Biological Activities of Cream Containing *Curcuma mangga* Extract

Suthasinee Srirod

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Pharmacy in Pharmaceutical Sciences

Prince of Songkla University

2018

Biological Activities of Cream Containing *Curcuma mangga* Extract

Suthasinee Srirod

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Pharmacy in Pharmaceutical Sciences

Prince of Songkla University

2018

Thesis Title	Biological Activities of Cream Containing *Curcuma mangga* Extract
Author	Miss Suthasinee Srirod
Major Program	Pharmaceutical Sciences

Major Advisor

.....

(Assoc. Prof. Dr. Supinya Tewtrakul)

Examining Committee :

.....Chairperson

(Asst. Prof. Dr. Chatchai Wattanapiromsakul)

.....Committee

(Assoc. Prof. Dr. Supinya Tewtrakul)

.....Committee

(Assoc. Prof. Dr. Thanaporn Amnuaikit)

.....Committee

(Dr. Teeratad Sudsai)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Pharmacy Degree in Pharmaceutical Sciences

.....

(Prof. Dr. Damrongsak Faroongsarng)

Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

.....Signature
(Assoc. Prof. Dr. Supinya Tewtrakul)
Major Advisor

.....Signature
(Miss Suthasinee Srirod)
Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

..... Signature

(Miss Suthasinee Srirod)

Candidate

ชื่อวิทยานิพนธ์ ฤทธิ์ทางชีวภาพของครีมที่มีส่วนผสมของสารสกัดขมิ้นขาว
ผู้เขียน นางสาวสุธาศิณี ศรีรอด
สาขาวิชา เภสัชศาสตร์
ปีการศึกษา 2561

บทคัดย่อ

ขมิ้นขาว (*Curcuma mangga* Valeton & Van Zijp) เป็นพืชล้มลุกอยู่ในวงศ์ Zingiberaceae การแพทย์พื้นบ้านใช้เหง้าขมิ้นขาวในการรักษาโรคผิวหนัง ช่วยกระชับมดลูกและใช้เป็นยาอายุวัฒนะ สารบริสุทธิ์ในขมิ้นขาว ได้แก่ diterpenes และ curcuminoids ซึ่งสารดังกล่าวมีรายงานเกี่ยวกับฤทธิ์ลดการอักเสบ การศึกษา การเตรียมตำรับครีมที่มีส่วนผสมของสารสกัดขมิ้นขาวและศึกษาฤทธิ์ทางชีวภาพของตำรับครีม โดยเลือกครีมเบส (สูตรที่ 2) ที่มีความคงตัวจากครีมเบสทั้ง 3 สูตร มาเตรียมเป็นตำรับครีมที่มีส่วนผสมของสารสกัดขมิ้นขาว 2-10% w/w จากนั้นทำการทดสอบคุณสมบัติทางกายภาพ ความคงตัวทางเคมี และฤทธิ์ทางชีวภาพของตำรับครีมประกอบด้วย ฤทธิ์ต้านอนุมูลอิสระ ต้านการอักเสบ และสมานแผลในเซลล์เพาะเลี้ยง จากการศึกษาพบว่า ครีมที่มีส่วนผสมของสารสกัดขมิ้นขาวเป็นเนื้อเดียวกัน มีความเป็นกรดเล็กน้อย (pH 5-6) และมีความคงตัวหลังเก็บที่อุณหภูมิสูงสลับต่ำ การทดสอบฤทธิ์ทางชีวภาพพบว่า ครีมที่มีส่วนผสมของสารสกัดขมิ้นขาวมีฤทธิ์ต้านอนุมูลอิสระเล็กน้อย (IC_{50} >100.0 ไมโครกรัม/มิลลิลิตร) เมื่อทดสอบด้วยวิธี DPPH และ H_2O_2-induced oxidative stress ส่วนการทดสอบฤทธิ์ต้านการอักเสบ โดยทดสอบในเซลล์เพาะเลี้ยงแมคโครฟาจ (RAW264.7) ที่ถูกกระตุ้นด้วย LPS พบว่าครีมผสมสารสกัดขมิ้นขาว 10% w/w ก่อนและหลังสภาวะเร่ง (IC_{50} = 34.1 และ 37.9 ไมโครกรัม/มิลลิลิตร) ต้านการอักเสบได้ดีที่สุด ถัดมาคือ ครีมผสมของสารสกัดขมิ้นขาว 5% (IC_{50} = 42.9 และ 44.7 ไมโครกรัม/มิลลิลิตร) และ 2% ก่อนและหลังสภาวะเร่ง (IC_{50} = 49.1 และ 49.6 ไมโครกรัม/มิลลิลิตร) ตามลำดับ และครีมดังกล่าวมีฤทธิ์ต้านการอักเสบที่ดีกว่า diclofenac gel (IC_{50} = 54.3 ไมโครกรัม/มิลลิลิตร) ครีมที่มีส่วนผสมของสารสกัดขมิ้นขาวที่ความเข้มข้น 1 และ 3 ไมโครกรัม/มิลลิลิตร ช่วยกระตุ้นการเจริญเติบโตของเซลล์เพาะเลี้ยง HDF cells (102.2-110.0%) ได้ดีกว่าที่ความเข้มข้น 10 และ 30 ไมโครกรัม/มิลลิลิตรเมื่อทดสอบโดยใช้วิธี MTT assay นอกจากนี้ครีมที่มีส่วนผสมของสารสกัดขมิ้นขาว 5% (ความเข้มข้น 3 ไมโครกรัม/มิลลิลิตร) ก่อนและหลังทดสอบสภาวะเร่ง ช่วยสมานแผลได้ดีที่ 36 ชั่วโมง (% การเคลื่อนที่เซลล์ = 80.1 and 75.2) ถัดมาคือ ครีมที่มีส่วนผสมของสารสกัดขมิ้นขาว 2% (% การเคลื่อนที่เซลล์ = 78.7 and 73.5) และ 10% (% การเคลื่อนที่เซลล์ = 76.4 and 72.8) จากการศึกษาเห็นได้ว่าตำรับครีมที่กล่าวมาน่าจะช่วยลดการอักเสบและกระตุ้นการเจริญเติบโตของเซลล์และช่วยสมานแผลได้

Thesis Title Biological Activities of Cream Containing *Curcuma mangga* Extract
Author Miss Suthasinee Srirod
Major Program Pharmaceutical Sciences
Academic Year 2018

ABSTRACT

Curcuma mangga Valeton & Van Zijp (Zingiberaceae family) is an annual plant. The rhizomes of this plant are traditionally used for the treatment skin diseases, womb healing and elixir. Pure compounds from *C. mangga* are diterpenes and curcuminoids which have anti-inflammatory property. In this study, the cream formulation was prepared by using the most stable cream base (formula 2) selected from the 3 preparations of cream base. The most stable cream base was chosen to mix with 2-10% w/w of *C. mangga* extract. After that, the physical and chemical properties of formulation were investigated as well as evaluated biological activities (anti-oxidant, anti-inflammatory and wound healing effects). The results showed that cream containing *C. mangga* showed good consistency, homogeneity, pH range of acid value (5-6) and stable after heating-cooling test. For antioxidant activity, cream containing *C. mangga* exhibited mild inhibition of DPPH radical scavenging (IC_{50} value >100.0 µg/ml) and cream containing *C. mangga* protected intracellular free radical from measuring with H_2O_2-induced oxidative stress. The effect of cream containing *C. mangga* were examined on LPS-responses in murine macrophages RAW264.7 cell line. Cream containing 10% w/w *C. mangga* before and after heating-cooling test (IC_{50} = 34.1 and 37.9 µg/ml) inhibited inflammation better than cream containing 5% (IC_{50} = 42.9 and 44.7 µg/ml) and 2% *C. mangga* (IC_{50} = 49.1 and 49.6 µg/ml), respectively. In addition, the anti-inflammatory effect of this formulation was better than the positive control (diclofenac gel) with IC_{50} value of 54.3 µg/ml. The result of wound healing found that cream containing *C. mangga* before and after heating-cooling test at 1 and 3 µg/ml increased HDF cells proliferation by MTT assay (102.2-110.0%) more than other concentrations (10, 30 µg/ml) while cream containing 5% w/w *C. mangga* extract before and after heating-cooling test at 3 µg/ml enhanced the cells movement at 36 h (% cell migration = 80.1 and 75.2) followed by 2% (% cell migration = 78.7 and 73.5) and 10% w/w (% cell migration = 76.4 and 72.8). Consequently, cream containing *C. mangga* extract could reduce inflammation and increased cell viability and recovered the wound.

ACKNOWLEDGEMENT

First, I would like to express my sincere appreciation to my research advisor Associate Professor Dr. Supinya Tewtrakul for the continuous support of my study and related research. Guidance of advisor helped me in all long of research and writing of this thesis. In addition, I would like to thank the rest of my thesis committee for their perceptive comments and encouragement. I would like to grateful for the assistance given by Dr. Teeratad Sudsai for RAW264.7 cell line and HDF cells in this research.

I am also grateful to the university staffs of Prince of Songkla University for their unfailing assistance and the Center of Excellence for Innovation in Chemistry (PERCH-CIC) as well as the Graduate school of Prince of Songkla University for funding.

I thank any academic staffs in my department who has given advice or support or assisted my research and grateful to the management of Pharmaceutical Sciences, Prince of Songkla University for providing all the necessary facilities for the successful completion of this study.

Finally, I wish to thank my family and friends who have helped me along the way and continuous encouragement throughout my study, through the process of research, writing this thesis and my life in general. I thank all who help me to finish of this thesis. This accomplishment would not have been possible without them. Thanks for all your support.

Suthasinee Srirod

CONTENTS

CONTENTS (continued)

CONTENTS (continued)

CONTENTS (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

BHT	butylated hydroxytoluene
Bis	bisdemethoxycurcumin
CM cream	cream containing *C. mangga* extract
cP	centipoise
Cur	curcumin
De	demethoxycurcumin
DMEM	dulbecco's modified eagle medium
DMSO	dimethyl sulfoxide
et al.	et alibi
etc.	et cetera
EtOH	ethanol
g	gram
h	hour
H_2O_2	hydrogen peroxide
HC	heating cooling test
HDF	human dermal fibroblast
HPLC	high performance liquid chromatography
IC_{50}	inhibitory concentration at 50% of tested subject
kg	kilogram
L	liter
LPS	lipopolysaccharide
mg	milligram
min	minute
ml	Milliliter
mM	millimolar
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NO	nitric oxide
O/W	oil in water
P	*p*-value
pH	potential of hydrogen

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

q.s.	quantity sufficient
rpm	round per minute
RPMI	Roswell Park Memorial Institute 1640 medium
S.E.M.	standard error of the mean
v/v	volume by volume
w/w	weight by weight
%	percentage
°C	degree of Celsius
µl	microliter

CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Wound is a destroy skin which often occur because of an accident or pain. Healing of the wound is an important process which is protects, restores and repairs functional of the tissues and organs after organs or tissue damage or was attacked from the foreign body and toxic environment. Period of healing depends on size of wound and health (Ghayempour et al. 2016). The wound repairing process is composed of inflammatory phase, proliferation phase and remodeling phase. Inflammation is a complex biological response of vascular tissue. This procedure is induced by inflammatory mediators (histamine, prostaglandins, bradykinin and nitric oxide) and white blood cells (mast cells, neutrophil, monocyte and macrophages) into the area of tissue damage. While macrophages eliminate foreign body by phagocytosis and it can induce free radical formation (Patrulea et al. 2015; Mittal et al. 2013; Sun et al. 2003).

Free radical is an unstable molecule and can cause cells damage. Generation of free radicals derived from cellular metabolites or environmental effect are substances that can be found in tobacco smoke, air pollution, alcohol and UV-ray. Free radicals are divided into several types for example reactive oxygen species (ROS) including superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2) and the reactive nitrogen species (RNS) such as nitric oxide radical ($NO^{\bullet}$) (Chung et al. 2013; Wirasorn et al. 2014; Halliwell 2009). Nitrogen monoxide or NO is a gas that is important to the body and affects the physiological processes, blood circulation and inflammation. It is pro-inflammatory mediator produced from L-arginine which is oxidized with nitric oxide synthase (NOS). L-arginine is converted to L-citrulline. Beside, H_2O_2 is another free radical, made from vascular cells and inflammatory mediator and induces oxidative stress. The long term consequences or high level of accumulated free radicals (oxidative stress) are often associated with the body harm, illness and several diseases such as inflammatory disease, cancer, cardiovascular disease, alzheimer disease and aging process. Inhibition of

excessive inflammation mediator or free radicals is another way to prevent and treat the disease (Barnes and Belvisi 1993; Joo et al. 2014).

In the current trends, people attend to use natural herbs for health care. In addition, herbals have been studied on biological activities and active compounds especially Zingiberaceous plants such as *Curcuma longa* rhizome contains volatile oil, curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) and these compounds showed many properties such as antioxidants, anti-inflammatory and anti-ulcer activities. *Curcuma mangga* rhizome is the one of medicinal plants and contains phenolic compounds (ferulic acid, *p*-coumaric acid, cinnamic acid and caffeic acid), curcuminoid and diterpenes such as 15,16 bisnorlada-8(17), 11-dien-13-one and (*E*)-15,15-diethoxylabda-8(17),12-dien-16-al. This plant has been used as an alternative medicine and showed good anti-inflammatory activity (Kaewkroek et al. 2010; Kaewkroek 2009). The aim of this research is using *C. mangga* rhizomes which present anti-inflammation to develop the cream formulation and evaluate anti-inflammatory, anti-oxidation and wound healing effects of cream.

1.2 Review of Literature

1.2.1 Healing of wound

The skin is a barrier to protect the body from infection and radiation. There are many types of wound such as instance abrasions, lacerations, crush, contused and incised wound (Patrulea et al. 2015). Wound is a destroy skin which often occur because of an accident or pain.

Healing is an existence mechanism to save normal function and structure of body. Would healing is a spontaneous process after tissue damage. It has protection, repair and recover process of organ tissue or skin injury which relate to many cells (fibroblasts, smooth muscle cells, endothelial cells, immune cells and keratinocytes), cytokines or growth factors (Grazul-bilska et al. 2003). This process is very important to protect and prevent organs of the body (Ghayempour et al. 2016). The process of wound healing begins after an injury and continues for several months or years (Mittal et al. 2013). The duration of wound healing depends on wound size and health of patient. The process of wound healing can be divided into 3 main stages for instance inflammatory phase,

proliferative phase and remodeling phase (Sudsai et al. 2013). The basic different phases of wound healing are listed in Table 1 and Figure 1.

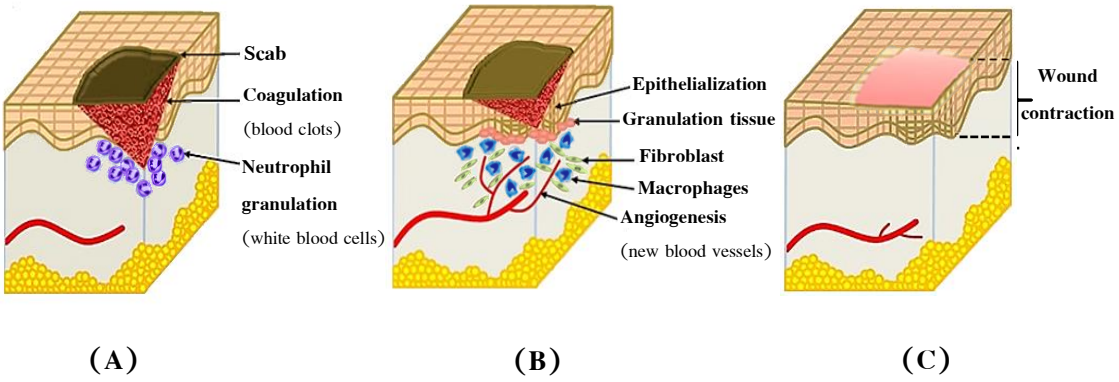

Figure 1 Stage of wound healing
(A) Inflammatory phase, (B) Proliferative phase and (C) Remodeling phase (Jayaraman et al. 2013)

Table 1 Phases of wound healing (Jayaraman et al. 2013)

Difference of the wound healing	Phase of wound healing		
	Hemostasis and inflammatory	Proliferative	Remodeling or maturation
Period of time	Immediately-Day 4	Day 4-3 weeks	3 weeks-2 years
Cells relate to heal	Platelets Neutrophils Macrophages	Macrophages Angiocytes Neurocytes Lymphocytes Fibroblasts Keratinocytes	Fibrocytes
Function	Clotting Phagocytosis and prepare for recover	Rebuild and increase new tissue	Develop wound tensile strength and new skin

1.2.1.1 Inflammatory phase (substrate or lag phase)

Inflammation is a mechanism to eliminate harmful stimuli, damaged cells, irritant substances or pathogens. It is the beginning phase of the healing process which includes hemostasis and inflammation and occurs immediately after tissue damage. It is a complex reaction and important processes of vascularized tissue to local injury or cellular degeneration and necrosis. Moreover, reactions of inflammation are changed to blood circulation, increase leukocyte and monocyte, eliminate or destroy foreign body that endanger normal tissue, release platelet derived growth factor (PDGF) and transforming growth factor beta (TGF-β) for migration and cells division which causes reparation or regeneration the new tissue (Patrulea et al. 2015; Mittal et al. 2013).

There are two causes of inflammation that are non-infectious factors such as physical trauma (surgery, trauma and ischemic damage to body tissue), chemical agents (toxin, chemicals irritants, extremes of heat and cold, alcohol), biological agent (damage cells) and psychological effect (nervousness). The second cause of inflammation is infectious factors including bacteria, viruses and fungi. At this stage, it can be found signs of inflammation that comprise of redness (rubor), swelling (tumor), heat (calor), pain or discomfort (dolor) and loss of function. Leucocytes secrete inflammatory mediators such as histamine, prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) and kinin to the wound area which causing pain. While the secretion of plasma or fluid exudate in vascular space around the wound causes swelling. In addition, heat and redness symptoms are caused by vasoconstriction (Sun et al. 2003; Medzhitov 2010).

1) The inflammatory response

The inflammatory response begins when tissues are damaged or attacked from the invader (bacteria, toxic). White blood cells (mast cells) are activated to release chemical mediators (histamine, prostaglandins and bradykinin) into the blood. These mediators cause vasodilation and increase capillary permeability of surrounding tissue injury. Some mediators lead to leak of fluid and enlist wandering macrophages (phagocytes) into the tissues which is the cause of swelling. Macrophages destroy pathogens resulting to the production of free radicals. Furthermore, some mediators

stimulate nerves result to pain and loss of function (Sun et al. 2003; Chen et al. 2018) (Figure 2).

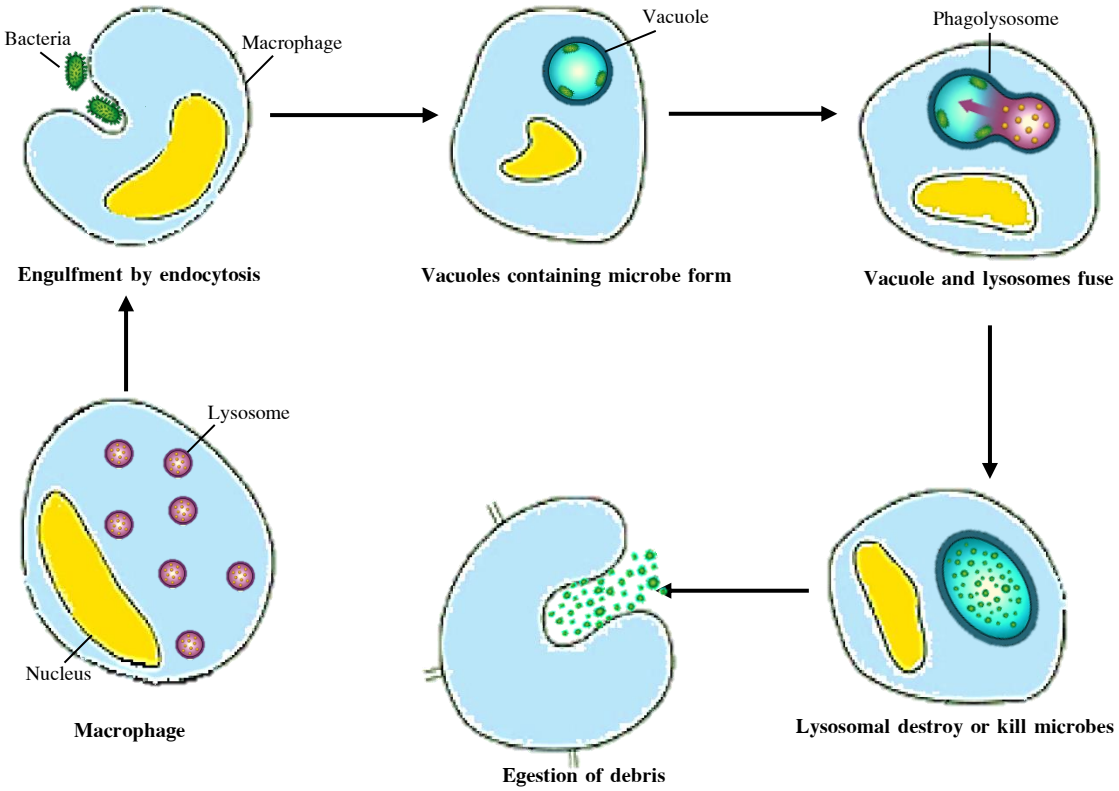

Figure 2 Procedure of a macrophage ingesting bacteria (Pathmakanthan and Hawkey 2000)

2) Inflammatory chemical mediators

Inflammatory mediators are produced from plasma and it is also secreted from certain cells. These chemical mediators attach to receptors on various cells that play a role of inflammation. The types of mediators and target cells cause different inflammatory responses. The chemical mediators involved inflammatory process including NO, prostaglandin E_2 (PGE_2) and tumor necrosis factor-alpha (TNF-α) (Guzik et al. 2003; Juhn et al. 2008). These inflammatory mediates are shown in Table 2.

Table 2 Sources and functions of inflammatory mediators (Guzik et al. 2003; Juhn et al. 2008)

Derived from	Groups	Chemical mediators	Sources	Functions
Cells	Preformed	1. Vasoactive amine - histamine - 5-hydroxytryptamine (serotonin)	Mast cells, Basophils, Platelets	Vasodilation, Vascular permeability, Endothelial activation
		2. Lysosomal compounds - proteases	White blood cell, Monocyte	White blood cell recruitment, Endothelial activation
	Newly synthesized	1. Prostaglandins - prostacyclin (PGI_2) - PGD_2, PGE_2, PGF_2	Mast cell, Leukocytes,	Vasodilation, Pain, Fever, Inhibits platelet aggregation
		2. Leukotrienes - thromboxane (TxA_2) - LTA_4, LTB_4, LTC_4 - LTC_4, LTD_4, LTE_4	Mast cells, Leukocytes	Vascular permeability, Platelet aggregation, Vasoconstriction
		3. Platelet activating factor (PAF)	Mast cells, Leukocytes	Vascular permeability, Platelet aggregation,
		4. Cytokines - interleukin-1 (IL-1) - tumor necrosis factor (TNF) - interleukin-8 (IL-8)	Lymphocyte, Macrophage, Mast cells, Endothelial cells	Fever, Endothelial activation, Pain
		5. Nitric oxide (NO)	Macrophage, Endothelium	Vasodilation, Killing of microbes, Vascular permeability
		6. Oxygen derived free radical - superoxide	Leukocytes	Vasoconstriction, Killing of microbes, Leukocyte activation, Tissue damage

Table 2 Sources and functions of inflammatory mediators (Continued) (Guzik et al. 2003; Juhn et al. 2008)

Derived from	Groups	Chemical mediators	Sources	Functions
Plasma	Immune system	1. Complement activation - C3a - C5a	Plasma in liver Macrophage	Vascular permeability, Leukocyte adhesion Activation, Chemotaxis
	Blood coagulation (Hageman factor activation)	1. Kinin system - bradykinin	Liver	Vascular permeability, Pain, Vasodilation
		2. Coagulation system	Liver	Adhesion molecules, Fibroblast proliferation
		3. Fibrinolysis system	White blood cell	Inhibit blood clotting

3) Categories of inflammation

Types of inflammation are divided into two types. Acute inflammation is a short duration which begins over seconds, minutes, hours and days after cell damage. Fundamental cells in this type are neutrophils, mast cells, platelets and basophils. Acute inflammatory process causes vascular change which includes vasodilation, increased blood flow due to dilation of blood vessels (arterioles), plasma proteins exudate and migration of neutrophils out of the vessel into interstitial spaces (Benly 2015).

Chronic inflammation is a long duration. These processes of inflammations coincide with tissue destruction and healing. The causes of chronic inflammation are persistent infection, prolonged exposure to injury and autoimmune disease. Moreover chronic inflammations are due to infiltration with macrophages, lymphocytes, plasma cells, antibody and fibroblasts which contribute to fibroblast proliferation (Zamora et al. 2000; Kaewkroek 2009).

1.2.1.2 Proliferative phase (fibroplasias)

Proliferative phase is the second phase of wound healing. In this phase, fibroblast cells around the wound begin to proliferate, migrate and generate collagen.

It composes of granulation tissue formation, collagen deposition, contraction of lesion, epithelialization and angiogenesis (Patrulea et al. 2015; Mittal et al. 2013).

Granulation tissue (endothelial cells, macrophages and fibroblasts) formation and angiogenesis are normal process of wound repair. It cause new blood vessel by vascular endothelial cell of blood vessel injury. In this step, macrophages stimulate growth factors (angiogenic factors) consist in fibroblast growth factor (FGF), vascular endothelial growth factor (VEGE), PDGF, transforming growth factor α (TGF-α) and TGF-β (Velnar, et al. 2009). Epithelialization is replication and movement by traverses and mitosis the lesion from epithelial cells for protect the body, destroy pathogen and cell death (Mittal et al. 2013).

1.2.1.3 Remodeling phase (maturation or differentiation phase)

The final phase of the wound repair is maturation or remodeling. The processes of this phase comprise of wound contract and collagen remodeling when closing of the wound. Macrophages, endothelial cells and fibroblast cells secrete matrix metalloproteinases (MMPs). Type III collagen (result from the proliferative phase) is degrading and replace with type I collagen which is stronger collagen (Gonzalez et al. 2016; Reinke et al. 2012). Collagen is a protein which occurs from fibroblasts in the body and increases the tensile strength of the scar, collagen form tight and development of new skin (Patrulea et al. 2015).

1.2.1.4 Factors that can affect with a delay in wound healing

The affecting of wound healing in the human body has several factors. Local factors of wound healing are direct affect the characteristics of the lesion such as lack of oxygen, infection and foreign body. Systemic factors include elderly age, disease (anemia, obesity, liver disease and diabetes). Regional factors comprise of venous insufficiency, arterial insufficiency and neuropathy. In addition, other factors consist of stress, alcoholism, smoking, drugs (nonsteroidal anti-inflammatory drugs or NSAIDs, chemotherapeutic drug and steroids) and nutrition (Mittal et al. 2013; Hunt et al. 2000; Burns et al. 2003). All these factors are important for improvement of the wound treatment. Furthermore, healing of the wound depends on wound size and patient strengths.

1.2.2 Free radicals

Free radical or oxidant is a molecule or ions with electrons surrounding. Molecules are unstable and susceptible to react in a chain reaction. It can react with biomolecules that are being built around and cause damage to various components of the cells within the body (Sarma et al. 2010; Pham-Huy et al. 2008).

Sources of free radicals in all kinds of living organisms will occur all the time. The free radicals are caused by internal body factors, the process of metabolism, metabolites, reaction of the formation and breakdown of molecules. The external body factor such as obtaining medicines, particular medicines in antimicrobial and anticancer drugs, radiation, cigarette smoke and ozone can cause free radicals in the body (Diaz et al. 2012). Free radicals exhibit an important part in cellular responses together with reactive oxygen species (ROS) [superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2)] and reactive nitrogen species (RNS) such as nitric oxide radical ($NO^{\bullet}$) and nitrogen dioxide radical ($NO_2^{\bullet}$) (Chung et al. 2013; Wirasorn et al. 2014; Halliwell 2009) (Figure 3).

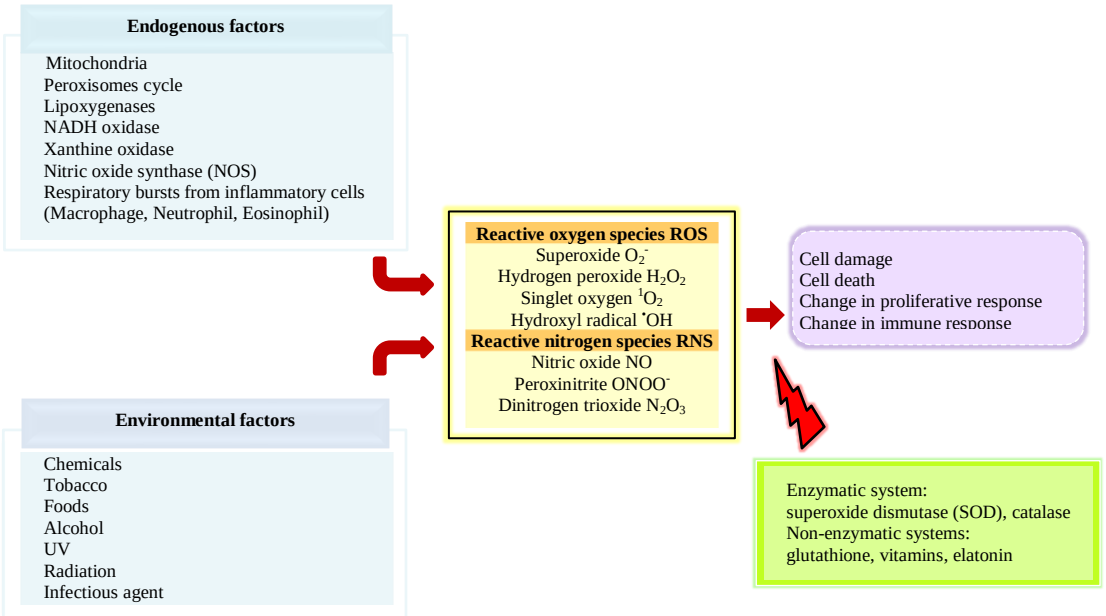

Figure 3 Sources of free radicals
(Thanan et al. 2015; Addor 2017)

1.2.2.1 NO

Nitric oxide (nitrogen monoxide, $NO^{\bullet}$) is a highly reactive radical, intermediate molecule, colorless gas at room temperature and is cytotoxic agent or causes cell injury. It consists of one atom of nitrogen which produced from terminal nitrogen in guanidine group of L-arginine and one atom of oxygen (Barnes and Belvisi 1993). The source of NO is an important signaling group of atoms that performs a crucial role in the pathogenesis of an inflammatory disease. It presents an anti-inflammatory effect underneath situation of normal physiology for instance blood pressure regulation, wound repair and host defence mechanism (Tatsutomi et al. 2016; Lechner et al. 2015). Whereas, NO is regarded as a pro-inflammatory mediator that persuade inflammation owing to overabundance in unnatural conditions. NO is accepted to influence vasodilatation in cardiovascular system and then induces tissue damage assorted with acute and chronic inflammations (Kotha et al. 2015; Sharma et al. 2007). In addition, NO is catalyzed from L-arginine which is reflected semi-essential amino acid in mammalian cells by oxidation of the enzyme nitric oxide synthase (NOS) (Barnes and Belvisi 1993; Joo et al. 2014).

There are three NOS isoforms from the amino acid L-arginine which operate in different parts of the body: neuronal NO synthase (nNOS, NOS I) expressed in keratinocytes and melanocytes, endothelial NO synthase (eNOS, NOS III) found in epidermis, fibroblasts, capillaries and exocrine glands. Macrophage or inducible NO synthase (iNOS, NOS II) influence in fibroblasts, keratinocytes, Langerhans and endothelial cells (Lechner et al. 2015; Yoshioka et al. 2016; Frank et al. 2002).

Macrophages are considerable constituents of immune system, protecting host cells from infection and production of several inflammatory mediators for example interferon, interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), prostaglandins together with the short-lived free radical NO (Nicholas et al. 2007). Interferon-gamma (INF-γ) and lipopolysaccharide (LPS) are stimulants of macrophages and involve the pro-inflammatory cytokines production. It is the reason that the inhibition of NO creation in LPS-activated RAW264.7 cells is used for anti-inflammatory activity assay (Joo et al. 2014).

1.2.2.2 H_2O_2

H_2O_2 is not a free radical and moderately involve in chemical reaction. It influences oxidative stress, made from vascular cells and inflammatory which is produced from NADPH oxidase pending inflammation process. H_2O_2 is transformed to water through enzyme catalyzed reaction (catalase and glutathione peroxidase) (Figure 4) (Coyle et al. 2006; Halliwell et al. 2000; Othman et al. 2015).

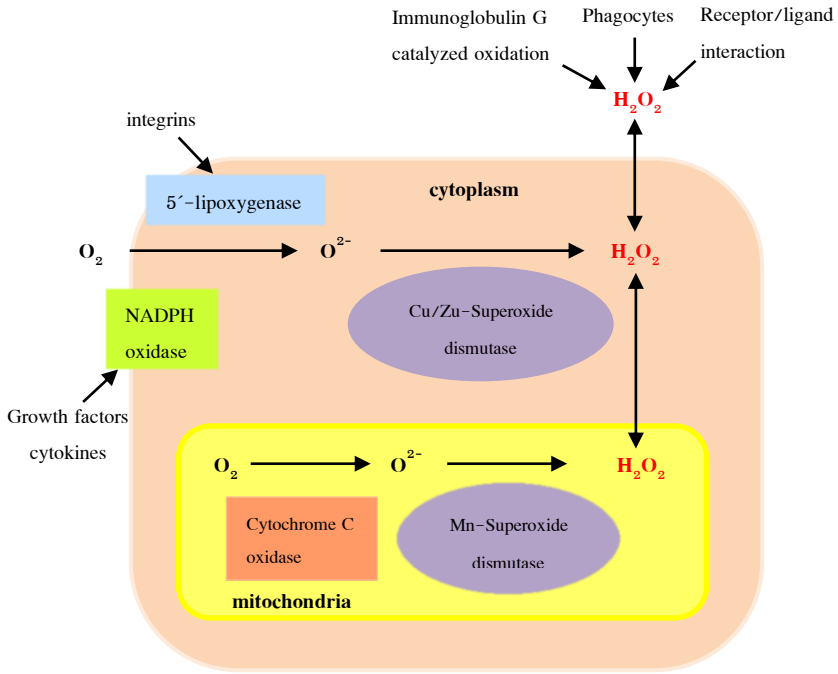

Figure 4 Sources of hydrogen peroxide (Veal et al. 2007; Coyle and Kader 2006)

1.2.2.3 Anti-oxidation

Antioxidant is a chemical compound that can prevent or slow down oxidation in the body. It has a mechanism to capture or scavenge radicals directly. Inhibition the generation of free radicals or the metal chelation can protect against free radicals and inhibit the enzyme as catalyst radicals.

Antioxidant can help strengthen the body and destroy free radicals. The antioxidants found in the body are catalase, glutathione peroxidase and superoxide dismutase or proteins compounds. These compounds control free radicals in the human

body. The imbalance between free radicals and antioxidants in the body causes oxidative stress that can damage the organs and tissues of the body.

The production of antioxidants in the body and eating antioxidants can control the amount of free radicals and the balance of free radicals. The mechanism of antioxidants can be divided into six mechanisms which it is shown in Table 3.

Table 3 The mechanism of antioxidants in the body (Jirum et al. 2011)

Mechanisms	Functions	Examples
Radical scavenging	Antioxidants give hydrogen atom or electrons to free radicals, resulting in the stability of free radicals.	Flavonoids, Lignans, Carotenoids, Phenolic acids, Ascorbic acids, Propyl gallate (PG), Tocopherols, Ubiquinone (Coenzyme Q), Butylated hydroxyanisole (BHA), Butylated hydroxytoluene or Dibutylhydroxytoluene (BHT), Tert-butylhydroquinone (TBHQ)
Singlet oxygen quenching ($^1O_2$)	Change singlet oxygen quenching to triplet oxygen and release energy	Carotenoids
Metal chelating	Prevent metal redox cycling	Fe^{2+}, Cu^{2+}, Flavonoids, Citric acid, Phosphoric acid
Enzyme inhibition	Inhibits the action of lipoxygenase and binding cofactor (iron ion)	Phenolic compounds (Flavonoids, Phenolic acid and Gallates)
Synergism	Supports function of antioxidants	α-Tocopherol, Ascorbic acid
Chain breaking	Protects cell membranes from lipid autooxidation and electron-acceptor antioxidants from peroxyl radicals ($ROO^{\bullet}$)	α-Tocopherol

1) Source of antioxidants

1.1) Synthetic antioxidant is antioxidants generally used in the food industry and inhibit the oxidation of fat which makes food taste, smell and color changes (Pokorny et al. 2001).

1.2) Natural antioxidants are safe to consume, rather than synthetic antioxidants. They are found in plants, animals and microorganisms by trapping free radicals but do not stimulate or cause oxidation (Pokorny et al. 2001).

1.2.3 Plant material (*Curcuma mangga* Valeton & Van Zijp)

1.2.3.1 Botanical characteristics

Curcuma in one of the largest general in the family Zingiberaceae which comprise rhizomatous plant approximately eighty species. It is extensively propagated in Asia from India to South China, Southeast Asia, and Northern Australia. In Thailand, it is discovered thirty-eight species in the evergreen forest.

Curcuma mangga Valeton & Van Zijp is a herbaceous plant in a Zingiberaceae family, generally known as mango ginger, mango turmeric and Thai people called khamin-khao (Sakunsom 2006). The smell of the rhizomes is similar to that of mango (Pujimulyani et al. 2013). The color of the rhizomes is yellowish-brown outside and pale yellow inside (Sirirugsa et al. 2007). Main rhizome of this species has round shape or ellipsoid but lateral rhizome is cylindrical and abundantly branch. Leaf sheath is superimposable and leaf blade (Nooheet 2014). Coma bracts are white or pink (Figure 5).

C. mangga is grown throughout Thailand. It is also locally grown in Java and Malay Peninsula (India, Myanmar, Vietnam, Thailand, Cambodia, and Sumatra) (Sirirugsa et al. 2007). *C. mangga* grown in sandy loam and thrives in rainy season. Furthermore people use this rhizome and shoot as vegetable and food that eat with chili sauce, pickled fish or a curry. In traditional medicine, it is the elixir and detoxify of poisonous plants (Sakunsom 2006), treatment of skin diseases such as red spot from itching and can reduce body heat caused by fever (Indis and Kurniawan 2016) and treatment of womb healing (Abas et al. 2005).

Figure 5 Botanical illustration of *C. mangga* Valeton & Van Zijp
(A) Leaf, (B) Flower and (C) Rhizome of *C. mangga*

Nutrition value of *C. mangga* rhizomes 100 g comprise of energy 47 kcal, protein 400 mg, fat 1,300 mg, carbohydrate 8,600 mg, fiber 1,100 mg, thiamine (vitamin B1) 0.03 mg, riboflavin (vitamin B2) 0.04 mg and ascorbic acid (vitamin C) 1.95 mg (Lim 2016).

1.2.3.2 Medical use and properties of *C. mangga* rhizomes

The compounds isolated from ethyl acetate and methanol extract of *C. mangga* rhizome which is labdane diterpenoids showed activity against free radical compared to BHT (butylated hydroxytoluene) and antioxidant activity using ferric thiocyanate method (FTC) compared with α-tocopherol (positive control) showed that ethyl acetate extract of *C. mangga* inhibited radicals at 67.8% (Sakunsom 2006) and all pure isolates from aqueous, methanol and ethyl acetate extracts of *C. mangga* at 25 μg/ml showed cytotoxic activity against human tumor cell lines, such as lung cancer (CI-H460), colon cancer (HCT-116), gastric cancer (AGS), CNS cancer (SF-268) and breast cancer (MCF-7) (Liu and Nair 2011).

Crude extract and compounds purified from *C. mangga* rhizome displayed anti-inflammatory activity. The chloroform fraction of *C. mangga* showed the highest activity by inhibition of NO production, followed by hexane, ethyl acetate and water fractions, respectively. The compounds purified from *C. mangga* rhizome were found to be (*E*)-15,15-diethoxylabda-8,12-dien-16-al (diterpene diethyl acetal) which inhibited NO production and demethoxycurcumin inhibited prostaglandin E_2 (PGE_2) release

in RAW264.7 cells. Demethoxycurcumin, diterpene diethyl acetal and bisdemethoxycurcumin were found to inhibit iNOS and COX-2 mRNA expression (Kaewkroek 2009). The *in vivo* study of ethanol extract of *C. mangga* and its fractions for example aqueous, chloroform, ethyl acetate and hexane fractions, especially chloroform and hexane fractions possessed analgesic effect as well as anti-inflammatory activity (Ruangsang et al. 2010).

Essential oil of *C. mangga* fresh rhizome was analysed by GC/MS. The major compounds were monoterpene group (myrcene, 3-phellandrene and trans-ocimene) with antibacterial and antifungal effects and non-volatile components separated by column chromatography contain labdane diterpene (15-ethoxyl-8, 12-labdadien-15, olide), norlabdane diterpene (15,16-bisnorlabda-8, 11-dien-13-one) and steroid (β-sitosterol and stigmasterol) (Makboon 2000; Baharudin et al. 2015; Kamazeri et al. 2012). Moreover, ethanolic and water extracts, together with volatile oils from *C. mangga* rhizomes have been reported to exhibit anti-allergic activity using a RBL-2H3 cell line (Tewtrakul and Subhadhirasakul 2007). The antecedent studies showed that the extract from *C. mangga* rhizome contain many compounds. The chemical components of *C. mangga* rhizomes are shown in Table 4.

Table 4 Purified compounds of *C. mangga* rhizome extract

Group	Compound	Reference
Coumarin	Scopoletin	Abas et al. 2005
Phenolic compounds	*p*-Hydroxycinnamic acid	
Monoterpenes	Myrcene	Baharudin et al. 2015
	Trans-ocimene	
Diterpenes	15,16 Bisnorlabda-8(17), 11 dien-13-one	Kaewkroek 2009; Kaewkroek et al. 2010 ; Nooheet 2014
	(*E*)-15,15-Diethoxylabda-8(17),12-dien-16-al	Kaewkroek 2009
	(*E*)-Labda-8(17)-12diene-15,16-dial	Liu and Nair 2011

Table 4 Purified compounds of *C. mangga* rhizome extract (Continued)

Group	Compound	Reference
Diterpenes	14,15,16-Trinor-labdan-8,12-diol	Liu and Nair 2011
	Calcaratarins A	
	Communic acid	
	Copallic acid	
	Zerumin A	
	Zerumin B	
	8-Methene-1,1,10-trimethyldelcalin	
	1, 1, 10-Trimethyl-decalin	
Curcuminiods	1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	Abas et al. 2005
	Demethoxycurcumin	Abas et al. 2005; Kaewkroek 2009; Kaewkroek et al. 2010
	Bisdemethoxycurcumin	Abas et al. 2005; Kaewkroek 2009; Kaewkroek et al. 2010
	Curcumin	Abas et al. 2005
Steroid	β-Sitosterol	Malek et al. 2011

1.2.4 Structure and function of the skin

Skin is a largest organ and barrier function in the human body for protection the body from external environment, pathogenic, protect organs and fluids, reduce friction and concussion of wounds, control temperature in body and prevents loss of water (Csizmazia 2011; Kielhorn et al. 2006). The layer of the skin can be divided into three major layers including the epidermis which is the external layer contains of keratinocyte, the dermis composed of hair roots, sweat glands, blood vessel and subcutaneous layer (Ng et al. 2015) (Figure 6).

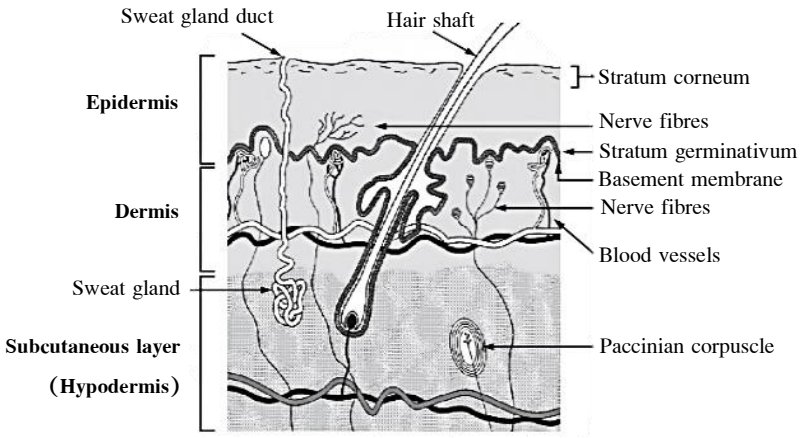

Figure 6 Structure of skin (Kielhorn et al. 2006)

1.2.4.1 Drug permeation routes of skin

Drug permeation is the passage of chemical or drug through the surface of the skin into the dermis and there are two major permeation pathways of stratum corneum (Csizmazia 2011; Volz et al. 2015).

1) Transepidermal penetration consists of two routes such as transcellular (intracellular) route and the intercellular route (Figure 7).

2) Appendageal route consist of sebaceous duct, hair follicle and sweat gland (Volz et al. 2015).

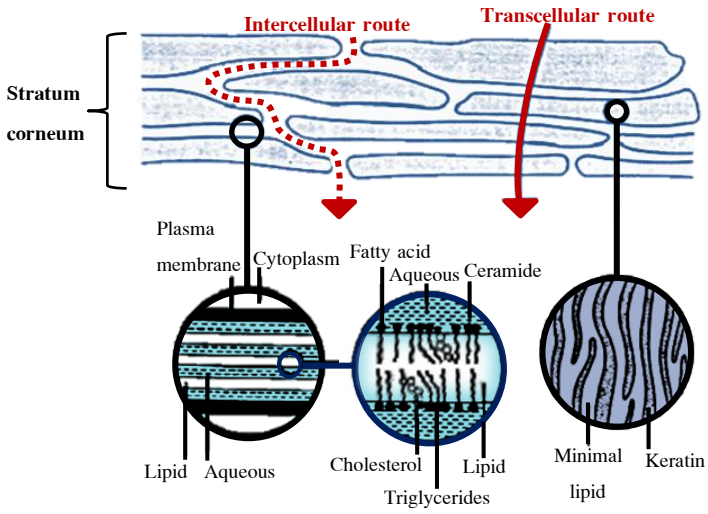

Figure 7 Route of drug permeation (Csizmazia 2011)

1.2.5 Pre-formulation and formulation development of semisolid dosage form

Pre-formulation studies is the first step of new formulation (prepared into pharmaceutical dosage form) and develop stable drug, safe and effective formulas associated with the characterization of the physical, physicochemical, stability, mechanical properties and compatibility of a new drug substance alone or when mixed with other ingredients (excipients) and packaging materials (Desu et al. 2015).

Semisolid dosage forms are topical dosage forms. Topical formulations are applied directly to an external body surface by dispersion, rubbing, infusion and spraying. The topical dosage forms used for the cosmetic, protective, therapeutic function and use to treat skin disorder. Semisolid dosage forms comprise many types (creams, lotion, gel or jellies, pastes, ointment) which are chosen for the research and development.

Cream is homogeneous and has softer consistency. There are two kinds of emulsion; hydrophobic creams or oil-in-water (o/w) creams (non-greasy), hydrophilic creams or water-in-oil (w/o) creams (greasy) (Ueda et al. 2009). A semisolid dosage form is advantageous in terms of its easy application and reduced the side effects because it is used to external body and local effect (Das et al. 2014). Creams are evaluated for various pharmacopeia and nonpharmacopeia tests to learn physicochemical, microbial screening, *in vitro* drug release studies and *in vivo* bioequivalence studies. These tests help in retentive quality (Mahalingam et al. 2007).

1.2.6 Pharmaceutical products containing herbal extracts

Plai (*Zingiber cassumunar* Roxb.) is a favorite plant used for muscles pain or injury. It was found that 14% Plai cream reduced delayed onset muscle soreness or analgesic better than 7% Plai cream (Manimmanakorn et al. 2016).

Cream containing 1% and 2% ethanol extract of *Curcuma aromatica* Salisb rhizome (oil in water and water in oil cream formulation) exhibited anti-inflammatory activity in arachidonic acid-induced ear inflammation. The percentage inhibition of inflammation was found to be 2% w/o cream formulation (0.05 g/ear) showed the highest inhibition of inflammation (68.8%) and it was compared to standard drug (phenidone 1mg/ear) (73.3%). The extract and cream from *Curcuma aromatica* found to play anti-inflammatory and wound healing activity in animal models (Kumar et al. 2009).

Methanolic extract of Fenugreek seed (*Trigonella foeum-graecum* L.) at doses 100, 200 and 400 mg/kg reduced the paw edema (using carrageenan-induced paw edema) in mice, beside cream containing 2-5% fenugreek seed extract (0.3 g) inhibited paw edema after topical administration (applied to the plantar surface of the left hind paw by softly scrubbing 50 times with the index finger) (Sharififara et al. 2009).

The cream containing with 1%, 2% and 4% w/w of *Daucus carota* L. root (Carrot) ethanol extract when applied topically in animal model decreased scar size and increased wound contraction on excision wound model (wounds size 300 mm^2 and 2 mm deepness) and on incision wound model increased in tensile strength, hydroxyproline and protein content (Patil et al. 2012).

The topical formulations comprise with *Ichnocarpus frutescens* (L.) R.br root methanol extract showed anti-inflammatory and analgesic effects by carrageenan-induced rat paw edema in rats and formalin-induced paw licking test in rats. Cream containing 1-6% w/w *Ichnocarpus frutescens* reduced paw edema when compared to the control group, especially the formulation containing 6% w/w of methanol extract of herbal root showed good inhibition of carrageenan-induced paw edema which was similar to the effect of 0.5% Piroxicam gel (Standard) (Kharat et al. 2010).

1.3 Objectives

1.3.1 To develop cream containing *C. mangga* extract.

1.3.2 To investigate antioxidant, anti-inflammatory and wound healing effects of cream containing *C. mangga* extract.

1.3.3 To study physical, chemical and biological stabilities of cream formulation containing *C. mangga* extract.

CHAPTER 2

RESEARCH METHODOLOGY

2.1 Equipment and Chemicals

2.1.1 Materials and Equipment

1) 96 well plate	Nunc, Denmark
2) 24 well plate	Nunc, Denmark
3) Centrifuge tube	SPL Life Sceinces
4) Pipettes	Pipetman, France
5) Pipette tips	KIRGEN
6) Microplate reader	BMG: SPECTRostar Nano
7) ESCO Class II biological safety cabinet	ISOCIDE
8) CO_2 incubator	Thermo scientific
9) Centrifuge	Hermle Z383K
10) Viscometer	Scientific promotion
11) pH meter	SN AMS1-063H

2.1.2 Chemicals

1) Ethanol	RCI Labscan, Korea
2) Roswell park memorial institute 1640 medium (RPMI-1640 medium)	Gibco, USA
3) 2,2-Diphenyl-1-picrylhdrazyl (DPPH)	Sigma, Germany
4) Fetal bovine serum (FBS)	Sigma, USA
5) Penicillin streptomycin	Gibco, USA
6) Butylatedhydroxytoluene (BHT)	Sigma, Germany
7) Dulbecco's modified eagle medium (DMEM)	Gibco, USA
8) Dimethyl sulfoxide (DMSO)	Invitrogen, USA

9) N-(1-Naphthyl) ethylenediamine	Sigma, USA
10) Lipopolysaccharide from *E. coli* (LPS)	Sigma, USA
11) Indomethacin	Sigma, USA
12) Hydrogen peroxide (H_2O_2)	Merck, USA
13) 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl tetrazolium bromide (MTT)	Gibco, USA
14) 0.25% Trypsin-EDTA	Gibco, USA
15) Sulfanilamide	Sigma, China
16) Phosphoric acid	Sigma, Germany
17) Trypan blue stain	Gibco, USA
18) Sodium hydrogen carbonate ($NaHCO_3$)	Sigma, USA

2.2 Methods

2.2.1 Preparation of *Curcuma mangga* extract

The plant used for this study is *C. mangga* Valeton & Van Zijp rhizomes (Zingiberaceae). The plant was collected from a plantation located in Na Mom district, Songkhla, Thailand. The voucher specimen number is SKP No. 206 03 13 01. It was deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Prince of Songkla University. Five kilograms of the fresh rhizomes were cleaned and discarded of any contamination, cut into small pieces and dried 48 h in hot air oven (55-60ºC), then ground into coarse powder and passed it from sieve #10. The powder (800 g) was extracted with ethanol (EtOH) (3×1.5 L) by maceration method for 5 days (3 times) at room temperature (Kaewkroek 2009; Ruangsang 2010), filtrated, and evaporated (65ºC) using a rotary evaporator to acquire the ethanol extract of *C. mangga*. The extract of *C. mangga* was 3.08% w/w (24.62 g). The crude extract was kept in a bottle in the refrigerator at 4ºC (Tewtrakul and Subhadhirasakul 2007).

2.2.2 Cells culture and harvesting

The murine macrophage cell or RAW264.7 cells ATCC No. TIB-71 was maintained and cultured in 10% FBS (v/v), containing a Roswell Park Memorial Institute

1640 medium (RPMI-1640 medium) supplemented with sodium bicarbonate 2 g, 1% penicillin and streptomycin. The cells were changed every 3 days and were harvested when the culture reached 80% confluent with 0.25% trypsin-EDTA and diluted to a suspension in a new medium. Human dermal fibroblast (HDF cells) were cultured in DMEM (ATCC® PCS-201-010) containing 10% FBS, sodium hydrogen carbonate 3.7 g, 1% antibiotic mixture (penicillin and streptomycin) under sterile conditions in humidified at 37°C and incubator for 5% CO_2 (Tewtrakul et al. 2008; Kaewkroek 2010; Sudsai et al. 2013; Girija et al. 2017).

2.2.3 Cream formulation

The preparation of cream base of three master formulas is oil in water (O/W) emulsion cream. The preservatives and other water soluble ingredients were melted into the aqueous phase and heated to 70-75ºC in a water bath. The emulsifier and other oil soluble components were melted in the oil phase and heated to 70-75ºC. After heating, the aqueous phase was added into the oil phase with uninterrupted stirring till cooling of the emulsifier took place and the cream was formulated, active ingredient was added in the cream and was stirred continuously until it congealed. The finished product was transferred to a suitable package. The study on physical, chemical and biological stabilities of products was conducted. Development of cream formulation was mixed with *C. mangga* extract. The ingredients of cream formulation used are explained in Table 5 (Matangi et al. 2014).

Table 5 The components of cream formulation

Part	Ingredients	Function	Master formula (g)		
			1	2	3
A	Water	Vehicle	q.s.100	q.s.100	q.s.100
	Disodium EDTA	Bind to metal ions	0.1	0.1	0.1
	Glycerin	Humectants	3.0	3.0	3.0
	Propylene glycol	Humectants	5.0	5.0	5.0
	Carbopol gel 1%	Thickening agent	2.5	2.5	2.5
	Paraben concentrate	Preservative	0.5	0.5	0.5

Table 5 The components of cream formulation (continued)

Part	Ingredients	Function	Master formula (g)		
			1	2	3
B	Stearic acid	Emollients	2.0	2.0	2.0
	Cremophor A6	Emulsifiers	1.0	1.0	1.0
	Cremophor A25	Emulsifiers	1.0	1.0	1.0
	Stearyl alcohol	Stiffening agent	1.0	1.0	1.0
C	*C. mangga* extract	Active ingredient	1.0-10.0	1.0-10.0	1.0-10.0

q.s.: Quantity sufficient

2.2.4 Evaluation of physical properties of cream base and cream containing *C. mangga*

2.2.4.1 Determination of organoleptic properties

The presentation of the cream is evaluated by its color, odor and texture (Dhase et al. 2014).

2.2.4.2 Determination of the pH

The pH meter was calibrated using a solution of standard buffer and pH of the prepared formula was measured using a digital pH meter (Saad et al. 2013).

2.2.4.3 Determination of viscosity

A Brookfield Dial Reading Viscometer (RVT) was used to measure the viscosity (in cP) of cream. The spindle (Helipathspindle set) was used for spindle number T-F. The spindle was rotated at 10 rpm. The determinations were carried out in triplicate and the mean of three readings was reported (Dhase et al. 2014).

2.2.4.4 Separation of cream

Cream was tested by weighing 10 g cream and put in a centrifuge tubes and then centrifuge at 5,000 rpm at 25ºC for 10 min.

2.2.5 Evaluation of the stability of cream base and cream containing *C. mangga*

The stability was tested in various conditions (6 cycles) of the heating cooling test: 45ºC (in incubator) 48 h alterations with 4ºC (in refrigerator) 48 h for 1 cycle (Leelapornpisid et al. 2014).

2.2.6 Evaluation of chemical property of cream base and cream containing *C. mangga*

The high performance liquid chromatography: HPLC (Shimadzu UFLC series) condition consisted of a reverse phase C_{18} analytical column. The mobile phase used 0.1% formic acid-acetonitrile (50:50, v/v) at a flow rate of 1.5 ml/min (20 min), with UV detection at 405 nm.

2.2.7 Biological properties of cream base and cream containing *C. mangga* extract

2.2.7.1 Anti-oxidation activity tests

1) 2,2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay

DPPH free radical scavenging method is one of antioxidant activity (Figure 8). The method was recited and modified for this study. The stock solution of the sample extract (10 mg/1,000 µl) is prepared in dimethyl sulfoxide (DMSO) and diluted to various concentrations (10, 30, 100 µg/ml) with absolute ethanol in 96 well plate. The reaction mixture contains 100 µl of sample at various concentrations and 100 µl of 0.1 mM DPPH in absolute ethanol. The commercial standard antioxidant such as butylated hydroxytoluene (BHT) was used as a positive control. The DPPH solution in the absence

of sample was used as a control and the absolute ethanol was used as a blank. After incubation for 30 min in the dark condition, the absorbance values were measured at 517 nm using a microplate reader and calculated the percentage of scavenging activity. The percentage of the sample against DPPH radical was followed equation and IC_{50} value, which showed the sample concentration at 50% of the DPPH radical scavenged (Unlu et al. 2003; Indis and Kurniawan 2016).

$$\% \text{ Inhibition} = [(A_{ctr} - A_{sample}) / A_{ctr}] \times 100$$

A_{ctr} = Absorbance of control − Absorbance of control blank

A_{sample} = Absorbance of sample − Absorbance of sample blank

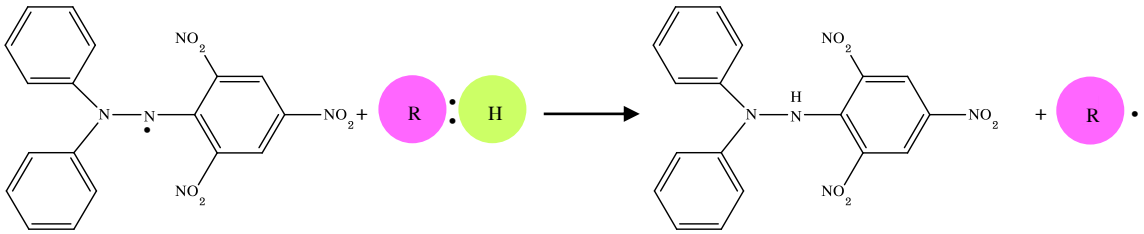

Figure 8 Reaction mechanism of DPPH with antioxidant (Liang and Kitts 2014)

2) Hydrogen peroxide induced oxidative stress assay (H_2O_2-induces oxidative stress)

H_2O_2 is a stable ROS. It is a considerable signaling molecule in vascular cells. H_2O_2-induced oxidative stress was determined by measuring cell viability by MTT assay. The methodology was modified from Sudsai et al. (2016). HDF cells are seeded in 96 well plate at 8×10^3 cells/well in 10% FBS mix with DMEM medium to confluence and then after 24 h, cells are treated with various concentrations (1-100 μg/ml) of extracts. After pretreatment with different concentrations for 1 h incubation at 37˚C in a humidified atmosphere containing 5% CO_2, the cells are co-incubated with 0.9 mM of H_2O_2 for another 24 h. At the end of the incubation, cell viability is determined by the MTT assay.

2.2.7.2 Anti-inflammation test (Anti-NO production assay)

Inhibitory effect on NO production by murine macrophage-like RAW264.7 cells was evaluated using a modified method from Tewtrakul and Subhadhirasakul (2008). Briefly, RAW264.7 cells were cultured in RPMI-1640 medium, seeded in 96 well plate with 1×10^5 cells/well and incubated 1 h at 37°C (5% CO_2). Next, the medium was replaced with a fresh medium containing lipopolysaccharide LPS from *E. coli* 1 μg/ml (endotoxin from Gram-negative bacteria) and samples were added at various concentrations (10-100 μg/ml) for the test group. After that the cells were incubated for 24 h. NO production of RAW264.7 cells was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent (Figure 9). The optical density was measured with a microplate reader at 570 nm. COX inhibitors (indomethacin and diclofenac gel) were used as positive controls. The percentage of inhibition was calculated using the following equation and IC_{50} values are determined graphically (n=3).

$$\% \text{ Inhibition} = [(A_{ctr} - A_{sample}) / A_{ctr}] \times 100$$

A_{ctr} = Absorbance of control - Absorbance of control blank

A_{sample} = Absorbance of sample - Absorbance of sample blank

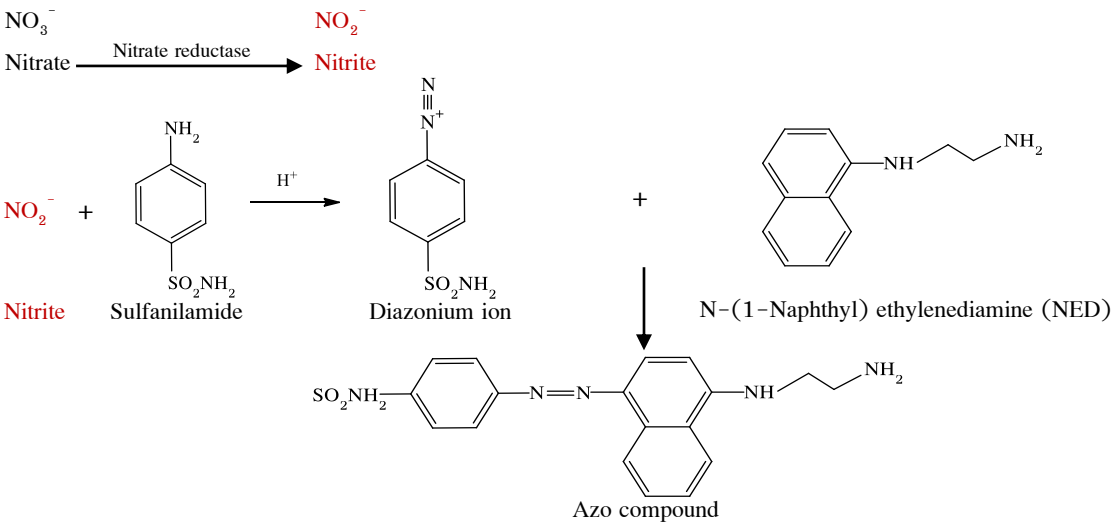

Figure 9 Principle of nitrite determination using the Griess reaction (Coneski and Schoenfisch 2012)

2.2.7.3 Cells viability test (MTT assay)

RAW264.7 cells were seeded in 96 well plate with 1×10^5 cells/well, added the test samples (10-100 μg/ml) and incubated 24 h. After that MTT solution (10 μl, 5 mg/ml in PBS) was added to the wells. Then 2 h incubation at 37ºC in a 5% CO_2, added DMSO 100 μl into each well to dissolve the formazan production in the cells. The absorbance of this purple solution was detected at 570 nm by microplate reader (Sudsai et al. 2013). The samples were determined the cytotoxicity when the optical density of the sample was less than 80% of the control group. The calculation was as follows. % Cell viability = (Absorbance of sample / Absorbance of control) ×100 (Sudsai et al. 2013)

2.2.7.4 Wound healing assay

1) Cells proliferation by MTT assay

The procedure reported by Gothai et al. (2016) and Sudsai et al. (2013) was used in this study with slight adjustment. HDF cells were seeded at 1×10^4 cells/well into 96 well plate with DMEM containing 10% FBS. For 24 h, 1-30 μg/ml of samples of various concentrations were added. Afterwards, this plate was incubated at 37°C with 5% CO_2 (48 h). 10 μl of 5 mg/ml MTT solution was added in each of the wells and then incubated for another 2 h at 37°C. The remaining MTT solution was dislodged and replaced with DMSO to dissolve the formazan crystal. The absorbance of the colored product was detected at 570 nm. The percentage of cell proliferation was evaluated and compared to negative control.

2) Cells migration (Scratch assay)

The procedure reported by Gothai et al. (2016) and Sudsai et al. (2013) was used in this study and the methodology was modified from with slight adjustment. 10% FBS in DMEM was cultured HDF cells. 5×10^4 cells/well of the HDF cells were seeded into every well of 24 well plates. After incubation at 37ºC with 5% CO_2 until the HDF cells grew a confluent monolayer, two vertical scratches (left and right) were

generated in every well by using a 1,000 µl sterile pipette tip. Any cellular debris was took away by washing with PBS and replacing with 1,000 µl of the fresh medium in the absence or presence of test sample. Photographs were taken on day 0 (0 h), then plates were incubated in 5% CO_2 at 37ºC and then more photographs were taken at 12 h, 24 h and 36 h. The images were analyzed using computing software (Image J). A percentage of the closed area was measured and collated with the value obtained previous treatment with the samples (0 h). An increase of the percentage of closed area exhibited the migration of cells (Sudsai et al. 2013).

2.2.7.5 Statistical analysis

Data values of the results were shown in mean ± S.E.M of three computations. Statistical significance of data analysis was carried out by Duncan test, using the SPSS statistics: one-way analysis of variance (ANOVA) and p-value <0.05 was investigated as significant.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1 Preliminary formulation study of cream containing *C. mangga* extract

Cream is emulsions that emerge in semisolid form and varying degree of viscosity. Three recipes of cream base were prepared and tested for stability with the heating-cooling method. Their physical appearances were then observed, the color, odor, pH, viscosity and separation of cream before and after heating-cooling test, and the best prepared formulation or suitable cream base was selected. The stability studies of the various parameters are shown in Table 6. The cream base formula 2 showed good physical properties. Thus, cream base 2 was selected for further development of cream formulation mixed with 2%, 5% and 10% w/w of *C. mangga* extract.

3.2 Evaluation of physical properties of cream containing *C. mangga* extract before and after heating-cooling test

The formulations of cream containing *C. mangga* extract were kept at heating-cooling condition (45ºC and 4ºC, 6 cycles). This was confirmed by visual appearance. It was found that no change in color and smell of cream compared with those of before heating-cooling test. The tone of color is dependent on the concentration of the extract in the formulation. The pH of cream containing *C. mangga* before and after heating-cooling test was found to be in range of 5.0-6.0 which is suitable for skin because the pH of the human skin is between 4.0-6.0 (Ali and Yosipovitch 2013) and a pH value of 5.5 in skin formulations is advised to be average (Lambers et al. 2006). The pH values of formulations cream containing *C. mangga* remained constant with slightly increased values. The viscosity before heating-cooling test was in the range of 8,100-8,500 cP while after heating-cooling test was in the range of 6,600-8,000 cP. It was indicated that after the heating-cooling test, the viscosity was slightly decreased and this may be affected by the ingredients in the cream base. The formulated cream containing *C. mangga* was found to be stable without any separation when it was kept under heating-cooling conditions. There were no differences of physical properties among the creams containing *C. mangga* (Table 7).

Table 6 Physical properties of cream base

Parameters	Base 1		Base 2		Base 3	
	Before	After	Before	After	Before	After
Physical appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Color	White & glossy	White & glossy	White & glossy	White & glossy	White & glossy	White & glossy
Texture	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Homogeneous and smooth	Not homogeneous	Not homogeneous
pH values	5.5 ± 0.0^{c}	5.6 ± 0.0	5.5 ± 0.0^{c}	5.6 ± 0.0	$5.7 \pm 0.1^{a,b}$	5.6 ± 0.0
Viscosity ($\times 10^{3}$ cP)	$7.9 \pm 0.4^{*b,c}$	$9.1 \pm 0.5^{*c}$	$8.9 \pm 0.4^{a,c}$	9.9 ± 0.4^{c}	$6.0 \pm 0.5^{*a,b}$	$5.0 \pm 0.1^{*a,b}$
Separation of cream	No	No	No	No	No	No

*Statistical significant difference from before heating-cooling test (Mean ± S.E.M. of three determinations), $p<0.05$

[a]Significant difference from formulation 1

[b]Significant difference from formulation 2

[c]Significant difference from formulation 3

Table 7 Physical properties of cream containing *C. mangga* extract

Parameters	2% CM cream		5% CM cream		10% CM cream	
	Before	After	Before	After	Before	After
Appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Color	Beige	No change	Brown	No change	Dark brown	No change
Smell	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Texture	Smooth and homogeneous	Smooth and homogeneous	Smooth	Smooth	Immiscible	Immiscible
pH values	5.5 ± 0.1	5.5 ± 0.0[b,c]	5.6 ± 0.1*	5.9 ± 0.0*[a,c]	5.6 ± 0.0*	5.9 ± 0.0*[a,b]
Viscosity ($\times 10^3$ cP)	8.2 ± 0.3	8.0 ± 0.5	8.33 ± 0.3	7.5 ± 0.5	8.5 ± 0.5*	6.7 ± 0.6*
Separation	No	No	No	No	No	No

*Statistical significant difference from before heating-cooling test (Mean ± S.E.M. of three determinations), $p<0.05$

[a]Significant difference from 2% CM cream, [b]Significant difference from 5% CM cream, [c]Significant difference from 10% CM cream, CM cream = Cream containing *C. mangga* extract

3.3 Determination of chemical property (marker compounds) of cream containing *C. mangga* before and after heating-cooling cycles by HPLC

HPLC chromatogram of cream formulation containing *C. mangga* 1-10% w/w were tested for the presence of marker compounds (curcumin, demethoxycurcumin and bisdemethoxycurcumin) in formulation and demethoxycurcumin showed good anti-inflammatory activity and is the highest content in *C. mangga*. Demethoxycurcumin was analyzed using Shimadzu UFLC series (Kyoto, Japan) on a GL Sciences Inc. inertSustain C(18) analytical column (4.6x250 mm, 5 micron) using 0.1% formic acid in distilled water and acetonitrile (50:50, v/v) as mobile phase at a flow rate of 1.5 ml/min (20 min) with detection at 405 nm. The result is shown in Table 8 and Figure 10. HPLC result showed that the % demethoxycurcumin of cream containing *C. mangga* was slightly decreased after heating-cooling test.

Table 8 HPLC chromatogram of cream containing 2%, 5% and 10% w/w of *C. mangga* extract before and after heating-cooling test

Compounds	Samples	%Curcuminoids of cream containing *C. mangga* extract (Mean ± S.E.M.)	
		Before heating cooling test	After heating cooling test
Curcumin	2% CM cream	10.3 ± 0.2	9.7 ± 0.5
	5% CM cream	10.2 ± 0.1	10.0 ± 0.1
	10% CM cream	10.4 ± 0.1	10.3 ± 0.0
Demethoxycurcumin	2% CM cream	76.6 ± 0.4*	74.4 ± 0.3*
	5% CM cream	76.3 ± 0.2*	74.8 ± 0.1*
	10% CM cream	76.8 ± 0.4*	75.4 ± 0.4*
Bisdemethoxycurcumin	2% CM cream	13.1 ± 0.2*	17.5 ± 1.2*
	5% CM cream	13.5 ± 0.3*	15.5 ± 0.3*
	10% CM cream	12.8 ± 0.3*	14.2 ± 0.4*

*Significant difference between before and after heating-cooling test (Mean ± S.E.M. of three determinations), $p<0.05$

CM cream = Cream containing *C. mangga* extract

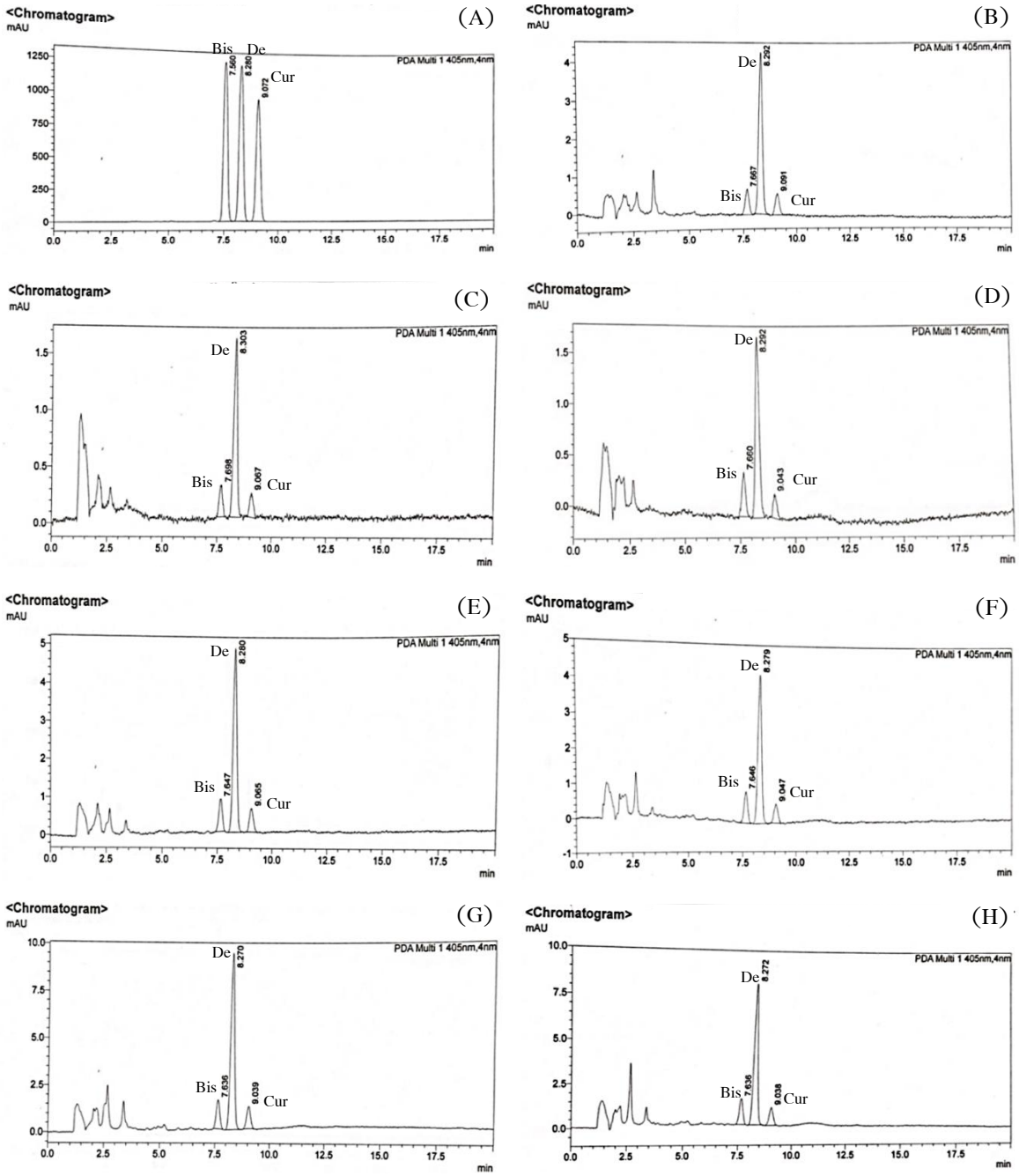

Figure 10 HPLC chromatogram of standard markers (A), *C. mangga* extract (B), cream containing 2% w/w *C. mangga* before heating-cooling (C), cream containing 2% w/w *C. mangga* after heating-cooling (D), cream containing 5% w/w *C. mangga* before heating-cooling (E), cream containing 5% w/w *C. mangga* after heating-cooling (F), cream containing 10% w/w *C. mangga* before heating-cooling (G) and cream containing 10% w/w *C. mangga* after heating-cooling (H)

(Bis = bisdemethoxycurcumin, De = demethoxycurcumin, Cur = curcumin)

3.4 Evaluation on biological activities of *C. mangga* extract, cream containing *C. mangga* extract before and after heating-cooling test

3.4.1 Anti-oxidations

3.4.1.1 DPPH assay

DPPH or $C_{18}H_{12}N_5O_6$ is a constant free radical. Molecular mass of DPPH is 394.33 g/mol. Hydrogen atom of antioxidant is adheres to odd electron (nitrogen atom) of DPPH and resulting in stable radical. DPPH radical scavenging assay is measured by spectrophotometer. The wavelength at 517 nm is strong absorption of DPPH. Solution and odd electron of this absorption show deep purple color in solution changing to yellow (Kedare and Singh 2011). DPPH assay is a rapid, easy method to estimate antioxidants, color measurement and this experiment can be beneficial to evaluate many samples at a time (Garcia et al. 2012). However this examination is not proper for testing in plasma as a result of plasma proteins were precipitated in the alcohol which is the solvent for dissolving compound (Boligon et al. 2014).

In this study, the potential DPPH free radical scavenging activity of *C. mangga* rhizome extract and positive control (BHT, synthetic antioxidant was used as food additives) were measured at different concentrations (10, 30 and 100 µg/ml) to investigate the antioxidant activity. The DPPH scavenging activity of *C. mangga* extract was the lowest activity when compared with a standard BHT. The IC_{50} value on DPPH inhibition of *C. mangga* extract was 78.7 ± 4.2 µg/ml while the IC_{50} of BHT was 47.9 ± 1.4 µg/ml (Table 9). *C. mangga* extract had slightly antioxidant DPPH scavenging activity using DPPH method. It was corresponding to the previous research by Widowati (2011).

The DPPH method was used to measure the antioxidant activity of the formulation containing *C. mangga* 1-10%. The pharmaceutical cream made from *C. mangga* extract before heating-cooling cycle test showed antioxidant activity with IC_{50} value of >100.0 µg/ml of *C. mangga* cream which were similar to those of after heating-cooling test (Table 10).

Table 9 Antioxidant activity of *C. mangga* extract on DPPH radical scavenging activities

Samples	% Inhibition at various concentrations (Mean ± S.E.M.) (μg/ml)				IC_{50}
	0	10	30	100	μg/ml
EtOH extract	0.0 ± 2.9	5.8 ± 4.5	24.9 ± 1.9*	57.2 ± 1.2*	78.7 ± 4.2[a]
BHT	0.0 ± 1.0	20.3 ± 4.8*	30.7 ± 1.0*	70.7 ± 1.8*	47.9 ± 1.4

*Significant difference from the control

[a]Significant difference from BHT, $p<0.05$ (Mean ± S.E.M. of three determinations)

Table 10 Antioxidant activity of cream containing *C. mangga* extract before and after heating-cooling cycles test on DPPH radical scavenging activities

Sample		% Inhibition at various concentrations (Mean ± S.E.M.) (µg/ml)				IC_{50}
		0	10	30	100	(µg/ml)
Before	Cream base	0.0 ± 2.9	-16.0 ± 2.7	-15.0 ± 1.0	-2.5 ± 1.7	>100
	2% CM cream	0.0 ± 2.1	-13.1 ± 1.2	-12.8 ± 1.3	-11.5 ± 0.6	>100
	5% CM cream	0.0 ± 2.1	-20.5 ± 0.7	-12.3 ± 1.7	-9.2 ± 2.6	>100
	10% CM cream	0.0 ± 2.1	-9.3 ± 2.9	-6.2 ± 2.7	5.2 ± 1.4	>100
After	Cream base	0.0 ± 0.4	-14.0 ± 0.9	-12.9 ± 0.9	-10.7 ± 1.1	>100
	2% CM cream	0.0 ± 2.3	-6.2 ± 0.7	-5.0 ± 0.4	1.9 ± 1.8	>100
	5% CM cream	0.0 ± 2.3	-2.6 ± 1.0	0.6 ± 1.4	5.1 ± 3.1	>100
	10% CM cream	0.0 ± 2.3	-1.1 ± 0.4	-0.5 ± 1.0	16.3 ± 1.8	>100

CM cream = Cream containing *C. mangga* extract

3.4.1.2 H_2O_2-induced oxidative stress

H_2O_2 is generated by inflammatory and vascular cells. It induces oxidative stress and can lead to cell death. H_2O_2 is a weak acid solution and is an oxidant that influenced cell damage, an important role in many disorders in cells and causes the production of free radicals (ROS radical). Amongst a great category of ROS, H_2O_2 has a crucial role because of it is created from approximately all sources of oxygen radicals (Jian et al. 2011; Sroka and Cisowski 2003). When H_2O_2 is added into culture medium, it cause various cells to injure or cell death. H_2O_2-induced oxidative stress was detected with HDF cells at various concentrations of H_2O_2 (0.25, 0.5, 0.7, 0.8, 0.9, 1 and 2.0 mM). HDF cells are cells in the dermis layer of human skin. Cell death from H_2O_2 induced oxidative depend on the concentration of H_2O_2. If low concentrations of H_2O_2 (<0.5 mM or 10 μM to 100 μM) was persuaded cell apoptosis while higher concentration of H_2O_2 (>0.5 mM or 1 mM to 10 mM) was urged necrosis (Teramoto et al. 1999; Clément and Pervaiz 2013). Percentage of cell viability in HDF cells was concentration dependent effect of H_2O_2 and is shown in Table 11. In this experiment, 0.9 mM of H_2O_2 was selected to detection of HDF cell death and MTT assay was studied the prevention of the sample at different concentrations. H_2O_2-induced oxidative stress is used for endogenous anti-oxidation (Jian et al. 2011).

The protective effects against HDF cell death induced by 0.9 mM H_2O_2 suggest that treatment with *C. mangga* extract at the concentration of 1, 3, 10, 30 and 100 μg/ml showed protection of fibroblasts against H_2O_2 71.5%, 72.5%, 65.0%, 71.2% and 57.4%, respectively (Table 12). While the percentage of cell viability untreated with the sample showed a protection of fibroblasts against 0.9 mM H_2O_2 at 55.8%.

The prevention effects of HDF cell death induced by 0.9 mM H_2O_2 showed the good treatment with cream containing *C. mangga* extract before and after heating-cooling cycles test. HDF cells were pretreated with 1, 3, 10, 30 and 100 μg/ml cream containing *C. mangga* extract for 1 h followed by exposure to 0.9 mM H_2O_2. HDF cells without H_2O_2 were officiated as a control group. The result of maximum protective effect (% cell viability) of cream base (10 μg/ml), 2% CM cream (30 μg/ml), 5% CM cream (10 μg/ml) and 10% CM cream (30 μg/ml) before heating-cooling test were found to be 61.1%, 71.4%, 70.9%, 69.1%, respectively. While the maximum percentage of cell viability of cream base (3 μg/ml), 2% CM cream (1 μg/ml), 5% CM cream (1

μg/ml) and 10% CM cream (10 μg/ml) after heating-cooling test were found to be 61.5%, 77.9%, 75.4%, 75.4%, respectively. For percentage of cell viability of incubation with 0.9 mM H_2O_2 (untreated sample) were 54.1%-56.3% (Table 13). It is not significant difference protection of fibroblasts against H_2O_2-induced cell membrane damage between before and after heating-cooling test (p<0.05).

In this study, All the test sample (*C. mangga* extract, cream base, 2% CM cream, 5% CM cream and 10% CM cream before and after heating-cooling test) were prevented HDF cells from H_2O_2-induced oxidative damage and displayed antioxidant activity. The effect of the MTT assay of all the test of samples could protect cells from oxidative damage induced by H_2O_2.

Table 11 Protective effect at various concentrations of (0.25-2 mM) H_2O_2-induced HDF cells death

	% Viability of various concentration of H_2O_2 in HDF cells (mM)							
H_2O_2	Control	0.25	0.50	0.70	0.80	0.90	1.00	2.00
%Viability	100.0 ± 2.1	79.3 ± 3.1	76.6 ± 1.8	52.8 ± 3.6	39.8 ± 1.0	29.3 ± 0.6	27.5 ± 0.2	16.9 ± 1.2

*Significant difference from the control, $p<0.05$ (Mean ± S.E.M. of three determinations)

Table 12 Protective effect of 0.9 mM H_2O_2-induced HDF cells death by *C. mangga* extract

Sample	% Viability of HDF cells at various concentrations (μg/ml)						
	Control	H_2O_2	1	3	10	30	100
EtOH extract	100.0 ± 0.8	55.8 ± 1.1	71.5 ± 7.8*	72.5 ± 7.6*	65.0 ± 7.4*	71.2 ± 4.9*	57.4 ± 3.1*

*Significant difference from the control, $p<0.05$ (Mean ± S.E.M. of three determinations)

Table 13 Protective effect of 0.9 mM H_2O_2-induced HDF cells death from cream containing *C. mangga* extract before and after heating-cooling cycles test

Sample		% Viability of HDF cells at various concentrations (μg/ml)						
		Control	H_2O_2	1	3	10	30	100
Before	Cream base	100.0 ± 0.6	54.1 ± 3.4	61.3 ± 4.3*,c	60.6 ± 2.0*,b,c	61.1 ± 2.4*	60.5 ± 2.1*	60.4 ± 2.5*,b,c
	2% CM cream	100.0 ± 0.6	54.1 ± 3.4	71.2 ± 3.4*	71.0 ± 2.6*	69.5 ± 4.0*	71.4 ± 1.8*	62.7 ± 4.8*,b
	5% CM cream	100.0 ± 0.6	54.1 ± 3.4	70.4 ± 3.0*	70.3 ± 3.4*	70.9 ± 5.0*	69.5 ± 5.7*	63.3 ± 5.0*,b
	10% CM cream	100.0 ± 0.7	55.4 ± 3.7	66.8 ± 4.0*	63.1 ± 1.1*,b	65.0 ± 0.6*	69.1 ± 1.3*	61.7 ± 1.9*,b,c
After	Cream base	100.0 ± 2.8	55.0 ± 4.4	60.9 ± 3.1*,c	61.5 ± 3.5*,b,c	60.5 ± 3.9*	59.2 ± 5.0*	61.5 ± 2.0*,b,c
	2% CM cream	100.0 ± 2.8	55.0 ± 4.4	77.9 ± 6.0*	77.6 ± 6.9*	77.0 ± 7.2*	77.8 ± 7.8*	64.8 ± 7.0*,b
	5% CM cream	100.0 ± 4.1	56.3 ± 3.1	75.4 ± 11.2*	74.9 ± 8.7*	72.2 ± 10.5*	71.8 ± 9.0*	62.3 ± 5.3*,b
	10% CM cream	100.0 ± 2.8	55.0 ± 4.4	72.6 ± 4.9*	73.0 ± 5.4*	75.4 ± 8.3*	74.1 ± 6.4*	68.4 ± 8.2*,b
	Vitamin C	100.0 ± 0.2	60.6 ± 0.2	76.1 ± 10.2*	79.9 ± 4.0*	77.3 ± 0.8*	66.7 ± 9.2*	12.0 ± 1.1*
	Vitamin E	100.0 ± 0.2	60.6 ± 0.2	78.4 ± 1.9*	77.4 ± 1.5*	77.6 ± 2.3*	74.6 ± 2.9*	76.5 ± 2.4*

*Significant difference from the control, $p<0.05$ (Mean ± S.E.M. of three determinations)

[a]Significant difference between before and after heating-cooling test, $p<0.05$, [b]Significant difference from vitamin C, $p<0.05$, [c]Significant difference from vitamin E, $p<0.05$

CM cream = Cream containing *C. mangga* extract

3.4.2 Anti-inflammation test (Anti-NO production assay)

NO is generated by immunocompetentcells (macrophage). It is accepted a free radical, mediator of inflammatory process. NO concentrations were evaluated with measuring quantity of nitrite by Griess reagent.

In the present study, ethanolic extract of *C. mangga* and indomethacin (NSAIDs, COX inhibitor) which is a positive control group were estimated for the inhibition of NO production. The various concentrations of *C. mangga* and LPS (1 µg/ml) were concurrently treated into the cells. The result of the extract showed less effect against NO production than indomethacin. The IC_{50} values of crude extract and indomethacin were 21.0 ± 1.4 µg/ml and 19.9 ± 1.1 µg/ml, respectively (Table 14). The IC_{50} value of the ethanolic extract of *C. mangga* nearby the previous report by Kaewkroek (2009) and Kaewkroek et al. (2010). The inhibitory effect of crude extract and indomethacin with MTT assay (cytotoxicity activity) were not due to toxic of cells (toxicity < 20%).

The inhibition of NO production in LPS-stimulated murine macrophages (RAW264.7 cells) were detected on nitrite production in culture medium of cream containing *C. mangga* (1-10%) compared with the standard (diclofenac gel). The 10% w/w of cream containing *C. mangga* extract before and after heating-cooling test showed the highest anti-NO production with IC_{50} values of 34.1 ± 2.1 µg/ml and 37.9 ± 0.6 µg/ml, followed by 5% cream containing *C. mangga* extract before and after heating-cooling test (IC_{50} values = 42.9 ± 1.0 µg/ml and 44.7 ± 1.9 µg/ml) and 2% cream containing *C. mangga* extract before and after heating-cooling test (IC_{50} values = 49.1 ± 0.9 µg/ml and 49.6 ± 0.6 µg/ml), respectively. All samples showed good activity than that of diclofenac gel (IC_{50} = 54.3 ± 1.1 µg/ml) and cream base showed less activity (Table 15).

Cream containing 1-10% w/w *C. mangga* extract were determined for their cytotoxicity of RAW264.7 cells by colorimetric MTT metabolic activity testing. This testing requires living cells to reduce MTT (a yellow tetrazolium compound) to an insoluble a purple formazan crystals by the action of mitochondrial reductase (Tonder et al., 2015). It was found that no cytotoxicity using LPS-stimulated RAW264.7 cells at different concentrations (10, 30 and 100 µg/ml) were observed.

Table 14 Inhibition on NO production of *C. mangga* extract

Sample	% Inhibition at various concentrations (Mean ± S.E.M.) (μg/ml)				IC_{50} (μg/ml)
	0	10	30	100	
EtOH extract	0.0 ± 2.7	29.4 ± 2.3*	60.1 ± 1.1*	92.0 ± 2.3*	21.0 ± 1.4
Indomethacin	0.0 ± 2.7	36.1 ± 1.3*	57.3 ± 1.5*	84.5 ± 2.7*	19.9 ± 1.1

*Significant difference from the control

[a]Statistical significance between indomethacin, $p<0.05$ (Mean ± S.E.M. of three determinations)

Table 15 Anti-NO production of cream base and cream containing *C. mangga* before and after heating-cooling test

Sample		% Inhibition at various concentrations (Mean ± S.E.M.) (μg/ml)				IC_{50}
		0	10	30	100	(μg/ml)
Before	Cream base	0.0 ± 2.7	13.4 ± 1.0	24.5 ± 1.6	72.3 ± 1.8	52.0 ± 0.6[a]
	2% CM cream	0.0 ± 1.6	11.0 ± 2.2	25.7 ± 1.5	75.1 ± 1.2	49.1 ± 0.9*
	5% CM cream	0.0 ± 2.7	8.5 ± 1.8	32.5 ± 2.0	79.3 ± 1.9	42.9 ± 1.0*
	10% CM cream	0.0 ± 2.5	20.5 ± 1.9	38.7 ± 2.9	83.5 ± 1.8	34.1 ± 2.1*,[a]
After	Cream base	0.0 ± 1.7	14.5 ± 2.0	22.0 ± 1.9	69.8 ± 2.0	56.6 ± 0.7 [a]
	2% CM cream	0.0 ± 2.4	9.6 ± 2.0	25.4 ± 2.2	75.1 ± 1.1	49.6 ± 0.6
	5% CM cream	0.0 ± 2.1	11.4 ± 1.8	28.9 ± 2.1	78.4 ± 2.1	44.7 ± 1.9*
	10% CM cream	0.0 ± 3.2	15.0 ± 1.7	37.4 ± 2.8	80.7 ± 1.7	37.9 ± 0.6*,[a]
Diclofenac gel		0.0 ± 2.7	4.2 ± 2.2	19.9 ± 2.6	74.6 ± 1.8	54.3 ± 1.1

*Statistical significant difference between diclofenac gel and samples, $p<0.05$ (Mean ± S.E.M. of three determinations)

[a]Significant difference between before and after heating-cooling test, $p<0.05$

CM cream = Cream containing *C. mangga* extract

3.4.3 Wound healing assay

3.4.3.1 Cell proliferation of HDF cells

The HDF cells were seeded on different samples with *C. mangga* extract, cream base and 2%, 5%, 10% w/w cream containing *C. mangga* before and after heating-cooling test and *Aloe vera* gel (commercial product contains saponin, aloe genin, glycoprotein such as aloctin A and B which has been reported topically for anti-inflammatory and wound healing activity) (Tangjitjareonkun et al. 2015) to test the viability of cell and cell growth by cell proliferation using MTT assay. The cell viability of all samples was compared to the control group which was untreated with the sample. HDF cells are discovered at connective tissues and originate from adult human primary dermal fibroblast of skin. Dermal fibroblasts play essential roles in healing of cutaneous wound and remodeling. It has many characters as proliferation and migration into the wound edge (Allahbakhshian-Farsani et al. 2014).

Cell proliferation is enlargement the number of cells or cell growth and cell division. Cell proliferation and cell migration are essential events for the cell recruitment to site of tissue damage for healing the wound (Maeda et al. 2013). Cells enhancement was detected by MTT assay after treatment with different concentrations of crude extract, cream containing *C. mangga* extract and *Aloe vera* gel. Crude extract increased cell proliferation at 1 µg/ml (109.7%), 3 µg/ml (106.4%), 10 µg/ml (104.9%) and 30 µg/ml (102.8%), respectively (Table 16).

After cream containing 2%, 5% and 10% w/w *C. mangga* extract before and after heating-cooling test were treated into the wells and observed the percent cells growth. It was found that cream containing 2%, 5% and 10% w/w of *C. mangga* extract before and after heating-cooling test (1 and 3 µg/ml) increased cell viability more than 100%, while a cell viability of the other concentrations (10 and 30 µg/ml) were less than 100%. Furthermore the cell proliferation of commercial product (*Aloe vera* gel) at 1 µg/ml (104.1 ± 1.9), 3 µg/ml (105.7 ± 4.9), 10 µg/ml (104.3 ± 5.8) and 30 µg/ml (107.5 ± 7.3) increased cell viability more than 100%. The results are shown in Table 17. Thus, these concentrations (1 and 3 µg/ml) of cream containing 2%, 5% and 10% w/w of *C. mangga* before and after heating-cooling test were chosen to measure cell migration (cell movement) by *in vitro* scratch assay.

Table 16 Effect of *C. mangga* extract on HDF cells viability

Sample	% Viability of HDF cells at various concentrations (μg/ml)				
	0	1	3	10	30
EtOH extract	100.0 ± 2.1	109.7 ± 2.7*	106.4 ± 3.8*	104.9 ± 3.7*	102.8 ± 1.9*

*Statistical significant difference from control, $p<0.05$ (Mean ± S.E.M. of three determinations)

Table 17 Effect of cream base and cream containing *C. mangga* before and after heating-cooling test on HDF cells viability

Sample		% Viability of HDF cells at various concentrations (μg/ml)				
		0	1	3	10	30
Before	Cream base	100.0 ± 1.7	89.9 ± 4.2[b]	91.8 ± 2.0[b]	90.7 ± 2.9[b]	81.8 ± 2.7*[,b]
	2% CM cream	100.0 ± 2.4	109.1 ± 4.2*	102.4 ± 2.7	97.9 ± 3.2	90.9 ± 3.4*[,b]
	5% CM cream	100.0 ± 2.9	109.9 ± 2.2*	107.3 ± 2.3*	98.8 ± 3.5	95.5 ± 3.1
	10% CM cream	100.0 ± 2.7	110.0 ± 2.6*	106.2 ± 3.4	98.7 ± 5.0	96.0 ± 2.0
After	Cream base	100.0 ± 1.8	91.4 ± 5.5[b]	90.0 ± 2.8[b]	87.7 ± 3.3[b]	88.5 ± 2.0[b]
	2% CM cream	100.0 ± 1.7	108.7 ± 2.9	105.0 ± 3.3	97.4 ± 2.1	91.1 ± 2.1[b]
	5% CM cream	100.0 ± 1.7	107.4 ± 3.5	102.2 ± 2.5	98.4 ± 2.1	93.1 ± 3.3[b]
	10% CM cream	100.0 ± 2.2	109.7 ± 1.8*	107.0 ± 2.8*	97.6 ± 1.7	96.1 ± 2.4
Aloe vera gel		100.0 ± 2.5	104.1 ± 1.9	105.7 ± 4.9	104.3 ± 5.8	107.5 ± 7.3*

*Statistical significant difference from control, $p<0.05$ (Mean ± S.E.M. of three determinations)

[a]Significant difference between before and after heating-cooling test, $p<0.05$

[b]Significant difference between *Aloe vera* gel, $p<0.05$

CM cream = Cream containing *C. mangga* extract

3.4.3.2 Cell migration assay (Scratch assay) of HDF cells

Heal of the wound is restored the body when there is the injury of skin. This part is migratory response, cells migration or cell movements which are important to repair new tissues.

The cell migration *in vitro* scratch assay (wound closure method) is a measurement the rate of cells to migrate or cell movement in wound area and wound closure made in a confluent dish of cells monolayer. The advantages of this assay are a simple, low-cost and can imitate cell movement of wound healing *in vivo*.

The cell migration assay was observed the *in vitro* wound closure effects of the *C. mangga* extract, cream base and 2%, 5%, 10% w/w cream containing *C. mangga* before and after heating-cooling test and *Aloe vera* gel (commercial product) at 0, 12, 24 and 36 h. In this study, the percentage movement of the cells was reported wound closure of all samples at 36 h.

The percent cell movements of *C. mangga* extract (36 h) at 1 and 3 µg/ml were 82.3% and 82.9%. The result is shown in Table 18 and Figure 11. For the wound healing activities of cream containing 2%, 5% and 10% w/w of *C. mangga* extract before and after heating-cooling test on HDF cells using scratch assay were measured by the percentage movement of cells into a wound, the results are shown in Tables 19-20 and Figures 12-13. The percentage movement of cream containing 5% w/w of *C. mangga* extract before heating-cooling test (at 3 µg/ml) showed the highest migration of HDF cells at 36 h by 80.1%, followed by 2% w/w (78.7%) and 10% w/w (76.4%), respectively. For after heating-cooling test, it was found that cream containing 5% w/w of *C. mangga* extract at 3 µg/ml enhanced migration of HDF cells at 36 h with 75.2%, followed by 2% (73.5%) and 10% w/w cream (72.8%), respectively when compared with that of the control group (61.1%). While different concentrations of *Aloe vera* gel at 1 µg/ml (60.6%), 3 µg/ml (59.0%), 10 µg/ml (55.6%) and 30 µg/ml (55.2%) showed less percentage movement than cream containing *C. mangga* extract both before and after heating-cooling test (Table 21, Figure 14).

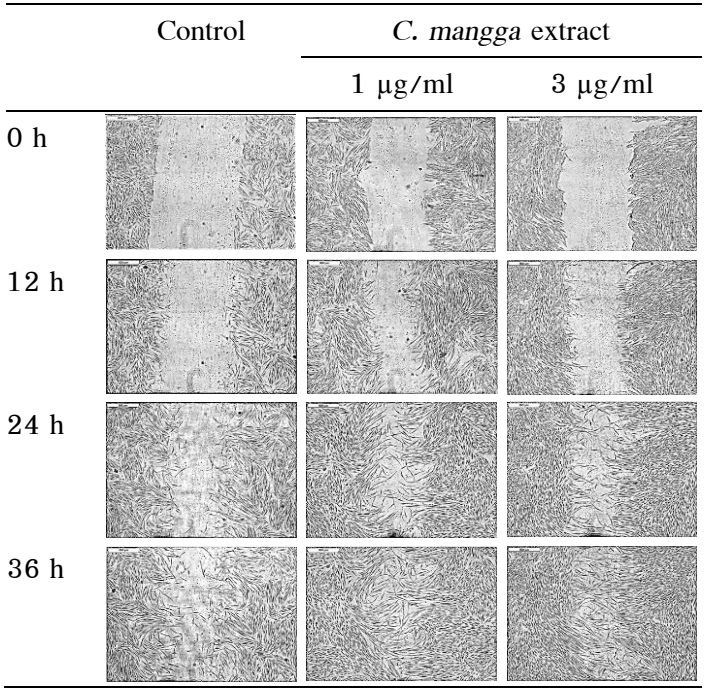

Figure 11 Effect of *C. mangga* (CM) extract on HDF cells (Migration assay)

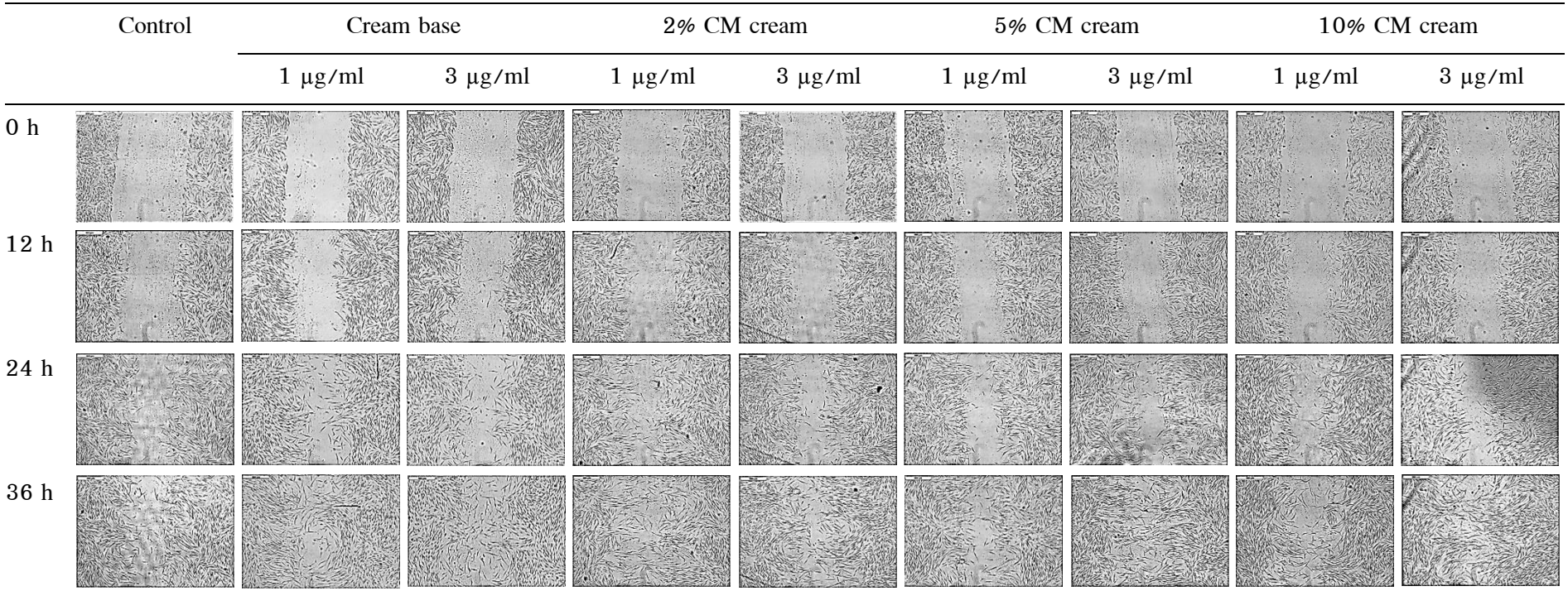

Figure 12 Effect of cream base and cream containing *C. mangga* (CM) extract on HDF cells (Migration assay) before heating-cooling test

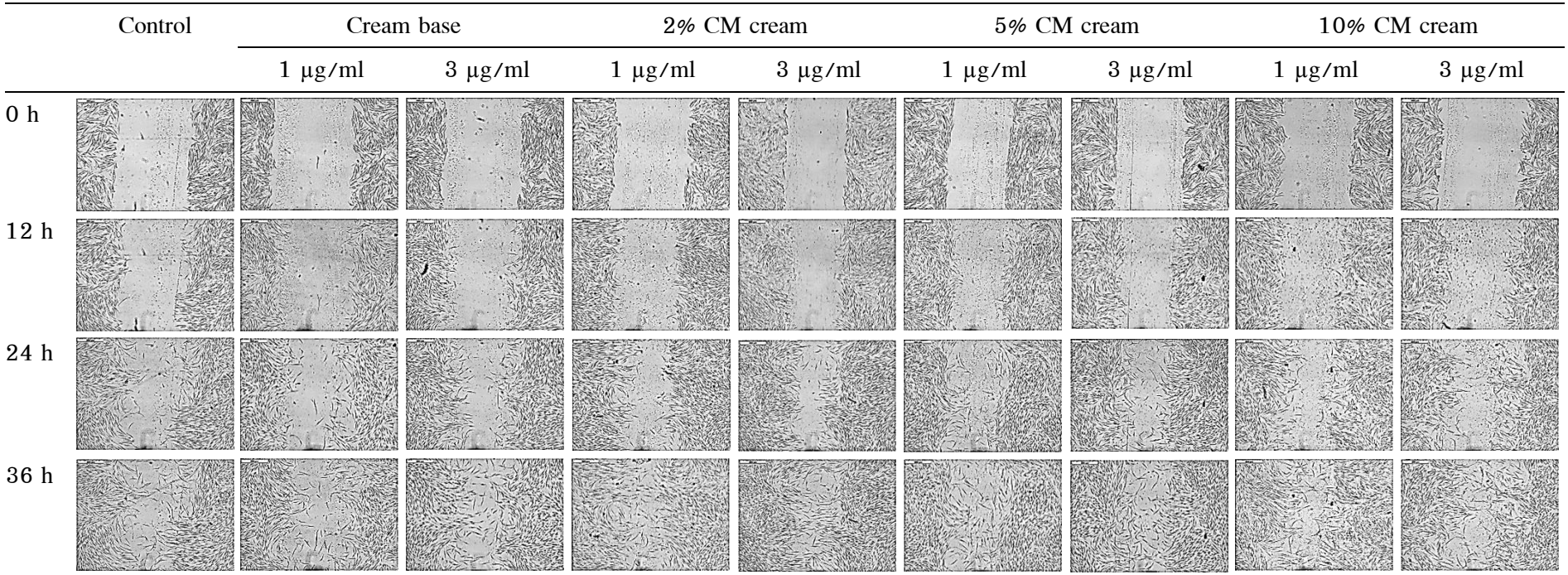

Figure 13 Effect of cream base and cream containing *C. mangga* (CM) extract on HDF cells (Migration assay) after heating–cooling test

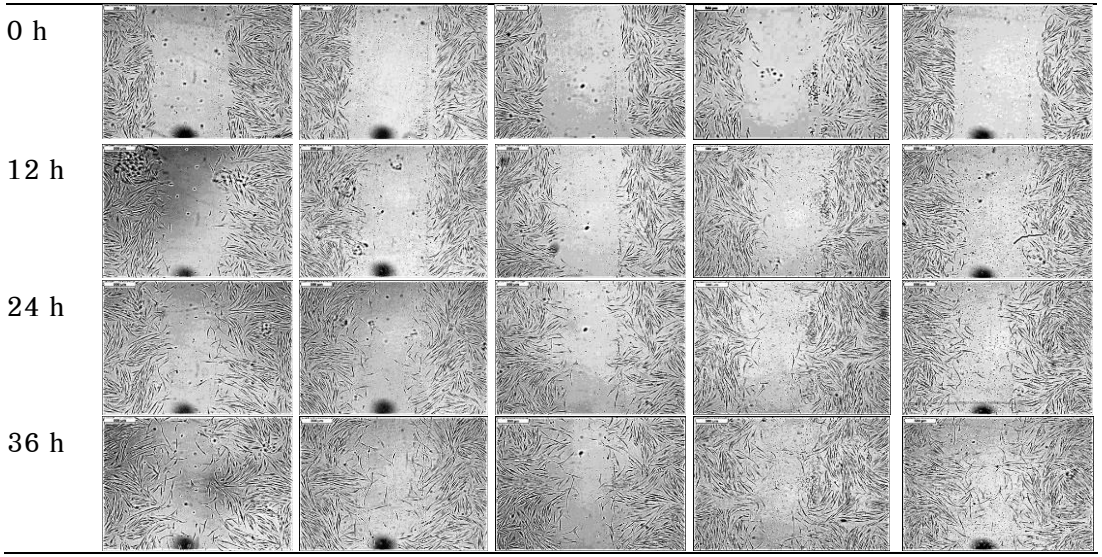

Figure 14 Effect of commercial product (*Aloe vera* gel) on HDF cells (Migration assay)

Table 18 Effect of *C. mangga* extract on HDF cells migration assay

Sample	Dose (μg/ml)	Length between the scratch (μm)				%Migration rate of cells		
		0 h	12 h	24 h	36 h	12 h	24 h	36 h
Control	–	1,432.1 ± 10.9	1,110.0 ± 18.2	712.6 ± 24.5	511.5 ± 26.2	22.5 ± 1.3	50.2 ± 1.7	64.3 ± 1.8
C. mangga extract	1	1,016.7 ± 17.0	662.4± 16.0	306.7 ± 11.5	179.6 ± 10.6	34.9 ± 1.6*	69.8 ± 1.1*	82.3 ± 1.0*
	3	1,074.7 ± 37.2	732.7 ± 3.3	363.3 ± 21.0	184.1 ± 10.2	31.8 ± 0.3*	66.2 ± 2.0*	82.9 ± 1.0*

*Significant difference from the control, $p<0.05$ (Mean ± S.E.M. of three determinations),

(−) = Not determined

Table 19 Effect of cream base and cream containing *C. mangga* extract on HDF cells before heating-cooling test (Migration assay)

Sample	Dose	Length between the scratch (μm)				%Migration rate of cells		
	(μg/ml)	0 h	12 h	24 h	36 h	12 h	24 h	36 h
Control	-	1,432.1 ± 10.9	1,110.0 ± 18.2	712.6 ± 24.5	511.5 ± 26.2	22.5 ± 1.3	50.2 ± 1.7	64.3 ± 1.8
Before Cream base	3	1,263.1 ± 28.8	956.8 ± 22.5	735.5 ± 31.3	393.7 ± 17.6	24.3 ± 1.8	41.8 ± 2.5	68.8 ± 1.4
	1	1,209.5 ± 17.4	875.7 ± 52.1	510.3 ± 37.2	425.4 ± 24.4	27.6 ± 4.3	57.8 ± 3.1	64.8 ± 2.0[a]
2% CM cream	3	1,250.3 ± 4.8	878.0 ± 18.5	467.5 ± 22.9	265.8 ± 23.0	29.8 ± 1.5	62.6 ± 1.8*	78.7 ± 1.8*
	1	1,282.7 ± 24.4	914.0 ± 30.6	521.7 ± 35.4	330.2 ± 51.2	28.7 ± 2.4	59.3 ± 2.8*	74.3 ± 4.0*
5% CM cream	3	1,187.7 ± 38.0	792.8 ± 18.5	421.0 ± 12.0	237.0 ± 30.9	33.2 ± 1.6*	64.6 ± 1.0*	80.1 ± 2.6*
	1	1,280.6 ± 10.9	823.7 ± 33.7	519.0 ± 30.2	305.9 ± 32.0	35.7 ± 2.6*	59.5 ± 2.4	76.1 ± 2.5*[a]
10% CM cream	3	1,231.5 ± 20.4	867.2 ± 34.0	482.8 ± 9.0	290.3 ± 19.6	29.6 ± 2.8	60.8 ± 0.7*	76.4 ± 1.6*
	1	1,266.0 ± 3.6	925.5 ± 23.2	633.4 ± 11.5	381.7 ± 26.4	26.9 ± 1.8	50.0 ± 0.9	69.9 ± 2.1

*Significant difference from the control, $p<0.05$ (Mean ± S.E.M. of three determinations)

[a]Significant difference between before and after heating-cooling test, $p<0.05$

(-) = Not determined,

CM cream = Cream containing *C. mangga* extract

Table 20 Effect of cream base and cream containing *C. mangga* extract on HDF cells after heating–cooling test (Migration assay)

Sample	Dose	Length between the scratch (μm)				%Migration rate of cells		
	(μg/ml)	0 h	12 h	24 h	36 h	12 h	24 h	36 h
Control	–	1,477.0 ± 40.0	1,209.9 ± 24.6	911.8 ± 20.7	574.5 ± 39.5	18.1 ± 1.7	38.3 ± 1.4	61.1 ± 2.7
After Cream base	3	1,503.1 ± 38.1	1,172.8 ± 24.1	782.0 ± 19.5	528.0 ± 21.0	22.0 ± 1.6	48.0 ± 1.3*	64.9 ± 1.4
	1	1,558.5 ± 15.2	1,257.5 ± 41.0	977.7 ± 43.5	660.3 ± 32.3	19.3 ± 2.6	37.3 ± 2.8	57.6 ± 2.1[a]
2% CM cream	3	1,193.9 ± 15.7	879.3 ± 35.8	575.3 ± 36.3	316.2 ± 36.0	26.4 ± 3.0*	51.8 ± 3.0*	73.5 ± 3.0*
	1	1,501.0 ± 31.7	1,108.1 ± 19.7	642.9 ± 32.5	485.1 ± 23.7	26.2 ± 1.3*	57.2 ± 2.2*	67.7 ± 1.6*
5% CM cream	3	1,354.8 ± 24.7	967.8 ± 10.7	611.6 ± 18.3	335.6 ± 16.1	28.6 ± 0.8*	54.9 ± 1.4*	75.2 ± 1.2*
	1	1,181.4 ± 22.0	951.8 ± 16.1	674.3 ± 22.4	379.7 ± 18.9	19.4 ± 1.4	42.9 ± 1.9	67.9 ± 1.6*[a]
10% CM cream	3	1,629.3 ± 36.3	1,203.3 ± 23.7	787.4 ± 10.3	443.3 ± 17.2	26.2 ± 1.5*	51.7 ± 0.6*	72.8 ± 0.9*
	1	1,372.5 ± 33.9	1,029.4 ± 14.4	655.2 ± 27.5	438.3 ± 17.9	25.0 ± 1.1*	52.3 ± 2.0*	68.1 ± 1.3*

*Significant difference from the control, $p<0.05$ (Mean ± S.E.M. of three determinations)

[a]Significant difference between before and after heating–cooling test, $p<0.05$

CM cream = Cream containing *C. mangga* extract

(–) = Not determined

Table 21 Effect of commercial product (*Aloe vera* gel) on HDF cells migration assay

Sample	Dose	Length between the scratch (μm)				%Migration rate of cells		
	(μg/ml)	0 h	12 h	24 h	36 h	12 h	24 h	36 h
Control	–	1,460.5 ± 75.9	1,135.5 ± 76.2	993.7 ± 62.4	648.5 ± 46.1	22.4 ± 1.3	32.0 ± 0.8	55.7 ± 1.6
Aloe vera gel	1	1,208.9 ± 49.4	946.0 ± 46.1	773.4 ± 42.0	476.1 ± 17.2	21.8 ± 1.1	36.1 ± 1.0	60.6 ± 1.4
	3	1,275.6 ± 61.9	1,051.0 ± 33.6	871.3 ± 25.1	523.6 ± 30.9	17.5 ± 1.4	31.6 ± 1.7	59.0 ± 0.5
	10	1,298.3 ± 63.3	1,016.0 ± 56.1	856.5 ± 63.4	575.4 ± 13.9	21.8 ± 1.0	34.2 ± 2.0	55.6 ± 1.2
	30	1,429.4 ± 27.7	1,214.2 ± 48.1	1,020.0 ± 71.4	640.9 ± 32.2	15.1 ± 2.1*	28.7 ± 4.0	55.2 ± 1.5

*Significant difference from the control, $p<0.05$ (Mean ± S.E.M. of three determinations), (−) = Not determined

CHAPTER 4

CONCLUSIONS

C. mangga rhizomes have been used for health (reduces stomach discomfort, gastric ulcer and postpartum care) and there are many reports on pharmacological effects including antibacterial, antifungal, anti-cancer, anti-inflammatory and analgesic effect. Therefore this research aimed to use *C. mangga* rhizomes which has been reported on biological activity as anti-inflammation for development of herbal cream.

In the present work, preparation of cream containing *C. mangga* extracts which is an O/W type of cream. This cream is brown to dark brown. When testing the stability of the cream product found that formula 2 is stable (no separation of cream, acidity and viscosity were slightly changed when compared to creams before heating-cooling test). It is considered to be acceptable and was chosen to mix with *C. mangga* extract. After that the physical, chemical and biological properties of cream base, cream containing *C. mangga* extracts (2%, 5% and 10% w/w) before and after heating-cooling test (6 cycles) including antioxidant activity (DPPH assay and H_2O_2-induced oxidative stress), anti-inflammatory activity and wound healing assay were evaluated.

Cream containing *C. mangga* showed good consistency, homogeneity and smooth, pH range of acid value (5-6). This cream was stable after heating-cooling test using HPLC technique because the percentages of standard compounds (curcumin, demethoxycurcumin and bisdemethoxycurcumin) were slightly decreased after heating-cooling test and still showed good anti-inflammatory activity.

The DPPH inhibition of *C. mangga* extract showed the lowest activity when compared with BHT and the antioxidant activity of the cream containing *C. mangga* extract inhibited DPPH free radicals at concentration greater than 100 µg/ml. H_2O_2 is one of the many free radicals that can be harmful for the cells. *C. mangga* extract, cream base and cream containing *C. mangga* extract with 0.9 mM H_2O_2 showed protective effect of cell death from endogenous antioxidant which is H_2O_2-induced oxidative stress.

The screening anti-inflammatory activity, 10% w/w of cream containing *C. mangga* extract before and after heating-cooling test (IC_{50} values = 34.14 µg/ml and 37.9 µg/ml) exhibited the most inhibitory activity against NO production using LPS-

stimulated RAW264.7 cell than 2% w/w (IC_{50} = 42.9 µg/ml and 44.7 µg/ml) and 5% w/w before and after heating-cooling test (IC_{50} = 49.1 µg/ml and 49.6 µg/ml). Furthermore, the anti-inflammatory effect of cream containing *C. mangga* extract was better than a positive control (diclofenac gel) with IC_{50} value of 54.3 µg/ml.

Wound healing (proliferation activity) of cream containing *C. mangga* extract before and after heating-cooling test found that the concentration at 1 and 3 µg/ml activated cell proliferation more than the other concentrations (10 and 30 µg/ml) and the control group while all concentration (1, 3, 10 and 30 µg/ml) of *Aloe vera* gel showed percentage of cell viability more than the control group. For scratch assay using HDF cells, the percentage movement of the cells showed apparent wound closure for all samples at 36 h. The 5% w/w cream containing *C. mangga* extract before and after heating-cooling test showed the highest percentage cells movement than 2% and 10% w/w. Cream containing *C. mangga* extract can stimulate fibroblast proliferation and migration, considered as important factors in dermis regeneration.

Hence, this study can be concluded that the development of cream containing *C. mangga* extract could reduce inflammation and increase cell proliferation and migration to the wound because of the presence of polyphenol that is curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) and phenolic compounds etc. In addition, this formulation should be detected on skin permeability test, efficiency and safety of cream formulation. It can be developed to other cosmetic, cosmeceutical or drug such as gel, lotion and toner as well as increase in value added and promote Thai herbal usage.

REFERENCES

Abas F, Lajis NH, Shaari K, Israf DA, Stanslas J, Yusuf UK, Raof SM. Labdane diterpene glucoside from the rhizomes of *Curcuma mangga*. Journal of Natural Products 2005; 68:1090-1093.

Ali SM, Yosipovitch G. Skin pH: from basic science to basic skin care. Acta Dermato-Venereologica 2013; 93:261-267.

Addor FAS. Antioxidants in dermatology. Anais Brasileiros De Dermatologia Journal 2017; 92:356-362.

Allahbakhshian-Farsani M, Abdian N, Ghasemi-Dehkordi P, Sadeghiani M, Saffari-Chaleshtori J, Hashemzadeh-Chaleshtori M, Khosravi-Farsani S. Cytogentic analysis of human dermal fibroblasts (HDFs) in early and late passages using both karyotyping and comet assay techniques. Cytotechnology 2014; 66:815-822.

Baharudin M, Hamid S, Susanti D. Chemical composition and antibacterial activity of essential oils from three aromatic plants of the Zingiberaceae family in Malaysia. Journal of Physical Science 2015; 26:71-81.

Barnes PJ, Belvisi MG. Nitric oxide and lung disease. Thorax 1993; 48:1034-1043.

Benly P. Role of histamine in acute inflammation. Journal of Pharmaceutical Sciences and Research 2015; 7:373-376.

Boligon AA, Machado MM, Athayde ML. Technical evaluation of antioxidant activity. Medicinal Chemistry 2014; 4:517-522.

Burns JL, Mancoll JS, Philips LG. Impairment to wound healing. Clinics in Plastic Surgery 2003; 30:47-56.

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2018; 9:7204-7218.

Chung J, Oh JU, Lee S, Kim S. Inhibition of inducible nitric oxide synthase, cycleooxygenase-2 and lipid peroxidation by methanol extract of *Pericarpium zanthoxyli*. Tropical Journal of Pharmaceutical Research 2013; 12:369-375.

Clément M, Pervaiz S. Intracellular superoxide and hydrogen peroxide concentrations: a critical balance that determines survival or death. Redox Report 2013; 6:211-214.

Coneski PN, Schoenfisch MH. Nitric oxide release: part III measurement and reporting. Chemical Society Reviews 2012; 41:3753-3758.

Coyle CH, Kader KN. Mechanisms of H_2O_2-induced oxidative stress in endothelial cells exposed to physiologic shear stress. ASAIO Journal 2006; 53:17-22.

Coyle CH, Martinez LJ, Coleman MC, Spitz DR, Weintraub NL, Kader KN. Mechanisms of H_2O_2-induced oxidative stress in endothelial cells. Free Radical Biology and Medicine 2006; 40:2206-2213.

Csizmazia E. Drug permeation study through biological membrane barriers [Ph.D. Thesis]. Szeged, Hungary: University of Szeged; 2011.

Das T, Debnath J, Nath B, Dash S. Formulation and evaluation of an herbal cream for wound healing activity. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6:693-697.

Desu PK, Vaishnavi G, Divya K, Lakshmi U. An overview on preformulation studies. Indo American Journal of Pharmaceutical Sciences 2015; 2:1399-1407.

Diaz P, Jeong SC, Lee S, Khoo C, Koyyalamudi SR. Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds. Chinese Medicine 2012; 7:1-9.

Dhase AS, Khadbadi SS, Saboo SS. Formulation and evaluation of vanishing herbal cream of crude drugs. American Journal of Ethnomedicine 2014; 1:313-318.

Frank S, Kampfer H, Wetzler C, Pfeilschifter J. Nitric oxide drives skin repair: Novel functions of an established mediator. Kidney International 2002; 61:882-888.

Garcia EJ, Oldoni TL, Alencar SM, Reis A, Loguercio AD, Grande RH. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Brazilian Dental Journal 2012; 23:22-27.

Ghayempour S, Montazer M, Rad MM. Encapsulation of *Aloe vera* extract into natural tragacanth gum as a novel green wound healing product. International Journal of Biological Macromolecules 2016; 93:344-349.

Girija DM, Kalachaveedu M, Subbarayan R, Jenifer P, Rao SR. *Aristolochia bracteolata* enhances wound healing *in vitro* through anti-inflammatory and proliferative effect on human dermal fibroblasts and keratinocytes. Pharmacognosy Journal 2017; 9:s129-s136.

Gonzalez ACO, Costa TF, Andrade ZA, Medrado ARAP. Wound healing-A literature review. Anais Brasileiros de Dermatologia 2016; 91:614-620.

Gothai S, Arulselvan P, Tan WS, Fakurazi S. Wound healing properties of ethyl acetate fraction of *Moringa oleifera* in normal human dermal fibroblasts. Journal of Intercultural Ethnopharmacology 2016; 5:1-6.

Grazul-bilska AT, Johnson AL, Bilski JJ, Redmer DA, Reynolds LP, Abdullah A, Abdullah KM. Wound healing: The role of growth factors. Drug of Today 2003; 39:787-800.

Guzik TJ, Korbut R, Adamek-Guzik T. Nitric oxide and superoxide in inflammation and immune regulation. Journal of Physiology and Pharmacology 2003; 54:469-487.

Halliwell B, Clement MV, Ramalingam J, Long LH. Hydrogen peroxide. ubiquitous in cell culture and *in vivo*?. IUBMB Life 2000; 50:251-257.

Halliwell B. The wonderings of a free radical. Free Radical Biology and Medicine 2009; 46:531-542.

Hunt TK, Hopf H, Hussain Z. Physiology of wound healing. Advance skin wound care 2000; 13:6-11.

Indis NA, Kurniawan F. Determination of free radical scavenging activity from aqueous extract of *Curcuma mangga* by DPPH method. Journal of Physics 2016; 710:1-5.

Jayaraman P, Nathan P, Vasanthan P, Musa S. Govindasamy V. Stem cells conditioned medium: a new approach to skin wound healing management. Cell Biology International 2013; 37:1-7.

Jian Z, Li K, Liu L, Zhang Y, Zhou Z, Li C, Gao T. Heme oxygenase-1 protects human melanocytes from H_2O_2-induced oxidative stress via the Nrf2-ARE pathway. Journal of Investigative Dermatology 2011; 131:1420-1427.

Jirum J, Srihanam P. Oxidants and antioxidants: sources and mechanism. Academic Journal Kalasin Rajabhat University 2011; 1:59-70.

Joo T, Sowndhararajan K, Hong S, Lee J, Park S, Kim S, Jhoo J. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells by stem bark of *Ulmus pumila* L. Saudi Journal of Biological Sciences 2014; 21:427-435.

Juhn SK, Jung M, Hoffman MD, Drew BR, Preciado DA, Sausen NJ, Jung TK, Kim BH, Park S, Lin J, Ondrey FG, Mains DR, Huang T. The role of inflammatory mediators in the pathogenesis of otitis media and sequelae. Clinical and Experimental Otorhinolaryngology 2008; 1:117-138.

Kaewkroek K, Wattanapiromsakul C, Tewtrakul S. Anti-inflammatory mechanism of compounds from *Curcuma mangga* rhizomes using RAW264.7 macrophage. Natural Product Communications 2010; 5:1547-1550.

Kaewkroek K. Study on anti-inflammatory activity of *Curcuma mangga* rhizomes [Master thesis]. Songkhla, Thailand: Prince of Songkla University; 2009.

Kamazeri T, Samah OA, Taher M, Susanti D, Qaralleh H. Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma mangga*, and *Zingiber cassumunar* from Malaysia. Asian Pacific Journal of Tropical Medicine 2012; 5:202-209.

Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology 2011; 48:412-422.

Kharat N, Shylaja H, Viswanatha GL, Lakshman K. Anti-inflammatory and analgesic activity of topical preparation of root extracts of *Ichocarpus frutescent* (L.) R.BR. International Journal of Applied Biology and Pharmaceutical Technology 2010; 1:1101-1106.

Kielhorn J, Melching-Kollmuss S, Mangelsdorf I. Environmental health criteria for dermal absorption. Geneva: World Health Organization; 2006.

Kotha K, Szczesniak RD, Naren AP, Fenchel MC, Duan LL, McPhail GL, Clancy JP. Concentration of fractional excretion of nitric oxide (FENO): A potential airway biomarker of restored CFTR function. Journal of Cystic Fibrosis 2015; 14:733-740.

Kumar A, Chomwal R, Kumar P, Sawal R. Anti-inflammatory and wound healing activity of *Curcuma aromatica* Salisb extract and its formulation. Journal of Chemical and Pharmaceutical Research 2009; 1:304-310.

Lambers H, Piessens S, Bloem A, Pronk H, Finkel P. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. International Journal of Cosmetic Science 2006; 28:359-370.

Lechner M, Lirk P, Rieder J. Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. Seminars in Cancer Biology 2015; 15:277-289.

Leelapornpisid P, Mungmai L, Sirinthunyalug B, Jiranusornkul S, Peerapornpisal Y. A novel moisturizer extract from freshwater macroalga [*Rhizoconium hieroglyphicum* (C.Agardh) Kützing] for skin care cosmetic. Chiang Mai Journal Science 2014; 41:1195-1207.

Liang N, Kitts DD. Antioxidant property of coffee components: assessment of methods that define mechanisms of action. Molecules 2014; 19:19180-19208.

Lim TM. Edible medicinal and non-medicinal plants, modified stems, roots, bulbs. Cham, Switzerland: Springer international publishing; 2016 [cited 2017 October 10]. Available from: https://www.springer.com/gp/book/9783319260648.

Liu Y, Nair MG. Labdane diterpenes in *Curcuma mangga* rhizomes inhibit lipid peroxidation, cyclooxygenase enzymes and human tumour cell proliferation. Food Chemistry 2011; 124:527-532.

Maeda H, Wada N, Tomokiyo A, Monnouchi S, Akamine A. Prospective potency of TGF-β1 on maintenance and regeneration of periodontal tissue. International Review of Cell and Molecular Biology 2013; 304:283-367.

Mahalingam R, Li X, Jasti BR. Semisolid dosages: ointments, creams, and gels. United States of America: John Wiley & Sons, Inc.; 2007.

Makboon K. Chemical constituents of *Curcuma mangga* rhizome [Master thesis]. Bangkok, Thailand: Chulalongkorn University; 2000.

Malek SA, Lee GS, Hong SL, Yaacob H, Wahab NA, Weber FJ, Shah S. Phytochemical and cytotoxic investigations of *Curcuma mangga* rhizomes. Molecules 2011; 16:4539-4548.

Manimmanakorn N, Manimmanakorn A, Boobphachart D, Thuwakum W, Laupattarakasem W, Hamlin MJ. Effects of *Zingiber cassumunar* (Plai cream) in the treatment of delayed onset muscle soreness. Journal of Integrative Medicine 2016; 14:114-120.

Matangi SP, Mamidi SA, Gulshan MD, Raghavamma STV, Nadendla RR. Formulation and evaluation of anti-aging poly herbal cream. International Journal of Pharmaceutical Sciences Review and Research 2014; 24:133-136.

Medzhitov R. Inflammation 2010: New Adventures of an Old Flame. Cell 2010; 140:771-776.

Mittal A, Sardana S, Pandey A. Herbal boon for wounds. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5:1-7.

Mulay S, Borade A, Bele A, Khale A. Aloe Vera - A Review. International Journal of Pharmaceutical and Phytopharmacological Research 2013; 3:203-211.

Ng KW, Lau WM. Skin deep: the basics of human skin structure and drug penetration. Percutaneous penetration enhancers chemical methods in penetration enhancement: Drug Manipulation Strategies and Vehicle Effects. 2015; 1:3-11. doi:10.1007/978-3-662-45013-0_1.

Nicholas C, Batra S, Vargo MA, Voss OH, Gavrilin MA, Wewers MD, Guttridge DC, Grotewold E, Doseff AI. Apigenin blocks lipopolysaccharide-induced lethality *in vivo* and proinflammatory cytokines expression by inactivating NF-kappaB through the suppression of phosphorylation. Journal of Immunology 2007; 179:7121-7127.

Nooheet W. Inhibition of pathogenic bacteria contaminating exposed surfaces by extracts of Zingiberaceae family [Master thesis]. Songkhla, Thailand: Prince of Songkla University; 2014.

Othman SB, Yabe T. Use of hydrogen peroxide and peroxyl radicals to induce oxidative stress in neuronal cells. Reviews in Agricultural Science 2015; 3:40-45.

Pathmakanthan S, Hawkey CJ. A lay doctor's guide to the inflammatory process in the gastrointestinal tract. Postgraduate Medical Journal 2000; 76:611-617.

Patil MK, Kandhare AD, Bhise SD. Pharmacological evaluation of ethanolic extract of *Daucus carota* Linn root formulated cream on wound healing using excision and incision wound model. Asian Pacific Journal of Tropical Biomedicine 2012; 2:S646-S655.

Patrulea V, Ostafe V, Borchard G, Jordan O. Chitosan as a staring material for wound healing applications. European Journal of Pharmaceutics and Biopharmaceutics 2015; 97:417-426.

Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. International Journal of Biomedical Science 2008; 4:89-96.

Pokorny J, Yanishlieva N, Gordon M. Antioxidants in food: practical applications. British Journal of Nutrition 2001; 87:391.

Pujimulyani D, Raharjo S, Marsono Y, Santoso U. The effect of size reduction and preparation duration on the antioxidant activity of White Saffron (*Curcuma mangga* Val.). Journal of Food and Pharmaceutical Sciences 2013; 1:18-21.

Reinke JM, Sorg H, Wound repair and regeneration. European Surgical Research 2012; 49:35-43.

Ruangsang P, Tewtrakul S, Reanmongkol W. Evaluation of the analgesic and anti-inflammatory activities of *Curcuma mangga* Val and Zijp rhizomes. Journal of Natural Medicines 2010; 64:36-41.

Saad AH, Ahmed SN, Moahmed EB. Formulation and evaluation of herbal cream from *Ziziphus spina* leave extract. International Research Journal of Pharmacy 2013; 4:44-48.

Sakunsom P. Plants for anticancer and their mechanism [Master thesis]. Nakorn Prathom, Thailand: Silpakorn University; 2009.

Sarma AD, Mallick AR, Ghosh AK. Free radicals and their role in different clinical conditions: an overview. International Journal of Pharma Sciences and Research 2010; 1:185-192.

Sharififara F, Khazaelia P, Alli N. *In vivo* evaluation of anti-inflammatory activity of topical preparations from fenugreek (*Trigonella foenum-graecum* L.) seeds in a cream base. Iranian Journal of Pharmaceutical Sciences Summer 2009; 5:157-162.

Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammopharmacology 2007; 15:252-259.

Sirirugsa P, Larsen K, Maknoi C. The genus *Curcuma* L. (Zingiberaceae): distribution and classification with reference to species diversity in Thailand. Gardens' Bulletin Singapore 2007; 59:203-220.

Sroka Z, Cisowski W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food and Chemical Toxicology 2003; 41:753-758.

Sudsai T, Wattanapiromsakul C, Nakpheng T, Tewtrakul S. Evaluation of the wound healing property of *Boesenbergia longiflora* rhizomes. Journal of Ethnopharmacology 2013; 150:223-231.

Sudsai T, Wattanapiromsakul C, Tewtrakul S. Wound healing property of isolated compounds from *Boesenbergia kingii* rhizomes. Journal of Ethnopharmacology 2016; 184:42-48.

Sun J, Zhang X, Broderick M, Fein H. Measurement of nitric oxide production in biological systems by using griess reaction assay. Sensors 2003; 3:276-284.

Tangjitjareonkun J, Supabphol R. Application of *Aloe vera* on wound healing. Journal of Medicine and Health Sciences 2015; 22:53-67.

Tatsutomi M, Jo J, Tabata Y. Preparation of a nitric oxide imaging agent from gelatin derivative micelles. Regenerative Therapy 2016; 5:64-71.

Teramoto S, Tomita T, Matsui H, Ohga E, Matsuse T, Ouchi Y. Hydrogen peroxide-induced apoptosis and necrosis in human lung fibroblasts: protective roles of glutathione. The Japanese Journal of Pharmacology 1999; 79:33-40.

Tewtrakul S, Subhadhirasakul S. Anti-allergic activity of some selected plants in the Zingiberaceae family. Journal of Ethnopharmacology 2007; 109:535-538.

Tewtrakul S, Subhadhirasakul S. Effects of compounds from *Kaempferia parviflora* on nitric oxide, prostaglandin E_2 and tumor necrosis factor alpha productions in RAW264.7 macrophage cells. Journal of Ethnopharmacology 2008; 120:81-84.

Thanan R, Oikawa S, Hiraku Y, Ohnishi S, Ma N, Pinlaor S, Yongvanit P, Kawanishi S, Murata M. Oxidative stress and its significant roles in neurodegenerative diseases and cancer. International Journal of Molecular Sciences 2015; 16:193-217.

Tonder AV, Joubert AM, Cromarty AD. Limitations of the 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC Research Notes 2015; 8:1-10.

Ueda CT, Shah VP, Derdzinski K, Ewing G, Flynn G, Maibach H, Marques M, Rytting H, Shaw S, Thakker K, Yacobi A. Topical and transdermal drug products. Pharmacopeial Forum 2009; 35:750-764.

Unlu GV, Candan F, Sokmen A, Dafefera D, Polissiou M, Sokmen E, Donmez E, Tepe B. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey. Var. *pectinatus* (Lamiaceae). Journal of Agricultural and Food Chemistry 2003; 51:63-66.

Veal EA, Day AM, Morgan BA. Hydrogen peroxide sensing and signaling. Molecular Cell 2007; 26:1-14.

Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. The Journal of International Medical Research 2009; 37:1528-1542.

Volz P, Boreham A, Wolf A, Kim T, Balke L, Frombach J, Hadam S, Afraz Z, Rancan F, Blume-Peytavi U, Vogt A, Alexiev U. Application of single molecule fluorescence microscopy to characterize the penetration of a large amphiphilic molecule in the stratum corneum of human skin. International Journal of Molecular Sciences 2015; 16:6960-6977.

Widowati W, Sardjono CT, Wijaya L. Free radicals scavenging activities of spices and curcumin. Proceeding of the 2nd International Symposium on Temulawak; 2011 May 24-29; Bogor, Indonesia. 178-181.

Wirasorn K, Klarod K, Hongsprabhas P, Boonsiri P. Oxidative stress, antioxidant and cancer. Srinagarind Medical Journal 2014; 29:207-219.

Yoshioka Y, Sugino Y, Tozawa A, Yamamuro A, Kasai A, Ishimaru Y, Maeda S. Dopamine inhibits lipopolysaccharide-induced nitric oxide production through the formation of dopamine quinone in murine microglia BV-2 cells. Journal of Pharmacological Sciences 2016; 130:51-59.

Zamora R, Vodovotz Y, Billiar TR. Inducible nitric oxide synthase and inflammatory disease. Molecular Medicine 2000; 6:347-473.

VITAE

Name Miss Suthasinee Srirod

Student ID 5910720019

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Oriental Medicine	Rangsit University	2559

Scholarship Awards during Enrolment

1. Center of Excellence for Innovation in Chemistry (PERCH-CIC)

List of Publication and Proceedings

1. Srirod S, Tewtrakul S. Biological activities of cream containing *Curcuma mangga* extract. the 5th International Conferences on Current Drug Development 2018 (CDD 2018) and the 3rd International Conferences on Herbal and Traditional Medicine 2018 (HTM2018); 2018 May 23rd – 25th; Songkhla, Thailand.
2. อนุสิทธิบัตร เรื่อง สูตรตำรับครีมต้านการอักเสบจากสารสกัดขมิ้นขาว เลขที่คำขอ: 1803001259 วันที่ยื่นคำขอ 1 มิถุนายน พ.ศ.2561.