasham *et al*., [5] suggested that *F. deltoidea* possessed anti-ageing activity *via* the prevention of inflammation and recovery of decreased collagen synthesis.

Nowadays, high demands for the production of new natural products lead to the birth of researches on herbal plants. Numerous number of phytochemical compounds were found in *F. deltoidea* such as polyphenol, flavonoid, phenolic acid, tannins and vitamin C, which contributed to the anti-inflammatory effect, anti-ageing, anti-cancer antioxidant and several other activities [6, 7].

Skin damages can cause numerous problems such as microbial infections, depending on the degrees of damages [8]. Damaged skins like burns, cuts and scratches need to be treated and healed in a short period of time in order to prevent severe infections. *F. deltoidea* meets these criteria as it has the potential of healing the wounds as it has been used traditionally to treat wounds and rheumatism by the Malay [9].

In a previous study, Abdulla *et al*., [10] have used *F. deltoidea* extract for the topical treatment of the wounded area on rats. Their findings included the promotion of wound healing of the extract. However, to the best of our knowledge, there is no report on the effect of *F. deltoidea* extract in *in vitro* wound healing assay.

Therefore, this study was carried out in order to investigate the effectiveness of the wound healing activity in Human Skin Fibroblast (HSF 1184) cell using *F.deltoidea* leaf extract.

2.0 EXPERIMENTAL

2.1 Preparation of *F.deltoidea* Leaf Extract

F. deltoidea leaf (5 kg) were extracted using boiling water (80 L) for 2 h. After the removal of the solid parts by filtration, the extracted solution was spray-dried using a pilot spray dryer (Niro A/S, GEA Group, Soeborg Denmark). The resultant powder was used to determine the proliferative and the wound healing effects.

2.2 Cell Culture

The Human Skin Fibroblast (HSF 1184) cells were grown in a Dulbecco's modified essential medium (DMEM), containing 10% of Fetal Bovine Serum (FBS), and 1% of penicillin-streptomycin antibiotic in a T-flask. The cells were maintained at 37°C in a humidified atmosphere of 5% carbon dioxide (CO_2). The media were purchased from Life Technologies Bio Diagnostic, Selangor, Malaysia.

2.3 Radical Scavenging Activity (DPPH Assay)

DPPH assay was conducted according to the method described by Blois [11] with some modifications. Each well of the 96 micro-well plate was filled with 100-200 µL of samples (mixed with methanol to obtain the desired concentrations (µg/mL). Then, 20 µL of DPPH solution (a dissolved solution of 1.5 g DPPH and 3 mL methanol) was added to each well. The mixture was then left to incubate for 30 minutes in a dark environment at room temperature. The optical density (OD) of the wells was read by using the ELISA reader at 517 nm. The percentage of inhibition was calculated according to the following equation:

$$\%\ Inhibition = \frac{1 - OD\ (DPPH + Sample)}{OD\ (DPPH + MeOH)} \times 100$$

2.4 MTT Assay

The fibroblast cells were seeded in 96-well microplates at a density of 1×10^5 cells/well with the volume of 100 µg/well DMEM complete medium and incubated overnight to allow the cells to confluence in the wells. Then, the medium was discarded and replaced with 150 µL medium containing various concentrations of treatments (ascorbic acid and *F. deltoidea* leaf extract) in respective wells. Those wells without treatment served as control. The plate was then incubated for 48 hours. Each concentration was prepared and tested in triplicate. 3-(4,5- Dimethyl-2-thiazolyl)-2, 5-diphenyl- 2*H*-tetrazolium bromide (MTT) assay was used as described previously by Mosmann [12]. The cell viability was determined when the enzyme of living cells were able to reduce the yellow MTT dye into the blue-purple coloured insoluble formazan. The MTT solution (a stock solution of 5 mg/mL in PBS) (Sigma, Subang Jaya, Selangor, Malaysia) was then added (20 µL in each well) and incubated for 3-4 hours. After that, the mixture was discarded and 150 µL of DMSO was filled in each well and left at room temperature for 10-15 minutes. The absorbance of the supernatant was measured at 550 nm using ELISA microplate reader. The following cell viability was calculated using the following equation:

$$Cell\ Viability\ (\%) = \frac{Sample}{Control} \times 100$$

2.5 Scratch Assay

The fibroblast cells were grown at a density of 3×10^5 cells/well in a 6-well plate and cultured in DMEM complete media for 24 hours. The fibroblast cells with ≥80% of confluence were scratched using the sterilized pipette tip (yellow tip; volume 2-200 µL, 53 mm). The medium was then removed and the cells

were washed with Phosphate Buffered Saline (PBS). The cells were cultured in serum-free DMEM media (as control), 5 µg/mL ascorbic acid and *F. deltoidea* leaf extract for 24 hours. The pictures of wound closure were taken in intervals of 0, 6, 12 and 24 hours. The percentage of wound closure was calculated by using this equation:

% Wound closure = $(W_{0h} - W_{xh}) / W_{xh} \times 100\%$
W_{0h} = Wound at 0 hour
W_{xh} = Wound at 'x' hour ; 'x' = 0, 6, 12 and 24 hours

2.6 Statistical Analysis

ImageJ™ software was used to analyze the image of the scratched area. An assessment of the statistical significance was performed by using Statistical Package for the Social Sciences (SPSS 15.0) software for Student's t-test. All results were represented as the average ± SD of the combined data from replicate experiments.

3.0 RESULTS AND DISCUSSION

3.1 Effect of Radical Scavenging Activity of *F. deltoidea* Leaf Extract

The scavenging activity (DPPH assay) was performed on *F. deltoidea* leaf extract with ascorbic acid serving as the positive control. The results were presented as percentage of the scavenging activity in Figure 1.

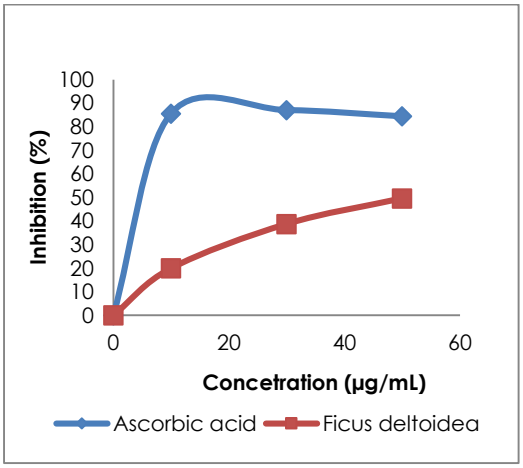


Figure 1 The effect of DPPH scavenging activity in *F. deltoidea* leaf extract with ascorbic acid used as control.

The antioxidant activity of the *F. deltoidea* leaf extract was determined by the DPPH assay. The results were compared with the standard ascorbic acid (Figure 1) which was an efficient antioxidant agent. Different concentrations of *F. deltoidea* leaf extract were used in the DPPH free radical scavenging assay at the concentration of 10 to 50 µg/mL (Figure 1). The *F. deltoidea* leaf extract showed significant effect in scavenging the DPPH free radical, reaching up to 49.59% at the concentration of 50 µg/mL. These results were in agreement with the findings by Wahid *et al.*, [2] which also reported a significant inhibition of the DPPH free radical following the treatment with *F. deltoidea* extracts. Furthermore, *F. deltoidea* is believed to have an abundance source of naturally occurring antioxidants. This is due to the flavonoids and phenolic compounds present in *F. deltoidea* which may contribute to its strong activity in wound healing and anti-inflammations on the human epidermal [13].

3.2 *F. deltoidea* Leaf Extract Induces Cell Viability

MTT assay was used to assess the effect of *F. deltoidea* leaf extract on cell proliferative activity (Figure 2). This assay is a preferable approach to investigate the cell growth and cell cytotoxicity as it measures the integrity of cell membrane by determining the mitochondrial activity through enzymatic reaction on reduction of MTT to formazan [5,14].

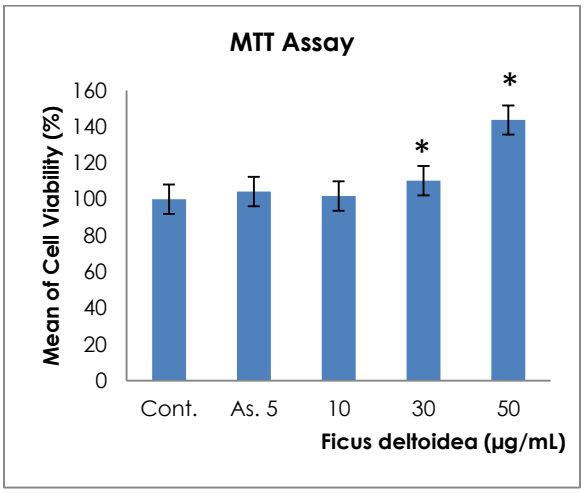


Figure 2 The effects of different concentrations of treatments (µg/mL) on cell viability (%). *$p < 0.05$ indicating a significant difference compared to the control; Control indicated as (Cont.); Ascorbic acid of 5 µg/mL was indicated by (As. 5)

The treatment of *F. deltoidea* leaf extract on different concentrations of 10 to 50 µg/mL induced the cell viability in cultured fibroblast cells up to 40% in a dose-dependent manner (Figure 2). Ascorbic acid was used as the positive control in promoting cell growth. This result shows that *F. deltoidea* leaf extract significantly increased the cell growth of fibroblast cell lines when compared to ascorbic acid. This is in agreement with Nor Azurah *et al.*, [14] who reported that the cell viability increased with the increased of *F.deltoidea* concentrations up to an optimum concentration.

3.3 Effect of *F.deltoidea* Leaf Extract on Fibroblast Cells in Wound Scratch Test

The effect of *F. deltoidea* leaf extract on the migration of fibroblast were tested in an *in vitro* wound healing model, in which scraped wounds were generated in a confluent cell cultured. Cells with or without *F. deltoidea* treatment were allowed to migrate into the denuded area from 0-24 hours at 37°C. The scratch test results were shown in Figure 3 and 4.

From Figure 3 and 4, it was demonstrated that cells treated with *F. deltoidea* started to migrate into the denuded area at 6 hours of treatment and scratch closured was almost completed at 24 hours when treated with 50 µg/mL concentration of extract. In contrast, untreated cells showed less motility as indicated by less migrated cells in the denuded area at 24 hours of incubation.

The 50 µg/mL concentration of *F. deltoidea* leaf extract reduced the wound area faster than the other concentrations. These results supported the hypothesis that high antioxidant and proliferative activity of *F. deltoidea* were associated with a high healing property. This is in agreement with Abdulla *et al.*, findings which revealed the wound healing effect of *F. deltoidea* extracts in *in vivo* studies [10]. The effectiveness of the wound healing effects might be due to the regulation of pro-collagen 1 expression and anti-inflammatory activities of *F. deltoidea* as reported by Hasham *et al*. [5].

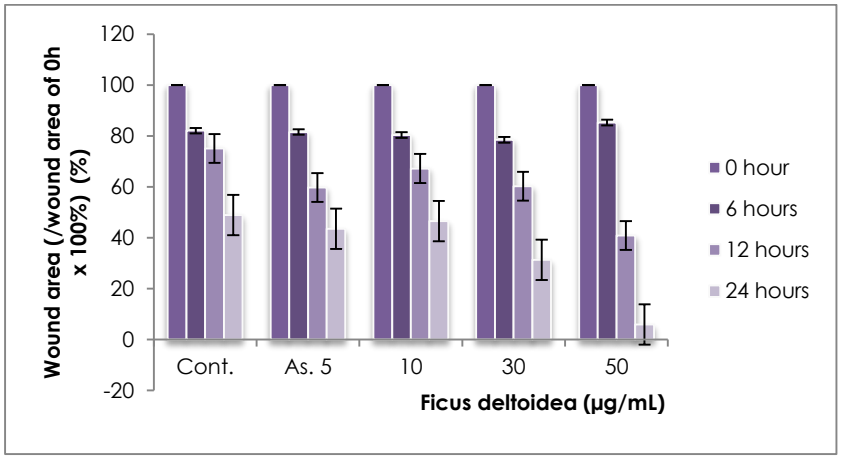


Figure 3 The effect of various concentrations (µg/mL) of *F.deltoidea* leaf extract on the percentage of wound closure (%) of scratched fibroblast cells; Control indicated by (Cont.); Ascorbic acid indicated by (As. 5)

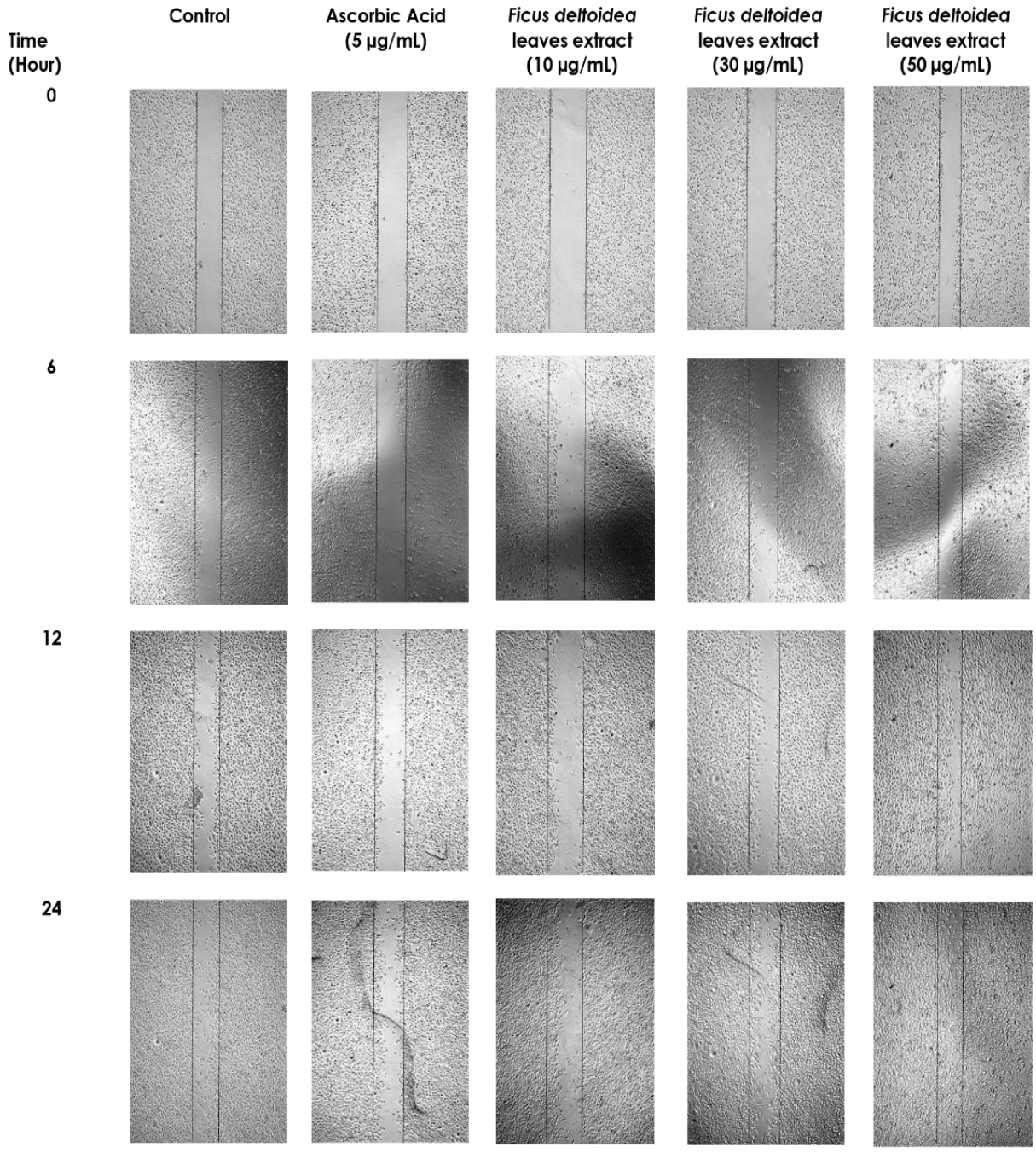


Figure 4 The digital images showed the effect of different concentrations of *F. deltoidea* leaf extract on fibroblast cells migration in wound scratch test assay (the dotted lines represented the scratched area): (a) control without treatment; (b) 5 µg/mL ascorbic acid; (c) 10 µg/mL *F. deltoidea* leaf extract; (d) 30 µg/mL *F. deltoidea* leaf extract; (e) 50 µg/mL *F. deltoidea* leaf extract. The migration of the fibroblast cells were captured by using inverted light microscope attached to a digital camera with themagnification lens of 5x

4.0 CONCLUSION

F. deltoidea leaf extract was demonstrated to contain a rich source of naturally occurring antioxidants as it showed a significant effect in inhibiting DPPH radical at optimum concentrations. *F. deltoidea* leaf extract was also shown to induce cell viability and promoting cell growth in fibroblast cells. The presence of *F. deltoidea leaf* extract promoted the wound healing activity in *in vitro* fibroblast cells.

In conclusion, 50 µg/mL concentration of *F. deltoidea* was the best concentration for treatments

of wound healing activity as it showed the fastest wound closure compared to the other concentrations of treatments used. The present study demonstrated that *F. deltoidea* leaf extract stimulated fibroblast cell growth and played an important role in repairing cell tissue. This study supported the claim that *F. deltoidea* has the wound healing properties. Finally, these findings suggested that further research on identifying the relevant bioactive compounds that leading to the wound healing activity should be carried out as *F. deltoidea* leaf extract showed a great potential for the pharmaceutical industries.

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