IN VITRO EVALUATION AND WOUND HEALING CREAM FORMULATION OF *Acalypha Indica Linn* ETHANOLIC EXTRACT

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To my father may Allah bless and have mercy upon his soul. To my beloved mother for her prayers. To my brothers for their support.

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ABSTRACT

Plant-based drugs were used by ancient civilizations for treating various diseases. It is shown scientifically in recent studies that some remedies are effective. *Acalypha indica Linn*, is an annual shrub known in Malaysia as 'Kucing Galak'. It is traditionally used by practitioner for wound-healing. In this study, *Acalypha indica Linn* aerial parts and roots were extracted using 30% ethanol. The extracts were standardized based on protein and polysaccharide content prior to further biochemical and cell tests. Aerial parts extract exhibited higher antioxidant ability compared to roots extract with IC_{50} of 62 µg/mL and 206 µg/mL, respectively. In cell studies, *In vitro* cytotoxicity was carried out on human skin fibroblast cells (HSF 1189). Both aerial parts and roots extracts showed low cytotoxicities towards (HSF 1189) with 753 µg/mL LD_{50} for aerial parts extract and undetected on root extract. Further investigation on wound healing showed higher wound closure percentage for both extracts compared to untreated control with 75% at1 µg/mL for aerial parts, 70% at 0.1 µg/mL for root extract and only 59% closure percentage for untreated control after 48h of the study. Finally, the extracts were formulated into oil-in-water (O/W) cream and then the physicochemical properties such as stability, viscosity and pH were determined.

ABSTRAK

Ubatan berasaskan tumbuhan telah digunakan oleh orang dahulu kala untuk megubati pelbagai penyakit. Kajian saintifik terbaru telah menunjukkan keberkesanan ubatan-ubatan tersebut. *Acalypha indica Linn* ialah tumbuhan renek yang lebih dikenali di Malaysia sebagai 'Kucing Galak'. Tumbuhan ini telah digunakan sebagai ubatan tradiosional untuk penyembuhan luka. Dalam kajian ini, bahagian batang dan daun, dan akar pokok *Acalypha indica Linn* telah diekstrak menggunakan 30% etanol. Sebelum menjalankan ujian biokimia dan sel, ekstrak ini telah diselaras berdasarkan kandungan protin dan polisakarida. Ekstrak bahagian batang dan daun mempunyai antioksida yang lebih tinggi berbanding ekstrak bahagian akar dengan masing-masing menunjukkan nilai IC_{50} 62 µg/mL dan 206 µg/mL. Ujian toksik telah dijalankan keatas sel kulit manusia fibroblast (HSF 1189). Ekstrak bahagian batang dan daun menunjukkan nilai toksik terhadap sel HSF 1189 yang rendah dengan nilai LD_{50} 753 µg/mL. Ekstrak akar pokok pula tidak menunjukkan sebarang toksik terhadap sel HSF 1189. Ujian lanjut keatas penyembuhan luka menunjukkan bahawa ekstrak bahagian batang dan daun, dan akar pokok mempunyai peratusan penutupan luka yang tinggi berbanding kumpulan yang tidak menerima ekstrak. Masing-masing menunjukkan nilai penutupan luka 75% pada kepekatan 1 µg/mL untuk ekstrak bahagian batang dan daun, 70% pada kepekatan 0.1 µg/mL untuk ekstrak akar pokok dan 59% untuk kumpulan yang tidak menerima sebarang ekstrak. Yang terakhir, ekstrak ini diformulasi kepada krim minyak-dalam-air (O/W) dan diuji dengan ciri-ciri fizikokimia seperti kestabilan, kelekitan dan pH.

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LIST OF ABBREVIATION/ SYMBOLS

Abc	- Absorbance
ANOVA	- Analysis of Variance
BFF	- Basic Fibroblast Factor
^oC	- Degree Celsius
CO_2	- Carbon Dioxide
DMEM	- Dulbecco's Modified Eagle's Medium
DMSO	- Dimethylsulfoxide
DPPH	- 2,2-diphenyl-1-picrylhydrazyl
ECM	- Extracellular Matrix
EGF	- Epidermal Growth Factor
FBS	- Foetal Bovine Serum
FGF	- Fibroblast Growth Factor
g	- Gram
h	- Hour
H_2SO_4	- Sulfuric Acid
HCl	- Hydrochloric Acid
HSF 1184	- Human Skin Fibroblast
IC_{50}	- 50% Inhibitory Concentration
LD_{50}	- 50% Lethal Concentration
mg	- Milligram
mg/L	- Milligram per Liter

min	-	Minute
MMPs	-	Matrix Metalloproteinases
MTT	-	Methyl Tetrazolium
nm	-	Nanometer
PBS	-	Phosphate Buffered Saline
PDGF	-	platelet-derived Growth Factor
ROS	-	Reactive Oxygen Species
TGF	-	Transforming Growth Factor
UV	-	Ultraviolet
pH	-	Hydrogen Concentration
Pen Strep	-	Penicillin Streptomycin mixtures
RPM	-	Revolutions Per Minute
SD	-	Standard Deviation
SEM	-	Standard Error of the Mean
SPSS	-	Statistical Package for the Social Sciences
VEGF	-	Vascular Endothelial Growth Factor
µg	-	Microgram
µL	-	Microliter
WHO	-	World Health Organization
%	-	Per cent
>	-	Greater Than
<	-	Less Than

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CHAPTER 1

INTRODUCTION

1.1 Study Background

Prior to this time, the healing process was considered to be a passive process with respect to the physician (Boateng *et al*., 2008). This theory was summed up by Ambroise Pare, who said "I dressed the wound, God heals it" (Keynes, 1951). The healing process was firstly studied by Winter, and others in 1960s (winter and Scales, 1963), since then much more information has been revealed about cellular and biochemical components of wound healing and factors affecting this process. It is now known that healing is not a passive process, but rather can be accelerated and enhanced by the use of specific wound care/dressing techniques and products (Boateng *et al*., 2008).

For hundreds of years, plant-based treatments were used by ancient civilizations for curing diseases. Recently most of the research conducted in traditional medicines has shown that some traditional remedies are effective in spite of the fact that there is no clear justification. The greater part of the traditional therapy involves the use of plant extracts on their active principle (WHO, 1993).

Acalypha indica Linn is a herb traditionally used in wound healing management (Upadhyay *et al*., 2007). New researches conducted on this herb *in vivo* have found evidences that support the wound healing properties of this herb (Reddy *et al*., 2002; Ganeshkumar *et al*., 2012). The anti- oxidant properties and the protein

and polysaccharide content of ethanolic extract of this herb have major contribution in wound healing properties. Formulating wound healing cream from the extract of *Acalypha indica Linn* would supplement the wound healing products available in the market with new promising biopharmaceutical product with an active wound healing properties that accelerate the wound healing process.

1.2 Problem Statement

Patients suffering from chronic wounds like diabetes, vascular disease, and pressure ulcer are increasing in numbers. According to American Diabetes Association (2013), 25.8 million Americans suffer from diabetes. Likewise in 2012, two studies conducted by Vascular Disease Foundation and The National Pressure Ulcer Advisory Panel respectively showed that half a million Americans have ulcers on their legs caused by diseased veins, and 2.5 million US residents develop pressure ulcers every year. In Malaysia, according to world health organization 942,000 Malaysian diagnosed with diabetes in 2000 and the number expected to increase to be 2.479.000 in 2030 WHO (2000). A recent study conducted by Mazlina *et al*. (2011) showed that 47.1% of diabetic patient would develop foot ulcers.

Traditional wound management products including cotton wool, natural or synthetic bandages and gauzes do not provide the optimum environment to facilitate the healing process; they only dress the wound and have no biological activity to accelerate wound healing.

In this study, the formulated cream from *Acalypha indica Linn* extracts made use of the wound healing properties of this herb especially the anti-oxidant (Ganeshkumar *et al*., 2012). The healing process of the ethanolic extract was evaluated *In vitro* on human skin fibroblast cell line (HSF 1189). On the other hand, the moisture provided by the cream itself accelerates the healing process (winter and Scales, 1963). cream will also act as a barrier preventing infection caused by bacteria (Boateng *et al*., 2008).

1.3 Significance of The Study

Herbs based biopharmaceutical products can be potential agents for wounds healing and are in great demand due to their wide availability, less toxicity, less side effect and cost effective. *Acalypha indica* is a common herb in tropical regions; it is also used traditionally in wound healing management.

Formulating *Acalypha indica* extract in the form of cream will carry the active ingredient of the extract. On the other hand, cream will prevent infection that might happen when applying plants, directly, or as a crude extract, as the crude extract might act as potential source for microorganisms.

1.4 Objectives of The Study

1. To extract and standardize the *Acalypha indica* aerial parts and roots with 30% ethanol, based on protein and polysaccharide content.
2. To evaluate the anti-oxidant properties of *Acalypha indica* extract.
3. To evaluate *In vitro* cytotoxicity activity and wound healing properties of *Acalypha indica* extracts on fibroblast (HSF 1189) cell line.
4. To formulate a wound healing cream comprising *Acalypha indica* extracts into oil-in water (O/W) cream.

1.5 Scope of The Study

This study were conducted to standardize the ethanolic extract based on protein and polysaccharide content, evaluate the anti-oxidant properties of the ethanolic extract of *Acalypha indica*, evaluate the healing process of the extract *In*

vitro on (HSF 1189) Fibroblast cell line, and formulate a wound healing cream from this extract. All results were statistically analysed.

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