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# In vitro fibroblasts viability and migration stimulation of *Acalypha indica*: an insight on wound healing activity

Ammar Mahmoud Ibrahim<sup>1\*</sup> , Mariani Abdul Hamid<sup>2</sup>, Rama Ali Althiab<sup>1</sup>, Amir Husni Mohd Shariff<sup>1</sup> and Razauden Mohamed Zulkifli<sup>1\*</sup>

## Abstract

**Background:** The current study investigates the antioxidant activity of *Acalypha indica* aerial parts and root ethanolic extracts and explore whether these extracts will stimulate fibroblasts viability and ability to migrate.

**Results:** Aerial parts extract exhibited higher DPPH scavenging activity compared to root extract with IC<sub>50</sub> of 62 µg/mL and 206 µg/mL, respectively. Both aerial parts and root extracts showed low cytotoxicity towards fibroblasts with 753 µg/mL LD<sub>50</sub> for aerial parts and undetected LD<sub>50</sub> for root extract. Additionally, aerial parts extract significantly induces fibroblasts proliferation up to 134%. Wound closure investigation showed a significant closure percentage for aerial parts compared to untreated control with 75% at 1 µg/mL and high closure percentage with 70% at 0.1 µg/mL for root extract compared to only 59% closure percentage for untreated control after 48 h of the study.

**Conclusions:** This study provided evidence for *A. indica* to have great wound healing potential. The finding builds the scientific background in future to utilise the high antioxidant activity of *A. indica* and its ability to stimulate fibroblasts migration and proliferation for further applications.

**Keywords:** In vitro, *Acalypha indica*, Wound healing, Cytotoxicity, Antioxidant

## Background

Ancient civilisations exploited plant-based treatments as the primary source for remedies to cure diseases [1]. Through decades, those traditional remedies have proved efficacy even without any scientific justification. New researches on traditional medicines are now revealing rational shreds of evidence on efficacy, safety, and therapeutic mechanisms of these remedies and proposing safe dosing, suitable formulations, and feasible routes of administration [2].

Winter and Scales were the first to study the healing process in the 1960s [3]. Since then, science has been revealing much more about systemic factors affecting

wound healing on biochemical, cellular, and molecular levels. Our current understanding of the healing process associates it with different biological activities. To consider herbal extract as a wound healing therapeutic, it should have a favorable effect on at least two of the following mechanisms: effect on fibroblast or keratinocyte cells; formation of collagen; and anti-inflammatory, antimicrobial, or antioxidant activity [4].

*Acalypha indica* (Family: *Euphorbiaceae*) is a small annual shrub found in tropical areas and throughout Polynesia. Over the centuries, traditional healers used *A. indica* for wound healing management [5]. Studies on *A. indica* identified abundant varieties of second metabolites such as glycosides, triterpenes, amides, tannins, cyanogenic glucoside acaliphin, including antioxidant entities such as Acalyphamide (flavonoid) and Acalyphine (alkaloid) [6–9]. Many in vivo studies on herb extracts

\*Correspondence: ammaibr@gmail.com; razauden@fbb.utm.my  
<sup>1</sup> Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia  
Full list of author information is available at the end of the article

proved its wound healing properties [10–14]. The findings attribute its wound healing property to different mechanisms, including anti-inflammatory activity [8, 15, 16], antimicrobial activity [12, 17], analgesic effects [16], and antioxidant activity [6, 11, 14, 16]. However, there is still a need for more investigations on the safety and efficacy of this herb on live cells: like fibroblasts and keratinocytes, which have vital roles in the healing process.

When wounding occurs, it triggers fibroblasts remodelling as myofibroblasts (ultrastructural morphology fibroblasts) that are crucial entities in the proliferative phase of the healing process. Activated myofibroblasts interact with epithelial cells to develop the granulation tissue; proliferate and migrate allowing granulation tissue to contract and mature; stimulate the production of growth factors, collagen, and extracellular matrix proteins [18].

This study aims to validate the antioxidant activity of *Acalypha indica* aerial parts and root ethanolic extracts and explore whether these extracts will stimulate fibroblasts viability and ability to migrate. Further awareness of *A. indica* impact on fibroblasts will grant us more insight into triggered healing mechanisms. Moreover,

Bovine Serum (FBS), Trypan blue, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), PBS (Phosphate Buffered Saline) (Sigma-Aldrich). ABS (Bovine Serum Albumin) (Vivantis) Pen Strep (Penicillin and streptomycin) (Gibco). Animal tissue culture lab in the Faculty of Sciences, Department of Bioscience in the corresponding university provided the Human Skin Fibroblast cell line (HSF 1189).

#### Antioxidant DPPH free radical-scavenging activity

We evaluated the antioxidant activity based on the scavenging activity of the aerial parts and root ethanolic extracts on DPPH [20, 21]. Using methanol as solvent, we prepared 0.5 mg/mL ascorbic acid as standard and (0.04% w/v) DPPH as a stock solution. DPPH scavenging activity was studied on concentration gradient of the extracts (500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.63 µg/mL and 7.81 µg/mL). DPPH solution in methanol was used as a control, and pure methanol was used as blank. After incubation for 30 min at room temperature in the dark, the absorbance was taken at 517 nm, and the antioxidant activity was expressed as IC<sub>50</sub> (µg/mL). The ability to scavenge the DPPH radicals was calculated using the following equation:

$$\% \text{Radical scavenging} = \left\{ \frac{[\text{Absorbance of control} - (\text{Absorbance of sample} - \text{Absorbance blank})]}{\text{Absorbance of control}} \right\} \times 100$$

validating its efficiency and safety in vitro on fibroblasts will push research forward to explore *A. indica*'s modes of action on live cells avoiding the ethical and economical hindrances of the in vivo studies [4, 19].

## Methods

### Plant material and preparation of the extract

In the present study, we examined two extracts of *Acalypha indica*: aerial parts and root extracts. *Acalypha indica* was planted at the School of Biomedical Engineering and Health Sciences at the corresponding university. The whole fresh plants were washed then dried at room temperature for seven days. Dry plants were then separated into aerial parts and roots. Each of the two partitions was chopped into small pieces and extracted for 24 h with 30% ethanol using a Soxhlet extractor. The solvent containing the extracted compounds was then concentrated using a rotary evaporator before freeze-drying the extracts for 48 h.

### Chemicals

Ethanol (96%), Methanol, DMSO (Dimethyl Sulfoxide) (Merck). DPPH (2,2-diphenyl-1-picrylhydrazyl), L-ascorbic acid, Dulbecco's Modified Eagle's Medium (DMEM), Fetal

The assay was performed in triplicates, and the results were averaged. A dose–response curve was plotted with the percentage of inhibition against the concentration of crude extracts on a log scale. The IC<sub>50</sub> value was then obtained from the graph.

### Cell viability (MTT assay)

The cell viability effect of *A. indica* aerial parts and root ethanolic extracts on fibroblasts was carried out using MTT assay [22, 23]. Human skin fibroblast cells (HSF 1189) were seeded in a 96-well plate at a density of  $2 \times 10^5$  cells/well and supplemented with DMEM (containing 10% FBS and 1% pen strep). After incubation for 24 h at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere, we treated ~80% confluence–cells with concentration gradient of the extracts (1000 µg/mL, 100 µg/mL, 10 µg/mL, 1 µg/mL, and 0.1 µg/mL) dissolved in DMSO and left one well untreated: DMEM only. After treating the cells, we incubated them again for 24 h. Then, we added MTT 10% to each well, and the cells were further incubated for 4 h. After that, we removed the media and added DMSO to dissolve the formed formazan. The absorbance was measured at 575 nm and 655 nm. The absorbance of formazan in untreated cells was considered as 100% proliferation or

viability. Cell viability percentage of the samples was calculated according to the equation:

$$\%Cell\ viability = \left\{ \frac{Absorbance\ of\ Treated\ cells}{Absorbance\ of\ Untreated\ cells} \right\} \times 100$$

The assay was performed in nine replicates, and the results were averaged. A dose–response curve was plotted with the viability percentage against the concentration of crude extracts on a log scale.

**Wound scratch assay**

In vitro wound healing activity of *A. indica* aerial parts and root, ethanolic extracts were determined by using scratch assay [22, 24, 25]. Fibroblasts were seeded in a 6-well plate at a density of 3 X 10<sup>5</sup> cells/well and incubated for 24 h to get 80% of cell confluence. We then made an artificial wound using 200 µL pipette tips. The treatment was then done by applying five different concentrations (1000 µg/mL, 100 µg/mL, 10 µg/mL, 1 µg/mL and 0.1 µg/mL) and one untreated well, which only contain growth medium (DMEM). The wound area was monitored under an inverted microscope for up to 48 h to observe fibroblasts migration. Images of the wound area were captured using an optical microscope (at 4 × magnification) to determine the percentage of the wound area. Duplicate wells were used per condition, and two fields per well were captured at each time point. ImageJ 1.48v software was used to analyse the images and calculate the wound area.

$$\%Wound\ closure = \left\{ \frac{Average\ scratch\ area\ (0\ h) - Average\ scratch\ area\ (48\ h)}{Average\ scratch\ area\ (0\ h)} \right\} \times 100$$

**Table 1** IC<sub>50</sub> values of the extracts as determined by DPPH assay

	IC <sub>50</sub> (µg/mL)
Aerial parts	62
Root	206
Ascorbic acid	1.9

IC<sub>50</sub> values of the free radicals scavenging activities of extracts along with ascorbic acid as standard (n = 3)

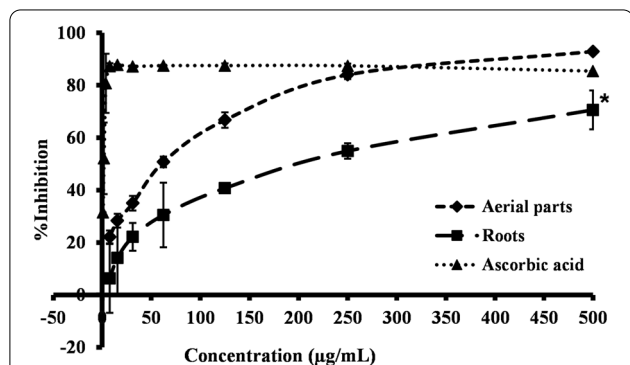
**Statistical analysis**

The statistical evaluation was studied by using Microsoft Excel 2010 and IBM SPSS Statistics version 20. The normality test was completed at a 0.95 confidence level, and the data were considered normally distributed when *p* > 0.05. One-way analysis of variance (ANOVA) and Kruskal–Wallis tests for parametric and non-parametric data were respectively used to determine the mean differences between the variables. The differences were considered statistically significant when *p* < 0.05. For the Post-Hoc test of ANOVA, Dunnett *t* was used for multiple data comparisons with homogeneous variances, whereas Games-Howell was used for data with non-homogeneous variances. Mann–Whitney test was used to study the differences in non-parametric data.

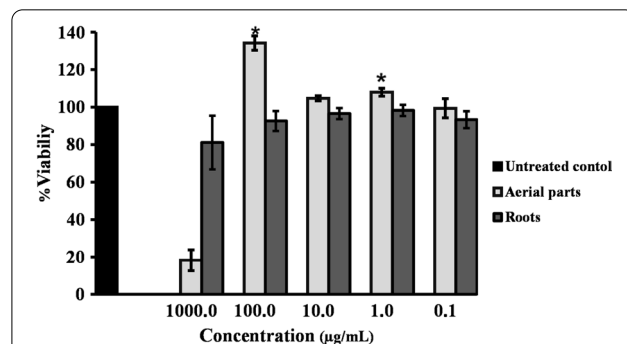
**Results**

**Antioxidant DPPH free radical-scavenging activity**

Figure 1 shows that at high concentration (500 µg/mL), aerial parts extract had no significant difference



**Fig. 1** Scavenging effect of DPPH. Values are expressed as mean DPPH inhibition% ± SD (n = 3). Statistical analysis was carried out using one-way ANOVA followed by *t* test. \*Statistically significant between aerial parts/root extracts in each concentration vs. ascorbic acid at \**p* < 0.05



**Fig. 2** % Viability of HSF 1189 with different concentrations of *A. indica* extracts. Values are expressed as mean ± SEM (n = 9). Statistical analysis was carried out using one-way ANOVA followed by *t* test. \*Statistically significant between extract concentrations vs. the untreated control at \**p* < 0.05

( $p > 0.05$ ) DPPH scavenging activity as compared to ascorbic acid, while root extract had significantly lower ( $p < 0.05$ ) DPPH scavenging activity as compared to ascorbic acid. Referring to  $IC_{50}$  values, Table 1 also confirms higher antioxidant activity against DPPH for aerial parts extract compared to root extract.

#### Cell viability (MTT assay) of *A. indica* extracts on human skin fibroblast cells (HSF 1189)

Figure 2 illustrates fibroblasts viability after 24 h of treatment according to MTT assay. Both aerial parts and root extracts showed low cytotoxicity towards fibroblast cells with 753  $\mu\text{g}/\text{mL}$   $LD_{50}$  for aerial parts and undetected  $LD_{50}$  for root extract. Figure 2 also confirms that at 100  $\mu\text{g}/\text{mL}$  and 1  $\mu\text{g}/\text{mL}$  aerial parts extract significantly ( $p < 0.05$ ) induces fibroblasts proliferation up to 134% and 107.9% respectively.

#### Wound scratch assay of *A. indica* extracts on human skin fibroblast cells (HSF 1189)

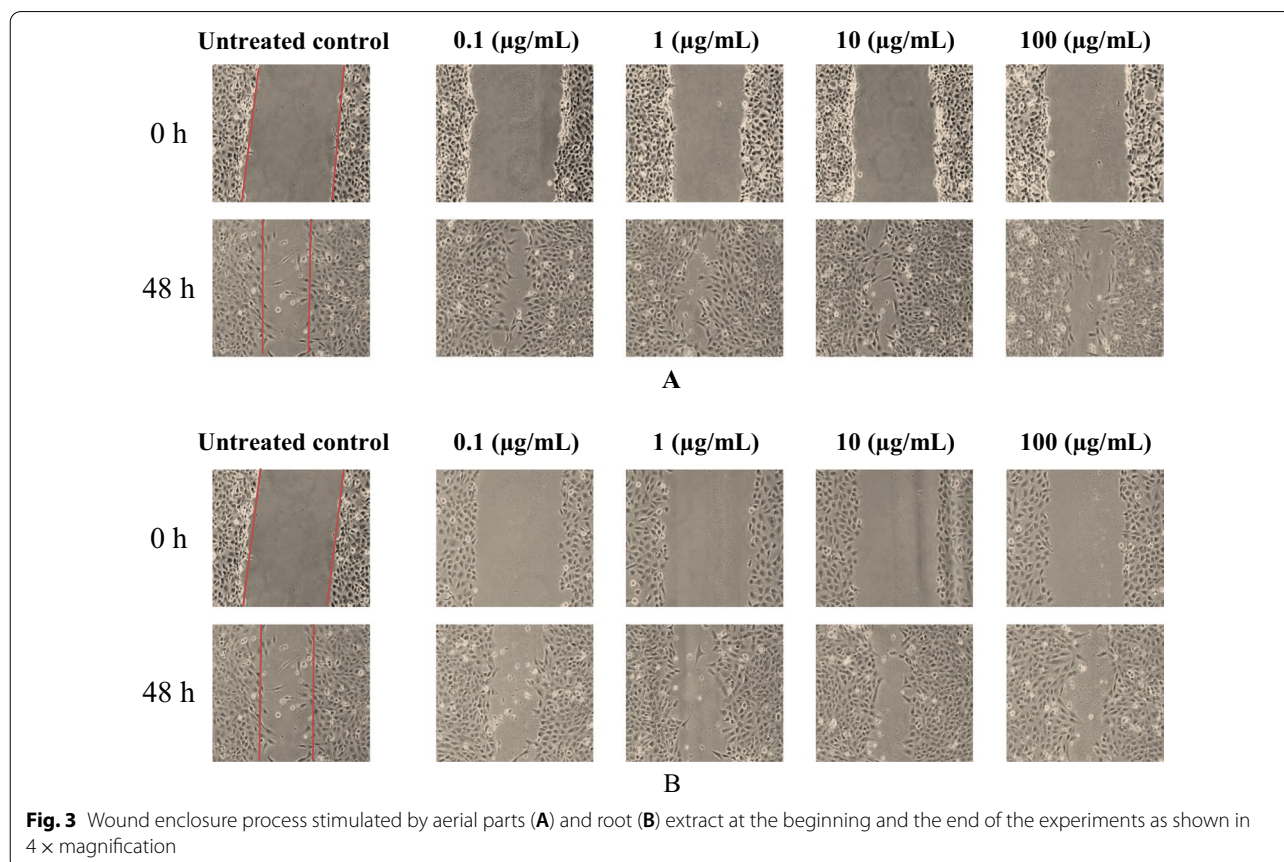
To assess the in vitro wound healing effect of *A. indica*, we monitored fibroblasts migration concerning the closure of the uncovered scratched area. Figure 3 illustrates

the enclosure process under stimulation of *A. indica* areal parts and root extracts.

Figure 4A confirms that low concentrations of aerial parts extract highly promote closure of the scratched area up to 70%; however, 1  $\mu\text{g}/\text{mL}$  significantly ( $p < 0.111$ ) increases the closure up to 75% compared to the untreated control, which made only 59% closure after 48 h of the study. On the other hand, higher concentrations showed a low closure percentage with only 48% (less than untreated control) for 100  $\mu\text{g}/\text{mL}$ , while cells in 1000  $\mu\text{g}/\text{mL}$  died after 6 h of treatment. Similarly, Fig. 4B confirms that low concentrations of root extract promote closure of the scratched area up to 70%. While 1000  $\mu\text{g}/\text{mL}$  showed lower closure as compared to untreated control with only 48%.

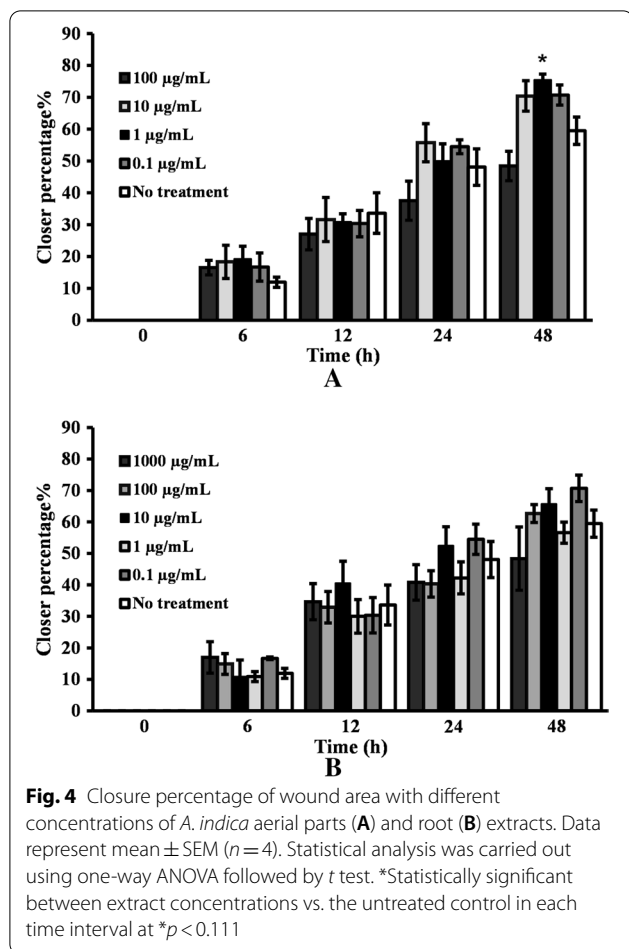
#### Discussion

We found that ethanolic extracts of both aerial parts and root have high antioxidant activity, support fibroblasts viability, and accelerate fibroblasts migration. These mechanisms target multiple phases of the dynamic wound healing process, making them, along with the previously reported anti-inflammatory and antimicrobial



**Fig. 3** Wound enclosure process stimulated by aerial parts (A) and root (B) extract at the beginning and the end of the experiments as shown in 4 × magnification





activities, the main factors to manage wounds by *A. indica*.

Many studies proved the beneficial effect of plant-based antioxidants on the wound repair process. San Miguel et al. [26] showed in their study that antioxidants lower inflammatory markers and facilitate wound healing by promoting fibroblast migration. Additionally, applying plant-based antioxidants to wound area accelerates the wound healing process because they scavenge free radicals that damage live tissues and delay the wound healing process [27, 28].

Previous studies reported the antioxidant activity of different *A. indica* aerial parts extracts (methanol, hexane, acetone, and chloroform); the methanolic extract showed the highest antioxidant activity [29, 30]. Our study reported the antioxidants activity of the aerial parts and root ethanolic extracts (Fig. 1 and Table 1). Dineshkumar et al. [7] and Nahrstedt et al. [6] attribute the antioxidant activity of aerial parts to the presence of antioxidant entities such as acalyphamide and acalyphine. However, further investigation is needed to explain the low antioxidant activity of the root compared to

aerial parts. We need to identify whether the low antioxidant activity of the root extract is because aerial parts are richer in phytochemicals-especially phenolic compounds-or other compounds are responsible for the antioxidant activity of the root extract [6, 7].

Upon skin injury, cells respond to the disruption in cell-cell contact by releasing cytokines, growth factors, and chemotactants, which trigger different types of cells [31]. Fibroblasts start to proliferate and migrate when triggered, promoting the newly formed connective tissue to contract [32, 33]. Figures 2, 3, 4 support our hypothesis of using-both aerial parts and root-*A. indica* ethanolic extracts to manage wounds by facilitating fibroblasts viability and migration. We noticed that the extract of the aerial parts showed a significant closure percentage compared to the root extract after 48 h of study, and aerial parts extract showed higher DPPH scavenging activity compared to root extract. On the other hand, while only aerial parts extract promoted fibroblasts proliferation, both aerial parts and root extracts showed no cytotoxicity on fibroblasts.

## Conclusions

In this study, we found that *A. indica* boosts the healing process not only by-previously reported-anti-inflammatory, antioxidant, and antimicrobial activities but also by stimulating fibroblasts viability and migration. Thus, we can use fibroblasts to explore further the healing mechanisms attributed to *A. indica*. We also associate the high healing properties for the aerial parts with the high antioxidant activity compared to the root, which has low antioxidant activity; thus, low healing properties.

## Abbreviations

DPPH: 2,2-Diphenyl-1-picrylhydrazyl; DMSO: Dimethyl sulfoxide; DMEM: Dulbecco's modified Eagle's medium; FBS: Fetal bovine serum; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS: Phosphate buffered saline; ABS: Bovine serum albumin; Pen Strep: Penicillin and streptomycin; HSF 1189: Human skin fibroblast cell line.

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## Plant authentication

The plant species were scientifically authorised in the Institute of Bioscience, Universiti Putra Malaysia (Voucher No. SK 3146/17).

## Authors' contributions

AI investigated and drafted the work. MAH done substantial contribution in analysis of the data. RA edited and revised the manuscript. AS made substantial contribution in acquisition of raw material and revision of the study. RZ conceptualised, administrated the project, and received funding. All authors have read and approved the manuscript.

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**Availability of data and materials**

All data and material are available on request.

**Declarations****Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

<sup>1</sup>Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia. <sup>2</sup>School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia.

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