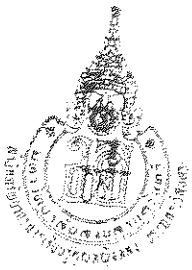

An Antibacterial Patch Made from Mangosteen Peel Extract

Turawat Phadungkarn

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

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ชื่อวิทยานิพนธ์ แผ่นแปะต้านเชื้อแบคทีเรียจากสารสกัดเปลือกมังคุด

ผู้เขียน นาย ธุรวัตร ผดุงการ

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ปีการศึกษา 2553

บทคัดย่อ

มังคุดเป็นไม้ยืนต้น ปลูกมากทางภาคตะวันออกและภาคใต้ของไทย มีชื่อทางวิทยาศาสตร์ว่า *Garcinia mangostana* L. อยู่ในวงศ์ Guttiferae จากตำรับยาแผนโบราณระบุว่าเมื่อนำเปลือกผลมังคุดมาบดเป็นผง หรือชง หรือต้มรับประทาน จะมีฤทธิ์ในการแก้ท้องเสีย หากนำมาฝนกับน้ำปูนใสใช้ทาบาดแผลจะมีฤทธิ์แก้แผลเปื่อยพุพอง แผลเป็นหนอง โดยมีการศึกษาฤทธิ์ทางชีวภาพของสารสกัดจากเปลือกมังคุด พบว่า สารสกัดหยาบจากเปลือกมังคุด มีฤทธิ์ต้านอนุมูลอิสระ, ต้านการอักเสบ, ต้านเชื้อแบคทีเรีย *Staphylococcus aureus*, *Helicobacter pyroli*, *Propionibacterium acnes* และ *Staphylococcus epidermidis* แต่ในปัจจุบันยังไม่มีการพัฒนาสูตรตำรับเพื่อใช้ในการรักษาแผล ฝีหนอง จากสารสกัดเปลือกมังคุดอย่างจริงจัง ดังนั้นวัตถุประสงค์ในงานวิจัยนี้คือ พัฒนาสูตรตำรับแผ่นแปะจากสารสกัดเปลือกมังคุดเพื่อต้านเชื้อแบคทีเรีย โดยในงานวิจัยนี้ได้สกัด α-mangostin จากเปลือกมังคุด (*Garcinia mangostana* L.) โดยใช้ 95% เอทานอล และแบ่งส่วนโดยใช้วิธีโครมาโทกราฟีแบบคอลัมน์ ซึ่งพบว่าสารสกัดมีปริมาณ α-mangostin เท่ากับ 77.86% โดยน้ำหนัก จากนั้นจึงนำสารสกัดจากเปลือกมังคุดไปทดสอบฤทธิ์ต้านเชื้อแบคทีเรีย 3 ชนิด ประกอบด้วย *S. epidermidis*, *S. aureus* และ *P. acnes* พบว่าสารสกัดจากเปลือกมังคุดสามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียได้ โดยความเข้มข้นต่ำสุดที่สามารถยับยั้งเชื้อได้ (MIC) ต่อเชื้อ

ทั้ง 3 ชนิดเท่ากับ 2.0, 4.0 และ 4.0 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ เมื่อนำสารสกัดจากเปลือกมังคุดมาพัฒนาเป็นสูตรตำรับแผ่นแปะเพื่อต้านเชื้อแบคทีเรียโดยใช้ เพคติน, อะการ์ และโซเดียมอัลจิเนต เป็นส่วนประกอบ พบว่า แผ่นแปะที่ประกอบด้วย อะการ์ 1 กรัม, โซเดียมอัลจิเนต 1 กรัม, เพคติน 1 กรัม, พลอพิลีนไกลคอล 20 มิลลิลิตร และ กลีเซอรีน 1.2 กรัม และแผ่นแปะที่ประกอบด้วย อะการ์ 1.5 กรัม, โซเดียมอัลจิเนต 1 กรัม, เพคติน 1 กรัม, พลอพิลีนไกลคอล 20 มิลลิลิตร และ กลีเซอรีน 1.05 กรัม โดยปริมาณตัวยาที่เหมาะสมในแผ่นแปะจะเท่ากับ 0.231 มิลลิกรัมต่อตารางเซนติเมตร แผ่นแปะที่ได้มีลักษณะ เรียบ มีความเป็นเนื้อเดียวกัน เมื่อดูพื้นผิวโดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด สารสกัดในแผ่นแปะมีความคงตัวดีเมื่อเก็บไว้ในอุณหภูมิ 45 °C/75% RH เป็นเวลา 4 เดือน โดยเมื่อนำแผ่นแปะมาทดสอบกับเชื้อโดยวิธี disc diffusion พบว่า แผ่นแปะมีฤทธิ์ต้านการเจริญเติบโตของเชื้อ *S. epidermidis*, *S. aureus* และ *P. acnes*

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Author	Mr. Turawat Phadungkarn
Major Program	Pharmaceutical Sciences
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ABSTRACT

Mangosteen is perennial plant which has grown in many areas in eastern and southern Thailand. The scientific name of mangosteen is *Garcinia mangostana* L. in the Guttiferae family. Traditional medicine recipes stated that when mangosteen was ground to be a powder and brew or boil. It will be effective in treating diarrhea. If dry mangosteen peel was ground and mixed with limewater, it can treat abscess, wound and ulcer. The study of biological effect of mangosteen extracts showed that extracts from rough mangosteen peel has antioxidant activity, anti-inflammatory activity, antibacterial such as *Staphylococcus aureus*, *Helicobacter pyroli*, *Propionibacterium acnes* and *Staphylococcus epidermidis.* It is popularly applied to cosmetic and pharmaceutical products. However, there were no formulations of mangosteen peel extract for treating abscess and ulcer. Thus, the aim of this study was to formulate the antibacterial patch, which made from mangosteen peel extract. Mangosteen peel concentrate extract was prepared from the fruit peel of *G. mangostana* by maceration using 95% ethanol and partition using column chromatography. The results showed that the amount of α-mangostin from mangostin peel extract was 77.86±1.13% w/w. Antibacterial activity determination of extract showed the mangosteen peel concentrate extract was capable of inhibiting the growth

of *S. epidermidis, S. aureus* and *P. acnes,* with MIC values of 2.0, 4.0 and 4.0 μg/ml, respectively. Formulation of antibacterial patch containing mangosteen peel concentrate extract was prepared and examined. It was found that appropriate formulation of patch was formulation 7, which consists of agar 1 g, sodium alginate 1 g, pectin 1 g, propylene glycol 20 ml and glycerin 1.2 g and formulation 12, which consist of agar 1.5 g, sodium alginate 1 g, pectin 1 g, propylene glycol 20 ml and glycerin 1.05 g. The appropriate concentration of mangosteen peel extract was 0.231 mg/cm^2. The antibacterial patch was then subjected to evaluation of stability test. The results indicated that α-mangostin in the antibacterial patch had a good stability when stored in 45 °C/75% RH for 4 months. The cross section of patch formulations 7 and 12 containing mangosteen peel extract were studied by scanning electron microscopy. The results showed that the cross-section of both formulations were homogeneous and smooth morphology. Finally, the antibacterial activity of patch was test by using disc diffusion method. The results showed that patch, which made from mangosteen peel extract, had antibacterial activity against *S. epidermidis*, *S. aureus* and *P. acnes*.

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Turawat Phadungkarn

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LIST OF ABBREVIATIONS AND SYMBOLS

α-	=	Alpha-
β-	=	Beta-
γ-	=	Gamma-
λ	=	Lambda
μg	=	Microgram
μl	=	Microliter
μm	=	Micrometer
°	=	Degree
AR	=	Analytical reagent
BHI	=	Brain heart infusion
CFU	=	Colony forming Unit
CH_2Cl_2	=	Dichloromethane
cm	=	Centimeter
cm^2	=	Square centimeter
°C	=	Degree Celsius
DMSO	=	Dimethyl sulfoxide
DPPH	=	2, 2-diphenyl-1-picrylhydrazyl
EB	=	Elongation at break
ELISA	=	Enzyme-linked immunosorbent assay
EtOAc	=	Ethyl acetate
EtOH	=	Ethanol
g	=	Gram

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

G. mangostana	=	*Garcinia mangostana*
HCl	=	Hydrochloric acid
HM	=	High molecular weight
H_2O_2	=	Hydrogen peroxide
HPLC	=	High-performance liquid chromatography
IC_{50}	=	Concentration of 50 percent inhibition
IR	=	Infrared
Kg	=	Kilogram
LM	=	Low molecular weight
LOD	=	Limit of detection
LOQ	=	Limit of quantitation
m	=	Meter
mA	=	Milliamp
MBC	=	Minimum bactericidal concentration
mg	=	Milligram
MHA	=	Mueller-hinton agar
MHB	=	Mueller-hinton broth
MIC	=	Minimum inhibitory concentration
min	=	Minute
ml	=	Milliliter
mm	=	Millimeter
m.p.	=	Melting point

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

MRSA	=	methicillin-resistant *Staphylococcus aureus*
N/mm^2	=	Newton per Square Millimeter
NaOH	=	Sodium hydroxide
NMR	=	Nuclear magnetic resonance
NBT	=	Nitroblue tetrazolium dye
PBS	=	Phosphate buffer solution
P. acnes	=	*Propionibacterium acnes*
P. aeruginosa	=	*Pseudomonas aeruginosa*
ROS	=	Reactive oxygen species
r^2	=	Coefficient of determination
RH	=	Relative humidity
RSD	=	Relative standard deviation
SEM	=	Scanning electron microscopy
SGOT	=	Serum glutamic oxaloacetic transaminase
SGPT	=	Serum glutamic pyruvic transaminas
S. aureus	=	*Staphylococcus aureus*
S. epidermidis	=	*Staphylococcus epidermidis*
TLC	=	Thin layer chromatography
TNF-α	=	Tumor necrosis factor-alpha
TS	=	Tensile strength
UV	=	Ultraviolet

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

VRE	=	Vancomycin resistant *Enterococci*
w/w	=	Weight by weight
XRD	=	X-ray diffraction

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Thailand is a country located in the tropical area which has the suitable environment and climate for growth of various species of bacteria. Nowadays problems of antibiotics drug resistance in Thailand has increased. So, finding new drug is one of many answers for solving this problem. One of the most important methods is by taking extracts from herbal with antibacterial activities and been used in folk medicine. One of many kinds of Thai herbs which have this property is mangosteen (บวร เอี่ยมสมบูรณ์, 2518; พระเทพวิมลโมลี, 2524; Chen *et al.*, 2008; Chomnawang *et al.*, 2005; Chomnawang *et al.*, 2007; Itoh *et al.*, 2008; Kosem *et al.*, 2008; Mahabusarakum *et al.*, 1983).

Mangosteen is perennial plant which has grown in many areas in eastern and southern Thailand. The scientific name of mangosteen is *Garcinia mangostana* L. in the Guttiferae family. In traditional medicine, mangosteen peel is ground to powder and brew or boil. The resulting solution is used to treat diarrhea. Dried powder of mangosteen peel after mixing with limewater can be used to treat abscess, wound and ulcer (บวร เอี่ยมสมบูรณ์, 2518; พระเทพวิมลโมลี, 2524). Biological

effect of mangosteen extracts showed that extracts from mangosteen peel have antioxidant activity, cytoprotective activity, and antibacterial against *Staphylococcus aureus, Helicobacter pyroli, Propionibacterium acnes* and *Staphylococcus epidermidis* (Chen *et al.*, 2008; Chomnawang *et al.*, 2005; Chomnawang *et al.*, 2007; Itoh *et al.*, 2008; Kosem *et al.*, 2008; Mahabusarakum *et al.*, 1983).

The chemical study found important compounds in mangosteen peel which belong to a group of xanthones (Asai *et al.*, 1995; Chin *et al.*, 2008; Govindachari *et al.*, 1971) such as α-mangostin, β-mangostin, γ-mangostin, mangostatin and gartanin. α-Mangostin (Figure 1.1) is the most content and displayed good biological effects. It has anti-inflammatory, histamine H1 receptor antagonist, antibacterial activity against *H. pylori,* methicillin-resistant *S. aureus* (MRSA) and vancomycin resistant *Enterococci* (VRE) (Chairungsrilerd *et al.*, 1996; Chen *et al.*, 2008; Sakagami *et al.*, 2005).

Nowadays formulation development for use in skin drug delivery, widespread especially, anti-fungal and antibacterial formulations are of interest. The products are available on the market such as gatifloxacin eye drops, antifungal shampoo, cream, tincture and patch. Patch can be divided into 2 types. The first type is patch containing active drug. Another type is patch which has no drug but used to protect wound from water and disease. The first type can be classified into 2 types (1.) patch which deliver drugs through skin to give systemic action, called transdermal patch and (2.) patch which deliver drugs to only area of skin, called topical patch (Shah *et al.*, 1992). Generally, patches could be divided to another model according to

paste style (1.) adhesive device, this model composes of the backing layer and the drug is in adhesive layer. The advantages of this model are able to determine the dose to the skin surface area in time frame. (2.) Monolithic device, this model consists of backing layer, a layer of drug which distribute in polymer and a layer of adhesive. The advantage of this model is release rates of the drug from polymer based on prolonged drug release pattern during the sheets stuck to skin and (3.) reservoir device, these models consist of backing layer, layer containing drug, membrane to control the release rate of drug and adhesive layer. The advantage of this model is that the drug can be used in solution or gel forms by using membrane plate to control the release of drug. The release rate is constant all at the times if the drug solution is still presence in a reservoir layer continues to be full (Figure 1.2) (Hadgraft and Guy, 1989).

Today's the extracts from mangosteen peel (mangosteen extract) was widely used in cosmetics such as liquid soap, soap bars and shampoo. However, no patch products from mangosteen peel extract that uses for the treatment of abscess is available. So that it is the good point for researcher to formulate the patch which contain the extracts from the mangosteen peel, which have high-content of α-mangostin, by using biodegradable polymer include sodium alginate, pectin and agar. In this study, a topical formulation antibacterial patch for the treatment abscess and prevent bacterial infection of skin area and in form of adhesive device was developed. Because the drug from patch can attack bacteria directly, the amount of drug to areas of skin can be determined in time range and the drug will be released enough quantities to inhibit bacteria after skin that is pasted by patch.

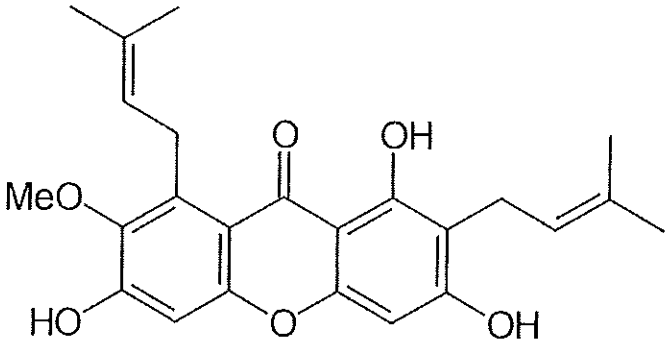


Figure 1.1 Chemical structure of α-mangostin

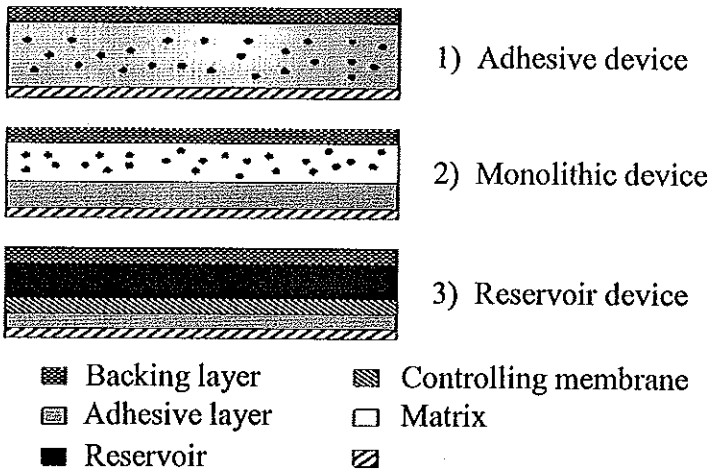


Figure 1.2 Three types of skin patch; 1. Adhesive device, 2. Monolithic device and 3. Reservoir device (ชัชฎา โพธิพุกกณะ, 2552).

1.2 Objectives of Research

The aims of this present study were as follow:

1. To prepare antibacterial patch made from mangosteen peel extract.
2. To study the anti-bacterial activity of patch.
3. To study the physical properties of the antibacterial patch.

CHAPTER 2

LITERATURE REVIEW

The topic of literature review in this thesis can be divided into 2 groups; *Garcinia mangostana* and component of the patch include pectin, agar and sodium alginate.

2.1 *Garcinia mangostana* L.

2.1.1 Botanical description of *G. mangostana*

Mangosteen, *G. mangostana* is a medicinal plant in the Guttiferae family (Figure 2.1). The original place of this plant is unknown but many people believed that it was originated at the Sunda Islands and the Moluccas which have wild trees in the forests of Kemaman, Malaya. Moreover, the tree may have been first domestic in Thailand or Burma (Corner, 1988). It has a number of ordinary names in particular : mangkhut (Thailand), mangosteen (English), mangostan or mangostin (Spanish), mangoustan (French), manggis (Indonesia, Malaysia), manggustan or manggis (Philippines), mongkhut (Cambodia), mangkhud (Laos) and cay mang cut (Vietnam) (Morton, 1987).

Figure 2-1 Mangosteen tree (from http://mthaifruit.igetweb.com)

The growing of mangosteen tree is very slow, upstanding, with a pyramidal crown; about 7-25 m in height, has dark-brown or almost black, flaking bark, the inner bark consist of yellow, gummy, bitter latex. The evergreen, adverse, short-stalked leaves are ovate-oblong or elliptic, leathery, and thick, dark-green, slightly glossy above, yellowish-green, and dull beneath; about 9-25 cm in long, 4.5-10 cm in wide, with outstanding, pale midrib. Its new leaves are rosy. Flower is about 4-5 cm in wide, and fleshy, may be male or hermaphrodite on the same tree (Figure 2.2). The branch form in clusters of 3-9 tips which has 4 sepals and 4 ovate, thick, fleshy petals, green with red spots and yellowish-red on the outside and inside,

respectively. The tips of young branchlets, the hermaphrodites are borne singly or in pairs; their petals are quickly shed and may be yellowish-green edged with red or mostly red (Morton, 1987).

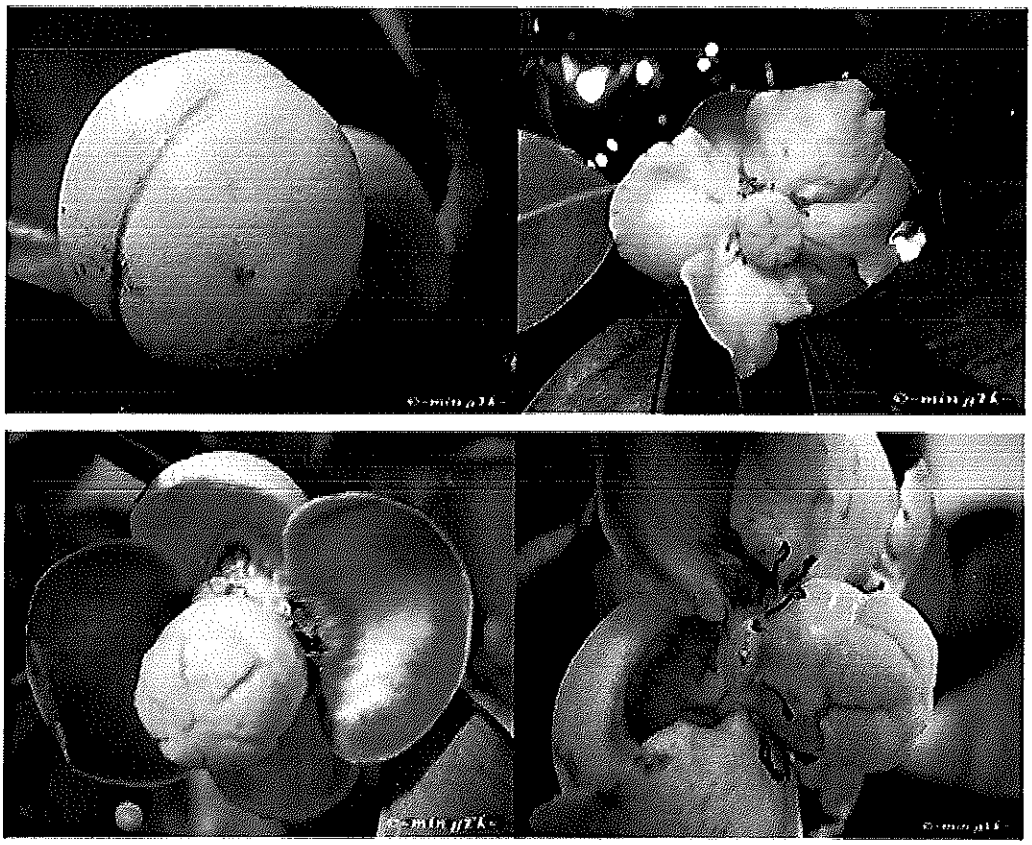


Figure 2.2 Flower of mangosteen (*G. mangostana*) (Min gEk, 2008)

The fruit is capped by the notable calyx at the stem end and with 4-8 triangular, flat remnants of the stigma in a rosette at the apex, is round, dark-purple to red purple and smooth externally; about 3.4-7.5 cm in diameter (Figure 2-3). The thick of rind is 6-10 mm, red in cross-section, purplish-white on the inside. It contains bitter yellow latex and a purple, staining juice. Snow-white pulp has 4-8 triangular segments, juicy, soft flesh (actually the arils of the seeds). The fruit have 1-5 fully developed seeds or may be seedless, ovoid-oblong, somewhat flattened which has 2.5

cm in long and 1.6 cm in wide, that cling to the flesh. The flesh is slightly acid and mild to clearly acid in flavor and is acclaimed as minutely luscious and delicious (Morton, 1987).

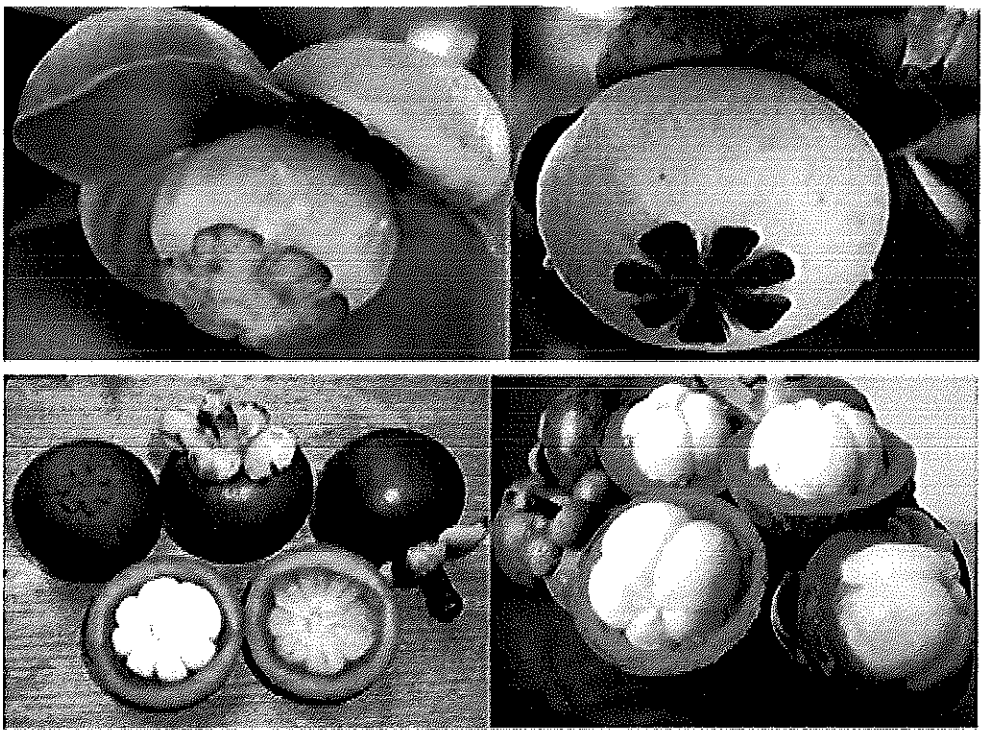


Figure 2-3 Fruits of mangosteen (*G. mangostana*)

(from http://www.montosogardens.com; http://asianlife.igetweb.com)

2.1.2 Ethnomedical Uses

The medicinal properties of *G. mangostana* in Thai traditional text books are described in the following paragraph.

Pericarp (Peel): used for wound healing (พระเทพวิมลโมลี, 2524; Aajsalee, 1979; 1981; Kitikajorn, 1983; Phongboonrod, 1976; School of Thai

traditional medicine, 1981; Songbandit, 1978), dysentery (Aajsalee, 1979; 1981; Kitikajorn, 1983; Phongboonrod, 1976; School of Thai Traditional Medicine, 1981; Songbandit, 1978); treatment of infected wounds and suppurations (Chewakakomarapach, 1974; Iamsomboon, 1975; Jainpanick, 1979; Kitikajorn, 1983; School of Thai Traditional Medicine, 1981; Songbandit, 1978) and chronic ulcers (Koonthon, 1981; Maraproeksavan, 1978).

2.1.3 Traditional Recipes:

Antidiarrheal

1. Boil the dried peel of fruit with a saturated calcium hydroxide solution, consequently the extract is taken to drink (Medical Science Department, Research section, 1983).

2. Special stone rub the dried fruit peels using water as a solvent. The obtained suspension is taken orally for antidiarrhea (Koonthon, 1981).

3. The dried fruit peel is boiled with water. The children dose is 1-2 teaspoonfuls for every four hours. The dose which appropriate for adult is one tablespoonful for every four hours (Medical Science Department, Research section, 1983).

Treatment of wounds

The fruits are dried and rubbed using saturated calcium hydroxide solution. The suspension is applied the wound areas (หมอชีวกโกมารทัจจ์. 2517; Iamsomboon, 1975).

2.1.4 Chemical constituents of *G. mangostana*

It has been stated that the bark, seed, and rind of *G. mangostana* consist of different chemical components in particular; the bark contains tannin (Abbiw, 1990); vitamin C (Quisumbing, 1978). The seeds contain 3% oil (Burkill, 1994); the fruit rind contains 7-13% tannin, pectin, and a resin as well as a yellow crystalline bitter principle called mangostin ($C_{20}H_{22}O_5$) or mangosim as major components (Nadkarni and Nadkarni, 1999; Burkill, 1994). Jayaweera (1981) declared that the flesh fruit (aril) contains 10.8% saccharose, 1% dextrose, and 1.2% kerrelose. A methanolic extract of *G. mangostana* leaves include a flavor compound, 2-ethyl-3-methylmaleimide *N-βD*-glucopyranoside (Krajewski *et al.*, 1996).

G. mangostana is rich in a variety of secondary metabolites, such as oxygenated and prenylated xanthones (Peres and Nagem, 1997; Peres *et al.*, 2000; Suksamrarn *et al.*, 2002). They are biologically active compounds receiving increasing interest in the studies of pharmacologic for different kinds of health benefits. Over 200 xanthones have been identified in nature. In the rind of *G. mangostana* fruit, over 40 xanthones were found. This unprecedented number of xanthone makes the fruit which contains the highest concentration of xanthone on the

planet, *G. mangostana* fruit.

Xanthones, is a class of polyphenolic compounds, are a naturally occurring compound and have been shown to have widespread biological and pharmacological activities. The body's immune system can be supported and enhanced by xanthone. Moreover, they also demonstrate strong antioxidant activity. Many research stated that xanthones were beneficial in helping with plenty conditions consist of: allergies, infections (microbial, fungus, viral), cholesterol levels, inflammation, skin disorders, gastro-intestinal disorders, and fatigue (Gopalakrishnan *et al.*, 1997; Chen *et al.*, 1996; Iinuma *et al.*, 1996; Chin and Kinghorn, 2008). The ability of one single xanthone is to neutralize multiple free radicals making them stopping the chain reaction of free radicals and harmless to the body. The xanthones have other biological activity in particular anti-cancer properties and anti-inflammatory properties (Akao *et al.*, 2008; Chen *et al.*, 2008).

2.1.5 Chemical and physical properties of α-mangostin

Structure of α-mangostin showed in figure 1.1. α-Mangostin is a yellow amorphous material with 180-181 °C of melting point (Yu *et al.*, 2007). There were many reports stated that α-mangostin is a bright yellow, optically inactive, phenolic, crystalline material, and m.p. at 182-183 °C (Schmid, 1855; Dragendorff, 1930, and Murakami, 1932). The UV spectrum of α-mangostin presented maximum absorption bands at λ_{max} 205, 244, 258, 316, and 358 nm, indicating that this compound was a xanthone derivative. The IR spectrum of α-mangostin exhibited

absorption bands at 3380 cm^{-1}, 1646 cm^{-1} and 1594 cm^{-1} for hydroxyl group, conjugated carbonyl group and a conjugated carbon of aromatic ring, respectively. The ^{13}C NMR spectrum of α-mangostin showed 24 resonances for 24 carbon atoms: thirteen quaternary carbons, four methine carbons, and five methyl carbons (Pongcharoen, 2004).

2.1.6 Pharmacological activities of *G. mangostana*

There were a number of pharmacological activities of *G. mangostana* have been reported such as anti acne, antibacterial, anti-cancer, antifungal, anti-inflammatory, antioxidant, antiviral activities, effect on central nervous system, cytotoxic activity, activity against leukemia and antihistamine activity. However, this review will be focused only on anti acne and antibacterial activities.

2.1.6.1 Anti acne

Mechanism of *G. mangostana* extract as anti acne activity can be divided in to 2 types; anti-inflammatory and antibacterial against *P. acnes* and *S. epidermidis.*

Chomnawang and co-workers (2007) reported anti-inflammatory activity of *G. mangostana* peel extract caused by free radical scavenging and cytokine reducing properties. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and Nitroblue tetrazolium dye (NBT) reduction assay were used to determine for anti-

inflammatory activity of *G. mangostana* peel extract. The result showed that the ethanol extract of *G. mangostana* peel reduced reactive oxygen species (ROS) production and had the most significant antioxidant activity. In addition, the tumor necrosis factor-alpha (TNF-α) production determined by enzyme-linked immunosorbent assay (ELISA) could be reduced by *G. mangostana* peel ethanolic extracts. *G. mangostana* peel ethanolic extract was highly effective in scavenging free radicals and was able to reduce the production of pro-inflammatory cytokines. Thus, this study identified that *G. mangostana* peel extract was promising source of anti-inflammatory agent which could be used to treated acne vulgaris.

S. epidermidis and *P. acnes* have been recognized as pus-forming bacteria and induce inflammation in acnes. Antibacterial activities of *G. mangostana* peel extract against *S. epidermidis* and *P. acnes* were tested by disc diffusion and broth dilution methods. The results showed the minimum inhibitory concentration (MIC) values against two bacterial species were the same (0.039 mg/ml) and the minimum bactericidal concentration (MBC) values were 0.039 mg/ml against *P. acnes* and 0.156 mg/ml against *S. epidermidis,* respectively (Chomnawang *et al.*, 2005).

2.1.6.2 Antibacterial activities

The aqueous and ethanolic extracts of *G. mangostana* peel were evaluated to inhibit 35 hospital isolates of methicillin resistant *Staphylococcus aureus* (MRSA). The results showed that the ethanolic extract of *G. mangostana* peel could

inhibit the growth of MRSA. The MICs and MBCs for MRSA isolates were 0.05-0.4 and 0.1-0.4 mg/ml, respectively (Voravuthikunchai and Kitpipit, 2005).

Sakagami and co-workers (2005) found that α-mangostin had antibacterial activity against vancomycin resistant *Enterococci* (VRE) and MRSA, with MIC values of 6.25 and 6.25-12.5 μg/ml, respectively. In addition, Suksamrarn *et al.* (2003) found that α-mangostin exhibited the potent inhibitory effect against *Mycobacterium tuberculosis*, with an MIC of 6.25μg/ml.

Phongpaichit and co-workers (1994) reported the antibacterial activity of extract from *G. mangostana* peel. 49 Isolates of MRSA from patients in Songklanagarind Hospital were evaluated by broth dilution method and 50 isolates of MRSA and 13 isolates of *Enterococcus* spp. from patients in Maharaj Nakorn Chiang Mai Hospital were tested by agar dilution method. The minimum inhibitory concentrations, MIC_{90} of α-mangostin on MRSA by these two methods were 3.1 and 3.7 μg/ml, respectively and inhibited the growth of all *Enterococcus* spp. with the MIC_{100} value was 1 μg/ml.

The antibacterial properties of α-mangostin and its derivatives were studied by Sundaram and co-workers (1983). They found that *Proteus* sp., *Klebsiella* sp. and *Escherichia coli* were only moderately susceptible to xanthones, whereas *S. aureus*, *P. aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis* were highly susceptible to them. The minimum inhibitory concentration of α-mangostin against *Proteus* sp., *Klebsiella* sp., *Escherichia coli*, *S. aureus*, *P. aeruginosa*, *Salmonella*

typhimurium and *Bacillus subtilis* were between 12.5 and 50 μg/ml.

2.1.7 Stability of α-mangostin in *G. mangostana* peel dichloromethane extract

Physical and chemical stability of *G. mangostana* peel dichloromethane extract was examined. It was found that when the extract were stored in various conditions such as in various temperatures such as 4 °C, 30 °C and 45 °C, they did not have an effect on the stability of the extract within 120 storage days, However, the experiment found that when keeping these extracts in exposures to light, the mangosteen peel extract become dark color after 45 days and the amount of α-mangostin was decreased significantly after 30 days of storage. Furthermore, *G. mangostana* peel extract was also examined under acidic, alkaline and oxidative conditions for 30 days at room temperature. The result showed that α-mangostin content was decreased significantly after 4 days when kept in 0.01 N HCl and decreased significantly after 2 days when kept in 3% H_2O_2. However, when *G. mangostana* peel extracts were kept in 0.01, 0.1, and 1 N NaOH at room temperature for 30 days. The results indicated that the amounts of α-mangostin in *G. mangostana* peel extracts were not significantly decreased (Yodhnu *et al.*, 2009).

2.1.8 Toxicological study of α-mangostin

The experiment was tested by using guinea pig. The amount of serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase

(SGPT) enzyme were evaluated. These enzymes are normally contained within liver cells. If the liver is injured, the liver cells spill the enzymes into blood, raising the enzyme levels in the blood and signaling the liver damage (Hepatitis Central, 2011). In the experiment, α-mangostin substance was injected into abdomen of guinea pig (200 mg per kg body weight). As a result, the amount of SGOT and SGPT increased significantly after received α-mangostin substance for 12 hours. But when compared with paracetamol, the amount of SGOT and SGPT enzyme in guinea pig which received paracetamol were increased more than the guinea pig which received α-mangostin. Moreover, the amount of total protein in the liver of guinea pig which received α-mangostin did not change (Sornprasit *et al.*, 1987).

2.1.9 Method of determination of α-mangostin in mangosteen peel extract

Yodhnu *et al.* (2009) reported that the high performance liquid chromatographic (HPLC) method was developed and validated to assay alpha-mangostin in plant extract using a 5 micron, C18 column (125x4 mm) and mobile phase consisting of 70% acetonitrile in 0.2% formic acid in water delivered isocratically at a flow rate of 1 ml/min with UV detection at 240 nm. The assay was fully validated and shown to be linear ($r^2 > 0.999$), sensitive (LOD = 0.02 μg/mL and LOQ = 0.08 μg/ml), accuracy (recovery rates > 95.8%), precision (intra-day variation < 1.8%, inter-day variation < 4.3%) and specific. Total analysis was about 8 min with typical retention time of α-mangostin of about 6 min.

2.1.10 Stability studies of α-mangostin in *G. mangostana* peel extract film

Yodhnu (2008) reported that films containing *G. mangostana* peel extract were prepared and kept in well-closed containers, protected from light using aluminium foil. Samples were then stored in a refrigerator at 4 °C, room temperature (30±2 °C) and 45 °C with 75% RH for 120 days. The content of α-mangostin was determined with the HPLC method. In order to perform the determination, about 100.0 mg of the films were cut and weighed and transferred to 15 ml centrifuge tube, 5 ml of methanol was then added. All samples were shaken for 10 s and then sonicated for 15 min at room temperature. The samples were centrifuged at 10 °C at 4000 rpm for 10 min. The supernatant was transferred to 25 ml volumetric flask. This procedure was repeated twice with the corresponding supernatants transferred to the corresponding 25 ml volumetric flask. The sample was then diluted to the final volume with methanol. Prior to injection, each sample was filtered through a 0.45 μm nylon membrane filter. The results showed that physical and chemical stability studies of the film indicated that the temperature (4 °C, 30 °C and 45 °C) did not have any effect on the stability of α-mangostin the film during 120 days of storage.

2.2 The main components of patch; Agar, pectin and sodium alginate

2.2.1 Agar

Agars are known as water-soluble, gel-forming polysaccharide which was extracted from certain seaweeds of the Rhodophyceae class. It is a complex mixture of polysaccharides composed of two major fractions-agarose, a neutral polymer, and a charged, sulfated polymer agaropectin. Agars usually composed of repeating agarobiose units alternating between 3- linked β-D-galactopyranosyl (G) and 4-linked 3,6-anhydro-α-L-galactopyranosyl (LA) units. This disaccharide regularity may be marked or modified in a number of ways by substitution of hydroxyl groups with sulfate hemiesters and methyl ethers in various combination and more rarely with a cyclic pyruvate ketal as 4,6-O-[(R)-1-carboxyethylidene] acetal and sometimes by additional monosaccharides. Structural of the agar group of polysaccharides are shown on Figure 2.4 (Usov, 1998).

Agar is insoluble in cold water, but it swells considerably, absorbing as much as twenty times its own weight of water. It dissolves readily in hot water above 85 °C and the gel is setting when the preparation is cooled down to temperatures close to 35 °C. The gels are able to withstand temperatures below 80 °C. The gels set again as the temperature is decreased to temperatures close to 35 °C. Moistened agar can be flocculated by ethanol, 2-propanol or acetone, or salted out by high concentrations of electrolytes.

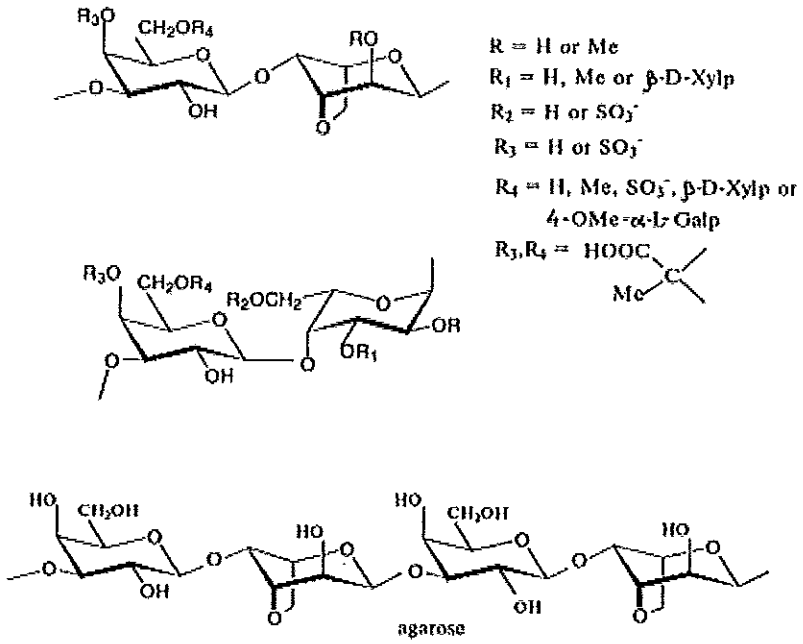


Figure 2.4 Structural features of the agar group of polysaccharides (Usov, 1998)

2.2.2 Pectin

Pectin is a family of complex polysaccharides that contain D-galacturonic acid (GalA) units, joined in chains by means of α-(1-4) glycosidic linkage. These uronic acids have carboxyl groups, some of which are naturally present as methyl esters and others which are commercially treated with ammonia to produce carboxamide groups (Figure 2.5).

Pectin is soluble in pure water. Pectinic and pectic acids's monovalent cation (alkali metal) salts are also soluble in water. Moreover, di- and trivalent cations salts are insoluble or weakly soluble. Dry powdered pectin has a tendency to hydrate

very rapidly and forming clumps when is added to water. These crumps , contained in an envelope of highly hydrated outer coating, include semidry packets of pectin. Further solubilisation of such crumps is so slow. Dry mixing pectin powder can help to prevent clump formation by using pectin having improved dispersibility through special treatment during manufacturing (Sriamornsak, 2003).

Dissolved pectin can be decomposed spontaneously by de-esterification as well as de-polymerisation. Furthermore, the rate of decomposition depends on water activity, pH and the temperature. Generally, pH 4 is the maximum stability for pectin. The pectin solution consists of sugar has a certain protective effect while elevated temperatures rise the rate of degradation. Hydrolysis of glycosidic linkages is observed as a result of low pH-values and elevated temperatures. Low pH favor with de-esterification. A high molecular weight (HM)-pectin becomes slower setting or gradually adapts low molecular weight (LM)-pectin characteristics by de-esterification. HM-pectin is stable at room temperature, at near-to-neutral pH (5-6). A so-called elimination starts, as the temperature (or pH) increases, which results in chain cleavage and very rapid loss of viscosity and gelling properties. At these conditions, LM-pectins show a somewhat better stability. Even at room temperature, alkaline pH-values pectin is rapidly de-esterified and degraded (Sriamornsak, 2003).

Recently, Jitpukdeebodintra *et al.* (2009) reported the wound healing effect of pectin film. The pectin film prepared from lime pectin of 10-20 %w/w with 10% w/w glycerin as plasticizer. The pectin films were tested on mice's skin. After 30 days treated, the wounds' length on the pectin treated side were shorter 3.3±1.2% than

the originated one, and reduce significantly more than the controlled untreated with pectin film with 95% confident.

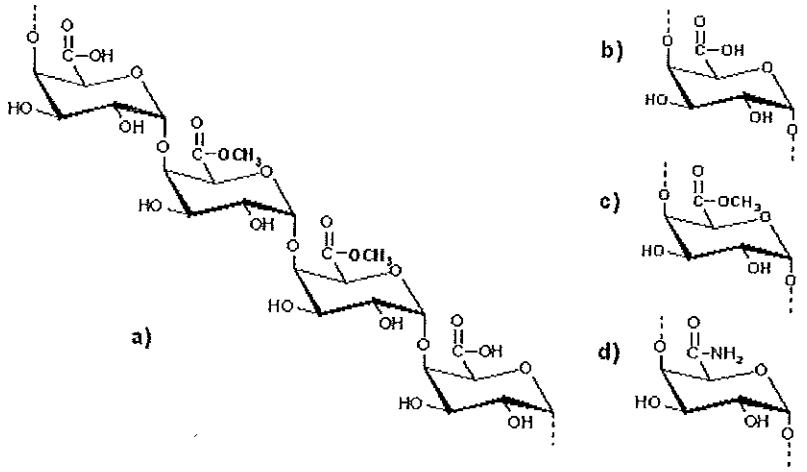


Figure 2-5 (a) A repeating segment of pectin molecule and functional groups: (b) carboxyl; (c) ester; (d) amide in pectin chain (Sriamornsak, 2003)

2.2.3 Sodium alginate

Sodium alginate is a purified carbohydrate product extracted from brown seaweeds by the use of dilute alkali. It is sodium salt of alginic acid, consists of 1,4-linked β-D-mannuronate (M) and α-L-guluronate (G) residues (Figure 2.6). The chemical formula of sodium alginate is $(C_6 H_7 NaO_6)_n$, it contains not less than 90.8 percent and not more than 106.0 percent of sodium alginate of average equivalent weight 222.00, calculated on the dried basis (Ravichandran, 2009).

Alginate is one of interest as a potential biopolymer film or coating component because of its unique colloidal properties, which include thickening,

stabilizing, suspending, film forming, gel producing, and emulsion stabilizing. CAS number of sodium alginate is 9005-38-3. It dissolves slowly in water, forming a viscous solution and insoluble in ethanol and ether. It is incompatible with strong acids, strong bases and strong oxidizing agents.

A study in six healthy adults fed a daily intake of 8 g sodium alginate for seven days does not interfere with the calcium absorption in normal healthy adults (Millis *et al.*, 1947). The other work is done with five healthy male volunteers fed daily intake of 175 mg/kg body weight of sodium alginate for 7 days, followed by a daily intake of 200 mg/kg body weight of sodium alginate for a further 16 days, showed no significant adverse effects (Rowe *et al.*, 2003).

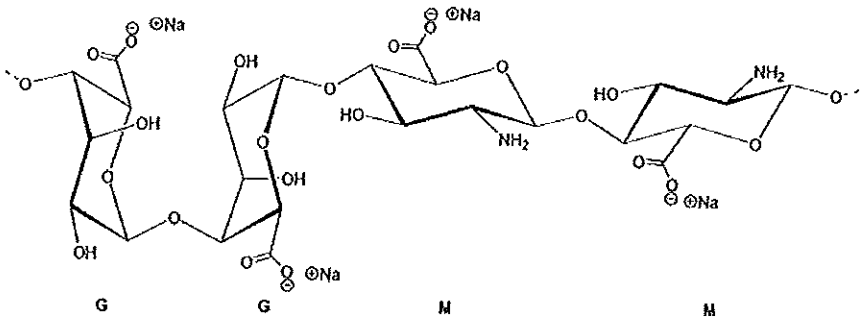


Figure 2-6 Sodium alginate consists of 1,4-linked β-D-mannuronate (M) and α-L-guluronate (G) residues (Volker83, 2008)

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and reagents

3.1.1 Quantitative analysis

The reference standard of α-mangostin was purchased from Chromadex Inc. (Santa Ana, CA; Purity 98.5%), while the secondary standard of α-mangostin was supported by Asst. Prof. Dr. Chatchai Wattanapiromsakul, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Acetonitrile, (HPLC grade), methanol (AR grade), hydrochloric acid 37% (HCl) were purchased from Labscan Asia Co., Ltd. (Bangkok, Thailand), formic acid was obtained from MAY & BAKER Ltd. (Dagenham, England).

3.1.2 Extraction of α-mangostin from the fruit peel of *G. mangostana.*

Petroleum ether, dichloromethane (CH_2Cl_2), 95% ethanol (EtOH) and ethyl acetate (EtOAc) were commercial grade and were purchased from High Science distributor, Thailand. These solvents were used for extraction of α-mangostin from the dried fruit peel of *G. mangostana.*

3.1.3 Antibacterial activity study

The test organisms used in this study were as followed: *S. aureus* (ATCC 25923), *S. epidermidis* (TISTR517) and *P. acnes* (DMST 14916). These bacteria were obtained from Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. Mueller-Hinton agar (MHA) and Brain Heart Infusion (BHI) were purchased from Merck. Standard neomycin and standard neomycin paper disc were purchased from Oxoid Ltd. (England). Sodium chloride (NaCl) was AR grade was purchased from Labscan Asia Co., Ltd. (Bangkok, Thailand) and di-methyl sulfoxide (DMSO) was purchased from Riedelde Haen (Germany).

3.1.4 Preparation of patch containing mangosteen peel extract

Ethanol extract of *G. mangostana* peel (α-mangostin, 71.55%, w/w), propylene glycol USP and glycerin (S. Tong Chemicals Co., Ltd, Thailand), pectin from citrus peel (Sigma-Aldrich, Denmark), sodium alginate (Merck, Germany), agar (Sigma-Aldrich, Denmark) and distilled water were used for preparation of patch containing *G. mangostana* peel extract.

3.2 Preparation of *G. mangostana* peel extracts

G. mangostana used in this experiment were collected from Pha Toh district, Chumphon Province in August 2007.

Dried mangosteen peel about 2 kg were ground and then macerated in 3 liters of ethanol for 7 days. After that, the solution was evaporated by rotary evaporator. The maceration was repeated 3 times to get total crude extract. After that, the mangosteen peel extract (30 g) was mixed with silica gel (400 g) The example of these dry substances were prepared by dissolve the substances extraction by a few ethanol and grind with silica gel at 30 g until getting the dry substances. Column (30x5 cm) was packed by taking silica gel which are previously mixed with mobile phase added (petroleum ether and ethyl acetate for 7:3). After finishing this step, the mixer was added in column and taped column in order to adjust the surface of silica gel to be smooth. After that, this powder was taking to spread on the surface of silica gel in the column. The elution volume of each fraction was 50 ml. For the next step, these fractions were evaporated by rotary evaporator and then were analyzed by thin layer chromatography (TLC). The high α-mangostin content fraction was combined by using α-mangostin standard to comparison. %Yield of α-mangostin content was determined by HPLC methods.

3.3 Determination of α-mangostin content in the concentrated extracted

The amount of α-mangostin in the concentrated extract was analyzed by using reverse phase high performance liquid chromatography (Agilent 1100 series, Palo Alto, CA). The column was Hypersil® BDS C18, 125 x 4 mm, 5 µm. The mobile phases were acetonitrile: 0.2% formic acid = 70:30 v/v, flow rate at 1.0 ml/min, UV Detector was set at 240 nm, injection volume was 20 µl (Yodhnu *et al.*, 2008).

Firstly, the standard solutions of α-mangostin were prepared to have concentrations of 1.0, 5.0, 10.0, 15.0 and 20.0 μg/ml by using methanol as a solvent. After that, these standard solutions of α-mangostin were taken to analyze by HPLC to construct standard curve between the real concentrations of α-mangostin and peak areas. After that, the solution of the concentrated extracts was prepared by dissolved in methanol to give the solution having concentration 5.0, 10.0 and 15.0 μg/ml. Then, these samples were taken to analyze by using HPLC and calculated to determine the amount of α-mangostin of the concentrated extracts by using α-mangostin standard graph.

3.4 Minimum Inhibitory Concentration (MIC) Test

A broth microdilution assay was performed for the determination of the MIC. *S. epidermidis* and *S. aureus* were cultured in Mueller-Hinton Agar (MHA) and *P. acnes* was cultured in Brain heart infusion broth. A sequential two-fold dilution method was used in MIC test. There extract was diluted in DMSO with the concentration of 1 mg/ml and diluted with media to the concentration of 64 μg/ml. Neomycin, a positive control, was diluted in sterile water to a concentration of 64 μg/ml and filtered through 0.45 μm sterile filter paper. The test was performed in 96-well plate. The stock solution of neomycin was diluted with media to give the final concentrations of 0.125 to 32 μg/ml (two-fold dilution serial). The stock solution of the extract was diluted with media to give the final concentration of 0.25 to 64 mg/ml. The inoculum was prepared and adjusted to contain 10^8 CFU/ml, by adjusting the turbidity of saline culture to match the McFarland 0.5 standard. It was then further

diluted 1:100 in Mueller-Hinton broth (MHB) to contain 10^6 CFU/ml and 2 µl of the adjusted inoculum was added to each well. The cultures of *S. epidermidis* and *S. aureus* were then incubated at 37 °C under aerobic conditions for 24 hour. *P. acnes* culture was incubated at 37 °C under anaerobe conditions for 48 hour. The MIC was calculated as the highest dilution showing complete inhibition of the test strains (Lorian, 1996).

3.5 Minimum Bactericidal Concentration (MBC) Test

The MBC was measured as the lowest concentration of the compound to kill microorganisms. The incubation mixtures aerobic and anaerobic bacteria that showed positive result of inhibitory effect were streaked on MHA, BHI plate, respectively and incubate. The lowest concentration that did not show any growth was taken as the MBC (Lorian, 1996).

3.6 Preparation an antibacterial patch containing the mangosteen peel extract

For the first stage, agar 0.5, 1 and 1.5 g were dissolved in boiling water and sodium alginate 1 g and pectin 1 g was added following agar and then the solution was mixed to be homogeneous. The mangosteen peel extract amount 16, 20, 24, 28, and 32 g were dissolved in propylene glycol 20 ml and then added to film base mixture and continued stirring to be united. The amount of mangosteen peel extract calculated to be concentration were 0.231, 0.288, 0.346, 0.403 and 0.461 mg/cm^2,

respectively. Glycerin was added in formulations 3-7, 9-13. After that leaving it out until no air bubble. For the third stage, this mixture was added into the preparing plate (25x30 cm^2) and let gelation. Finally, gelation was heated at 50 °C for 24 hours. The combinations of patch formulations are shown in table 3-1.

Table 3-1 The compositions of patch

Formulations	Sodium alginate (g)	Pectin (g)	Agar (g)	propylene glycol (ml)	Glycerin (g)
1	1	1	0.5	20	0
2	1	1	1	20	0
3	1	1	1	20	0.03
4	1	1	1	20	0.3
5	1	1	1	20	0.6
6	1	1	1	20	0.9
7	1	1	1	20	1.2
8	1	1	1.5	20	0
9	1	1	1.5	20	0.035
10	1	1	1.5	20	0.35
11	1	1	1.5	20	0.7
12	1	1	1.5	20	1.05
13	1	1	1.5	20	1.4

3.7 Physical characterization

3.7.1 Moisture uptake study

The patch was cut into 1x1 cm size. It was then placed in a dessicator containing silica gel for 24 hours followed by weighing (Ws) the patch was then to put in a dessicator containing solution saturated of NaCl which humidity was 75% at room temperature after waiting until the patch was saturated that the weight was not increased. The patch was taken out weight (Wm) moisture uptake capacity was calculated based on the equation (Amnuaikit *et al.*, 2005).

$$\text{Moisture uptake capacity} = \{(Wm-Ws)/Ws\}x100$$

3.7.2 Thickness study

The thickness of patch was measured by using micrometer (Series 102-139, Mitutoyo, Japan) which measure thickness from 3 positions and then measured the average of the thickness (Amnuaikit *et al.*, 2005).

3.7.3 Study the surface morphology by using scanning electron microscopy

Feature cross section of the patch's surface was tested by using scanning electron microscopy (SEM). This patch was cut to small pieces (0.5x0.5 cm)

and then was placed on the grid and fixed with adhesive tape. Then, it was covered by the gold and analyzed by using scanning electron microscope (JSM-5200, JEOL, Japan) (Dong *et al.*, 2006).

3.7.4 Study the appearance of the patch

In this study, the observed characteristics of the patch were determined as color of patch and homogenicity under microscope (CH2 Model, Olympus, Japan) and observed with naked eyes.

3.8 Study the amount of drug contained in the patch and distribution of the drug in a patch.

The amount of drug contained in the patch and distribution of the drug in a patch were studied by using methanol extracted the drug from the patch. Then, the amount of α-mangostin was analyzed by HPLC comparing with the standard curve of α-mangostin standard.

The 2x1 cm^2 of patch size was cut into small pieces and were added into a 15 ml centrifuge tube. After that, methanol 5 ml was added into the tube and vortex for about 15 seconds and sonicated for 15 minutes and centrifuged at 4000 rpm about 5 minutes. Supernatant obtained from extraction was taken into volumetric flask size 25 ml after that extracted patch with the same method again, and then supernatant obtained from extraction of the second was taken into volumetric flask with solution

obtained in the first and adjust volume to 25 ml with methanol. This experiment was performed 3 times and the entire solutions were injected into the system HPLC.

The consistency of the drug in the patch were checked by re-experimented at least 3 sheets and 3 points each and then used means ± SD for calculated percent relative standard deviation (RSD) following equation.

$$\text{Percent RSD} = (\text{SD/means}) \times 100$$

3.9 Study the antibacterial activity of patch

The experiment was performed by the disc diffusion method with some modification. Microorganisms tested were adjusted the turbidity to yield approximately 10^8 CFU/ml with 0.85% sodium chloride compared to turbidity of McFarland No. 0.5. The prepared inoculum was streaked on the surface of agar base with cotton stick. The patch was applied with forceps and press down with slight pressure on the agar. Controlled discs were the patch without the extract, and standard neomycin disc (30 µg/disc) as a positive control. For *S. aureus* and *S. epidermidis*, the plates were incubated at 37 °C under aerobic conditions for 24 hour, and *P. acnes* were incubated at 37 °C under anaerobic conditions for 48 hour. The antibacterial activity was expressed as the mean of inhibition diameter (mm, including the diameter of disc) (Lorian, 1996).

3.10 *In vitro* release study

The releasing of the α-mangostin from the patch was studied by using a modified Franz diffusion cell (Hanson Research Corporation, California, USA). For the first stage, patch was cut into 3.14 cm^2 and was placed on the receptor cell which the receptor compartment contained isotonic phosphate buffer solution (PBS) pH 7.4 amount to 11 ml. The contact area of patch was 1.77 cm^2. Temperature of the modified Franz diffusion cell was controlled during experiments by using a circulating water bath at 37 °C. The receptor compartment was stirred all time by using the magnetic stirrer, speed 200 rpm and then the sample was collected for 0.5 ml at 15 minutes, 30 minutes, one, two, three, four, six, eight, and 12 hours with fresh volume of PBS (0.5 ml) was filled into immediately after sampling. After that, the samples were filtered through 0.45 μm membrane then analyzed by HPLC and calculated using the following equation (Amnuaikit *et al.*, 2005).

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i$$

Where Q_t was the cumulative α-mangostin release, C_t was the α-mangostin concentration of the receptor fluid at each sampling time, C_i was the α-mangostin concentration of the i^{th} sample, and V_r and V_s were the volumes of the receptor fluid and the sampling volume, respectively.

3.11 Stability of patch

The patch was stored at 4 °C, ambient temperature and 45 °C, under 75% RH for 4 months in zip plastic bag, protected from light using aluminium foil. The amount of α-mangostin remaining in the patch was measured by using HPLC at 0, 1, 2, 3 and 4 months. The physical changes of the patch such as color and appearance of the patch were observed during storage period.

3.12 Mechanical properties of patch

The patch was fixed between two clamps of universal testing machine (LR10K, Lloyd Instrument Limited., UK) using 5 kg load cell. After that, the patch was slowly pulled by using the speed 30 mm per min. Tensile strength (TS) and elongation break (EB) was calculated using by the following equation.

$$TS = \text{Breaking force/Cross sectional area } (N/mm^2)$$

$$EB = (\text{Increase in length/Original length}) \times 100(\%)$$

3.13 X-ray diffraction study

X-ray diffraction (XRD) was used in the experiment for determination the nature of the crystallinity of the powder extracted from the mangosteen, the patches with and without contained mangosteen peels extracts. The test samples were placed into the glass sheet and analyzed by using X-ray Diffractrometer (X'Pert MPD,

Philips) with Nickel-filtered Cu Kα radiation at 40 kV and 50 mA during 2 of 5°-40° (Dong *et al.*, 2006).

3.14 Statistical Analysis

Each test was repeated at least three times which the results were reported as mean (X) ± SD and differences of significance were calculated by ANOVA and multiple comparison tests by using the SPSS version. 15.0 Program.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preparation of *G. mangostana* peel extracts

From first step, 181 g of crude substance of mangosteen peel extract was obtained from 2 kg of dried mangosteen peels. It was 9.05% w/w when compared with dried mangosteen peel weight. For the next step, crude substance was pre-purified by using column chromatography method. Each fraction was kept and analyzed by TLC using α-mangostin as standard. Fraction 27-42 was combined because of high content of α-mangostin (Figure 4.1). Results from this extraction found that 30 g of crude substance provided 4.53 g of mangosteen peel concentrate extract in yellow powder. It was 1.37% w/w when compared with dried mangosteen peel weight and 15.4% w/w when compared with crude substance weight.

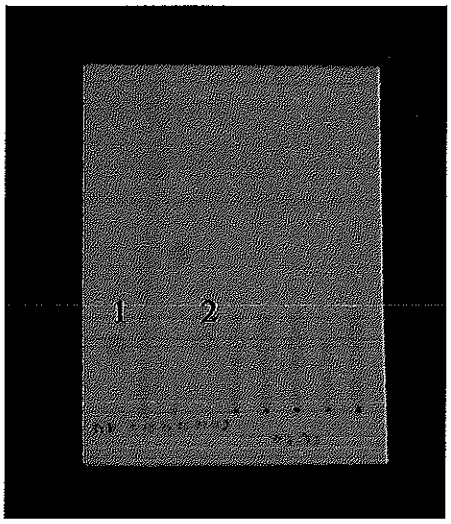


Figure 4.1 TLC of fraction of crude mangosteen peel extract: α-mangostin standard (1), mangosteen peel extract fractions 27-42 (2)

4.2 Analysis to define the amount of α-mangostin in the concentrate extract

α-Mangostin content of mangosteen peel concentrate extract was examined by using HPLC method. HPLC chromatogram of mangosteen peel concentrate extract showed in figure 4.2. In the first step, ethanol was used to extract α-mangostin from dried mangostin peel and then pre-purified by using column chromatography method and kept high α-mangostin contented fractions. The result showed that the amount of α-mangostin from mangosteen peel concentrate extract was 77.86±1.13% w/w. This concentration is higher than the concentration from the study of Jujun et al. (2009). In addition, Jujun reported that concentration of mangostin in crude extract was 11.45% w/w.

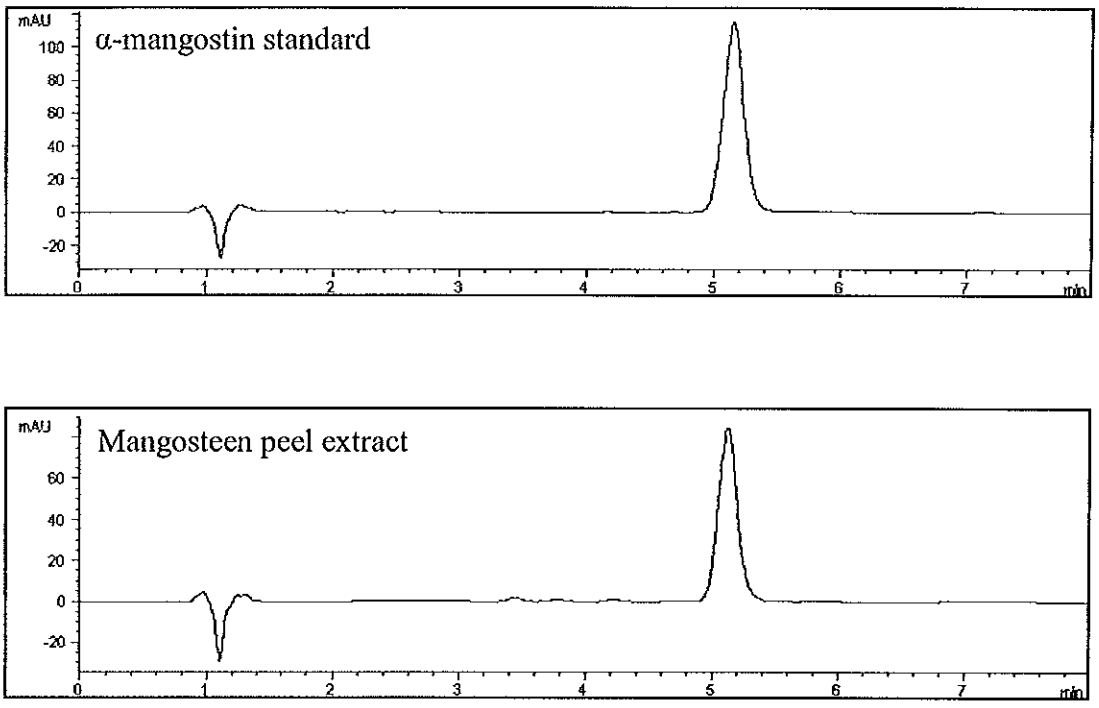


Figure 4.2 HPLC chromatogram of α-mangostin standard and high yield α-mangostin in mangosteen peel extract

4.3 Evaluation of antibacterial activity of mangosteen peel extract against *S. aureus, S. epidermidis*, and *P. acnes*

Since, α-mangostin was found as a major ingredient of *G. mangostana* (Sen *et al.*, 1982; Parveen and Khan, 1988; Mahabusarakam *et al.*, 1987; Sakai *et al.*, 1993; Chairungsrilerd *et al.*, 1996). There are many reports showed antibacterial activity of α-mangostin against *S. aureus, S. epidermidis*, and *P. acnes.* In this study, mangosteen peel extract gave the high α-mangostin content as well as strong antibacterial activities against *S. aureus, S. epidermidis*, and *P. acnes.* The MIC values demonstrated that the mangosteen peel extract showed strong antibacterial activities against *S. aureus, S. epidermidis*, and *P. acnes* with MIC values of 4.00,

2.00 and 4.00 μg/ml, respectively (Table 4-1). In addition, neomycin inhibited the growth of *S. aureus, S. epidermidis*, and *P. acnes* with MIC values of 0.5, 0.5, and 1.00 μg/ml, respectively. These results indicated that mangosteen peel extract could inhibit the growth of gram-positive bacteria effectively. But in this experiment, bactericidal activity of mangosteen peel extract against *S. aureus, S. epidermidis*, and *P. acnes* were not observed at concentration lower than 32 μg/ml. From this result, determined that the mangosteen peel extract don't have bactericidal activity when the concentration of extract lower than 32 μg/ml.

Table 4-1 The MIC values of mangosteen peel extract against *S. aureus, S.epidermidis* and *P. acnes*.

Extract/antibiotic	MIC (μg/ml)*		
	S. aureus	*S. epidermidis*	*P. acnes*
Garcinia mangostana L.	4	2	4
Neomycin	0.5	0.5	1

*All values are means as obtained by triplicate analyses (n=3).

4.4 Appearance of patch without mangosteen peel extract

In this experiment, 13 formulations of patch without mangosteen peel extract were prepared. Formulation and component of patch are shown in table 3-1.

The specification for the best appearance of the patches was clear, smooth surface, uniformity and not cracked. Figure 4.3 showed the patches of formulations 1-13 without mangosteen peel extract. After preparation, the patches of all formulation were transparent and very light yellow color. The patches from formulations 1, 5-7 and 11-13 were smooth but patch from formulation 1 was very easy torn. The patches from formulations 2-4 and 8-10 were crumpled and the edges of all patches were shrunk. After physical properties of the patch was tested, there were 6 formulation patches included formulation 5-7 and 11-13 to be used for preparation the antibacterial patch.

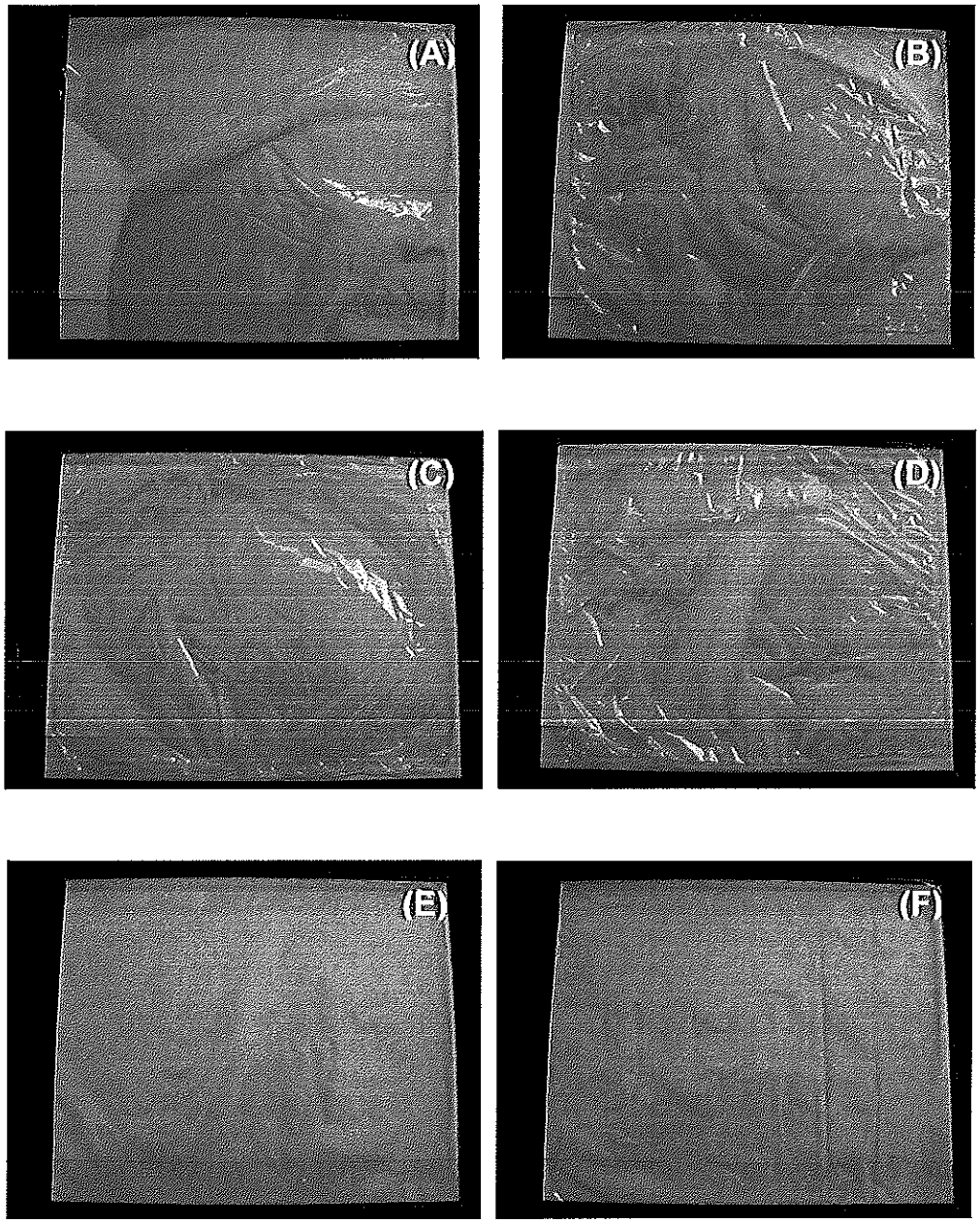


Figure 4.3 Pictures of patches without mangosteen peel extracts: (A) Formulation 1; (B) Formulation 2; (C) Formulation 3; (D) Formulation 4; (E) Formulation 5; (F) Formulation 6; (G) Formulation 7; (H) Formulation 8; (I) Formulation 9; (J) Formulation 10; (K) Formulation 11; (L) Formulation 12 and (M) Formulation 13 (continue).

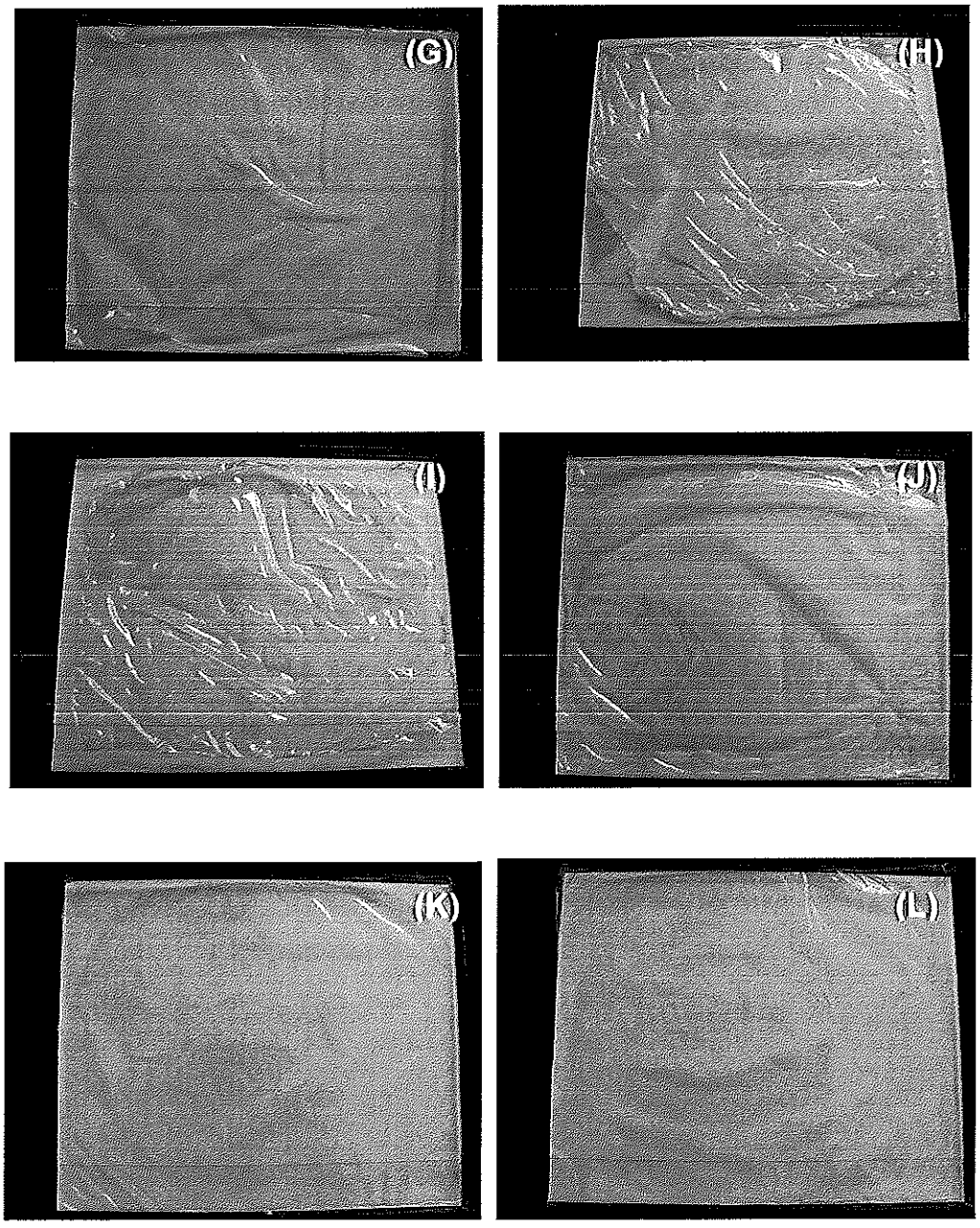


Figure 4.3 Pictures of patches without mangosteen peel extracts: (A) Formulation 1; (B) Formulation 2; (C) Formulation 3; (D) Formulation 4; (E) Formulation 5; (F) Formulation 6; (G) Formulation 7; (H) Formulation 8; (I) Formulation 9; (J) Formulation 10; (K) Formulation 11; (L) Formulation 12 and (M) Formulation 13 (continue).

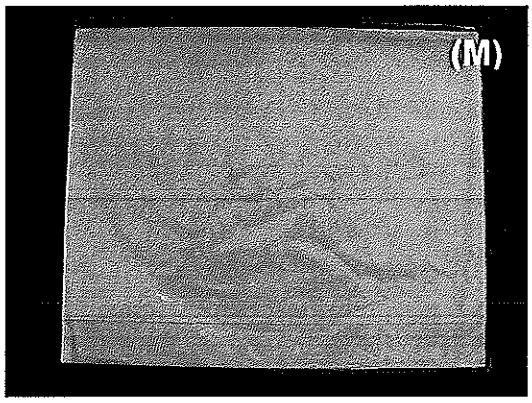


Figure 4.3 Pictures of patches without mangosteen peel extracts: (A) Formulation 1; (B) Formulation 2; (C) Formulation 3; (D) Formulation 4; (E) Formulation 5; (F) Formulation 6; (G) Formulation 7; (H) Formulation 8; (I) Formulation 9; (J) Formulation 10; (K) Formulation 11; (L) Formulation 12 and (M) Formulation 13 (continue).

4.5 Physical characterizations

4.5.1 Moisture uptake study

Moisture uptake capacities of all patches are presented in table 4-2. From these results, moisture uptake capacity of patch formulation 1 was 22.15%, it was the highest moisture uptake capacity in this experiment. There were different significantly between moisture uptake capacity of patch formulation 1 and patch formulation 2-13 ($p<0.05$) because of the effect of hydrophilic polymer including pectin and sodium alginate. In formulation 1, the amount of agar was less than another formulation; therefore, the pectin and sodium alginate could absorb moisture more than another formulation. When amount of agar was changed from 0.5 g to 1

and 1.5 g in patch formulation 2-7 and 8-13, the moisture uptake capacity was significant decreased ($p<0.05$).

The effect of plasticizer to moisture uptake capacity showed in figure 4.4. They were compared within the group in formulations 2-7 and 8-13. When glycerin was added more than or equal to 20% w/w of amount of polymer in patch, the moisture uptake capacity of patches were increased significantly more than patch containing glycerin less than 20% ($p<0.05$). However, the percent of glycerin in formulation gave the highest moisture uptake capacity at 20% w/w whereas moisture uptake capacity of patches containing glycerin 30% and 40% w/w of amount of polymer was not different from 20% of glycerin ($p\geq0.05$).

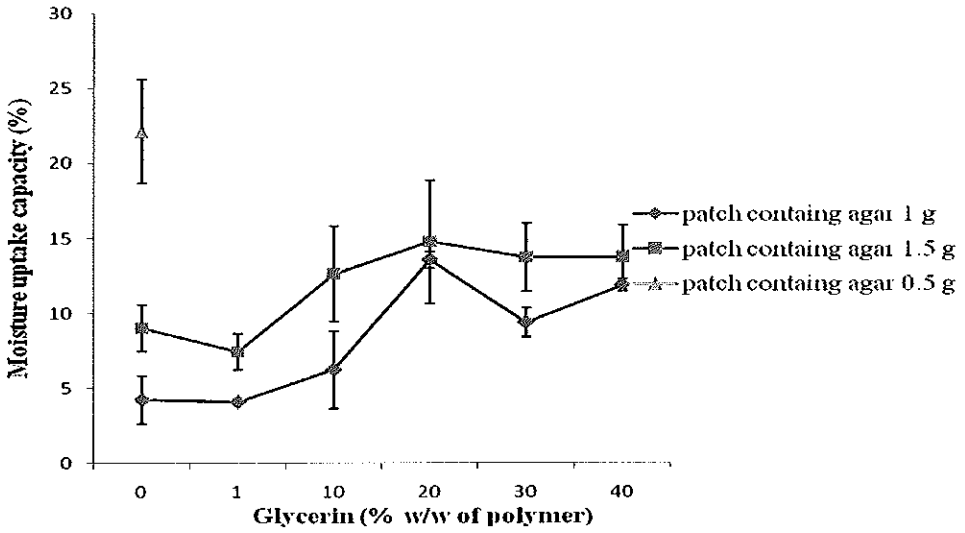


Figure 4.4 Moisture uptake capacity (%) of the patches at different concentration of glycerin.

Table 4-2 Moisture uptake capacity of the blank patches

Formulations	Moisture uptake capacity (%)*
1	22.15±3.47[a]
2	4.25±1.59
3	4.09±0.18
4	6.22±2.58
5	13.54±0.56[b]
6	9.37±0.96[b]
7	11.85± 0.41[b]
8	9.02±1.57
9	7.42±1.20
10	12.61±3.16
11	14.75±4.11[c]
12	13.71±2.27[c]
13	13.73±2.09[c]

*All values are mean±SD as obtained by triplicate analyses (n=3).

a Effect of agar concentrations on moisture uptake capacity compared with all formulations (formulation 2-13)

b,c Effect of glycerin on moisture uptake capacity compared with patches without glycerin (patch formulation 2 and 8)

a,b,c Significant at $p<0.05$

4.5.2 Thickness study

The thickness of all patch are shown in table 4-3. The prepared patch had thickness in the range of 0.031-0.051 mm. The thickness of patch in this experiment is in normal range between 0.035-0.070 mm (Maior *et al.*, 2008; Mukherjee *et al.*, 2005; Padula *et al.*, 2003; Sriamornsak *et al.*, 2008; Wang *et al.*, 2007). The thicknesses of patches depend on both polymer and plasticizer that were added in patch, in this experiment was glycerin. According to the results the thickness of patch, which glycerin was added below 20% w/w of polymer were not different compared with the patch without glycerin ($p \geq 0.05$). Contrary to patch containing the amount of glycerin more than 20% w/w of polymer, the thickness of patches were increased significantly ($p < 0.05$).

Table 4-3 Thickness of the blank patches

Formulations	Thickness (mm)*
1	0.049±0.004
2	0.037±0.005
3	0.035±0.004
4	0.031±0.002
5	0.039±0.002
6	0.043±0.003[a]
7	0.044±0.002[a]
8	0.044±0.002
9	0.040±0.000
10	0.041±0.002
11	0.041±0.002
12	0.050±0.000[b]
13	0.051±0.002[b]

*All values are mean±SD as obtained by triplicate analyses (n=3).

a,b Effect of glycerin to thickness of patches compared with patches without glycerin (patch formulation 2 and 8)

a,b Significant at $p<0.05$

4.6 Mechanical properties of patch

The tensile strength values of patches were summarized in Table 4-4. Tensile strength of patch varied from 25.98 to 60.00 N/mm^2 depending on the component of the patch. From these results, showed that the tensile strength of blank patch without glycerin (formulations 1, 2 and 8) were increased significantly when the amount of agar in patch were increased. Even if agar is also hydrophilic, however, the strength believe to be more complex range of poly saccharide chain [α(1-3), β(1-4)] than pectin and sodium alginate. When compared within the group; patches from formulations 2-7 and formulations 8-13, the tensile strength of patches were decreased significantly when glycerin was added more than or equal to 20% w/w of polymer ($p<0.05$).

The elongations at break of all patches are presented in table 4-4. Patch formulation 1 had the highest elongation at break but the lowest tensile strength therefore it was the most easily break. The elongation at break of patches when compared within the group; formulations 2-7 and formulations 8-13, were increased significant when the quantity of glycerin was added more than or equal to 30% w/w of polymer in the patch.

The effect of glycerin to the tensile strength and elongation at break showed when the amount of glycerin was added more than or equal to 20% and 30% w/w of polymer in the patch, respectively.

Mechanism of plasticizer to reduce tensile strength and increase elasticity is to reduce intermolecular friction between the polymer molecules. This theory has been presented in different ways, but the idea persisting in all of them is that when a plastic part is flexed, the polymer molecules have to slip over each other. The plasticizer acts by lubricating the movement of the molecules and reducing their internal resistance to sliding (Marcilla and Beltrán, 2004).

Table 4-4 Tensile strength and elongation at break of the blank patches

Formulations	Tensile strength (N/mm^2)*	Elongation at break (%)*
1	25.98±2.70	25.86±3.59
2	49.41±4.54	8.71±1.91
3	49.51±3.27	10.88±1.08
4	42.45±5.84	10.31±1.31
5	35.44±5.51[a]	11.08±1.95
6	39.08±3.33[a]	19.99±3.39[c]
7	48.50±3.82	25.23±3.90[c]
8	54.04±6.08	14.78±1.33
9	54.70±6.23	16.16±2.96
10	60.00±3.94	17.31±0.75
11	44.52±4.62[b]	13.32±1.53
12	44.43±4.13[b]	22.07±3.39[d]
13	42.57±2.80[b]	24.46±2.06[d]

*All values are mean±SD as obtained by triplicate analyses (n=3).

a,b Effect of glycerin to tensile strength of patches compared with patches without glycerin (patch formulation 2 and 8)

c,d Effect of glycerin to elongation at break of patches compared with patches without glycerin (patch formulation 2 and 8)

a,b,c,d Significant at $p<0.05$

4.7 Study the effect of concentration of mangosteen peel extract on the antibacterial activity of patch

This study was determined for comparison between antibacterial activity and concentrations of mangosteen peel concentrate extract on the patches. In this experiment, patch formulation 5 was chosen by randomization for tested patch. After that, antibacterial patches were prepared with different concentration of mangosteen peel concentrate extract; 0.231, 0.288, 0.346, 0.403 and 0.461 mg/cm^2. Antibacterial activity of patches was tested with 3 bacteria including *S. aureus, S. epidermidis* and *P. acnes*. The results of antibacterial of patches are presented in table 4-5. These results showed that control patch had no antibacterial activity (no inhibition zone). The range of inhibition diameter of all patch to *S. aureus, S. epidermidis* and *P. acnes* were 0.93-0.98, 0.94-1.02 and 1.10-1.24 cm, respectively. There were not significantly increased the inhibition diameter of patches even if the concentration of mangosteen peel extract of the patch increased. The diffusion from patch was not depend on concentration of mangosteen peel extract because water penetrated into the patch equally and eluted mangosteen peel extract from the patch in same concentration. Therefore the concentration of patch, that was equal to 0.231 mg/cm^2, was chosen for prepared antibacterial patches for stability test of the patch formulations 5-7 and 11-13.

Table 4-5 Antibacterial activity of the patches with different concentrations of the mangosteen peel extract in the patches.

Formulations	Amount of the extract/area(mg/cm^2)	*S. aureus* (cm)*	*S. epidermidis* (cm)*	*P. acnes* (cm)*
1	0.231	0.93±0.11	0.99±0.03	1.24±0.10
2	0.288	0.92±0.03	0.94±0.07[a]	1.10±0.02[b]
3	0.346	0.94±0.09	1.02±0.07	1.14±0.03
4	0.403	0.96±0.08	0.99±0.02	1.12±0.01[b]
5	0.461	0.98±0.07	0.94±0.01[a]	1.17±0.07
neomycin	30 µg/disc	1.84±0.07	2.48±0.07	3.23±0.04

*All values are mean±SD as obtained by triplicate analyses (n=3).

a Significant at p<0.05 when compared with patch containing mangosteen peel extract 0.231 mg/cm^2 against *S. epidermidis*

b Significant at p<0.05 when compared with patch containing mangosteen peel extract 0.231 mg/cm^2 against *P. acnes*

4.8 Study the amount and distribution of mangosteen peel extract contained in the patch

In this experiment, antibacterial patches were formulated by using component of patch formulations 5, 6, 7, 11, 12 and 13 and concentration of mangosteen extract in patch were prepared at 0.231 mg/cm^2. The physical appearances of patches are presented in figure 4.5. The colors of all of patches were slightly yellow. When observed by microscope, the patches were clear. It can be determined that the antibacterial patch made from mangosteen peel extract had homogeneously.

The amounts of mangosteen peel extract content in the patches aer presented in table 4-6. The method for separated extract from patch had percent remaining in the range of 90.51-101.38%. Percent RSD of extract in patches were calculated from equation in USP30&NF25. Percent RSD of extract in the patch of patch formulations 5, 6, 7, 11, 12 and 13 were 2.36, 2.69, 2.76, 2.04, 2.34 and 1.73, respectively. Percent RSD of extract of all patch less than 6 concluded that the mangosteen peel extract in the patch has good uniformity (Authority of the United States Pharmacopeial Convention, 2007).

Table 4-6 The amount of mangosteen peel extract contents in the patch analyzed by HPLC

Formulations	Concentration of extract in patch ($\mu g/cm^2$)*	Percent remaining*	Percent RSD
5	217.96±5.15	101.38±2.40	2.36
6	215.24±5.80	101.05±2.72	2.69
7	193.68±5.34	90.51±2.50	2.76
11	205.33±4.18	96.40±1.96	2.04
12	205.30±4.80	95.93±2.24	2.34
13	199.31±3.45	93.14±1.61	1.73

*All values are mean±SD as obtained by triplicate analyses (n=3)

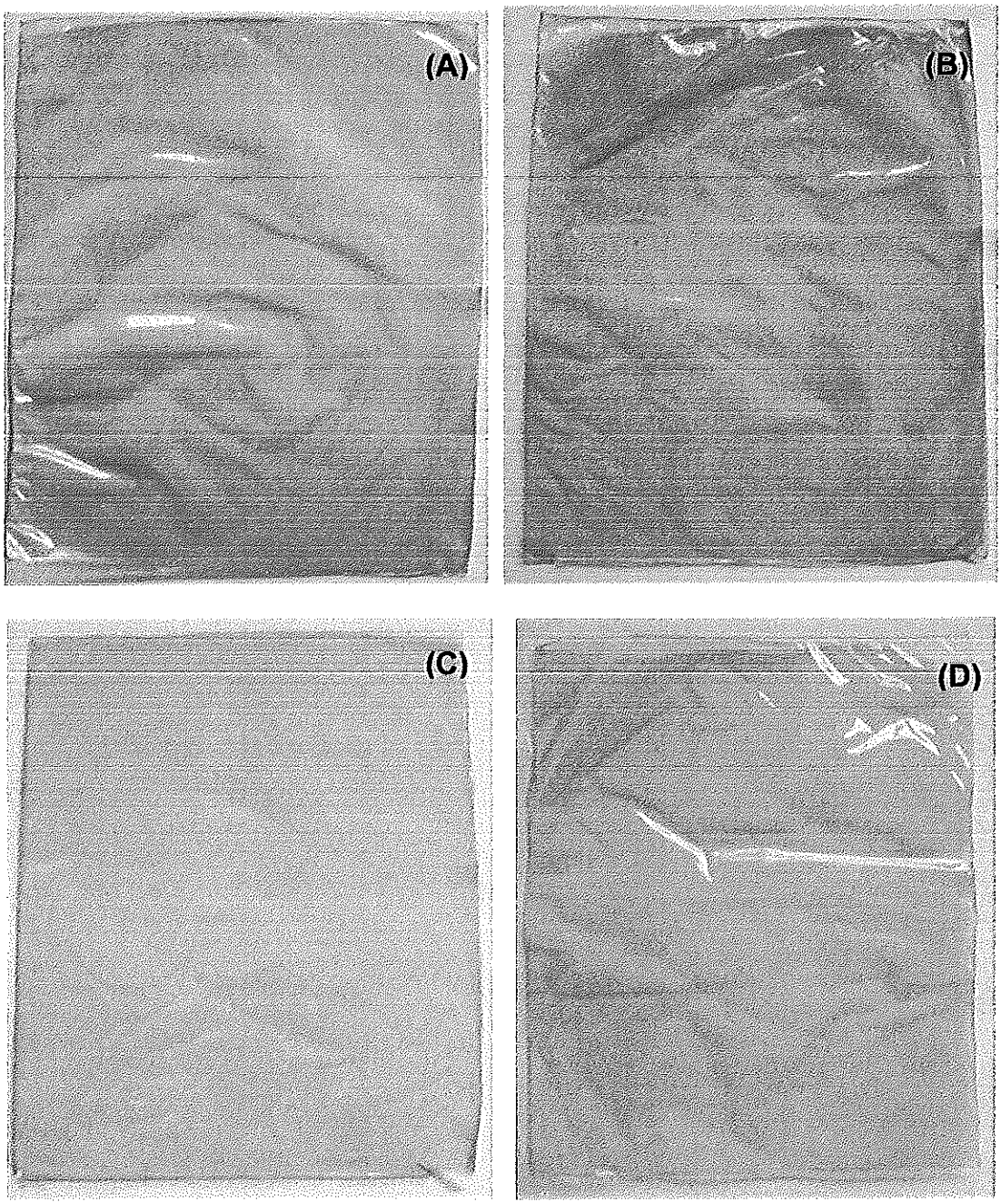


Figure 4.5 Picture of the antibacterial patches containing mangosteen peel extract: (A) Formulation 5; (B) Formulation 6; (C) Formulation 7; (D) Formulation 11; (E) Formulation 12 and (F) Formulation 13.

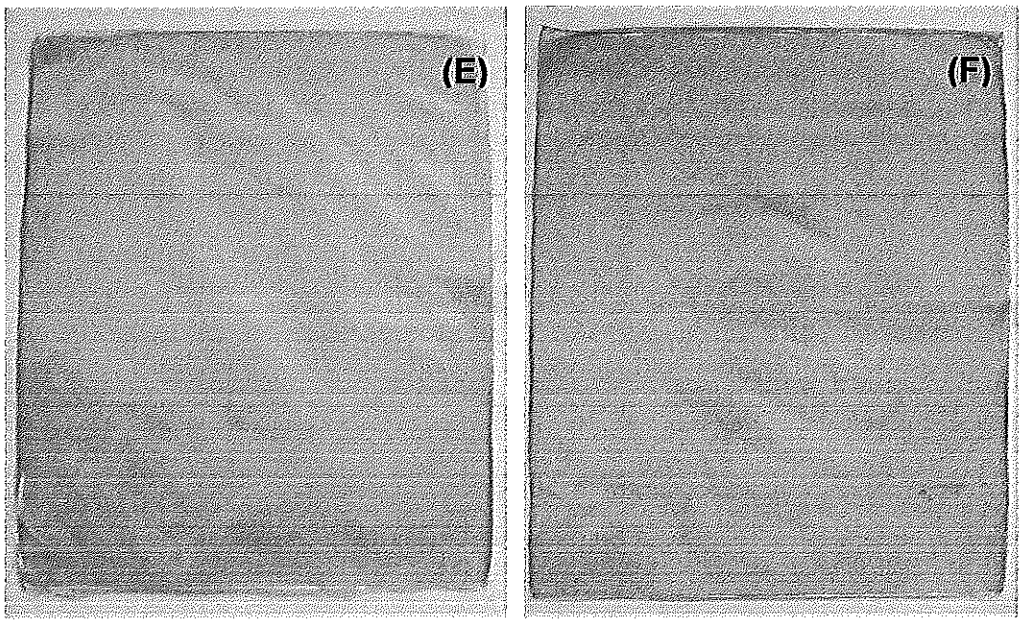


Figure 4.5 Picture of the antibacterial patches containing mangosteen peel extract: (A) Formulation 5; (B) Formulation 6; (C) Formulation 7; (D) Formulation 11; (E) Formulation 12 and (F) Formulation 13 (continued).

4.9 Stability of patch

Stability test modified from Yodhnu's thesis, The antibacterial patch was stored at 4 °C, 30 °C and 45 °C 75% RH in zip code container, protected from light using aluminium foil. This modification used for evaluation that stability of antibacterial patches affected environment.

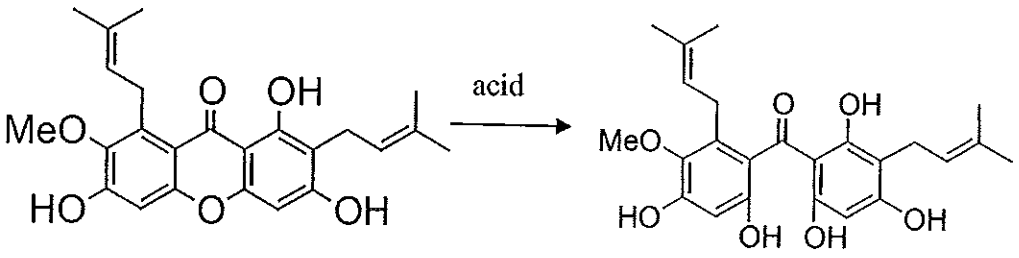
Stability of the patch containing mangosteen peel extract was determined under light protected condition at 4 °C, 30 °C, and 45 °C/75% RH for 120 days. Physical appearance of the film and the α-mangostin content were determined at intervals of 0, 30, 60, 90 and 120 days. The results showed that at 45 °C/75% RH, the

color of the patches gradually changed from light yellow to dark yellow when compared to the film kept at 4 °C and 30 °C during 120 days. The percent remaining of amount of α-mangostin in tested patches change from original whether the samples were stored at 4 °C, 30 °C or 45 °C/75% RH during 120 days of the storage (Tables 4-7, 4-8 and 4-9).

At 45 °C, after patches were stored for 4 months, it found that percent remaining of mangosteen peel extract in patch formulations 6, 7, 8, 11, 12 and 13 were 71.78, 75.48, 81.90, 63.06, 82.85 and 70.48, respectively. From the results, found that there are only 2 formulations of patch; formulation 7 and formulation 12, still had amount of α-mangostin more than 80% (table 4-8). The result indicated that formulation 7 and formulation 12 were appropriate formulation for formulated antibacterial patches made from mangosteen peel extract.

After patches were stored for 4 months, the percent remaining of α-mangostin significantly decreased because of acid hydrolysis. Acid functional group from agar and sodium alginate may be hydrolyzed ether group of α-mangostin. This process might give the derivative of α-mangostin called benzophenone derivative (figure 4.6).

When antibacterial patch made from mangosteen peel extract are prepared to be as commercial product for good stability, it should be kept in well-closed container to protect from light.



α-mangostin benzophenone derivative

Figure 4.6 Acid hydrolysis of α-mangostin

Table 4-7 α-mangostin content of patches after stored at 4 °C for 120 days

Time (days)	% Remaining*					
	Formulation 5	Formulation 6	Formulation 7	Formulation 11	Formulation 12	Formulation 13
0	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
30	96.81±4.07	90.50±2.59#	98.00±0.51#	98.77±1.38	93.79±1.14#	82.35±1.28#
60	92.42±5.42#	86.46±0.44#	92.14±0.97#	97.29±3.22#	92.62±2.51#	81.70±2.53#
90	85.74±3.18#	80.38±1.33#	86.96±2.21#	90.06±1.16#	92.37±3.66#	80.46±1.55#
120	88.00±3.59#	77.00±7.41#	87.64±2.84#	90.47±3.44#	88.68±2.11#	75.72±4.49#

*All values are mean±SD as obtained by triplicate analyses (n=3)

#Significance at p<0.05 when compared with the α-mangostin content at initial time.

Table 4-8 α-mangostin content of patches after stored at 30 °C for 120 days

Time (days)	% Remaining*					
	Formulation 5	Formulation 6	Formulation 7	Formulation 11	Formulation 12	Formulation 13
0	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
30	89.42±4.00#	91.98±4.55#	98.41±1.64	95.29±2.04#	88.19±5.55#	95.50±1.56#
60	86.52±4.05#	85.24±4.29#	92.37±4.36#	93.12±2.21#	85.55±5.36#	92.18±4.10#
90	76.40±4.01#	81.40±1.67#	88.30±5.88#	88.41±1.81#	80.73±4.99#	91.61±3.32#
120	74.43±5.55#	78.70±2.53#	83.91±6.50#	81.61±1.44#	80.32±2.48#	91.01±3.09#

*All values are mean±SD as obtained by triplicate analyses (n=3)

#Significance at p<0.05 when compared with the α-mangostin content at initial time.

Table 4-9 α-mangostin content of patches after stored at 45 °C/75% RH for 120 days

Time (days)	% Remaining*					
	Formulation 5	Formulation 6	Formulation 7	Formulation 11	Formulation 12	Formulation 13
0	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00
30	94.45±0.82#	91.34±6.05#	99.82±1.52	90.27±1.17#	89.50±3.99#	90.06±3.29#
60	90.01±0.95#	84.21±1.17#	93.58±1.05#	78.65±2.75#	85.29±2.48#	87.06±2.60#
90	78.44±4.79#	83.69±1.70#	88.23±1.50#	67.71±1.48#	83.57±0.62#	75.74±6.97#
120	71.78±5.77#	75.48±1.01#	81.90±1.18#	63.06±1.81#	82.85±2.45#	70.48±1.46#

*All values are mean±SD as obtained by triplicate analyzes (n=3)

#Significance at p<0.05 when compared with the α-mangostin content at initial time.

4.10 *In vitro* release study

The release data of α-mangostin from patch formulation 7 and patch formulation 12 are showed in figure 4.7. All α-mangostin release tests were carried out in triplicate and the results are presented as average and standard variation. The mechanism of the release of mangosteen peel extract from the patch might be dissolution of the patch, diffusion of the α-mangostin or combination of both. Mean cumulative amounts of α-mangostin extract released from the patch after 12 hours were found to be 16.90 and 18.77 $\mu g/cm^2$ in case of the formulation 7 and formulation 12, respectively.

There were no significantly differences between the α-mangostin release from formulation 7 and formulation 12 ($p \geq 0.05$). From this data, it concluded that an antibacterial patch could prepared by choose component of patch formulation 7 or formulation 12.

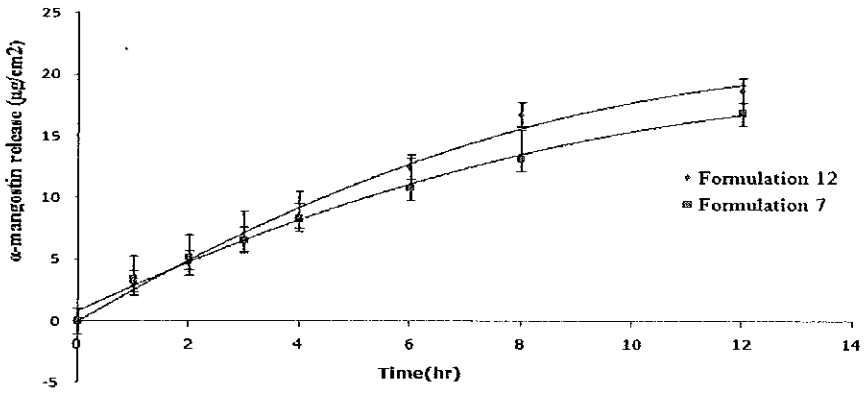


Figure 4.7 The in vitro release profiles of α-mangostin from antibacterial patches: (■) Formulation 7 and (◆) Formulation 12.

4.11 Study the surface morphology by using scanning electron microscope

The SEM micrographs of blank patch, antibacterial patch formulation 7 and formulation 12 before and after release test are presented in figure 4.8. SEM of the patch without mangosteen peel extract (figure 4.8(A)) showed homogeneous and smooth morphology. It could be resulted from the blend of pectin, sodium alginate and agar showed good compatibility. Figure 4.8(B) showed SEM of patch formulation 7 and figure 4.8(D) showed SEM of patch formulation 12 containing mangosteen peel extract, they showed the cross-section of antibacterial patch formulations 7 and 12 were homogeneous and smooth morphology liked SEM of patch without the extract. The results obtained here indicated good compatibility between the matrix and the mangosteen peel extract. Figures 4.8(C) and 4.8(E) showed SEM of the patch after release tested. They showed that the width of cross section of the patches decreased.

α-Mangostin is hydrophobic so the release of α-mangostin from the patch depend on solution of pectin and alginate. These results confirmed that one mechanism of the mangosteen peel extract released was the dissolution of the patch controlled.

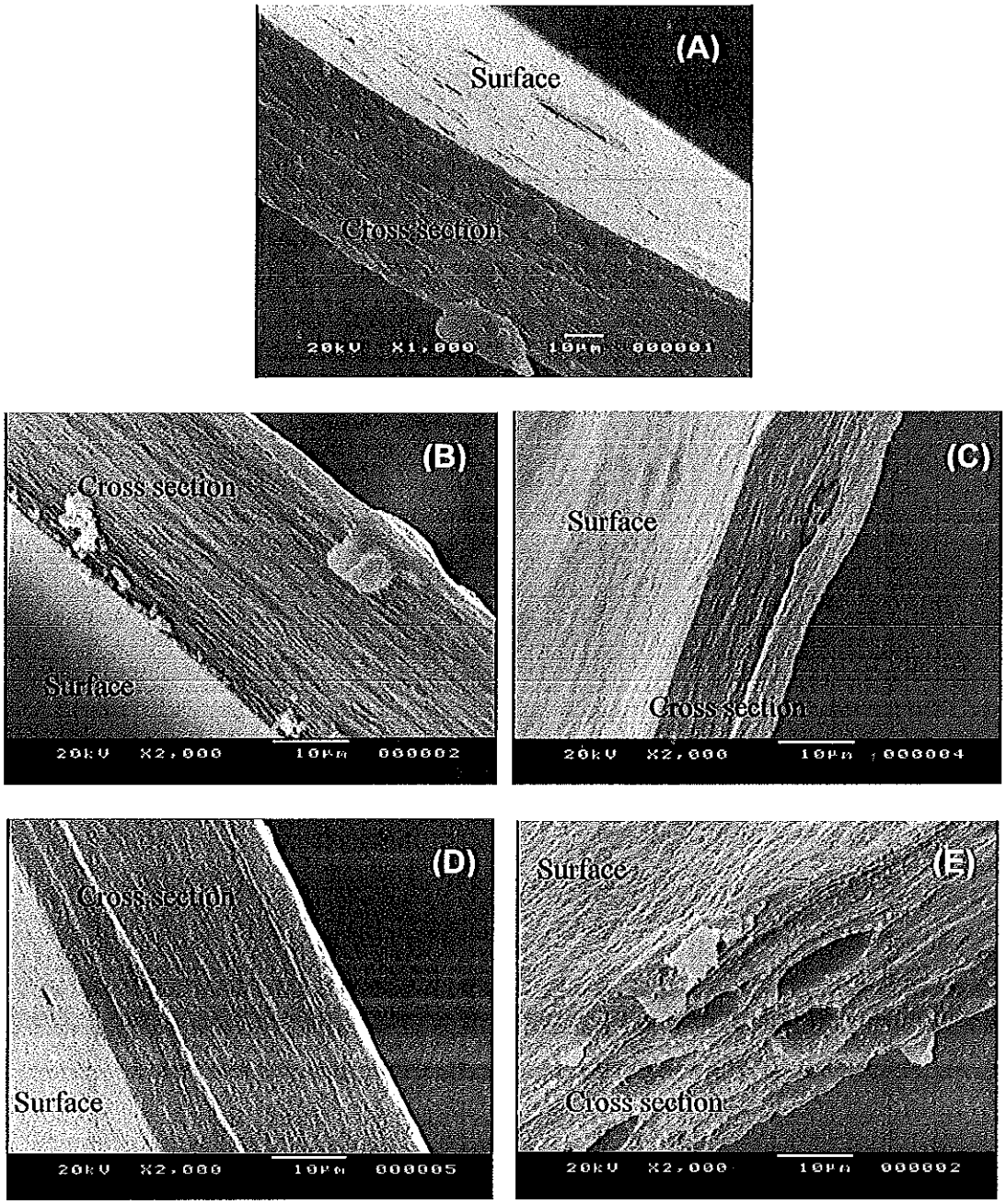


Figure 4.8 Picture of scanning electron micrograph of cross section of antibacterial patch made from mangosteen peel extract: (A) patch without mangosteen peel extract; (B) Formulation 7 before in vitro release tested; (C) Formulation 7 after in vitro release tested; (D) Formulation 12 before in vitro release tested; (E) Formulation 12 after in vitro release tested.

4.12 X-ray diffraction study

The X-ray diffractograms of mangosteen peel extract, blank patch, patch formulations 7 and 12 with mangosteen peel extract are shown in figure 4.9. The pattern of blank patch, as shown in figure 4.9 (D), had a very weak broad profile, indicating that the blank patch that content sodium alginate, agar and pectin were an amorphous material. As observed, the mangosteen peel extract was in crystalline state with many diffraction peaks between 0° to 40.0°. The diffraction peaks of patch formulation 7 and 12 were shown in figure 4.9 (C) and figure 4.9 (D), respectively. There were 2 diffraction peaks of mangosteen peel extract at about 2° and 12.5° Because of amount of mangosteen peel extract in the patch were very small when compared with the amount of polymer in the patch. Therefore, there were only 2 strong peaks of mangosteen peel extract showed in these diffractograms. It seems that the peaks did not change the position and shape. From these results, the mangosteen peel extracts remaining in the patch and was the crystalline forms.

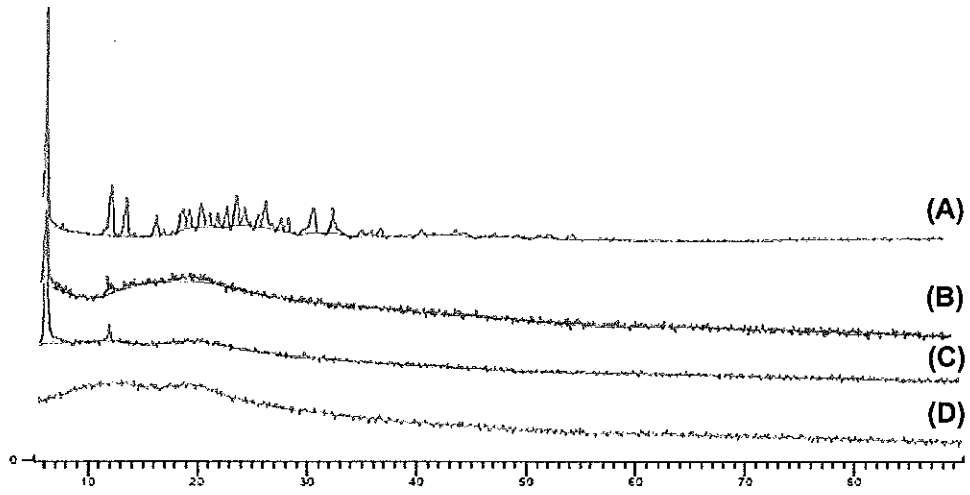


Figure 4.9 X-ray diffraction patterns: (A) high yield mangosteen peel extracts; (B) Formulation 12; (C) Formulation 7 and (D) patch without mangosteen peel extract.

4.13 Study the antibacterial activity of the patch

This experiment was tested to confirm the antibacterial activity of patches. The 5 concentration of mangosteen peel extracts were added in selected formulation; formulation 7 and formulation 12. The varied concentration of mangosteen extract of the patch include 0.231, 0.288, 0.34, 0.403 and 0.461 mg/cm^2, were tested antibacterial activity against 3 bacterial; *S. aureus, S. epidermidis* and *P. acnes*. The results of antibacterial of patches are presented in Table 4-10. All of the patches had antibacterial activity that showed the range of inhibition diameter to *S. aureus, S. epidermidis and P. acnes* were 0.87-1.01, 0.92-1.02 and 1.15-1.30 cm, respectively.

From the results of antibacterial activity of patches against *S. aureus, S. epidermidis and P. acnes*, it could be seem even though concentration of mangosteen peel extract in patch increased but the inhibition zone of patch to bacterial did not significant increased. On the contrary, the results showed that the inhibition zone of patch formulation 7/1, amount of mangosteen peel extract per patch equal to 0.231 mg/cm^2, had significantly wider than the inhibition zone of patch formulation 7/2 against *S. aureus* and the inhibition zone of patch formulation 7/3 against *S. epidermidis.* The results of the antibacterial activity same as patch formulation 12, the results showed that the inhibition zone of patch formulation 12/1 had significantly wider than the inhibition zone of patch formulation 12/2, 12/3 and 12/4 against *S. aureus* and the inhibition zone of patch formulation 12/2 and 12/3 to *S. epidermidis.* It can be summarized that the appropriate concentration of mangosteen peel extract in the patch in this experiment was 0.231 mg/cm^2.

Table 4-10 Antibacterial activity of the patches with different concentration of the mangosteen peel extract in the patches.

Formulations	Amount of the extract/area(mg/cm^2)	*S. aureus* (cm)*	*S. epidermidis* (cm)*	*P. acnes* (cm)*
7/1	0.231	0.94±0.04	1.02±0.02	1.30±0.05
7/2	0.288	0.87±0.02[a]	1.01±0.02	1.28±0.02
7/3	0.346	0.95±0.01	0.93±0.01[b]	1.27±0.04
7/5	0.461	0.94±0.03	1.00±0.01	1.30±0.03
12/1	0.231	0.99±0.01	0.98±0.15	1.19±0.06
12/2	0.288	0.94±0.01	0.93±0.01	1.17±0.06
12/3	0.346	0.96±0.01	0.92±0.03	1.22±0.04
12/4	0.403	0.94±0.03	0.96±0.01	1.15±0.06
neomycin	30 µg/disc	2.29±0.02	2.48±0.07	3.07±0.15

*All values are mean±SD as obtained by triplicate analyses (n=3)

a Significant at p<0.05 when compared with patch containing mangosteen peel extract 0.231 mg/cm^2 against *S. aureus*

b Significant at p<0.05 when compared with patch containing mangosteen peel extract 0.231 mg/cm^2 against *S. epidermidis*

CHAPTER 5

CONCLUSIONS

From this research work the following conclusions can be drawn:

1. Mangosteen peel concentrate extract was extracted from the fruit peel of *G. mangostana* by maceration using 95% ethanol and partition using column chromatography by taking silica gel mix with mobile phase which consisted of petroleum ether and ethyl acetate for 7:3. The results showed that the amount of α-Mangostin from mangostin peel extract was 77.86±1.13 % w/w.

2. The mangosteen peel extract from the fruit peel of *G. mangostana* was tested for antibacterial activities against *S. aureus, S. epidermidis* and *P. acnes* using broth microdilution methods. The results showed the mangosteen peel concentrate extract was capable of inhibiting the growth of *S. aureus, S. epidermidis* and *P. acnes,* with MIC values of 4.0, 2.0, and 4.0 µg/ml, respectively.

3. Patches were prepared using 13 different formulations with consist of sodium alginate 1 g, pectin 1 g, agar in the range of 0.5-1.5 g, propylene glycol 20 ml and glycerin in the range of 0-40% w/w of total weight. After physical characterization test, there were only 6 formulation of patches, with consist of sodium alginate 1 g, pectin 1 g, agar in the range of 1.0-1.5 g, propylene glycol 20 ml and

glycerin in the range of 20-40 %w/w of total weight, were chosen for preparation the patches containing mangosteen peel extract.

4. For 6 formulation of patches containing mangosteen peel extract were prepared and stability of the patches were determined under light protected condition at 4 °C, 30 °C, and 45 °C/75% RH for 120 days. At 45 °C, after patches were stored for 3 months, the results found that there were only 2 formulations of patch; formulation 7 and formulation 12, still amount of α-mangostin more than 80%. The result indicated that formulation 7 and formulation 12 were appropriate formulation for formulated antibacterial patch made from mangosteen peel extract.

5. *In vitro* release study of α-mangostin from patch formulation 7 and patch formulation 12 were evaluated. The results showed that there were no significant differences between the extract released from formulation 7 and formulation 12.

6. Patch formulation 7 and 12 containing mangosteen peel extract obtained from scanning electron microscopy showed that the cross section were homogeneous and smooth morphology. Moreover, X-ray diffractrogram also found that remaining mangosteen peel extract was crystalline forms. However, interaction between the polymer in patch with α-mangostin will be further studied by means of several technique such as differential scanning calorimetry or infrared spectroscopy.

7. The varied concentration of mangosteen peel extract of the patch include 0.231, 0.288, 0.34, 0.403 and 0.461 mg/cm^2 were tested antibacterial activity against *S. aureus, S. epidermidis* and *P. acnes.* The results found that the inhibition zone of patches to bacterial did not significantly different even though the concentrations of mangosteen peel in the patches were increased. It can determined that the appropriate concentration of mangosteen peel extract in this experiment were 0.231 mg/cm^2.

8. Finally, it can be concluded that the best of choice of the potential to develop for commercial products as antibacterial is patch formulation 7 and 12. The main composition of patch formulation 7 is agar 1 g, sodium alginate 1 g, pectin 1 g, propylene glycol 20 ml and glycerin 1.2 g and formulation 12 is agar 1.5 g, sodium alginate 1 g, pectin 1 g, propylene glycol 20 ml and glycerin 1.05 g at the concentration of mangosteen peel extract was 0.231 mg/cm^2, which the prepared plate was 25x30 cm^2. The antibacterial patches should be kept in well-closed container to protect from light for good stability.

BIBLIOGRAPHY

ชัชฎา โพธิพุกกณะ. 2552. อีกครั้งกับ Transdermal drug delivery System. *R&D Newsletter.* 16(2): 18-26.

บวร เอี่ยมสมบูรณ์. 2518. ดงไม้. โรงพิมพ์รุ่งเรืองธรรม. กรุงเทพมหานคร: 247.

พระเทพวิมลโมลี. 2524. ตำรายาพื้นบ้าน. โรงพิมพ์มงกุฎราชวิทยาลัย. กรุงเทพมหานคร: 80.

หมอชีวกโกมารทัจจ์. 2517. ตำรายาวิเศษ. ชัยมงคลการพิมพ์, กรุงเทพมหานคร: 27.

Aajsalee, S. 1979. Traditional medicine for the household. Pitayakaan press. Bangkok.

Aajsalee, S. 1981. Herbal medicine. Pitayakaan press. Bangkok.

Abbiw, D.K. 1990. Useful plants of Ghana West African use of wild and cultivated plants. Intermediate technology publications and the royal botanic gardens kew, Ghana.

Akao, Y., Nakagawa, Y., Iinuma, M. and Nozawa, Y. 2008. Anti-cancer effects of xanthones from pericarps of mangosteen. *International Journal of Molecular Sciences*, 9: 355-370.

Amnuaikit, C., Ikeuchi, I., Ogawara, K., Higaki, K. and Kimura, T. 2005. Skin permeation of propranolol from polymeric film containing terpene enhancers for transdermal use. *International Journal of Pharmaceutics*, 289: 67-178.

Anonymous. 2007. *Garcinia mangostana* (Clusiaceae) [Online]. Available from URL: http://www.montosogardens.com/garcinia_mangostana.htm [Accessed December 23, 2007].

Anonymous. 2008. มังคุดช่วยทำลายเซลล์มะเร็ง [Online]. Available from URL: http://asianlife.igetweb.com/index.php?mo=3&art=101327 [Accessed November 5, 2007].

Anonymous. 2010. ต้นมังคุด 3. [Online]. Available from URL: http://mthaifruit.igetweb.com/index.php?mo=18&display=view_single&pid=518235 [Accessed January 14, 2011].

Asai, F. Tora, H., Tanaka, T. and Iinuma, M. 1995. A xanthone from pericarps of *Garcinia mangostana*. *Phytochemistry*, 39(4): 943-944.

Authority of the United States Pharmacopeial Convention, 2007. The United States Pharmacopia: USP30: the National Formulary: NF25. Pharmacopeial Convention Inc. Rockville, MD: United States.

Bagyalakshmi, J., Vamsikrishna, R.P., Manavalan, R., Ravi, T.K. and Manna, P.K. 2007. Formulation development and *In Vitro* and *In Vivo* evaluation of membrane-moderated transdermal systems of ampicillin sodium in ethanol : pH 4.7 buffer solvent system. *AAPS PharmSciTech*, 8(1): Article 7.

Burkill, H.M., 1994. The useful plants of West Tropical Africa. 2nd ed. Vol. 2. Families E-I. Royal Botanic Gardens Kew, London.

Chairungsrilerd, N., Furukawa, K.N., Ohta, T., Nozoe, S. and Ohizumi, Y. 1996. Pharmacological properties of α-mangostin, a novel histamine H1 receptor antagonist. *European Journal of Pharmacology*, 314(3): 351-356.

Chairungsrilerd, N., Takeuchi, K., Ohizumi, Y., Nozoe, S. and Ohta, T. 1996. Mangostanol, a prenyl xanthone from *Garcinia mangostana*. *Phytochemistry*, 43(5): 1099-1102.

Chen, L., Yang, L. and Wang, C. 2008. Anti-inflammatory activity of mangostins from *Garcinia mangostana*. *Food and Chemical Toxicology*, 46(2): 688-693.

Chen, S.X., Wan, M. and Lon, B.N. 1996. Active constituents against HIV-1 protease from *Garcinia mangostana*. *Planta Medica*, 62(4): 381-382.

Chin, Y.W. and Kinghorn, A.D. 2008. Structural Characterization, Biological Effects, and Synthetic Studies on Xanthones from Mangosteen (*Garcinia mangostana*), a Popular Botanical Dietary Supplement. *Mini-Reviews in Organic Chemistry*, 5: 355-364.

Chin, Y.W., Jung, H.A., Chai, H., Keller, W.J. and Kinghorn, A.W. 2008. Xanthones with quinone reductase-inducing activity from the fruits of *Garcinia mangostana* (Mangosteen). *Phytochemistry*, 69(3): 754-758.

Chomnawang, M.T., Suvimol, S., Nukoolkarn, V.S. and Gritsanapan, W. 2005. Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. *Journal of Ethnopharmacology*, 101(1-3): 330-333.

Chomnawang, M.T., Suvimol, S., Nukoolkarn, V.S. and Gritsanapan, W. 2007. Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium acnes*. *Fitoterapia*, 78: 401-408.

Clancy, C.J., Yu, V.L., Morris, A.J., Snydman, D.R. and Nguyen, M.H. 2005. Fluconazole MIC and the fluconazole dose/MIC ratio correlate with therapeutic response among patients with candidemia. *Antimicrobial Agent and Chemotherapy*, 49(8): 3171-3177.

Corner, E.J.H. 1988. Mangosteen family. In: Wayside Trees of Malaya. *Malayan Nature Society*, Kuala Lumpur. 1: 349-357.

Dong, Z., Wang, Q. and Du, Y. 2006. Alginate/gelatin blend films and their properties for drug controlled release. *Journal of Membrane Science*, 280: 37-44.

Dragendorff, O. 1930. Uber das Harz von *Garcinia mangostana* L. *Liebigs Annalen*, 482: 280-301.

Ghaffari, A., Navaee, K., Oskoui, M., Bayati, K. and Tehrani, M.R. 2007. Preparation and characterization of free mixed-film of pectin/chitosan/Eudragit® RS intended for sigmoidal drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, 67(1): 175-186.

Gopalakrishnan, G., Banumathi, B. and Sures, G. 1997. Evaluation of the Antifungal Activity of Natural Xanthones from Garcinia mangostana and Their Synthetic Derivatives. *Journal of Natural Products*. 60: 519-524.

Govindachari, T.R., Kalyanaraman, P.S., Muthukumaraswamy, N. And Pai, B.R. 1971. Xanthones of *Garcinia mangostana* Linn. *Tetrahedron*, 27(16): 3919-3926.

Hadgraft, J. and Guy, R.H. 1989. Transdermal drug delivery. Vol. 35. New York: Marcel Dekker, Inc. 293-298.

Hepatitis Central. 2011. Liver Blood Enzymes [Online]. Available from URL: http://www. hepatitis-central.com/hcv/labs/liverenzymes.htm [Accessed February 10, 2011].

Hiorth, M., Tho, I. and Sande, S.A. 2003. The formation and permeability of drugs across free pectin and chitosan films prepared by a spraying method. *European Journal of Pharmaceutics and Biopharmaceutics*, 56(2): 175-181.

Iamsomboon, B. 1975. Plants from the forests. Roongroengtham press, Bangkok.

ICH. 1995. Q2 (R1) Validation of analytical procedures: text and methodology. International Conference on Harmonization, Canary Wharf, London.

Iinuma, M., Tosa, H., Tanaka, T., Asai, F., Kobayashi, Y., Shimano, R. and Miyauchi, K.I. 1996. Antibacterial activity of xanthones from guttiferaerous plants against methicillin-resistant *Staphylococcus aureus*. *Journal of Pharmacy and Pharmacology*, 48(8): 861-865.

Itoh, T., Ohguchi, K., Iinuma, M., Nozawa, Y. and Akao, Y. 2008. Inhibitory effect of xanthones isolated from the pericarp of *Garcinia mangostana* L. on rat basophilic leukemia RBL-2H3 cell degranulation. *Bioorganic & Medicinal Chemistry*, 16: 4500-4508.

Jainpanick, K. 1979. Selected articles on medicinal plants. Paper distributed on the opening ceremony of the traditional medicine museum, Department of Pharmacology, Faculty of medicine (Siriraj), Mahidol University, Bangkok.

Jayaweera, D.M.A., 1981. Medicinal plants used in Ceylon part 3. National Science Council of Sri Lanka. Colombo.

Jitpukdeebodintra, S. and Jangwang, A. 2009. Preliminally Lime Pectin Film for Wound Healing. The 19th Thaksin University Annual Conference: Research and Development for Thai Society, 334-335.

Jujun, P., Pootakham, K., Pongpaibul, Y., Tharavichitkul, P. and Ampasavate, C. 2009. HPLC Determination of Mangostin and Its Application to Storage Stability Study. *Chiang Mai University Journal of Natural Sciences,*8(1): 43-53.

Kitikajorn, S. 1983. Uses of medicinal plants, the traditional medicine. Aksornthai press. Bangkok.

Kongchanmitkul, W. 2002. Preparation and evaluation antibacterial throat sprays from mangosteen crude extract. Master's Thesis. Chiang Mai University, Chiang Mai, Thailand

Koonthon, K. 1981. Folkloric medicine in house. Preecha press, Bangkok.

Kosem, N., Han, Y. and Moongkarndi, P. 2007. Antioxidant and cytoprotective activities of methanolic extract from *Garcinia mangostana* Hulls. *Science Asia*, 33: 283-292.

Krajewski, D., Toth, G. and Schreier, P. 1996. 2-Ethyl-3-methylmaleimide *N-β-D* glucopyranoside from the leaves of mangosteen (*Garcinia mangostana*). *Phytochemistry*, 43(1): 141-143.

Lorian, V. 1996. Antibiotics in laboratory medicine. 4th ed. Baltimore: Williams & Wilkins.

Mahabusarakum, W., Phongpaichit, S., Jansukul, C. and Wiriyachitra, P. 1983. Screening of antibacterial activity of chemicals from *Garcinia mangostana*. *Songklanakarin Journal of Science and Technology*, 5: 337-339.

Mahabusarakam, W., Wiriyachitra, P. and Taylor, W.C. 1987. Chemical constituents of *Garcinia mangostana*. *Journal of Natural Products*, 50(3): 474-478.

Maior, J.F.A.S., Reis, A.V., Muniz, E.C. and Cavalcanti, O.A. 2008. Reaction of pectin and glycidyl methacrylate and ulterior formation of free films by reticulation. *International Journal of Pharmaceutics,* 355: 184-194.

Maraproeksavan, B. 1978. Thai traditional pharmacists. Suekarnkha press, Bangkok.

Marcilla, A. and Beltrán, M. 2004. Mechanisms of Plasticizers Action [Online]. Available from URL: http://doc.isiri.org.ir/c/document_library/get_file?p_l_id =18476&folderId=21113 &name=DLFE-20410.pdf [Accessed May 4, 2011].

Medical Science Department, Research section. 1983. Folk medicine Vol. I. Ministry of Public Health, Bangkok.

Millis, J. and Reed, F.B. 1947. The Effect of Sodium Alginate on the Absorption of Calcium. *Biochemical Journal*, 41: 273-275.

Min gEk, 2008. *Garcinia mangostana* L. [Online]. Available from URL: http://flickr.com/photos /mingek/tags/garcinia/ [Accessed January 25, 2008].

Morton, J.F. 1987. Mangosteen. In: Fruit of Warm Climates. Miami, FL. 301-304.

Mukherjee, B., Mahapatra, S., Gupta, R., Patra, B., Tiwari, A. and Arora, P. 2005. A comparison between povidone-ethylcellulose and povidone-eudragit transdermal dexamethasone matrix patches based on in vitro skin permeation. *European Journal of Pharmaceutics and Biopharmaceutic,* 59: 475-483.

Murakami, M. 1932. Uber die Konstitution des Mangostins. *Liebigs Annalen*, 496: 122-155.

Nadkarni, K.M. and Nadkarni, A.K. 1999. Indian materia medica-with Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home remedies. Popular Prakashan Private Ltd., Bombay, India.

Ofori-Kwakye, K. and Fell, J.T. 2003. Leaching of pectin from mixed films containing pectin, chitosan and HPMC intended for biphasic drug delivery. *International Journal of Pharmaceutics*, 250(1): 251-257.

Organization for Economic Co-operation and Development. 2002. OECD Guidelines for testing of chemicals, Volume 2, Section 4: Health Effects. Acute dermal irritation/corrosion. Test Guideline No. 404.

Padula, C., Colombo, G., Nicoli, S., Catellani, P.L., Massimo, G. and Santi, P. 2003. Bioadhesive film for the transdermal delivery of lidocaine: *in vitro* and *in vivo* behavior. *Journal of Controlled Release*, 88: 277-285.

Parveen, M. and Khan, N.U.D. 1988. Two xanthones from *Garcinia mangostana*. *Phytochemistry*, 27(11): 3694-3696.

Peres, V. and Nagem, T.J. 1997. Trioxygenated naturally occurring xanthones. *Phytochemistry*, 44(2): 191-214.

Peres, V., Nagem, T.J. and Oliveira, F.F.D. 2000. Trioxygenated naturally occurring xanthones. *Phytochemistry*, 55(2): 683-710.

Phongboonrod, S. 1976. Mai thed mueong Thai, Folkloric uses of Thai and foreign medicinal plants. Chaiwat press Co., Bangkok.

Pongcharoen, W. 2004. Chemical constituents from the fruit of *Garcinia bancana* and *Garcinia cowa*. *Master of Science Thesis in Organic chemistry, Prince of Songkla University*. 105-108, 250-255.

Phongpaichit, S., Ongsakul, M., Nilrat, L., Tharavichitkul, P., Bunchoo, S., Chuaprapaisilp, T. and Wiriyachitra, P. 1994. Antibacterial activities of extracts from *Garcinia mangostana* pericarps on methicillin-resistant *Staphylococcous aureus* and *Enterococcous* species. *Songklanakarin Journal of Science and Technology*, 16(4): 399-405.

Pranoto, Y., Salokhe, V.M. and Rakshit, S.K. 2005. Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. *Food Research International*, 38(3): 267-272.

Quisumbing, E. 1978. Medcinal plants of the Philippines. Katha Publishing Company. IMC Press, Quezon City, Philippines.

Ravichandran, R. 2009. Sodium alginate [Online]. Available from URL: http://www.uspbpep. com/usp28 /v28230/usp28nf23s0_m75750.htm [Accessed January 20, 2009].

Rhim, J. 2004. Physical and mechanical properties of water resistant sodium alginate films. *Lebensmittel-Wissenschaft und-Technologie*, 37(3): 323-330.

Rowe, R.C., Shesdey, P.J. and Weller, P.J. 2003. Sodium alginate. Handbook of pharmaceutical excipients 4th edition. Pharmaceutical Press and American Pharmaceutical Association, 543-545.

Sakagami, Y., Iinuma, M., Piyasena, K.G.N.P. and Dharmaratne, H.R.W. 2005. Antibacterial activity of α-mangostin against vancomycin resistant Enterococci (VRE) and synergism with antibiotics. *Phytomedicine*, 12(3): 203-208.

Sakai, S.I., Katsura, M., Takayama, H., Aimin, N., Chokethaworn, N. and Suttajit, M. 1993. The structure of Garcinone E. *Chemical & Pharmaceutical Bulletin*, 41(5): 958-960.

Schmid, W. 1855. Isolation of Mangostin from *Garcinia mangostana* Linn. *Liebigs Annalen*, 93: 83-89.

School of Thai traditional medicine. 1981. Principle of Thai traditional pharmacy. Prachettuphon wimolmangkalaramrajworawiharn temple press, Bangkok.

Sen, A.K., Sarkar, K.K., Mazumder, P.C., Banerji, N., Uusvueri, R. and Hase, T.A. 1982. The structures of garcinones A, B and C: Three new xanthones from *Garcinia mangostana. Phytochemistry,* 21(7): 1747-1750.

Shah, V.P., Behl, C.R., Flynn, G.L., Higuchi, W.I. and Schaefer, H. 1992. Principles and criteria in the development and optimization of topical therapeutic products. *Journal of Pharmaceutical Sciences*, 81(10): 1051-1054.

Songbandit, K. 1978. Ethnomedical uses of Thai herbal medicines from the records on palm leaves. Silpabanakhaan Press, Bangkok.

Sornprasit, A., Sripiyarattanakul, K., Chuay-Yim, P. and Tanakittithun, P. 1987. Preliminary toxicological study of mangostin. *Songklanakarin Journal of Science and Technology*, 9: 51-57.

Sriamornsak, P. 2003. Chemistry of pectins and its pharmaceutical uses: a review. *Silpakorn University International Journal*, 3(1-2): 206-228.

Sriamornsak, P. Use of citrus pectin in pharmaceutical production and drug delivery [Online]. Available from URL: http://www.scisoc.or.th/stt/30/invited/paper/11.pdf [Accessed April 1, 2008].

Sriamornsak, P., Wattanakorn, N., Nunthanid, J. and Puttipipatkhachorn, S. 2008. Mucoadhesion of pectin as evidence by wettability and chain interpenetration. *Carbohydrate Polymers,* 78: 458-467.

Sundaram, B.M., Gopalakrishnan, C., Subramanian, S., Shankaranarayanan, D., Kameswaran L. 1983. Antimicrobial activities of Garcinia mangostana. *Planta Medica*, 48: 59-60.

Suksamrarn, S., Suwannapoch, N., Ratananukul, P., Aroonlerk, N. and Suksamrarn, A. 2002. Xanthones from the green fruit hulls of *Garcinia mangostana. Journal of Natural Products*, 65(5): 761-763.

Usov, A.I. 1998. Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloids,* 12: 301-308.

Volker83. 2008. File:sodium alginate.jpg [Online]. Available from URL: http://commons. wikimedia.org/wiki/File:Sodium_alginat.jpg [Accessed January 15, 2011].

Voravuthikunchai, S.P. and Kitpipit, L. 2005. Activity of medicinal plant extracts against hospital isolates of methicillin-resistant *Staphylococcus aureus. Clinical Microbiology and Infection,* 11(6): 510-512.

Wang, Q., Dong, Z., Du, Y. and Kennedy, J.F. 2007. Controlled release of ciprofloxacin hydrochloride from chitosan/polyethylene glycol blend films. *Carbohydrate Polymers,* 69: 336-343

Yodhnu, S. 2008. Development of the Extraction Method for α-Mangostin from the Fruit Peel of Mangosteen and Stability Study in the Extract and Antibacterial Film. The Degree of Master of Pharmacy, Pharmaceutical Sciences, Prince of Songkla University.

Yodhnu, S., Sirikatitham, A. and Wattanapiromsakul, C. 2009. Validation of liquid chromatography for the determination of an antibacterial agent; α-mangostin in mangosteen peel extract: A tool for quality assessment of *Garcinia mangostana* L. *Journal of Chromatographic Science*, 47(3): 185-189.

Yu, L., Zhao, M., Yang, B., Zhao, Q. and Jiang, Y. 2007. Phenolics from hull of *Garcinia mangostana* fruit and their antioxidant activities. *Food Chemistry,* 104(1): 176-181.

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Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Pharmacy	Prince of Songkla University	2005

Scholarship Award during Enrolment

Academic Excellent Program in Pharmaceutical Sciences, Prince of Songkla University, 2006.

List of Publications and Proceedings

An Antibacterial Patch Made from the Mangosteen Peel Extract. Phadungkarn, T., Amnuaikit, T. and Wattanapiromsakul, C. Oral presented at The 14th National Graduate Research Conference, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand, 10-11 September 2009.