

# Wound Healing Effects of *Litsea garciae* Extracts: *In vitro* Study on Fibroblast Cells

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# Wound Healing Effects of *Litsea garciae* Extracts: *In vitro* Study on Fibroblast Cells

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## **DECLARATION**

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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### ABSTRACT

Wound healing is a complex physiological process that involves regeneration of new cells and substitution of damaged cells after the onset of lesion. Wound healing involves the collaboration of many cell types and categorised into four overlapping main phases: haemostasis, inflammation, proliferation and remodelling. Although various wound healing treatments are currently available, the long-term usage of the medications has caused some adverse effects. To date, many traditional medicinal plants have been studied and widely used for various diseases due to their high efficacy, safety, and low cost. Litsea garciae (L. garciae) has many traditional medicinal uses and has been used by the indigenous people of Borneo Island to treat various diseases. However, its wound healing potential in skin wound therapy has not yet been investigated. Therefore, this study aimed to investigate whether the lipid and methanolic extracts of *Litsea garciae* could facilitate the process of *in vitro* wound healing and has a beneficial effect on the proliferation and migration of fibroblasts during in vitro wound healing. Total lipids were extracted using two different solvents: petroleum ether (PE) and Bligh and Dyer (BD). In addition, the crude methanolic extracts were prepared using 80% methanol. Firstly, the fatty acids composition of the lipid pulp and seed extracts of L. garciae were analysed using gas chromatography-mass spectrometry (GC-MS). The antimicrobial activities of the L. garciae lipid and methanolic pulp and seed extracts were evaluated through disk diffusion and broth microdilution assays. For the investigation of the potential wound healing properties of the L. garciae extracts, an in vitro study on the migration and chemotactic motility of the human dermal fibroblasts were conducted using wound scratch assay and transwell assay respectively. The effects of L. garciae extracts on the stimulation of the collagen type I, epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) expression levels were evaluated using

enzyme-linked immunosorbent assays (ELISA). This study showed that the prominent fatty acids in the L. garciae lipid seed extracts were lauric acid, palmitic acid, oleic acid, and linoleic acid. While for the L. garciae lipid pulp extracts, the predominant fatty acids were palmitic acid, stearic acid, oleic acid, and linoleic acid. All the L. garciae extracts exhibited mild antibacterial activities against the Gram-positive bacteria while Gram-negative bacteria exerted a weaker susceptibility towards the L. garciae extracts. The lipid pulp extracts were found to exhibit a stronger antimicrobial activity than the methanolic pulp extract while methanolic seed extract showed higher inhibition effect as compared to the lipid seed extracts. As for the wound healing property investigations, there were enhancement in the migration and chemotactic motility of fibroblasts treated with all the L. garciae pulp and seed extracts, but not with the methanolic seed extract. The expression levels of the collagen type I, EGF and VEGF by the wounded fibroblasts were ameliorated in the presence of the L. garciae extracts. These findings validated the ability of L. garciae extracts in promoting the wound healing process, which might be attributed to the presence of phytochemicals and fatty acids in the extracts. This study suggested that the L. garciae pulp and seed extracts might be a potent medication for skin wound healing.

Keywords: Wound healing, Litsea garciae, fibroblast, fatty acids, antimicrobial

## Kesan-kesan Penyembuhan Luka bagi Ekstrak Litsea garciae: Kajian in vitro pada Sel Fibroblas

#### ABSTRAK

Penyembuhan luka merupakan proses fisiologi yang kompleks di mana ia melibatkan regenerasi sel-sel baru dan penggantian sel-sel yang rosak selepas kelukaan. Penyembuhan luka melibatkan kerjasama banyak jenis sel dan dikategorikan kepada empat tahap utama yang bertindih: hemostasis, inflamasi, proliferasi, dan pembentukan semula. Walaupun kini terdapat pelbagai cara bagi rawatan luka, tetapi penggunaan ubat-ubatan dalam jangka masa yang lama telah membawa kesan sampingan. Pada masa ini, banyak tumbuhtumbuhan yang digunakan dalam perubatan tradisional telah dikaji dan digunakan secara meluas untuk merawat pelbagai penyakit disebabkan oleh keberkesanannya yang tinggi, keselamatan dan kos yang rendah. Litsea garciae (L. garciae) mempunyai banyak kegunaan dalam perubatan tradisional telah digunakan oleh bumiputera di Pulau Borneo untuk merawat pelbagai penyakit. Walau bagaimanapun, potensi tumbuhan ini dalam penyembuhan luka belum lagi dikaji. Oleh itu, kajian ini bertujuan untuk menyelidik sama ada ekstrak lipid dan metanol L. garciae dapat memudahkan proses penyembuhan luka dan mempunyai kesan yang bermanfaat terhadap proliferasi dan migrasi fibroblas dalam penyembuhan luka in vitro. Lipid diekstrak dengan menggunakan dua pelarut yang berbeza: eter petroleum (PE) dan "Bligh and Dyer" (BD). Selain itu, ekstrak metanol mentah disediakan dengan menggunakan 80% metanol. Pertamanya, komposisi asid lemak bagi ekstrak isi dan biji L. garciae yang diekstrak dengan PE dan kaedah BD telah dianalisa dengan menggunakan kromatografi gas-spektrometri jisim (GC-MS). Aktiviti antibakteria bagi ekstrak lemak dan metanolik L. garciae telah dinilai melalui ujian difusi cakera dan ujian mikrodilusi larutan. Bagi penyiasatan potensi ekstrak L. garciae atas sifat

penyembuhan luka, kajian in vitro pada migrasi dan motiliti kemotaktik bagi fibroblas kulit manusia telah dijalankan dengan menggunakan ujian goresan luka dan ujian "transwell" masing-masing. Kesan-kesan ekstrak L. garciae pada peningkatan tahap ekspresi kolagen jenis I, faktor pertumbuhan epidermis (EGF) dan faktor pertumbuhan endothelial vaskular (VEGF) telah dinilai melalui esei imunoserapan berkaitan enzim (ELISA). Hasil daripada penyelidikan ini mendapati bahawa asid lemak yang menonjol dalam ekstrak biji lipid L. garciae ialah asid laurik, asid palmitik, asid oleik dan asid linoleik. Manakala untuk ekstrak isi lipid L. garciae, asid lemak yang dominan ialah asid palmitik, asid stearik, asid oleik dan asid linoleik. Semua ekstrak L. garciae menunjukkan aktiviti antibakteria yang sederhana terhadap bakteria Gram-positif manakala bakteria Gram-negatif menunjukkan kerentanan yang lebih lemah terhadap ekstrak L. garciae. Ekstrak isi didapati mempunyai aktiviti antibakteria yang lebih kuat daripada ekstrak isi metanolik manakala ekstrak biji metanolik menunjukkan kesan perencatan yang lebih tinggi berbanding dengan ekstrak biji lipid. Bagi penyiasatan sifat penyembuhan luka, terdapat peningkatan dalam migrasi dan motiliti kemotaktik untuk fibroblas yang dirawat dengan semua ekstrak isi dan biji L. garciae, kecuali ekstrak biji metanolik. Tahap ekspresi kolagen jenis I, EGF dan VEGF yang dihasilkan oleh fibroblas telah meningkat apabila dirawat dengan ekstrak L. garciae. Dapatan kajian ini mengesahkan keupayaan ekstrak L. garciae dalam meningkatkan proses penyembuhan luka. Hal ini mungkin disebabkan oleh kewujudan fitokimia dan asid lemak dalam ekstrak tersebut. Kajian ini mencadangkan potensi ekstrak isi dan biji L. garciae sebagai ubat yang berkesan untuk penyembuhan luka kulit.

Kata kunci: Penyembuhan luka, Litsea garciae, fibroblas, asid lemak, antibakteria

## TABLE OF CONTENTS

		Page
DECI	LARATION	i
ACK	NOWLEDGEMENT	ii
ABST	TRACT	iii
ABST	RAK	v
TABI	LE OF CONTENTS	vii
LIST	OF TABLES	xii
LIST	OF FIGURES	xiv
LIST	OF ABBREVIATIONS	xviii
CHA	PTER 1 INTRODUCTION	1
1.1	Background of Study	1
1.2	Problem Statement	2
1.3	Objectives of Research	4
CHA	PTER 2 LITERATURE REVIEW	5
2.1	Structure of Skin	5
2.1.1	Wounds and Types of Wounds	6
2.1.2	Mechanism of Wound Healing	8
2.1.3	Wound Healing Treatment	14

2.1.4	Medicinal Plants with Wound Healing Properties	17
2.2	Litsea garciae	22
2.2.1	Geographical Distribution	23
2.2.2	Botany and Morphology	24
2.2.3	Applications	25
2.2.4	Chemical and Nutritional Compositions	27
2.2.5	Pharmacological Properties	35
2.3	Other Indigenous Plants in Sarawak	41
CHAI	PTER 3 MATERIALS AND METHODS	43
3.1	Chemicals and Reagents	43
3.2	Equipment	45
3.3	Litsea garciae	46
3.3.1	Litsea garciae Fruits Collection	46
3.3.2	Methanolic Extraction of ground Pulp and Seed of Litsea garciae	48
3.3.3	Total Lipid Extraction of Litsea garciae	49
3.3.4	Collection of Litsea garciae Pulp and Seed Extracts by Rotary Evaporation	51
3.4	Fatty Acid Analysis	52
3.5	Antimicrobial Assay	54
3.5.1	Preparation of Mueller-Hinton, Nutrient and Blood Agar Medium and Nutrient	
	Broth	54

3.5.2	Isolation of S. aureus, S. epidermidis, E. coli and P. aeruginosa Pure Culture	55
3.5.3	Preparation of Inoculum using Growth Method	56
3.5.4	Preparation of Diluted Sample	57
3.5.5	Disk Diffusion Assay	57
3.5.6	Broth Microdilution Assay	60
3.6	Cell Culture	62
3.6.1	Preparation for Cell Culture Work	62
3.6.2	Storage of Human Dermal Fibroblasts (HDF)	62
3.6.3	Human Dermal Fibroblasts (HDF) Culture	63
3.7	Cell Viability Analysis by MTT Assay	69
3.7.1	Solutions for MTT Assay	69
3.7.2	Cytotoxicity Tests for Extracts, Allantoin and DMSO	69
3.8	Investigation on the Wound Healing Potency of Litsea garciae Extracts	71
3.8.1	Wound Scratch Assay	71
3.8.2	Transwell Assay	72
3.8.3	Collagen 1 ELISA	75
3.8.4	Secretion of EGF	77
3.8.5	Secretion of VEGF	79
3.9	Statistical Analysis	81
CHA	PTER 4 RESULTS	82

4.1	Extraction of Samples	82
4.2	Fatty Acids Content	85
4.3	Antimicrobial Activities	95
4.3.1	Diameter of Zone of Inhibition	95
4.3.2	Minimum Inhibitory Concentration (MIC)	104
4.4	Cell Culture	110
4.5	Cell Viability	111
4.5.1	Effects of DMSO and allantoin on fibroblasts viability incubated in DMEM	
	containing 10% FBS	111
4.5.2	Effects of L. garciae Extracts on Fibroblasts Viability	113
4.6	Wound Healing Properties of L. garciae Extracts	117
4.6.1	Effects of L. garciae Extracts on Fibroblast Cells Migration	117
4.6.2	Effects of L. garciae Extracts on Fibroblasts' Chemotactic Motility	130
4.6.3	Effects of L. garciae Extracts on Wound Healing Markers	136
CHAI	PTER 5 DISCUSSION	144
5.1	Extraction Yield of Pulp and Seed of L. garciae	144
5.2	Characterisation of Fatty Acids Extracted from Pulp and Seed of L. garciae	146
5.3	Antimicrobial Effects of Lipids Extracted from Pulp and Seed of L. garciae	151
5.4	Human Dermal Fibroblasts Cell Culture	157
5.5	Effects of Pulp and Seed Extracts of L. garciae on Human Dermal Fibroblasts	
	Viability	159

5.6	Wound Healing Properties of Pulp and Seed Extracts of L. garciae	162
5.6.1	Effects of L. garciae Extracts on Fibroblast Cells Migration	162
5.6.2	Effects of L. garciae Extracts on Fibroblasts' Chemotactic Motility	164
5.6.3	Effect of L. garciae Extracts on Wound Healing Markers	167
5.7	Summary	170
CHAPTER 6 CONCLUSION		175
6.1	Conclusion	175
6.2	Limitation of study and Future Work	176
REFI	ERENCES	179
APPE	APPENDICES 2	

## LIST OF TABLES

Table 2.1	Growth factors involved in the wound healing process [Adapted from (Broughton et al., 2006; Enoch & Leaper, 2007)].	12
Table 2.2	Medicinal plants and their wound healing activities [Adapted from (Ning et al., 2022)].	20
Table 2.3	Nutrient contents of the <i>L. garciae</i> pulp/seed per 100 g pulp/seed portion.	28
Table 2.4	Phytochemical screening of <i>L. garciae</i> [Adapted from (Wulandari et al., 2018)].	32
Table 2.5	Total phenolic and flavonoid content of <i>L. garciae</i> [Adapted from (Wulandari et al., 2018)].	33
Table 2.6	The total phenolic, flavonoid and anthocyanin contents of different parts of <i>L. garciae</i> fruit extracts [Adapted from (Hassan et al., 2013)].	34
Table 2.7	The antioxidant properties of <i>L. garciae</i> fruit extracts from different parts using three different assays [Adapted from (Hassan et al., 2013)].	36
Table 2.8	Minimal Inhibitory Concentration (MIC) of <i>L. garciae</i> using antibacterial assay [Adapted from (Wulandari et al., 2018)].	38
Table 2.9	The percentage reduction of radial growth (mm) of <i>Colletotrichum</i> gloeosporioides ( $Cg$ ) and <i>Colletotrichum</i> capsici ( $Cc$ ) by varying concentrations of <i>L.</i> garciae leaf extracts in different solvents using antifungal assay [Adapted from (Johnny et al., 2010; Johnny et al., 2011)].	38
Table 2.10	Inhibitory concentration of barks and leaves methanolic extract on HeLa, MCF-7 and HT-29 [Adapted from Kutoi et al., (2012)].	39
Table 2.11	Lipoxygenase (LO), Hyaluronidase (HO) and Xanthine Oxidase (XO) inhibitory activities [Adapted from Kutoi et al., (2012)].	40
Table 3.1	List of materials used for the experiments.	43
Table 3.2	List of equipment used for the experiments.	45
Table 4.1	Extraction yield of the pulp and seed extracts of L. garciae.	84
Table 4.2	Fatty acid composition of the pulp and seed extracts of <i>L. garciae</i> .	90

- Table 4.3Antimicrobial activities of the pulp and seed extracts of L. garciae<br/>against Staphylococcus aureus, Staphylococcus epidermidis,<br/>Escherichia coli and Pseudomonas aeruginosa by disk diffusion<br/>assay.
- Table 4.4Antimicrobial activities of the pulp and seed extracts of L. garciae<br/>against Staphylococcus aureus, Staphylococcus epidermidis,<br/>Escherichia coli and Pseudomonas aeruginosa by broth microdilution<br/>assay.

105

98

## LIST OF FIGURES

Figure 2.1	The structure of skin [Adapted from (Voegeli, 2012)].	6
Figure 2.2	The wound healing stages involving cellular components [Adapted from (Wilkinson & Hardman, 2020)].	11
Figure 2.3	Transformation of the green, unripe <i>L. garciae</i> fruit into the red, ripe fruit.	22
Figure 2.4	Geographical distribution map of <i>L. garciae</i> [Adapted from (Google Maps, 2020)].	23
Figure 2.5	<i>L. garciae</i> tree bearing fruits (A) and flowers (B) [Adapted from (Bukbi, 2019)].	24
Figure 2.6	Different parts of the <i>L. garciae</i> fruit [Adapted from (Machabuca, 2008 and Herbs Encyclopedia, 2020)].	25
Figure 3.1	The sampling voucher of Litsea garciae (ZA-01).	47
Figure 3.2	Soxhlet (FAVORIT <sup>®</sup> ) extraction apparatus used in this study. Picture was taken by the author in the Biochemistry laboratory of Faculty of Medicine and Health Sciences, UNIMAS.	49
Figure 3.3	Streaking pattern for pure culture isolation of bacteria using four- streak method.	56
Figure 3.4	Procedure of the disk diffusion assay (Tankeshwar, 2022).	59
Figure 3.5	Measurement of the zone of inhibition diameter (American Society for Microbiology, 2016).	59
Figure 3.6	Illustration for the broth microdilution assay [Adapted from (Starlab, 2021)].	61
Figure 3.7	Morphology of fibroblasts under an inverted microscope (Olympus IX73) at (a) 40x, (b) 100x, (c) 200x magnification.	64
Figure 3.8	Illustration for one of the corners of a hemacytometer.	67
Figure 3.9	Illustration for the transwell migration assay (ThemoFisher Scientific, 2021).	74
Figure 4.1	Optical appearance of PE, ME and BD extracts of the pulp and seed of <i>L. garciae</i> . Pictures were taken at room temperature $(25^{\circ}C)$ and at $40^{\circ}C$ .	83

Figure 4.2	GC-MS chromatogram of (A) Petroleum ether and (B) Bligh and Dyer seed extracts of <i>L. garciae</i> .	88
Figure 4.3	GC-MS chromatogram of (C) Petroleum ether and (D) Bligh and Dyer pulp extracts of <i>L. garciae</i> .	89
Figure 4.4	Proportion (%) of saturated, monounsaturated and polyunsaturated fatty acids for (a) Petroleum ether and (b) Bligh and Dyer lipid pulp extracts of <i>L. garciae</i> .	91
Figure 4.5	Proportion (%) of saturated, monounsaturated and polyunsaturated fatty acids for (a) Petroleum ether and (b) Bligh and Dyer lipid seed extracts of <i>L. garciae</i> .	92
Figure 4.6	Proportion (%) of (a) saturated fatty acids, (b) monounsaturated fatty acids and (c) polyunsaturated fatty acids for petroleum ether and Bligh and Dyer lipid pulp and seed extracts of <i>L. garciae</i> .	94
Figure 4.7	Zone of inhibition of <i>L. garciae</i> pulp and seed extracts against <i>Staphylococcus aureus</i> .	100
Figure 4.8	Zone of inhibition of <i>L. garciae</i> pulp and seed extracts against <i>Staphylococcus epidermidis</i> .	101
Figure 4.9	Zone of inhibition of <i>L. garciae</i> pulp and seed extracts against <i>Escherichia coli</i> .	102
Figure 4.10	Zone of inhibition of <i>L. garciae</i> pulp and seed extracts against <i>Pseudomonas aeruginosa</i> .	103
Figure 4.11	Minimum Inhibitory Concentration (MIC) of <i>L. garciae</i> pulp and seed extracts against <i>Staphylococcus aureus</i> .	106
Figure 4.12	Minimum Inhibitory Concentration (MIC) of <i>L. garciae</i> pulp and seed extracts against <i>Staphylococcus epidermidis</i> .	107
Figure 4.13	Minimum Inhibitory Concentration (MIC) of <i>L. garciae</i> pulp and seed extracts against <i>Escherichia coli</i> .	108
Figure 4.14	Minimum Inhibitory Concentration (MIC) of <i>L. garciae</i> pulp and seed extracts against <i>Pseudomonas aeruginosa</i> .	109
Figure 4.15	Fibroblasts at different cell density were cultured in their growth media. For each cell density the mean absorptions $(n=3)$ were calculated after 24 h of incubation.	110
Figure 4.16	Viability of fibroblasts treated with DMSO and allantoin. Fibroblasts at $2.2 \times 10^4$ cells/well in 200 µL growth medium were incubated with (a) DMSO (0.05 - 10 %) and (b) Allantoin (50 - 500 µg/mL) for 24 h. The cell viability was measured by MTT assay. The data were	

	presented as mean $\pm$ SD (n = 6). Significance was indicated with p<0.05 (*) by independent t-test.	112
Figure 4.17	Viability of fibroblasts treated with petroleum ether, methanolic and Bligh and Dyer pulp and seed extracts. Fibroblasts at $2.2 \times 10^4$ cells/well in 200 µL growth medium were incubated with petroleum ether, methanolic and Bligh and Dyer (a) pulp and (b) seed extracts (31.25 - 500 µg/mL) for 24 h. The cell viability was measured by MTT assay. The data were presented as mean ± SD (n = 6). Significance was indicated with p<0.05 (*) by independent t-test.	115
Figure 4.18	Viability of fibroblasts treated with petroleum ether and Bligh and Dyer pulp and seed extracts. Fibroblasts at $2.2 \times 10^4$ cells/well in 200 $\mu$ L growth medium were incubated with (a) petroleum ether pulp extracts (7.5 - 31.25 $\mu$ g/mL), (b) petroleum ether and Bligh and Dyer seed extracts (7.5 - 31.25 $\mu$ g/mL) for 24 h. The cell viability was measured by MTT assay. The data were presented as mean $\pm$ SD (n = 6). Significance was indicated with p<0.05 by independent t-test and there was no significant difference (p>0.05).	116
Figure 4.19	Migration of fibroblasts without treatment for 48h.	120
Figure 4.20	Migration of fibroblasts after treatment with 31.25 $\mu$ g/mL petroleum ether pulp extract for 48h.	121
Figure 4.21	Migration of fibroblasts after treatment with (a) petroleum ether (7.5 - 31.25 $\mu$ g/mL) and (b) Bligh and Dyer (31.25 - 125 $\mu$ g/mL) pulp extracts as compared with the negative control for 48h. The data were expressed as mean $\pm$ SD (n = 3) and were analysed using one-way ANOVA followed by Tukey's HSD test. There was no statistically significant difference (p>0.05) between all pulp extracts and negative control.	122
Figure 4.22	Migration of fibroblasts after treatment with methanolic pulp extracts $(31.25 - 125 \ \mu\text{g/mL})$ as compared with the negative control for 48h. The data were expressed as mean $\pm$ SD (n = 3) and were analysed using one-way ANOVA followed by Tukey's HSD test. There was no statistically significant difference (p>0.05) between methanolic pulp extracts and negative control.	124
Figure 4.23	Migration of fibroblasts after treatment with (a) petroleum ether (7.5 - 31.25 $\mu$ g/mL), (b) Bligh and Dyer (7.5 - 31.25 $\mu$ g/mL) seed extracts as compared with the negative control for 48 h. The data were	

expressed as mean  $\pm$  SD (n = 3) and were analysed using one-way ANOVA followed by Tukey's HSD test. There was a statistical significant difference (\*p<0.05) between 31.25 µg/mL Bligh and Dyer seed extract and negative control at 24 h while the other seed extracts were statistically insignificant different (p>0.05).

127

- Figure 4.24 Migration of fibroblasts after treatment with methanolic seed extracts  $(31.25 125 \ \mu g/mL)$  as compared with the negative control for 48h. The data were expressed as mean  $\pm$  SD (n = 3) and were analysed using one-way ANOVA followed by Tukey's HSD test. There was no statistically significant difference (p>0.05) between methanolic seed extracts and negative control.
- Figure 4.25 Chemotactic motility of fibroblasts without treatment (growth media with and without FBS).
- Figure 4.26 Transwell assay to detect the chemotactic motility of fibroblasts. Cells were treated with  $31.25 \ \mu g/mL$  petroleum ether, Bligh and Dyer and methanolic pulp and seed extracts. The migrated cells on the lower chamber membrane were stained and counted. The number of cells were shown in the Figure 4.27.
- Figure 4.27 Chemotactic motility of fibroblasts after treatment with petroleum ether, methanolic and Bligh and Dyer (a) pulp and (b) seed extracts (31.25  $\mu$ g/mL) compared with the negative controls after 24 h incubation. The data were expressed as mean  $\pm$  SD (n = 3) and were analysed using one-way ANOVA followed by Tukey's HSD test. The statistical significant difference (p<0.05) between extracts and negative controls (No FBS and 10% FBS) were represented by (\*) and (#) respectively.
- Figure 4.28 The effect of petroleum ether, Bligh and Dyer and methanolic (a) pulp and (b) seed extracts on the expression of collagen I by wounded fibroblasts. The data were expressed as mean  $\pm$  SD (n = 3) and were analysed using one-way ANOVA followed by Tukey's HSD test. The statistical significant difference (p<0.05) between pulp and seed extracts and negative control were represented by (\*).
- Figure 4.29 The effect of petroleum ether, Bligh and Dyer and methanolic (a) pulp and (b) seed extracts on the secretion of EGF by wounded fibroblasts. The data were expressed as mean  $\pm$  SD (n = 3) and were analysed using one-way ANOVA followed by Tukey's HSD test. There was no statistical significant difference (p>0.05) between all pulp and seed extracts and negative control.
- Figure 4.30 The effect of petroleum ether, Bligh and Dyer and methanolic (a) pulp and (b) seed extracts on the secretion of VEGF by wounded fibroblasts. The data were expressed as mean  $\pm$  SD (n = 2) and were analysed using independent t-test. The statistical significant difference (p<0.05) between pulp extracts and negative control were represented by (\*) while seed extracts and negative control were statistically insignificant different (p>0.05).

128

132

134

135

141

143

138

## LIST OF ABBREVIATIONS

AAP	Auricularia auricula-judae
ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid
AEAC	Ascorbic acid equivalent antioxidant capacity
ALA	α-linolenic acid
ANOVA	Analysis of Variance
BD	Bligh and Dyer
BDPE	Bligh and Dyer pulp extract
BDSE	Bligh and Dyer seed extract
bFGF	Basic fibroblast growth factor
BHT	Butylated hydroxytoluene
BSC	Biological safety cabinet
CH <sub>3</sub> Cl	Chloroform
CHD	Coronary heart disease
CLSI	Clinical and Laboratory Standards Institute
CO <sub>2</sub>	Carbon dioxide
CTGF	Connective tissue growth factor
DCF	Digitaria ciliaris flower
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco's phosphate buffered saline
DPPH	2,2-Diphenyl-1-picryl-hydrazyl-hydrate
E. coli	Escherichia coli

ECM	Extracellular matrix
EFA	Essential fatty acids
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
Erk	Extracellular signal-regulated kinase
FabI	Enoyl-acyl carrier protein reductase
FAMEs	Fatty acid methyl esters
FBS	Foetal bovine serum
FD	Freeze-drying
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FRAP	Ferric reducing/antioxidant power
GC-MS	Gas chromatography-mass spectrometry
GML	Glycerol monolaurate
H <sub>2</sub> O	Water
HCl	Hydrochloric acid
HDF	Human dermal fibroblasts
НО	Hyaluronidase
HRP	Horseradish peroxidase
HSD	Honestly Significant Different
IC <sub>50</sub>	Half maximal inhibitory concentration
IL-1	Interleukin-1
KGF	Keratinocyte growth factor
L. garciae	Litsea garciae
LA	Linoleic acid

LCFAs	Long-chain fatty acids
LC-PUFA	Long chain polyunsaturated fatty acids
LC-SFA	Long-chain saturated fatty acids
LDL	Low-density lipoprotein
LNO	Lucuma nut oil
LO	Lipoxygenase
МАРК	Mitogen-activated protein kinase
MCFAs	Medium-chain fatty acids
ME	Methanolic
МеОН	Methanol
MEPE	Methanolic pulp extract
MESE	Methanolic seed extract
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant S. aureus
MSC	Mesenchymal stem cells
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MUFAs	Monounsaturated fatty acids
NaOH	Sodium hydroxide
NIST	National Institute of Standards and Technology
NO	Nitric oxide
NSAIDs	Nonsteroidal anti-inflammatory drugs
P. aeruginosa	Pseudomonas aeruginosa
PDGF	Platelet-derived growth factor

PE	Petroleum ether
PEPE	Petroleum ether pulp extract
PESE	Petroleum ether seed extract
PFA	Paraformaldehyde
PUFAs	Polyunsaturated fatty acids
RDFP	Red dragon fruit peel
S. aureus	Staphylococcus aureus
S. epidermidis	Staphylococcus epidermidis
SCFAs	Short-chain fatty acids
SD	Standard deviation
SFAs	Saturated fatty acids
SFM	Serum-free medium
SHSD	Superheated-steam drying
SPP	Saudi pomegranate peel
SPSS	Statistical Package for the Social Science
TFA	Trifluoroacetic acid
TGF	Transforming growth factor
TMB	3,3′,5,5′ -Tetramethylbenzidine
TNF	Tumour necrosis factor
UNIMAS	Universiti Malaysia Sarawak
v/v	Volume/volume
VEGF	Vascular endothelial growth factor
VLCFAs	Very long chain fatty acids
XO	Xanthine oxidase

### **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Background of Study**

Skin is a protective barrier against various pathogens and when this barrier becomes compromised, the body responds by initiating a sequential repair process known as wound healing. Wound healing is a complex and highly regulated biological process to replace and reestablishes impaired tissues. The normal wound healing event can be disrupted by some co-morbidities such as diabetes, obesity and malnutrition (Anderson & Hamm, 2014; Han & Ceilley, 2017). This may result in chronic wounds which brings a burden to the patients and health care system due to the high costing of chronic wound treatment.

Although there are a lot of wound healing treatments and medication options already available for wound healing, there is still a necessity to research new efficacious medicine, especially using a medicinal plant. The medications commonly used in wound care include antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs). However, there are adverse effects attributed to the long-term usage of antibiotics and NSAIDs. The popularity of researching medicinal plants could be due to the perception that plants will cause minimal unwanted side effects and are less costly and more efficacious.

*Litsea garciae* (*L. garciae*) is known locally as engkala. Previous studies showed that the bark, leaf and fruit of *L. garciae* possessed antioxidant, antimicrobial, antifungal, antiinflammatory, and anticancer properties. In addition, *L. garciae* also contained various phytochemical components such as alkaloids, butenolide, terpenes, flavonoids, amides, lignans, steroids and fatty acids. The indigenous people in Sarawak claimed that the leaves,