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รายงานวิจัยฉบับสมบูรณ์

การศึกษาประสิทธิภาพในการยับยั้งจุลชีพและการทดสอบประสิทธิภาพทางคลินิกในการยับยั้งการอักเสบของสิวของสารสกัดเปลือกมังคุดเพื่อใช้ในเครื่องสำอาง

ANTIMICROBIAL ACTIVITIES AND EFFICACY ON ACNE LESIONS OF EXTRACTS FROM *GARCINIA MANGOSTANA.*

โดย

นางสาวณัฐกานต์ ปัญญาจริวัฒน์ สำนักวิชาวิทยาศาสตร์เครื่องสำอาง

งานวิจัยนี้ได้รับทุนสนับสนุนจากมหาวิทยาลัยแม่ฟ้าหลวง
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EXECUTIVE SUMMARY

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Nattakan Panyachariwat
School of Cosmetic Science, Mae Fah Luang University

1. INTRODUCTION

In our effort to screen the cosmetic application of bioactive compounds, found in natural products of plant origin, we evaluated the crude extract from the fruit hulls of *Garcinia mangostana*. *Garcinia mangostana* L. of the family Clusiaceae (Guttiferae), commonly known as mangosteen, is a slow-growing tropical evergreen tree with leathery, glabrous leaves. The tree can attain 6-25 meters in height and is mainly found in India, Myanmar, Sri Lanka, Cambodia, Vietnam, and Thailand. Mangosteen is presumed to have originated in Southeast Asia, possibly in the Indonesian region, and was known in the western world as early as 1631.

In the present study, extracts of the fruit hulls of mangosteen, which have been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activities against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis,* and *Escherichia coli*. This study has also identified the promising source of anti-inflammatory agent which could be useful in treatment of acne vulgaris.

For many years, antibiotics have been used to treat acne vulgaris, however, antibiotic resistance has been increasing in prevalence within the dermatologic setting. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. In this study, extracts of the fruit hulls of mangosteen were also examined for biological properties against inflammatory acne induced by *Propionibacterium acnes (P. acnes*) and the efficacy on acne lesions.

1.1 Objectives

1. To study the biological activities of the crude extracts from the fruit hulls of mangosteen.
2. To evaluate the efficacy on acne lesions of cosmetics containing the crude extracts from the fruit hulls of mangosteen.
3. To confirm the claims in acne treatment of "Lanadene acne gel", the product of the school of cosmetic science.

1.2 Scope

The crude extracts of the fruit hulls of mangosteen were prepared and examined for antimicrobial activities against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes* using disk diffusion and agar dilution method. Zone of inhibition and MIC values were measured after applying mangosteen extracts for 24 hours. Their efficacy on acne lesions was also investigated and carried out by dermatologist responsible in this study under the same conditions, counted the retentional and inflammatory lesions on the entire face (except the nasal pyramid), at D0 and D28 after twice-daily used with 22 female volunteers at the age of 23±1.

2. COMPUTATIONAL THEORY

Garcinia mangostana has been documented in Thai traditional medicine in various aspects. Although many benefits have been claimed, few scientific reports are available in the literature. Phytochemical studies showed that the hulls of this plant contained various secondary metabolites, such as tannins, triterpenes, anthocyanins, xanthones, polysaccharides, phenolic compounds, vitamins B1, B2 and C, and other bioactive substances. Moreover, recent scientific studies reported that the hulls of *G. mangostana* possessed several biological and pharmacological properties such as antifungal [1], antimicrobial [2], antioxidant [3-6], antihistamine [7-8], anti-HIV-1 protease [9-10], and induction of apoptosis in cancer cell lines [11].

Although several properties of the fruit hulls of *G. mangostana* have been studied, few authentic scientific reports are available, when compared with other medicinal plants. The genus Garcinia (Guttiferae) is known to produce a variety of biologically active metabolites such as polyisoprenylated benzophenones and xanthones. The xanthones isolated from *G. mangostana* have been reviewed. Xanthones in the pericarp are composed of mangostione, α-mangostin, β-mangostin, γ-mangostin, gartinin, and garcinone E.

In the present study, extracts of mangosteen, which has been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activities against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and microorganism frequently involved in acne inflammation, *Propionibacterium acnes*.

3. RESEARCH METHODOLOGY

This research consists of 3 main parts including the quantitative analysis, antimicrobial susceptibility testing and clinical evaluation for anti-acne effect. The details of those steps are explained below.

3.1 Preparation of plant extracts

Garcinia mangostana hulls used in this study collected from various locations in Thailand. They were shade dried, powdered and extracted with 95% ethanol. The

ethanolic extract was standardized by quantifying the amount of α-mangostin, an active compound, using HPLC.

3.2 Antimicrobial susceptibility testing

3.2.1 Disc diffusion method

Antimicrobial tests were then carried out by disc diffusion method using 100 µl of suspension containing 10^8 CFU/ml of bacteria. This experiment was performed by the method of Hayes and Markovic (2002) [12] with some modifications.
Propionibacterium acnes was incubated in brain heart infusion medium (BHI) with 1% glucose for 72 h under anaerobic conditions and adjusted to yield approximately 1.0×10^8 CFU/ml. Aliquots of molten BHI with glucose agar were used as an agar base. A prepared inoculum was added to molten agar, mixed and poured over the surface of the agar base and left to solidity. A sterile paper disc was impregnated with test material and the disc was placed on the agar. Plates were then incubated at 37°C for 72 h under anaerobic conditions.

Staphylococcus aureus, Bacillus subtilis, *Escherichia coli* and *Salmonella albany* were incubated in tryptic soy broth (TSB), turbidity equivalent to McFarland No. 0.5, for 24 h at 37°C and adjusted to yield approximately 1.0×10^8 CFU/ml. The procedures were the same as mentioned above except the plates were incubated at 37°C for 24 h under aerobic conditions. All disc diffusion tests were performed in three separate experiments and the antibacterial activity was expressed as the mean of inhibition diameters (mm).

3.2.2 Determination of minimum inhibitory and bactericidal concentrations

The minimal inhibitory concentration (MIC) values were determined by agar dilution assay. The cultures were prepared at 24 h broth cultures of *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli* and *Salmonella albany* and 72 h broth cultures of *Propionibacterium acnes*. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms.

3.3 Preparation of "Mangosteen gel"

Water 67.00 g and glycerin 3.00 g were mixed and heated to 75°C. Then propylene glycol 2.50 g and methylparaben 0.10 g were dissolved in the mixture of water and glycerin and left at this temperature as Part A. Hydroxyethylcellulose 0.40 g was dispersed in Part A under turbine stirring and the mixture was then cooled to 50°C. Carbomer 1.00 g dispersed in water 19.00 g, was added to the mixture at 50°C under slow turbine stirring, following by the addition of 1.00 g triethanolamine (20% aqueous solution). Mangosteen extract 5.00 g dissolved in 1.00 g dimethicone copolyol, was added under the same turbine stirring to the mixture. Then the mixture was cooled with planetary mixing to room temperature to yield 100.00 g of "5% mangosteen gel".

3.4 Clinical examination of anti-acne effect

Clinical examination to evaluate the comedogenic potential of "5% mangosteen gel" on human subject was assessed by dermatologist. The experiment was conducted according to 2 objectives; to evaluate the effect and safety of cosmetic product on subjects with acne prone skin after 28 days of twice daily use, by clinical assessment by dermatologist and to determine the product organoleptic characteristics, efficacy and safety by analyzing the answers given by the volunteers to a subjective questionnaire. This was an open and intra-individual study and each subject is his/her own control.
On D0 and D28, the dermatologist counted the retentional (open comedones = blackheads and closed comedones = microcysts) and inflammatory lesions (papules and pustules) on the entire face (except the nasal pyramid).

3.4.1 Inclusion criteria

GENERAL CRITERIA

- Healthy subjects.
- Subjects having given their informed, written consent.
- Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by the clinical trial center could have been expected.

SPECIFIC CRITERIA

- Age: 18-35 years old.
- Subjects with greasy facial skin and acneic lesions.
- Type: Asian female.
- Phototype: III, IV or V.

3.4.2 Non-inclusion criteria

- Pregnant or nursing women.
- Cutaneous pathology on face, other than acne (eczema, etc).
- Serious or progressive diseases that the investigator judges may interfere with the study.
- Volunteers undergoing a topical or systemic treatment:
  - anti-inflammatories and/or anti-histamines during the previous week,
  - cough suppresants and/or corticoids during the four previous weeks,
  - retinoids and/or immuno-suppressors during the six previous months.
- Any acne treatment within the previous month by oral or local route.
- Unstable weight.
- Excessive exposure to sunlight or UV rays during study within previous month.
- Excessive use of alcohol or tobacco.

3.4.3 Trial organization: schedule

On D0

- Subjects came to the laboratory without having applied any product to their face since the previous evening.
- An information sheet was provided to remind them of the study details.
- They read and signed the information and consent forms in duplicate.
- Clinical examination of the initial state of the skin by the dermatologist and counting of the retentional and inflammatory lesions on the entire face except the nasal pyramid.
- Distribution of the products to the volunteers who apply them twice-daily to the whole face for 28 days
- Distribution of a safety grid, completed by the subjects everyday.

On D28

- Subjects returned to the laboratory without any application of the product to the face; the last application of the product was done the evening before.
- New clinical examination by the dermatologist who counted retentional and inflammatory lesions and evaluated the safety of the product.
- Subjects answered the subjective questionnaire on D28.
- Subjects bring their product and safety grid back to the laboratory in order to verify the compliance.

Ambient conditions during measurements were to have been:

- Ambient temperature: 25±1°C
- Relative humidity: between 40% and 60%.

4. RESULTS AND DISCUSSION

4.1 Quantitative analysis

In this study, α-mangostin, one of the major and interesting active compounds from *G. mangostana* was identified and quantitated with HPLC chromatography to standardize the quantity and quality of the extract. α-Mangostin was present in our GME at 32.56 ± 0.35 g/100 g of GME.

4.2 Antimicrobial activities

Antimicrobial activities of the ethanolic extracts of *Garcinia mangostana* (Guttiferae) against microorganisms (*Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis*, *Escherichia coli* and *Propionibacterium acnes*), examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter, and MIC values. The results were given in **Tables 1-2**.

Table 1 Antimicrobial activity of *Garcinia mangostana* extracts against the bacterial strains tested based on disc diffusion method.

Bacterial species	Inhibition zone in diameter (mm)	
	Garcinia mangostana extracts	Negative control
Staphylococcus aureus	18	-
Salmonella albany	10	-
Bacillus subtilis	14	-
Escherichia coli	8	-
Propionibacterium acnes	1	-

The maximal inhibition zones for bacteria strains; *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes* which were sensitive to *Garcinia mangostana* ethanol extracts, were 18, 10,14, 8 and 1 mm, respectively.

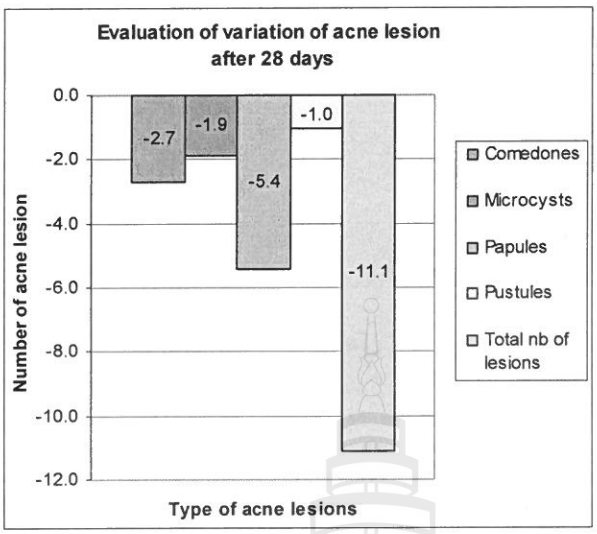
Table 2 The MIC values of *Garcinia mangostana* extracts against the bacterial strains tested based on agar dilution assay.

Bacterial species	*Garcinia mangostana* extracts
Gram positive	
Staphylococcus aureus	0.96 μg/ml
Bacillus subtilis	15.00 μg/ml
Gram negative	
Salmonella albany	3.87 μg/ml
Escherichia coli	1.93 μg/ml
Gram positive anaerobe	
Propionibacterium acnes	2.5 mg/ml

Mangosteen extracts had a minimum inhibitory concentration (MIC) of 0.96 μg/ml against strains of *S. aureus*, which was greater than that of the antibiotic vancomycin (MIC = 3.13-6.25 μg/ml). Extracts of mangosteen were also examined to determine the inhibitory effect against *S. albany*, *B. subtilis, E. coli and P. acne* with the minimum inhibitory concentration of 3.87 μg/ml, 15 μg/ml, 1.93 μg/ml and 2.5 mg/ml, respectively.

4.3 Clinical examination for anti-acne effect

The test was done on 22 female and 100% of subjects finished the study. The counting of acne lesions at the beginning of the study before any application and at the end of the study after 28 days of twice-daily use showed a statistically significant decrease of these lesions, particularly with the number of papules.



Under these study conditions, the product could be considered very well tolerated on the cutaneous level: the reported signs did not last more than five minutes after application and are normal with this kind of product.
70%, 91%, 74% and 61% of volunteers noticed an improvement effect on their acne in particular for the variation in the number of blackhead and a variation in the number, size and inflammation of pimples after this treatment, respectively.

5. CONCLUSION

The results showed that *Garcinia mangostana* extracts could effectively inhibit the growth of *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes*. Among those, the extracts of *Garcinia mangostana* showed strong inhibitory effects against *Staphylococcus aureus* (zone of inhibition ≥15 mm).
Subsequent experiments were conducted to determine inhibitory concentrations of *Garcinia mangostana* extracts. The MIC values against *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes* were 3.87 µg/ml, 15 µg/ml, 1.93 µg/ml and 2.5 mg/ml, respectively. The highest activity was shown against the Gram-positive bacterium *Staphylococcus aureus* with a MIC 0.96 µg/ml for the ethanol extract of the fruit hulls.
The efficacy on acne lesions of mangosteen extracts was also investigated. The efficacy on acne lesions was carried out by dermatologist responsible in this study under the same conditions, counted the retentional and inflammatory lesions on the entire face (except the nasal pyramid), at D0 and D28 after twice-daily used with 22 female volunteers at the age of 23±1. After used the "5 % Mangosteen gel" on acne lesions for 28 days, the number of acne lesions include of comedones, microcysts, papules and pustules showed a significant decreased from the beginning and for the cutaneous tolerance, the "5 % Mangoteen gel" could be considered very well tolerated on the cutaneous level. Globally, the volunteers appreciate the tested product according to evaluation questionnaires. A Majority of volunteers noticed an improvement effect on their acne in particular for the variation in the number of

blackhead and a variation in the number, size and inflammation of pimples after this treatment.

In conclusion, the obtained results confirm the presence of anti-bacterial principles in Mangosteen extract and demonstrate that these plant could represent a new source of anti-microbial agents, less expensive than the imported drugs. Mangosteen extracts are the novel compounds for cosmetic and pharmaceutical use in acne treatment according to their strong *in vitro* antimicrobial activity and their efficacy on acne lesions.

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การศึกษาประสิทธิภาพในการยับยั้งจุลชีพและการทดสอบประสิทธิภาพทางคลินิกในการยับยั้งการอักเสบของสิวของสารสกัดเปลือกมังคุดเพื่อใช้ในเครื่องสำอาง

ณัฐกานต์ ปัญญาจริวัฒน์ สำนักวิชาวิทยาศาสตร์เครื่องสำอาง มหาวิทยาลัยแม่ฟ้าหลวง

การทดสอบฤทธิ์ต้านเชื้อจุลินทรีย์ของสารสกัดหยาบจาก *Garcinia mangostana* (Guttiferae) โดยใช้วิธี disk diffusion และ agar dilution พบว่าสารสกัดสามารถยับยั้งการเจริญของ *Staphylococcus aureus Salmonella Albany Bacillus subtils Escherichia coli* และ *Propionibacterium acnes* โดยมีค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญของเชื้อ (MIC) เท่ากับ 0.96 ไมโครกรัมต่อมิลลิลิตร 3.87 ไมโครกรัมต่อมิลลิลิตร 15 ไมโครกรัมต่อมิลลิลิตร 1.93 ไมโครกรัมต่อมิลลิลิตร และ 2.5 มิลลิกรัมต่อมิลลิลิตร ตามลำดับ และจากการทดสอบประสิทธิภาพทางคลีนิกของสารสกัดเปลือกมังคุดในการยับยั้งสิวในอาสาสมัครหญิงจำนวน 22 คนที่มีอายุระหว่าง 22-24 ปี พบว่าเมื่อใช้เจลมังคุดที่มีปริมาณสารสกัดจากเปลือกมังคุด 5% เป็นเวลา 28 วัน สามารถลดปริมาณสิวชนิดต่าง ๆ ได้อย่างมีนัยสำคัญและไม่ก่อให้เกิดอาการแพ้ และจากการสำรวจความพึงพอใจด้วยแบบสอบถามพบว่า อาสาสมัครส่วนใหญ่มีความพึงพอใจในผลิตภัณฑ์ จึงทำให้สารสกัดเปลือกมังคุดเป็นที่น่าสนใจในการพัฒนาเป็นเครื่องสำอางและเวชสำอางในการรักษาสิว

คำสำคัญ: มังคุด ฤทธิ์ต้านเชื้อจุลินทรีย์ สิว สารสกัดเปลือกมังคุด

ANTIMICROBIAL ACTIVITIES AND EFFICACY ON ACNE LESIONS OF EXTRACTS FROM *GARCINIA MANGOSTANA*.

N. PANYACHARIWAT, School of Cosmetic Science, Mae Fah Luang University

Anti-microbial activities against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes* were shown by extracts of *Garcinia mangostana* (Guttiferae) using disk diffusion and agar dilution method. Zone of inhibition and MIC values were measured after applying mangosteen extracts for 24 hours. Mangosteen extracts had a minimum inhibitory concentration (MIC) of 0.96 μg/ml against strains of *S. aureus*, which was greater than that of the antibiotic vancomycin (MIC = 3.13-6.25 μg/ml). Extracts of mangosteen were also examined to determine the inhibitory effect against *S. albany*, *B. subtilis, E. coli and P. acne* with the minimum inhibitory concentration of 3.87 μg/ml, 15 μg/ml, 1.93 μg/ml and 2.5 mg/ml, respectively. The efficacy on acne lesions of mangosteen extracts was investigated. The efficacy on acne lesions was carried out by dermatologist responsible in this study under the same conditions, counted the retentional and inflammatory lesions on the entire face (except the nasal pyramid), at D0 and D28 after twice-daily used with 22 female volunteers at the age of 23±1. After used the "5 % Mangosteen gel" on acne lesions for 28 days, the number of acne lesions include of comedones, microcysts, papules and pustules showed a significant decreased from the beginning and for the cutaneous tolerance, the "5 % Mangoteen gel" could be considered very well tolerated on the cutaneous level. Globally, the volunteers appreciate the tested product according to evaluation questionnaires. A Majority of volunteers noticed an improvement effect on their acne in particular for the variation in the number of blackhead and a variation in the number, size and inflammation of pimples after this treatment. These results suggest that mangosteen extracts are the novel compounds for cosmetic and pharmaceutical use in acne treatment according to their strong in-vitro antimicrobial activity and their efficacy on acne lesions.

Keywords: *Garcinia mangostana*; anti-microbial; acne; mangosteen extracts.

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CHAPTER I
INTRODUCTION

Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their uses in traditional medicine. Thailand is rich in term of natural resources and herbal plant diversity and has a long story about traditional medicine. Since ancient times, plants have been widely used as drugs, foods and cosmetics. Mostly senior people have functioned to transfer such application knowledge and understanding to the new generations as part of their socialization within their families and communities. At present, patterns of medicinal plant application changed to business-based both domestically and internationally, affecting natural resource management. Scientific interest in the value of medicinal plant and their sustainable agricultural development has been growing up rapidly and continuously. Natural products have been recognized as an important tool in cosmetic industry throughout this century. Cosmetic consumers are changing their lifestyle and their attitudes towards life and the boundaries of the beauty and health markets are expanding more and more and becoming rich in new possibilities of exploration. In consumers' opinion, natural cosmetics from plants are more advantageous than chemical cosmetics due to their safety. In recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological activities of medicinal plants and to confirm the claims made about them in folklore medicines.

In our effort to screen the cosmetic application of bioactive compounds, found in natural products of plant origin, we evaluated the crude extract from the fruit hulls of *Garcinia mangostana*. *Garcinia mangostana* L. of the family Clusiaceae (Guttiferae), commonly known as mangosteen, is a slow-growing tropical evergreen tree with leathery, glabrous leaves. The tree can attain 6-25 meters in height as shown in **Figure 1-1** and is mainly found in India, Myanmar, Sri Lanka, Cambodia, Vietnam, and Thailand. Mangosteen is presumed to have originated in Southeast Asia, possibly in the Indonesian region, and was known in the western world as early as 1631.

Fig. 1-1 The mangosteen tree

Mangosteen has dark purple to red-purple fruits as shown in **Figure 1-2**. The edible fruit aril is white, soft, and juicy with a sweet, slightly acid taste and a pleasant aroma.

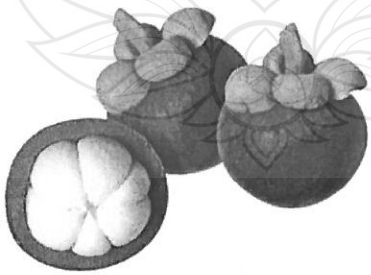


Fig. 1-2 The mangosteen fruit

Worldwide production of *G. mangostana* is about 150,000 tons per annum.[1] The pericarp of the fruit is about two thirds of the whole fruit by weight, bright red in colour,

and usually not eaten. People in these countries often use *G. mangostana* for traditional medicines including the treatment of abdominal pain, dysentery, diarrhoea, suppuration, infected wound, leucorrhoea and chronic ulcer and gonorrhoea.[2] Recently, products manufactured from *G. mangostana* have begun to be used as a botanical dietary supplement in the United States, because of their potent antioxidant potential.[3]

In the present study, extracts of the fruit hulls of mangosteen, which have been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activities against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis,* and *Escherichia coli*.

This study has also identified the promising source of anti-inflammatory agent which could be useful in treatment of acne vulgaris. For many years, antibiotics have been used to treat acne vulgaris, however, antibiotic resistance has been increasing in prevalence within the dermatologic setting.[4] The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. In this study, the extracts of the fruit hulls of mangosteen were also examined for biological properties against inflammatory acne induced by *Propionibacterium acnes (P. acnes*) and the efficacy on acne lesions.

Objective

1. To study the biological activities of the crude extracts from the fruit hulls of mangosteen.
2. To evaluate the efficacy on acne lesions of cosmetics containing the crude extracts from the fruit hulls of mangosteen.
3. To confirm the claims in acne treatment of "Lanadene acne gel", the product of the school of cosmetic science.

Research Output

The scientific data of anti-microbial activities against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes,* and the efficacy on acne lesions of crude extracts from the fruit hulls of mangosteen will be obtained.

Research Problem

Can the crude extracts from the fruit hulls of mangosteen be used as the novel compounds for cosmetic and pharmaceutical use in acne treatment considering from their *in-vitro* antimicrobial activity and their efficacy on acne lesions?

Conceptual Framework

Previous studies reported the inhibitory effects of mangostin and xanthone derivatives found in *Garcinia mangostana* (Guttiferae) against the growth of *Staphylococcus aureus*. However, the antimicrobial activities of crude extracts from the fruit hulls of mangosteen against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes* and the efficacy on acne lesions have not yet been investigated. Due to the convenience and economization, crude extracts were frequently used instead of pure compounds in cosmetic formulations. According to these reasons, antimicrobial activities of crude extracts from the fruit hulls of mangosteen against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes* and their efficacy on acne lesions were examined in this experiment.

Research Scope

The crude extracts of the fruit hulls of mangosteen were prepared and examined for antimicrobial activities against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes* using disk diffusion and agar dilution method. Zone of inhibition and MIC values were measured after applying mangosteen extracts *in vitro*. Their efficacy on acne lesions was also investigated and carried out by dermatologist responsible in this study under the same conditions, counted the retentional and inflammatory lesions on the entire face (except the nasal

pyramid), at D0 and D28 after twice-daily used with 22 female volunteers at the age of 23±1.

Research Period

From March 2007 to July 2008.

List of Abbreviations

GME	*Garcinia mangostana* extract
g	gram
µg	microgram
kg	kilogram
L	liter
mL	milliliter
M	mole
nM	nanomole
nm	nanometer
No	number
°C	degree Celsius
ESR	electron spin resonance
MeOH	methanol

Research Teams

Researcher	:	Nattakan Panyachariwat
Co-researcher	:	Pahol Sansomchai
Consultant	:	Assoc.Prof.Dr. Panvipa Krisdaphong

CHAPTER II
COMPUTATIONAL THEORY

Garcinia mangostana has been documented in Thai traditional medicine in various aspects. Although many benefits have been claimed, few scientific reports are available in the literature. The following studies demonstrate how the mangosteen could play a significant role in the treatment of inflammatory conditions, cancer of all types, heart disease, allergy/asthma, tuberculosis, central nervous system conditions, and human immunodeficiency virus (HIV). Additional studies show the anti-bacterial, anti-fungal and anti-viral properties of mangosteen.

Phytochemical studies showed that the fruit hulls of this plant contained various secondary metabolites, such as tannins, triterpenes, anthocyanins, xanthones, polysaccharides, phenolic compounds, vitamins B1, B2 and C, and other bioactive substances.[5] Moreover, recent scientific studies reported that the fruit hulls of *G. mangostana* possessed several biological and pharmacological properties such as antifungal,[6] antimicrobial,[7] antioxidant,[8-11] antihistamine,[12-13] anti-HIV-1 protease,[14-15] and induction of apoptosis in cancer cell lines.[16] The biological and pharmacological properties of extract from the fruit hulls of *Garcinia mangostana* are explained below.

The antifungal activity of several xanthones isolated from the fruit hulls of *Garcinia mangostana* and some derivatives of mangostin against three phytopathogenic fungi; *Fusarium oxysporum vasinfectum*, *Alternaria tenuis*, and *Dreschlera oryzae*, has been evaluated by Gopalakrishnan *et al.* in 1997.[6] The natural xanthones showed good inhibitory activity against the three fungi. Substitution in the A and C rings has been shown to modify the bioactivities of the compounds.

Prenylated xanthones, isolated from the fruit hulls, and the edible arils and seeds of *Garcinia mangostana*, were tested for their antituberculosis potential by Suksamrarn *et al.* in 2003.[7] α- and β-Mangostins and garcinone B exhibited strong inhibitory effect against *Mycobacterium tuberculosis* with the minimum inhibitory concentration (MIC)

value of 6.25 μg/ml. Tri- and tetra-oxygenated xanthones with di-C_5 units or with a C_5 and a modified C_5 groups are essential for high activities. Substitution in the A and C rings has been shown to modify the bioactivity of the compounds.

The study by Chanarat *et al.* in 1997 showed the anti-bacterial of mangosteen extract against intracellular Salmonella bacteria.[17] Polysaccharides from the pericarbs of mangosteen, *Garcinia mangostana* Linn., was obtained by treating the dried ground pericarbs with hot water followed by ethanol precipitation. The extract was fractionated by anion exchange chromatography on a DEAE-cellulose column as MDE1-5 fractions. The fractions of MDE3 and MDE4 composed of mainly D-galacturonic acid and a small amount of neutral sugar (L-arabinose as the major one and L-rhamnose and D-galactose as the minor ones) were studied for immunopharmacological activities by phagocytic test to intracellular bacteria (*Salmonella enteritidis*) and nitroblue tetrazolium (NBT) and superoxide generation tests. The results showed that the number of *S. enteritidis* in cultured monocyte with extract of pericarb of mangosteen (MDE3) was completly killed. Activating score (mean ± SD) of NBT test of 100 polymorphonuclear phagocytic cells were 145 ± 78, 338 ± 58, 222 ± 73, 209 ± 77, 211 ± 63, 372 ± 19, 369 ± 20, 355 ± 34 in normal saline control, phorbol myristate acetate (PMA), MDE3, MDE4, indomethacin (I), PMA + MDE3, PMA + MDE4 and PMA + I, respectively. Superoxide generation test was also done by color reduction of cytochrome C. Both MDE3 and MDE4 stimulate superoxide production. The number of *S. enteritidis* in cultured monocyte with extract of pericarb of mangosteen was killed. This paper suggests that polysaccharides in the extract can stimulate phagocytic cells and completly kill intracellular bacteria (*S. enteritidis*).

The antioxidant activities of 12 known xanthones, cudraxanthone G, 8-deoxygartanin, garcimangosone B, garcinone D, garcinone E, gartanin, 1-isomangostin, R-mangostin, γ-mangostin, mangostinone, smeathxanthone A, and tovophyllin A and two new highly oxygenated prenylated xanthones, 8-hydroxycudraxanthone G and mangostingone [7-methoxy-2-(3-methyl-2-butenyl)-8-(3-methyl-2-oxo-3-butenyl)-1,3,6-trihydroxyxanthone], found in a CH_2Cl_2-soluble extract of the mangosteen pericarp were determined by Jung *et al.* in 2006 using authentic and morpholinosydnonimine-

derived peroxynitrite methods.[9] Compounds 8-hydroxycudra-xanthone G, gartanin, R-mangostin, γ-mangostin, and smeathxanthone A were the most active.

In the study by Kosem *et al.* (2007), the methanolic extract from *G. mangostana* hulls (GME) was assessed for antioxidant and cytoprotective activities.[10] The results showed that GME contained phenolic compounds and possessed reducing power as well as Fe^{2+} chelating activity. The antioxidant properties were determined by scavenging DPPH, nitric oxide and lipid radicals in dose-dependent manners. In particular, the powerful scavenging activities were found against hydroxyl and superoxide radicals when investigated using ESR spectrometry. GME also enhanced the cell survival by decreasing the oxidative damage in ECV304 endothelial cells after H_2O_2 exposure. These data indicated that GME played a pivotal role on the antioxidant and chemopreventive activities *via* a reducing mechanism and inhibition of intracellular oxidative stress, respectively.

The antioxidant activities of three major phenolics; P_1 [1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl)], P_2 [1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl) xanthone] and P_3 (epicatechin), found in MeOH extract of the air-dried fruit hulls of *Garcinia mangostana* Linn., were evaluated by Yu *et al.* in 2007 using different tests, including the free radical scavenging capability and total antioxidant activity in a linoleic acid peroxidation.[11] These three phenolic compounds exhibited different antioxidant activities in these antioxidant tests. The hydroxyl radical and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capabilities and the activity against linoleic acid peroxidation of P_1 were greater than those of P_2 and P_3, while the superoxide anion radical scavenging activity of P_3 was greater than that of P_1, but was close to that of P_2 or α-tocopherol.

The study by Moongkarndi *et al.* in 2004 was designed to determine the antiproliferative, apoptotic and antioxidative properties of crude methanolic extract from the pericarp of *Garcinia mangostana* (family Guttiferae) using human breast cancer (SKBR3) cell line as a model system.[16] These investigations suggested that the methanolic extract from the pericarp of *Garcinia mangostana* had strong

antiproliferation, potent antioxidation and induction of apoptosis. Thus, it indicates that this substance can show different activities and has potential for cancer chemoprevention which was dose dependent as well as exposure time dependent.

A crude methanolic extract of the fruit hulls of *Garcinia mangostana* L. inhibited the contraction of the isolated rabbit aorta induced by histamine and serotonin. The extract has been fractionated by silica gel chromatography, monitoring the pharmacological activity to give active compounds; alpha-mangostin and gamma-mangostin. To define the pharmacological properties of alpha-mangostin, the effect of alpha-mangostin on both histamine H1 and H2 receptors, studied by Furukawa *et al.* in 1997, were examined by monitoring the mechanical responses of smooth muscles and measuring the radioligand binding to cultured vascular smooth muscle cells.[12] The results suggest that alpha-mangostin acts as a selective and a strong competitive histamine H1 receptor antagonist. The pharmacological actions of gamma-mangostin on 5-HT receptors were also investigated by using contractile response of vascular smooth muscle, platelet aggregation and radioligand binding studies. The results provide the evidence that gamma-mangostin is a selective and a strong competitive 5-HT_{2A} receptor antagonist. It is of great interest that the structures of alpha-mangostin and gamma-mangostin free from nitrogen atom are not resemble to the common structures of histamine and serotonin receptor antagonists. alpha-Mangostin and gamma-mangostin may become novel types of lead compounds for histamine and serotonin receptor antagonists.

The study by Nakatani *et al.* in 2002 was undertaken to examine the effects of mangosteen pericap extracts (100% ethanol, 70% ethanol, 40% ethanol and water) on histamine release and prostaglandin E2 synthesis.[13] It was found that the 40% ethanol extract of mangosteen inhibited IgE-mediated histamine release from RBL-2H3 cells with greater potency than the water extract of *Rubus suavissimus* that has been used as an anti-allergy crude drug in Japan. All extracts of mangosteen potently inhibited A23187-induced prostaglandin E2 synthesis in C6 rat glioma cells, while the water extract of *Rubus suavissimus* had no effect. The 40% ethanol extract of mangosteen inhibited the prostaglandin E2 synthesis in a concentration-dependent manner with

relatively lower concentrations than the histamine release. In addition, passive cutaneous anaphylaxis (PCA) reactions in rats were significantly inhibited by this ethanol extract as well as by the water extract of *Rubus suavissimus*. These results suggest that the 40% ethanol extract of mangosteen has potent inhibitory activities of both histamine release and prostaglandin E2 synthesis.

In 1996, Chen *et al.* reported that the ethanol extract of *Garcinia mangostana* L. (Guttiferae) showed fast, potent inhibitory activity against HIV-1 protease.[14] The activity-guided purification of the extract resulted in the isolation of two active, known compounds. The chemical structures of the isolated compounds were established by spectroscopic analyses as mangostin (IC_{50} = 5.12 ± 0.41 microM) and gamma-mangostin (IC_{50} = 4.81 ± 0.32 microM). The type of inhibition by both compounds is noncompetitive.

In 1998 Vlietinck *et al.* reviewed the inhibition of many compounds of plant origin in different stages in the replication cycle of human immunodeficiency virus (HIV):[15]

1) virus adsorption: chromone alkaloids (schumannificine), isoquinoline alkaloids (michellamines), sulphated polysaccharides and polyphenolics, flavonoids, coumarins (glycocoumarin, licopyranocoumarin), phenolics (caffeic acid derivatives, galloyl acid derivatives, catechinic acid derivatives), tannins and triterpenes (glycyrrhizin and analogues, soyasaponin and analogues);

2) virus-cell fusion: lectins (mannoseand N-acetylglucosamine-specific) and triterpenes (betulinic acid and analogues);

3) reverse transcription; alkaloids (benzophenanthridines, protoberberines, isoquinolines, quinolines), coumarins (calanolides and analogues), flavonoids, phloroglucinols, lactones (protolichesterinic acid), tannins, iridoids (fulvoplumierin) and triterpenes;

4) integration: coumarins (3-substituted-4-hydroxycoumarins), depsidones, *O*-caffeoyl derivatives, lignans (arctigenin and analogues) and phenolics (curcumin);

5) translation: single chain ribosome inactivating proteins (SCRIP's);

6) proteolytic cleavage (protease inhibition): saponins (ursolic and maslinic acids), xanthones (mangostin and analogues) and coumarins;

7) glycosylation: alkaloids including indolizidines (castanospermine and analogues), piperidines (1-deoxynojirimicin and analogues) and pyrrolizidines (australine and analogues);

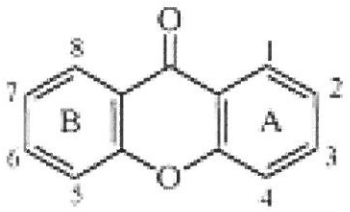
8) assembly/release: naphthodianthrones (hypericin and pseudohypericin), photosensitisers (terthiophenes and furoisocoumarins) and phospholipids.

The target of action of several anti-HIV substances including alkaloids (*O*-demethylbuchenavianine, papaverine), polysaccharides (acemannan), lignans (intheriotherins, schisantherin), phenolics (gossypol, lignins, catechol dimers such as peltatols, naphthoquinones such as conocurvone) and saponins (celasdin B, Gleditsia and Gymnocladus saponins), has not been elucidated or does not fit in the proposed scheme. Only a very few of these plant-derived anti-HIV products have been used in a limited number of patients suffering from AIDS viz. glycyrrhizin, papaverine, trichosanthin, castanospermine, *N*-butyl-1-deoxynojirimicin and acemannan.

The genus Garcinia (Guttiferae) is known to produce a variety of biologically active metabolites such as polyisoprenylated benzophenones,[18] and xanthones.[19]

Xanthones are secondary metabolites commonly occurring in a few higher plant families, and in fungi and lichens. Their taxonomic importance in such families and their pharmacological properties have aroused great interest.[20]

The symmetrical nature of the xanthone nucleus coupled with its mixed iogenetic origin in higher plants necessitates that the carbons be numbered according to a biosynthetic convention. Carbons 1-4 are assigned to the acetate-derived ring A, and carbons 5-8 to the shikimate-derived ring B.[19] The numbering system is based on xanthene-9-one as the basic skeleton,[21] and in cases where only ring B is oxygenated the lowest numbers are used, except for biosynthetic discussions.[19] The skeleton of xanthone **1** is shown in **Figure 2-1**.



Xanthone basic skeleton

Fig. 2-1 The basic skeleton of xanthone 1

The biosynthetic pathways to xanthones have been discussed in recent years. Initially, these attempted to interrelate the observed oxygen pattern of natural xanthones and correlate them with recognized oxygenation patterns. In general it seems that ring B and the attached CO group are provided by the shikimic acid pathway, whereas ring A arises *via* the acetate-malonate polyketide route.[22-24] Therefore Locksley *et al.* in 1967 reported the significance of maclurin in xanthone biosynthesis and the biogenetic-type synthesis of xanthones from their benzophenone precursors.[22,25] Gottlieb offered biogenetic proposals regarding xanthones.[21,26-27] Bhanu *et al.* related the biogenetic implications in the conversion of 4-phenylcoumarins into xanthones.[28] More recently; biosynthetic studies on tajixanthone and shamixanthone were reported by Ahmed *et al.* in 1992.[29] Some xanthones in lower plants have been proven to be totally acetate-derived, from seven acetate units.[30-31] However, the oxygenation patterns of all xanthones in higher plants suggest that these are formed by a mixed shikimate-acetate pathway. This involves the condensation of shikimate and acetate-derived moieties to form benzophenones or benzophenone-like intermediate **2**, which then react intramolecularly to form xanthone **3**. The biosynthesis of xanthone is summarized in **Figure 2-2**.

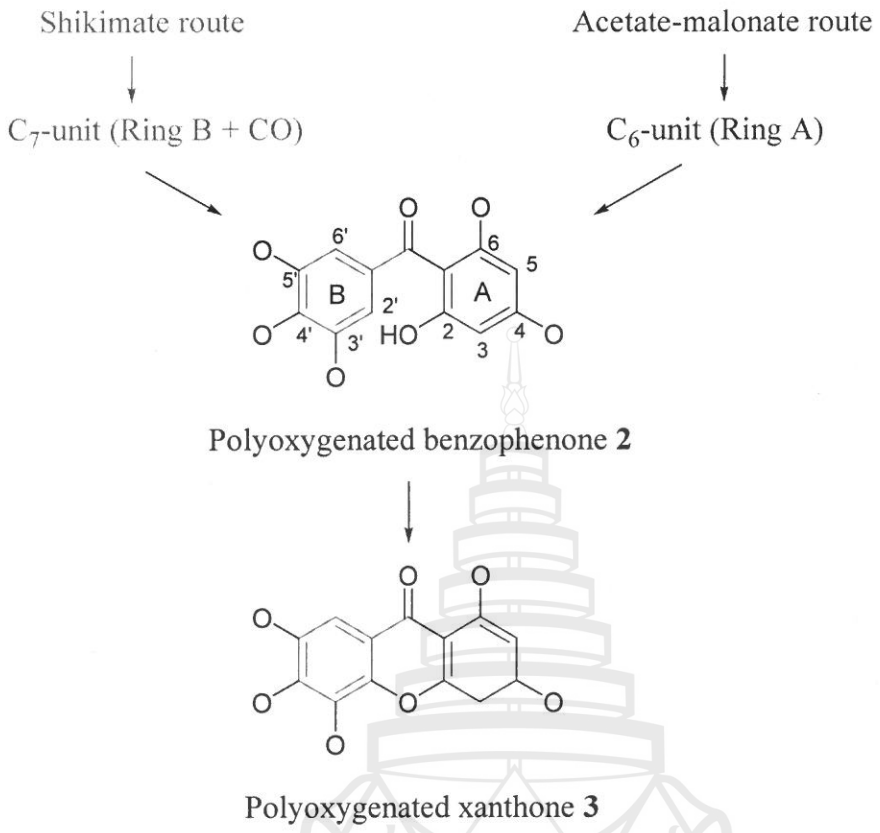


Fig. 2-2 The biosynthesis of xanthones

Mechanisms for this intramolecular reaction of benzophenone **2** to form xanthone **3** have been postulated involving either direct phenol oxidative coupling of 2,2′-dihydroxy benzophenone **5** *via* radical intermediate as shown in **Figure 2-3**,[32] intramolecular addition of hydroxyl groups in quinonoid intermediates **6** as shown in **Figure 2-4**,[33] dehydration between hydroxyl groups on the acetate and shikimate-derived rings **7** via suitably activated intermediates such as *O*-phosphates as shown in **Figure 2-5**,[34] or spirodienone **8** formation and subsequent rearrangement to form the xanthone as shown in **Figure 2-6**.[27,35]

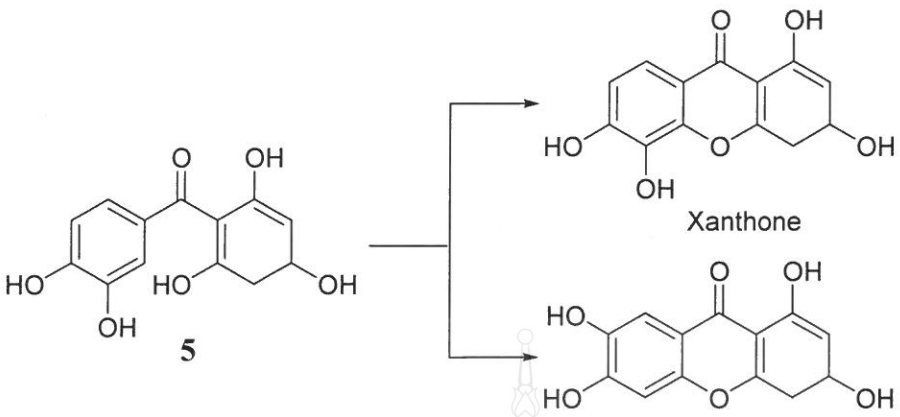


Fig. 2-3 Direct phenol oxidative coupling

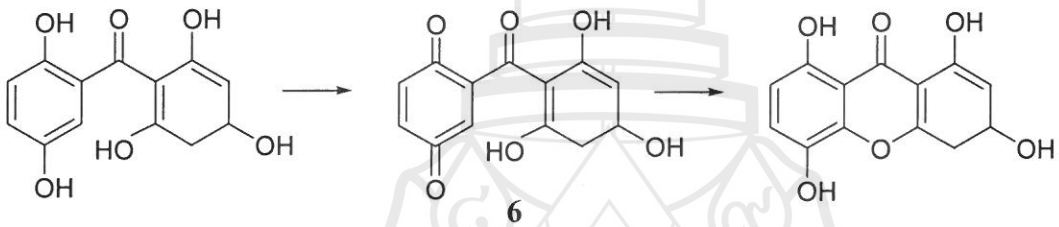


Fig. 2-4 Intramolecular addition

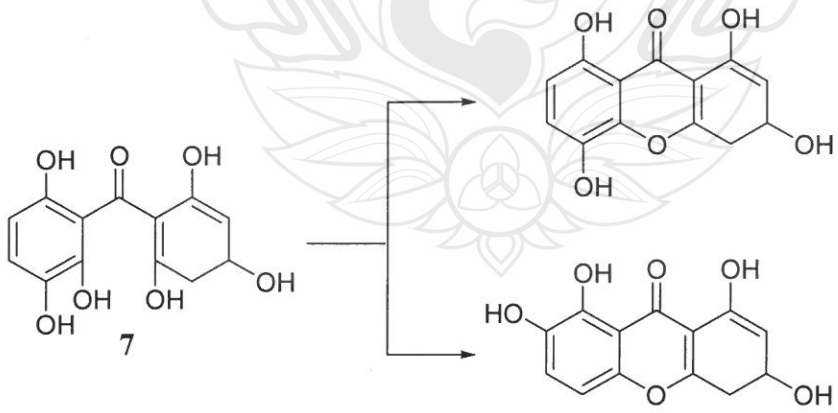


Fig. 2-5 Dehydration to form xanthone

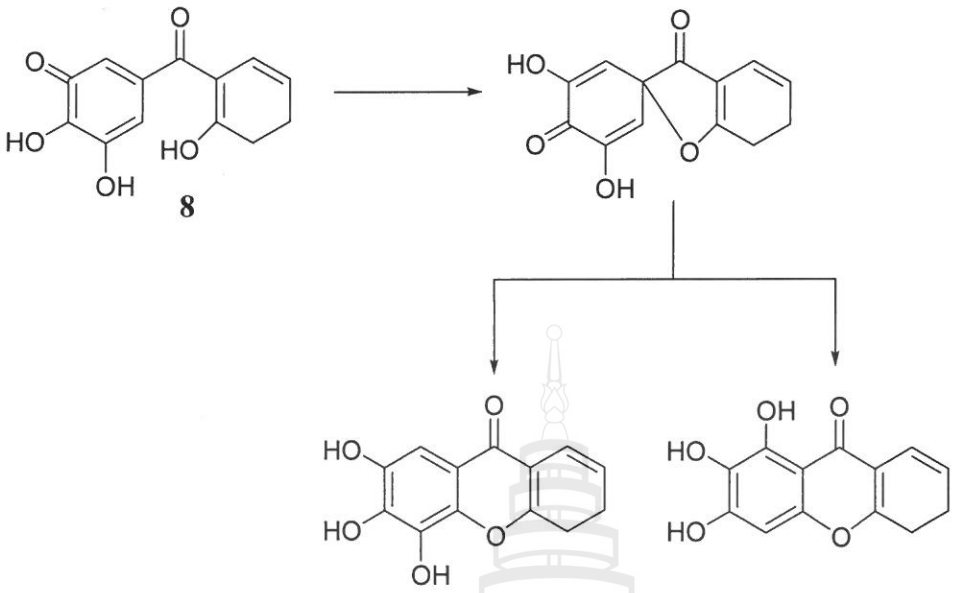


Fig. 2-6 Spirodienone formation and rearrangement

The above mechanisms had been discussed by Scheinmann *et al.* in 1969 and they concluded that "direct oxidative coupling of the benzophenone as in **Figure 2-3**, leads to a simpler explanation of the wide variety of oxidative patterns in (natural) 'xanthones' ".[36]

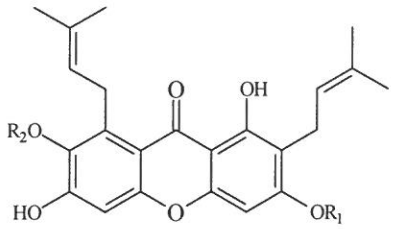
The xanthones isolated so far may be classified into five major groups: simple oxygenated xanthones, xanthone glycosides, prenylated and related xanthones, xanthonolignoids, and miscellaneous xanthones.[37]

Xanthones are commonly separated by silica gel chromatography using different solvent mixtures.[38] Xanthones are also separated and identified by comparison with authentic samples by TLC and by HPLC.[39-40] The structures of simple oxygenated xanthones have been established mainly from analysis of the UV, IR, MS and NMR spectroscopic data of these compounds.[24,41-43] Xanthones can be detected by their colour in UV light with and without ammonia, or by using a general phenolic spray.[38] The UV spectrum varies in a characteristic manner, depending on the oxygenation pattern, and with the availability of a considerable amount of data, assignments can be readily made. By the use of $AlCl_3$ shifts for chelated hydroxyls, as well as sodium acetate, sodium

hydroxide and boric acid shifts, considerable information on the position of hydroxyl groups can be obtained.[24,44] The use of IR spectroscopy in xanthone chemistry is generally limited to the detection of the carbonyl stretching frequency.[41,45] For example, the effect of chelation on the IR carbonyl frequency of hydroxy-xanthones may be a useful feature for analysis of spectra of substituted and extended xanthones.[46-47] The use of IR for detecting other functional groups, such as unchelated hydroxyl and methyl groups, has been described elsewhere.[41,48-50] The data obtained from 1H NMR spectroscopic analysis are of great value in characterizing and identifying naturally occurring xanthones.[51] Indeed, these have been used to determine the structure of substituents and for locating aromatic protons, through comparison with reference data and analysis of spin-spin coupling. Closer scrutiny of the chemical shifts of aromatic protons allows prediction of the oxygenation pattern,[52] and there are detailed NMR results for this class of compounds.[53] 1H NMR and 13C NMR spectroscopic analyses are the most useful tools in the structure elucidation of xanthones.[54] The 13C NMR spectra of a great number of naturally-occurring xanthones are reported, with all chemical shifts assigned.[53,55-56]

The xanthones isolated from *G. mangostana* have been reviewed.[24,37] Xanthones in the pericarp are composed of mangostione, α-mangostin **9**, β-mangostin **10**, γ-mangostin **11**, gartinin, and garcinone E.[57-59]

The xanthones, α-mangostin **9** and γ-mangostin **11**, are major bioactive compounds found in the fruit hulls of the mangosteen as shown in **Figure 2-7**.[60-62]



Xanthones
α-mangostin **9**: R_1=H, R_2=CH_3
β-mangostin **10**: R_1=R_2=CH_3
γ-mangostin **11**: R_1=R_2=H

Fig. 2-7 α-, β-, and γ-Mangostins

The biological activities of α-mangostin **9** have been confirmed to consist of a competitive antagonism of the histamine H1 receptor,[61] inhibition of oxidative damage by human low-density lipoproteins (LDL),[63-64] anti-cancer,[65] and antimicrobial activity against methicillin-resistant *Staphylococcus aureus*.[66] The details are explained below.

Chairungsrilerd *et al.* revealed in 1996 that, in the isolated rabbit thoracic aorta and guinea-pig trachea, α-mangostin **9** inhibited histamine-induced contractions in a concentration-dependent manner in the presence or absence of cimetidine, a histamine H_2 receptor antagonist.[61] But KCl-, phenylephrine- or carbachol-induced contractions were not affected by α-mangostin. The concentration-contractile response curve for histamine was shifted to the right in a parallel manner by α-mangostin. In the presence of chlorpheniramine, a histamine H_1 receptor antagonist, α-mangostin did not affect the relaxation of the rabbit aorta induced by histamine. In the guinea-pig trachea, α-mangostin had no effect on the relaxation induced by dimaprit, a histamine H_2 receptor agonist. α-Mangostin caused a concentration-dependent inhibition of the binding of [^{3}H]mepyramine, a specific histamine H_1 receptor antagonist to rat aortic smooth muscle cells. Kinetic analysis of [^{3}H]mepyramine binding indicated the competitive inhibition by α-mangostin. These results suggest that α-mangostin is a novel competitive histamine H_1 receptor antagonist in smooth muscle cells.

Williams *et al.* investigated in 1995 the possible antioxidant effects of mangostin **9**, isolated from *Garcinia mangostana*, on metal ion dependent (Cu^{2+}) and independent (aqueous peroxyl radicals) oxidation of human LDL.[63] Mangostin prolonged the lagtime to both metal ion dependent and independent oxidation of LDL in a dose dependent manner over 5 to 50 microM as monitored by the formation of conjugated dienes at 234 nm ($P < 0.001$). There was no significant effect of mangostin on the rate at which conjugated dienes were formed in the uninhibited phase of oxidation. Levels of thiobarbituric reactive substances (TBARS) generated in LDL were measured 4 and 24 hours after oxidation with 5 microM Cu^{2+} in the presence or absence of 50 microM or 100 microM mangostin. An inhibition of TBARS formation with 100 microM mangostin at 4 hours ($P = 0.027$) was observed but not at 24 hours ($P = 0.163$). Similar results were observed in the presence of 50 microM mangostin. Mangostin, at 100 microM, retarded the relative electrophoretic mobility of LDL at both 4 and 24 hours after Cu^{2+} induced oxidation. Mangostin (100 microM) significantly inhibited the consumption of alpha-tocopherol in the LDL during Cu^{2+} initiated oxidation over a 75 minute period ($P < 0.001$). From these results, we conclude that mangostin is acting as a powerful free radical scavenger to protect the LDL from oxidative damage in this *in vitro* system.

The results of the study by Mahabusarakam *et al.* in 2000 showed that structural modification of mangostin **9** can have a very profound effect on antioxidant activity.[64] Derivatisation of the C-3 and C-6 hydroxyl groups with either methyl, acetate, propane diol or nitrile substantially reduced antioxidant activity. In contrast, derivatisation of C-3 and C-6 with aminoethyl derivatives enhanced antioxidant activity, which may be related to changes in solubility. Cyclisation of the prenyl chains had little influence on antioxidant activity.

In 2003, Matsumoto *et al.* examined the effects of six xanthones from the pericarps of mangosteen, *Garcinia mangostana*, on the cell growth inhibition of human leukemia cell line HL60.[65] All xanthones displayed growth inhibitory effects. Among them, alpha-mangostin **9** showed complete inhibition at 10 microM through the induction of apoptosis.

The other xanthone derivative, γ-mangostin **11** has also been reported to have several pharmacological activities, such as being a potent inhibitor of animal Cdk-activating kinases (Cak), plant Ca^{2+}-dependent protein kinases (CDPK),[60] anti-inflammatory activities,[67-68] and a selective antagonist for 5-HT_{2A} receptors in smooth muscle cells and platelets.[69]

From the study by Jinsart *et al.* in 1992, the MeOH extract of the hull contained 4 inhibitors of plant Ca^{2+}-dependent protein kinase; 2 of which were purified and identified as the xanthones 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthen-9-one (mangostin **9**) and 1,3,6,7-tetrahydroxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthen-9-one (gamma-mangostin **11**).[60] Both xanthones also inhibited avian myosin light chain kinase and rat liver cyclic AMPdependent protein kinase. This is the first report of inhibition of plant and animal second messenger regulated protein kinases by plant-derived xanthones.

The effect of gamma-mangostin **11**, a tetraoxygenated diprenylated xanthone contained in mangosteen, on arachidonic acid (AA) cascade in C6 rat glioma cells was examined by Nakatani *et al.* in 2002.[67] gamma-Mangostin had a potent inhibitory activity of prostaglandin E2 (PGE2) release induced by A23187, a Ca^{2+} ionophore. The inhibition was concentration-dependent, with the IC_{50} value of about 5 microM. gamma-Mangostin had no inhibitory effect on A23187-induced phosphorylation of p42/p44 extracellular signal regulated kinase/mitogen-activated protein kinase or on the liberation of [14C]-AA from the cells labeled with [14C]-AA. However, gamma-mangostin concentration-dependently inhibited the conversion of AA to PGE2 in microsomal preparations, showing its possible inhibition of cyclooxygenase (COX). In enzyme assay *in vitro*, gamma-mangostin inhibited the activities of both constitutive COX (COX-1) and inducible COX (COX-2) in a concentration-dependent manner, with the IC_{50} values of about 0.8 and 2 microM, respectively. Lineweaver-Burk plot analysis indicated that gamma-mangostin competitively inhibited the activities of both COX-1 and COX-2. This study is a first demonstration that gamma-mangostin, a xanthone derivative, directly inhibits COX activity.

In 2004, the same study group (Nakatani *et al.*) also investigated the effect of gamma-mangostin **11** purified from the fruit hulls of the medicinal plant, *Garcinia mangostana*, on spontaneous prostaglandin E2 (PGE2) release and inducible cyclooxygenase (COX-2) gene expression in C6 rat glioma cells.[68] An 18-h treatment with gamma-mangostin potently inhibited spontaneous PGE2 release in a concentration-dependent manner with the IC_{50} value of about 2 microM, without affecting the cell viability even at 30 microM. By immunoblotting and RTPCR, it was shown that gamma-mangostin concentration-dependently inhibited lipopolysaccharide (LPS)-induced expression of COX-2 protein and its mRNA, but not those of constitutive COX-1 cyclooxygenase. Since LPS is known to stimulate IkappaB kinase (IKK)-mediated phosphorylation of inhibitor kappaB (IkappaB) followed by its degradation which in turn induces NF-kappaB nuclear translocation leading to transcriptional activation of COX-2 gene, the effect of gamma-mangostin on the IKK/IkappaB cascade controlling the NF-kappaB activation was examined. An *in vitro* IKK assay using IKK protein immunoprecipitated from C6 cell extract showed that this compound inhibited IKK activity in a concentration-dependent manner with the IC_{50} value of about 10 microM. Consistently gamma-mangostin was also observed to decrease the LPS-induced IkappaB degradation and phosphorylation in a concentrationdependent manner, as assayed by immunoblotting. Furthermore, luciferase reporter assays showed that gamma-mangostin reduced the LPS-inducible activation of NF-kappaB- and human COX-2 gene promoter region-dependent transcription. gamma-Mangostin also inhibited rat carrageenan-induced paw edema. These results suggest that gamma-mangostin directly inhibits IKK activity, and thereby prevents COX-2 gene transcription, an NF-kappaB target gene, probably to decrease the inflammatory agent-stimulated PGE2 production *in vivo*, and is a new useful lead compound for anti-inflammatory drug development.

Chairungsrilerd *et al.* shown in 1998 that γ-mangostin **11**, purified from the fruit hulls of the medicinal plant *Garcinia mangostana* caused a parallel rightwards shift of the concentration/response curve for the contraction elicited by 5-hydroxytryptamine (5-HT) in the rabbit aorta (pA_2 = 8.2) without affecting the contractile responses to KCl, phenylephrine (α_1) or histamine (H_1).[69] The perfusion pressure response of rat coronary

artery to 5-HT (5-HT_{2A}) was reduced concentration dependently by γ-mangostin (IC_{50} = 0.32 μM). 5-HT amplified, ADP-induced aggregation of rabbit platelets (5-HT_{2A}) was inhibited by γ-mangostin (IC_{50} = 0.29 μM), whereas that induced by thrombin was not affected, nor did γ-mangostin affect 5-HT-induced contraction of the guinea-pig ileum (5-HT_3) in the presence of 5-HT_1, 5-HT_2 and 5-HT_4 receptor antagonists. Furthermore, 5-HT-induced contraction of the rat fundus (5-HT_{2B}) and 5-HT-induced relaxation of the rabbit aorta in the presence of ketanserin (5-HT_1) and carbachol-induced contraction of the guinea-pig ileum (muscarinic M_3) were not affected by γ-mangostin (5 μM). γ-Mangostin inhibited [^{3}H]spiperone binding to cultured rat aortic myocytes (IC_{50} = 3.5 nM). The K_d for [^{3}H]spiperone binding was increased by γ-mangostin (3 nM) from 11.7 to 27.4 nM without affecting B_{max}. These results suggest that γ-mangostin is a novel competitive antagonist, free from a nitrogen atom, for the 5-HT_{2A} receptors in vascular smooth muscles and platelets.

Moreover, α- mangostins **9** and γ-mangostins **11** can inhibit both human immunodeficiency virus (HIV) infection,[14,15] and topoisomerases I and II.[70]

In the present study, extracts of mangosteen, which has been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activities against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis*, *Escherichia coli* and microorganism frequently involved in acne inflammation, *Propionibacterium acnes*. The previous antimicrobial activities of mangosteen extract are reviewed as followed.

Staphylococcus aureus

Staphylococcus aureus, shown in **Figure 2-8**, is the most common cause of staph infections. It is a spherical bacterium, frequently living on the skin or in the nose of a person. Approximately 20–30% of the general population are "staph carriers".[71] *S. aureus* was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses.[72] Each year some 500,000 patients in American hospitals contract a staphylococcal infection.[73] *S. aureus* is a facultatively anaerobic, Gram-positive coccus, which appears as grape-like clusters when viewed through a

microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates.[74] The golden appearance is the etymological root of the bacteria's name: *aureus* means "golden" in Latin.

Staphylococcus aureus can cause a range of illnesses from minor skin infections, such as pimples, impetigo (may also be caused by *Streptococcus pyogenes*), boils, cellulitis folliculitis, furuncles, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases, such as pneumonia, meningitis, osteomyelitis endocarditis, toxic shock syndrome (TSS), and septicemia. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the four most common causes of nosocomial infections, often causing postsurgical wound infections.

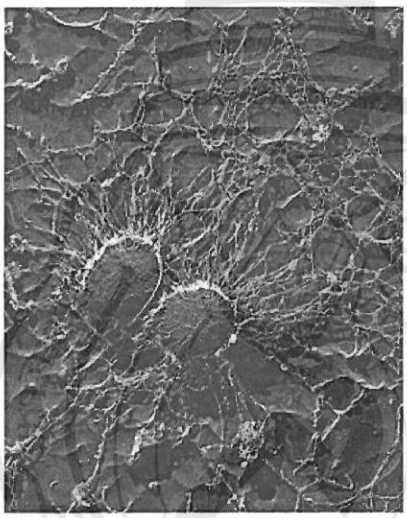


Fig. 2-8 *Staphylococcus aureus*

Today, *S. aureus* has become resistant to many commonly used antibiotics. In the UK, only 2% of all *S. aureus* isolates are sensitive to penicillin with a similar picture in the rest of the world, due to a penicillinase (a form of β-lactamase). The β-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin and flucloxacillin) were developed to treat penicillin-resistant *S. aureus* and are still used as first-line treatment. Methicillin was the first antibiotic in this class to be used (it was introduced in 1959), but only two years later, the first case of methicillin-resistant *S. aureus* (**MRSA**) was reported in England.[75]

Antimicrobial activities against *Staphylococcus aureus* of the mangosteen extracts are described below.

Mahabusarakum *et al.* reported in 1983 that mangostin, the major constituent from *G. mangostana* showed activity against *Staphylococcus aureus*, both normal and penicillin-resistant strains.[76] The minimum inhibitory concentration was 7.8 μg/mL. Other components also exhibited activity but at higher concentration.

Another investigation of antimicrobial activities of mangostin was performed by Sundaram *et al.* in 1983.[77] Mangostins, a xanthone and four of its derivatives; 3-*O*-methyl mangostin, 3,6-*O*-dimethyl mangostin, 1-isomangostin and mangostin triacetate, isolated from *Garcinia mangostana* L. (Guttiferae) were investigated for their *in vitro* antimicrobial properties. Eight bacteria and fourteen fungi comprising human pathogens, phytopathogens and saprophytes were tested by cup plate and streak plate methods. *S. aureus, P. aeruginosa, S. typhimurium* and *B. subtilis* exhibited high susceptibility to mangostin. A comparative analysis on the efficacy of mangostin and its derivatives by streak plate method can be summarized qualitatively as follow: mangostin > isomangostin > 3-*O*-methyl mangostin > 3,6-di-*O*-methyl mangostin > mangostin triacetate.

Mangostin **9**, gartanin, γ-mangostin **11**, 1-isomangostin, and 3-isomangostin isolated from *G. mangostana* (Guttiferae) were investigated for their *in vitro* activities against *Staphylococcus aureus* both normal and penicillin-resistant strains by Mahabusarakam *et al.* in 1986.[78] The best activity against both strains was found in mangostin. Mangostin **9**, γ-mangostin **11**, and gartanin showed no activity against *Candida albicans* and *Cryptococcus neoformans*, but exhibited moderate activities against *Trichophyton mentagrophytes* and *Microsporum gypseum*.

Iinuma *et al.* reported in 1996 the inhibitory effects of extracts of *Garcinia mangostana* (Guttiferae) against the growth of *S. aureus*.[66] NIHJ 209p were fractionated according to guidance obtained from bioassay and some of the components with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) were characterized. One

active isolate, alpha-mangostin **9**, a xanthone derivative, had a minimum inhibitory concentration (MIC) of 1.57-12.5 micrograms/mL. Other related xanthones were also examined to determine their anti-MRSA activity. Rubraxanthone, which was isolated from *Garcinia dioica* and has a structure similar to that of alpha-mangostin, had the highest activity against staphylococcal strains (MIC = 0.31-1.25 micrograms/mL), an activity which was greater than that of the antibiotic vancomycin (3.13-6.25 micrograms/mL). The inhibitory effect against strains of MRSA of two compounds when used in conjunction with other antibiotics was also studied. The anti-MRSA activity of alpha-mangostin was clearly increased by the presence of vancomycin; this behaviour was not observed for rubraxanthone. The strong *in vitro* antibacterial activity of xanthone derivatives against both methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* suggests the compounds might find wide pharmaceutical use.

In 2005, Sakagami *et al.* described the antibacterial activity of α- mangostin **9** against methicillin resistant *Staphylococcus aureus* (MRSA), with MIC values of 6.25 to 12.5 mg/ml.[79] These studies showed synergism between α-mangostin and vancomycin hydrochloride (VCM) against MRSA. These findings suggested that α-mangostin alone or in combination VCM against MRSA might be useful in controlling MRSA infections.

Extracts and pure compounds from the leaves and root bark of *Garcinia mangostana* were subjected to anti-microbial assay with special reference to methicillin resistant *Staphylococcus aureus* (MRSA) using an agar dilution method, exhibited by Tennakoon *et al.* in 2006.[80] Results indicated the activity of number of extracts against 15 MRSA strains. Activity guided fractionation showed that their activity is mainly due to the presence of γ-mangostin **11** and α-mangostin **9**. They showed activity at lower concentrations such as 0.39 and 1.56 µg/mL for 20 strains. According to the above experiments, other than α-mangostin **9**, γ-mangostin **11** also appears to hold promise as an anti-microbial agent in the treatment of infections with *S. aureus* including MRSA, and should be investigated further in appropriate *in vivo* models.

In 2006, Phongpaichit *et al.* showed the screening for antimicrobial activity in endophytic fungi isolated from surface sterilized leaves and branches of five Garcinia plants, *G. atroviridis, G. dulcis, G. mangostana, G. nigrolineata* and *G. scortechinii*, found in southern Thailand.[81] Fermentation broths from 377 isolated fungi were tested for antimicrobial activity by the agar diffusion method. Minimum inhibitory concentrations (MICs) were obtained for crude ethyl acetate extracts. Seventy isolates (18.6%) displayed antimicrobial activity against at least one pathogenic microorganism, such as *Staphylococcus aureus*, a clinical isolate of methicillin-resistant *S. aureus*, *Candida albicans* and *Cryptococcus neoformans*. The results revealed that 6-10%, 1-2% and 18% of the crude ethyl acetate extracts inhibited both strains of *S. aureus* (MIC 32-512 μg/mL), *Ca. albicans* and *Cr. neoformans* (MIC 64-200 μg/mL), and *Microsporum gypseum* (MIC 2-64 μg/mL), respectively. Isolates D15 and M76 displayed the strongest antibacterial activity against both strains of *S. aureus*. Isolates M76 and N24 displayed strong antifungal activity against *M. gypseum*. Fungal molecular identification based on internal transcribed spacer rRNA gene sequence analysis demonstrated that isolates D15 (DQ480353), M76 (DQ480360) and N24 (DQ480361) represented *Phomopsis sp., Botryosphaeria sp.* and an unidentified fungal endophyte, respectively. These results indicate that some endophytic fungi from Garcinia plants are a potential source of antimicrobial agents.

Salmonella albany

Salmonella is a major of *Enterobacteriaceae*. Human infection with *Salmolnella* is usually seen in the form of gastroenteritis, but occasionally extraintestinal focal infection and some restricted serovars cause enteric fever (such as typhoid or paratyphoid fever). The genus *Salmonella* is classified into two species, *S. enterica* and *S. bongori*. *S. enterica* is one of the most common causes of human gastroenteritis. *Salmonella* is the leading cause of human foodborne infections in Latin America, and poultry meat is one of the main vehicles. *Salmonella albany* or *Salmonella enterica subsp. enterica serovar albany* is a gram-negative, motile, non-sporing, non-capsulated bacillus that can be contracted through contaminated water, milk, food or fruits and vegetables or *via* convalescent or chronic carriers.

Bacillus subtilis

Bacillus subtilis, shown in **Figure 2-9**, is a Gram-positive, catalase-positive bacterium commonly found in soil.[82] A member of the genus *Bacillus*, *B. subtilis* has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. Unlike several other well-known species, *B. subtilis* has historically been classified as an obligate aerobe, though recent research has demonstrated that this is not strictly correct.[83] It has also been called Hay bacillus or Grass bacillus. It is bacillus because the bacterium is rod shaped or bacilli shaped. *B. subtilis* is not considered a human pathogen; it may contaminate food but rarely causes food poisoning. *B. subtilis* produces the proteolytic enzyme subtilisin. *B. subtilis* spores can survive the extreme heating that is often used to cook food, and it is responsible for causing *ropiness* - a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides - in spoiled bread dough.

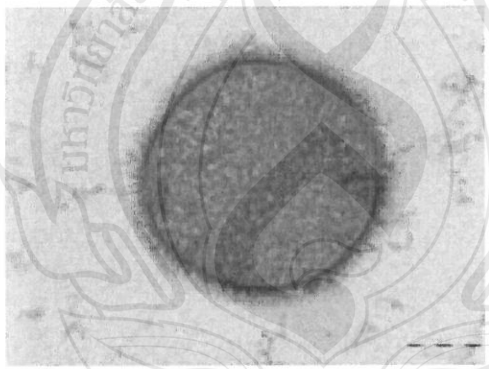


Fig. 2-9 *Bacillus subtilis*

Escherichia coli

Escherichia coli, shown in **Figure 2-10**, is a bacterium that is commonly found in the lower intestine of warm-blooded animals. Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for costly product recalls.[84-85] The harmless strains are part of

the normal flora of the gut, and can benefit their hosts by producing vitamin K_2,[86] or by preventing the establishment of pathogenic bacteria within the intestine.[87-88]

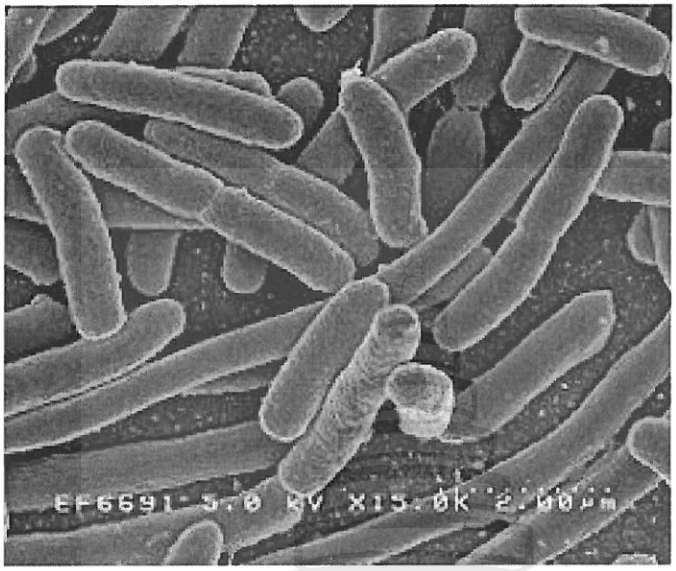


Fig. 2-10 ***Escherichia coli***

E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination.[89-90] The bacteria can also be grown easily and its genetics are comparatively simple and easily-manipulated, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology. *E. coli* was discovered by German pediatrician and bacteriologist Theodor Escherich in 1885,[89] and is now classified as part of the Enterobacteriaceae family of gamma-proteobacteria.

E. coli is Gram-negative, facultative anaerobic and non-sporulating. It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen-consuming organisms such as methanogens or sulfate-reducing bacteria.[91]

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for Haemolytic-uremic Syndrome (HUS), peritonitis, mastitis, septicemia and Gram-negative pneumonia.[92]

Propionibacterium acnes

Propionibacterium acnes is a relatively slow growing, typically aerotolerant anaerobic gram positive bacterium that cause chronic blepharitis and endophthalmitis, the latter particularly following intraocular surgery. The genome of the bacterium has been sequenced and a study has shown several genes that can generate enzymes for degrading skin and proteins that may be immunogenic (activate the immune system).

This organism has been implicated over other cutaneous microflora in contributing to the inflammatory response of acne. It acts as an immunostimulator which can produce a variety of enzymes and biologically active molecules, which are involved in the development of inflammatory acnes. These products include lipases, proteases, hyaluronidases, and chemotactic factors.[93] The main components of pilosebaceous unit on the skin, such as keratinocytes and sebocytes can be activated by *P. acnes* leading to the production of pro-inflammatory cytokines.[94] It has been reported that a secreted peptidoglycan of *P. acnes* can stimulate the production of the proinflammatory cytokines such as IL-1, IL-8, and tumor necrosis factor-alpha (TNF-α) by human monocytic cell lines and freshly isolated peripheral blood mononuclear cells from acne patients.[95]

Moreover, *P. acnes* significantly induces the interleukin-8 (IL-8) mRNA expression and selectively stimulates the expression of human beta-defensin-2 mRNA in keratinocytes.[96] Previous findings suggest that *P. acnes* has a major role in the inflammation of acne vulgaris by both antigenic and mitogenic reactions.[97] Additionally, *P. acnes* can evoke mild local inflammation by producing neutrophil chemotactic factors. As a consequence, neutrophils which are attracted to the acne lesion constantly release inflammatory mediators such as reactive oxygen species (ROS).[98] The ROS including superoxide radical anion, hydrogen peroxide and hydroxyl

radical generated play a critical role in irritation and disruption of the integrity of the follicular epithelium and are responsible for the progression of inflammatory acne.[99] This toxic ROS can also act as second messengers in the induction of several biological responses such as NF-kB and AP-1, the generation of cytokines. Removal of the ROS can significantly reduce cell damage that may occur during acne inflammation.[100]

This bacteria is largely commensal and thus present on most people's skin; and lives on fatty acids in the sebaceous glands on sebum secreted by pores. It may also be found throughout the gastrointestinal tract in humans and many other animals. It is named after its ability to generate propionic acid.

When a pore is blocked this anaerobic bacteria overgrows and secretes chemicals that break down the wall of the pore, spilling bacteria such as *Staphylococcus aureus* into the skin, and forming an acne lesion (folliculitis). It has also been found in corneal ulcers, and on very few occasions damaging heart valves leading to endocarditis, and infections of joints (septic arthritis) have been reported. Furthermore, propionibacterium have been found in ventriculostomy insertion sites, and areas subcutaneous to suture sites in patients that have undergone craniotomy.

P. acnes can be killed by benzoyl peroxide, tetracycline group and other antibiotics, and many antibacterial preparations. However, tetracycline-resistant *P. acnes* is now quite common. Clindamycin is also frequently used. New facts show that *P.acnes* are sensitive to some macrolides such as Azithromycin, which has a wide spectrum of action. It is normally prescribed 500 mg by mouth, three times weekly for 4 to 6 weeks. Azithromycin exhibits post-antibiotic effect by concentrating in the lung tissue for approximately 5 days. Indeed some antibacterial cream or ointment should be used during the therapy, giving a good local effect. Another antibiotic is Nadifloxacin from the group of so called 4-fluoroquinolones, such as Ciprofloxacin, Ofloxacin and Levofloxacin. It has action against *P. acnes* and some other microorganisms that also take part of the poly-infection.

Acne vulgaris is a skin disorder of the sebaceous follicles that commonly occurs in adolescence and in young adulthood. The major pathogenic factors involved are hyperkeratinization, obstruction of sebaceous follicles resulting from abnormal

keratinization of the infundibular epithelium, stimulation of sebaceous gland secretion by androgens, and microbial colonization of pilosebaceous units by *Propionibacterium acnes*, which promotes perifollicular inflammation. The clinical presentation of acne can range from a mild comedonal form to severe inflammatory cystic acne of the face, chest, and back. At the ultra-structural level, follicular keratinocytes in comedones can be seen to possess increased numbers of desmosomes and tonofilaments, which result in ductal hypercornification. The increased activity of sebaceous glands elicited by androgen causes proliferation of *P. acnes*, an anaerobe present within the retained sebum in the pilosebaceous ducts. The organism possesses a ribosome-rich cytoplasm and a relatively thick cell wall, and produces several biologically active mediators that may contribute to inflammation, for instance, by promoting leukocyte migration and follicular rupture. In inflamed lesions, numerous neutrophils and macrophages infiltrate around hair follicles and sometimes phagocytose *P. acnes*. The pathogenesis of acne is shown in **Figure 2-11**. Normal skin commensals including *Propionibacterium acnes*, *Propionibacterium granulosum*, *Staphylococcus epidermidis* and *Malassezia furfur*, proliferate rapidly during puberty and are often involved in the development of acne.[101] *Propionibacterium acnes* has been described as an obligate anaerobic organism. It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils. On contrary, *Staphylococcus epidermidis*, an aerobic organism, usually involves in superficial infections within the sebaceous unit.[102]

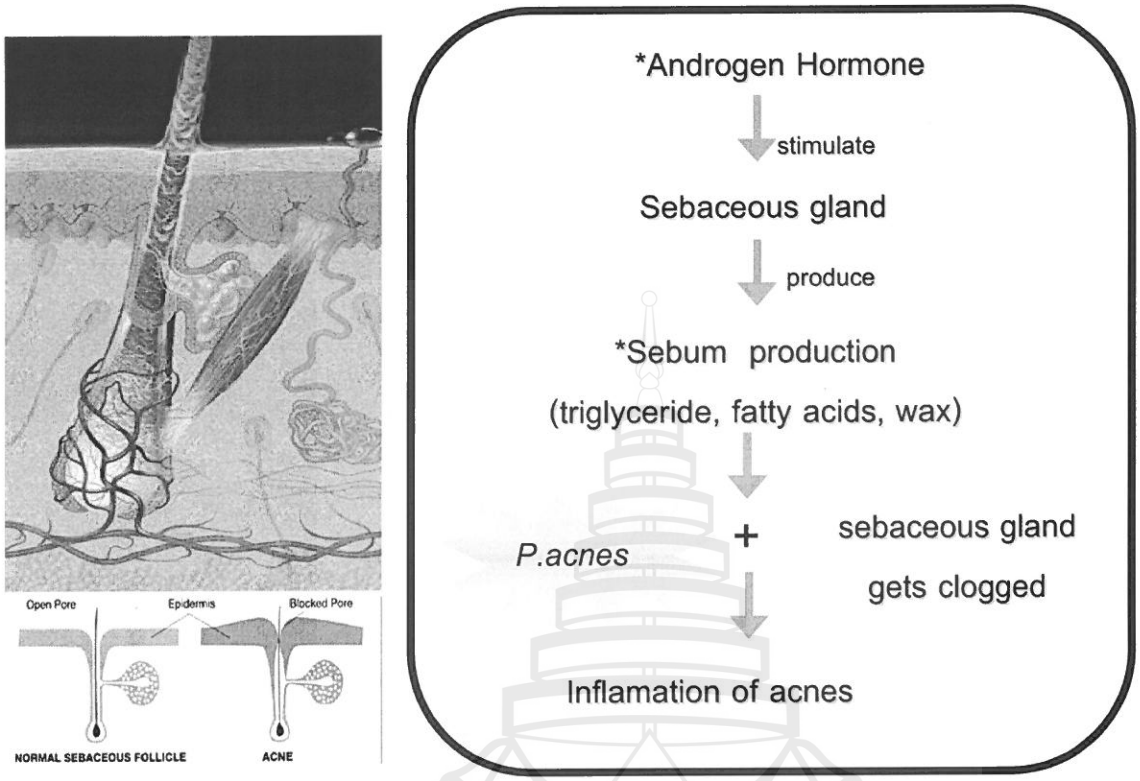


Fig. 2-11 Pathogenesis of acne

Current treatment for acne is mostly based on antibiotics such as clindamycin and tetracycline derivatives. However, antibiotic-resistant *P. acnes* are widely spread and become a critical problem worldwide. Therefore, an alternative treatment of acne must be studied and developed. This has led our interest in the possible effects of natural substances on anti-inflammation in acne lesions. Natural substances have been extensively studied in their biological activities especially during this decade.

In 2005, the study by Chomnawang *et al.* was conducted to evaluate antimicrobial activities of Thai medicinal plants against etiologic agents of acne vulgaris; *Propionibacterium acnes* and *Staphylococcus epidermidis*.[103] Crude extracts were tested for antimicrobial activities by disc diffusion and broth dilution methods. The results from the disc diffusion method showed that 13 medicinal plants could inhibit the growth of *Propionibacterium acnes*. Among those, *Senna alata*, *Eupatorium odoratum*, *Garcinia mangostana*, and *Barleria lupulina* had strong inhibitory effects.

Based on a broth dilution method, the *Garcinia mangostana* extract had the greatest antimicrobial effect. The MIC values were the same (0.039 mg/ml) for both bacterial species and the MBC values were 0.039 and 0.156 mg/ml against *Propionibacterium acnes* and *Staphylococcus epidermidis*, respectively. In bioautography assay, the *Garcinia mangostana* extract produced strong inhibition zones against *Propionibacterium acnes*. Antimicrobial activity from fractions of column chromatography revealed one of the active compounds in *Garcinia mangostana* could be mangostin, a xanthone derivative.

With the same study group (Chomnawang *et al.*), the study in 2007 was aimed to investigate the activity of Thai medicinal plants on inflammation caused by *Propionibacterium acnes* in terms of free radical scavenging and cytokine reducing properties.[104] *P. acnes* have been recognized as pus-forming bacteria triggering an inflammation in acne. Antioxidant activity was determined by DPPH scavenging and NBT reduction assay. The result showed that *Garcinia mangostana* possessed the most significant antioxidant activity and reduced reactive oxygen species production. *Houttuynia cordata*, *Eupatorium odoratum*, and *Senna alata* had a moderate antioxidant effect. In addition, *Garcinia mangostana* extracts could reduce the TNF-α production as determined by ELISA. *Garcinia mangostana* was highly effective in scavenging free radicals and was able to suppress the production of pro-inflammatory cytokines.

CHAPTER III
RESEARCH METHODOLOGY

3.1 Preparation of plant extracts

Garcinia mangostana hulls used in this study collected from various locations in Thailand. Fruits were cleaned with running tap water and fresh hulls were separated and chopped into pieces. They were dried under shade at room temperature for 5 days and the air-dried hulls were then ground to powder for extraction. An amount of 1 kg of powdered sample was extracted with 5 L of ethanol for a week with maceration at 37°C. The extract was then collected and filtered through Whatman No.1 filter paper in a Buchner funnel under vacuum. The filtrate was concentrated by evaporation with a vacuum rotary evaporator at 45°C to yield 183.8 g of dried ethanolic extract. The dried extract was stored at 4°C for further uses. The ethanolic extract was standardized by quantifying the amount of α-mangostin, an active compound, using HPLC equipped with the Cosmosil C18 column (46 mm x 250 mm). The extract solution (5 ml) was injected in each run with 0.2% formic acid and 70% acetonitrile in water as mobile phase at 1 ml/min, 25°C. The HPLC system was precalibrated with α-mangostin standard (5-500 µg/ml) to evaluate the linear correlation curve. The amount of α-mangostin was determined from the calibration curve of α-mangostin.

3.2 Microorganisms and media

The test organisms used in this study were as followed: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Salmonella albany* (ATCC 27853) and *Propionibacterium acnes* (ATCC 14916).

3.3 Antimicrobial susceptibility testing

3.3.1 Disc diffusion method

The dried plant extracts were dissolved in ethanol to a final concentration of 30 mg/ml and sterilized by filtration by 0.45 μm Millipore filters. Antimicrobial tests were then carried out by disc diffusion method using 100 μl of suspension containing 10^8 CFU/ml of bacteria.

This experiment was performed by the method of Hayes and Markovic (2002) with some modifications.[105] *Propionibacterium acnes* was incubated in brain heart infusion medium (BHI) with 1% glucose for 72 h under anaerobic conditions and adjusted to yield approximately 1.0×10^8 CFU/ml. Aliquots of molten BHI with glucose agar were used as an agar base. A prepared inoculum was added to molten agar, mixed and poured over the surface of the agar base and left to solidity. A sterile paper disc (6 mm in diameters) was impregnated with 10 μl of the 30 mg/ml extracts (300 μg/disc) placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Kanamycin (30 μg/disc) was used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial species tested. The disc was placed on the agar. Plates were then incubated at 37 ˚C for 72 h under anaerobic conditions.

Staphylococcus aureus, Bacillus subtilis, *Escherichia coli* and *Salmonella albany* were incubated in tryptic soy broth (TSB), turbidity equivalent to McFarland No. 0.5, for 24 h at 37 ˚C and adjusted to yield approximately 1.0×10^8 CFU/ml. The procedures were the same as mentioned above except the plates were incubated at 37 ˚C for 24 h under aerobic conditions and streptomycin (30 μg/disc) was used as positive reference standards. All disc diffusion tests were performed in three separate experiments and the antibacterial activity was expressed as the mean of inhibition diameters (mm).

3.3.2 Determination of minimum inhibitory concentrations (MIC)

The minimal inhibitory concentration (MIC) values were determined by agar dilution method. Extracts were tested against the reference strains for their inhibitory activity including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella Albany* and *Propionibacterium acnes*, in duplicate.

The test compounds, mangosteen extracts, were found to be completely soluble in DMSO. Inhibitory activity of DMSO was tested by making a series of DMSO in water from 1:2 dilution to 1:64 and inoculating each dilution with 1 drop of 106 mg/ml of *S. aureus* ATCC 25923. Visible growth of an organism was observed in a 1:16 dilution of DMSO in water. Therefore, a 1:15 DMSO:H_2O solvent system was used to determine the minimum inhibitory concentration (MIC) of the test compound. Dilutions of the test compounds were made in this solvent system.

Extracts from the dried fruit peel of *G. mangostana* dissolved in 1:15 dilution of DMSO:water were first diluted to the highest concentration (60 μg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 0.94 to 60 μg/ml. One milliliter of each dilution was mixed with 14 ml Mullar Hinton Agar and poured into a 10 cm petri dish and allowed to set on a level surface. These plates were used to inoculate an overnight inoculum of strains of *S. aureus*. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganisms.

MIC values of mangosteen extracts against *Bacillus subtilis*, *Escherichia coli* and *Salmonella albany* were determined under the same method mentioned above. MIC value of mangosteen extracts against *Propionibacterium acnes* was determined based on the same procedure as mentioned above except the serial two-fold dilutions were made in a concentration range from 1.75 to 80 mg/ml and the plates were incubated for 24 h.

3.4 Preparation of "Mangosteen gel"

Water 67.00 g and glycerin 3.00 g were mixed and heated to 75°C. Then propylene glycol 2.50 g and methylparaben 0.10 g were dissolved in the mixture of water and glycerin and left at this temperature as Part A. Hydroxyethylcellulose 0.40 g was dispersed in Part A under turbine stirring and the mixture was then cooled to 50°C. Carbomer 1.00 g dispersed in water 19.00 g, was added to the mixture at 50°C under slow turbine stirring, following by the addition of 1.00 g triethanolamine (20% aqueous solution). Mangosteen extract 5.00 g dissolved in 1.00 g dimethicone copolyol, was added under the same turbine stirring to the mixture. Then the mixture was cooled with planetary mixing to room temperature to yield 100.00 g of "5% mangosteen gel".

3.5 Clinical examination of anti-acne effect

Clinical examination to evaluate the comedogenic potential of "5% mangosteen gel" on human subject was assessed by dermatologist. The experiment was conducted according to 2 objectives; to evaluate the effect and safety of cosmetic product on subjects with acne prone skin after 28 days of twice daily use, by clinical assessment by dermatologist and to determine the product organoleptic characteristics, efficacy and safety by analyzing the answers given by the volunteers to a subjective questionnaire. This was an open and intra-individual study and each subject is his/her own control.

3.5.1 Assessment criteria

Primary criteria

After 28 days of twice-daily use, evaluation of:

- The anti-acne effect by a clinical examination (numeration of retentional and inflammatory lesions) by a dermatologist,
- The cutaneous tolerance by clinical evaluation and by collecting volunteers sensations.

Secondary criteria

- Analysis of the subjects' answers to a subjective evaluation questionnaire.

3.5.2 Procedures

3.5.2.1 Clinical examination

On D0 and D28, the dermatologist counted the retentional (open comedones = blackheads and closed comedones = microcysts) and inflammatory lesions (papules and pustules) on the entire face (except the nasal pyramid).

A variation in percentage is then calculated according to the following formula:

$$\frac{\text{Average No on D28 - Average No on D0}}{\text{Average No on D0}} \times 100$$

3.5.2.2 Cutaneous tolerance evaluation

On D28, the global tolerance of the product is assessed by clinical exam by the dermatologist. This evaluation takes into account the elements reported by the volunteer (functional and physical signs) as well as those noted by the dermatologist (clinical signs). The confrontation of these signs is used to conclude the final safety of the tested product.

On D0 and D28, the dermatologist assesses the following criteria:

	no	slight	moderate	severe	very severe
Erythema	☐	☐	☐	☐	☐
Edema	☐	☐	☐	☐	☐
Cutaneous dryness	☐	☐	☐	☐	☐
Desquamation	☐	☐	☐	☐	☐
Roughness	☐	☐	☐	☐	☐
Other :	☐	☐	☐	☐	☐

Define:.....

3.5.2.3 Subjective evaluation questionnaire

The answers given by the volunteers to a subjective evaluation questionnaire are used to evaluate the organoleptic characteristics, efficacy and safety of the tested product. These subjective criteria give accurate indication of product appreciation over time.

3.5.3 Trial organization: schedule

On D0

- Subjects came to the laboratory without having applied any product to their face since the previous evening.
- An information sheet was provided to remind them of the study details.
- They read and signed the information and consent forms in duplicate.
- Clinical examination of the initial state of the skin by the dermatologist and counting of the retentional and inflammatory lesions on the entire face except the nasal pyramid.
- Distribution of the products to the volunteers who apply them twice-daily to the whole face for 28 days
- Distribution of a safety grid, completed by the subjects everyday.

On D28

- Subjects returned to the laboratory without any application of the product to the face; the last application of the product was done the evening before.
- New clinical examination by the dermatologist who counted retentional and inflammatory lesions and evaluated the safety of the product.
- Subjects answered the subjective questionnaire on D28.
- Subjects bring their products and safety grid back to the laboratory in order to verify the compliance.

Ambient conditions during measurements were to have been:

- Ambient temperature: 25±1°C
- Relative humidity: between 40% and 60%.

3.5.4 Adverse Events/Serious Adverse Events

3.5.4.1 Definitions

An Adverse Event is defined as any expression or noxious and not wanted symptom suffered by subjects taking part in biomedical research, whether or not it relates to the tested product.

A Serious Adverse Event (SAE) is defined by one of the following criteria:

- Death,
- Life threatening,
- Hospitalization,
- Persistent or significant disability or incapacity,
- Congenital anomaly,
- Overdose,
- Cancer,
- Other event considered clinically significant by the investigator.

3.5.4.2 Documentation

Any or all Adverse Events related to the tested product (adverse reaction or effect) will be reported in the Case Report Form (CRF) and the study report. Any or all-concomitant treatment will be reported in the CRF and the study report. Any or all Serious Adverse Events will be reported in the CRF and the study report.

3.5.4.3 Early termination of the study

Test exit conditions are described as followed;

* In compliance with the Helsinki/Tokyo/Venice declaration and French law dated December 20, 1988 concerning the protection of subjects used in biomedical research, subjects had the right to exit from the study at any time and for any motive.

* The investigator also could have interrupted the treatment prematurely in the case of an intercurrent disease or undesirable effect.

* The sponsor could have demanded that any subject be excluded from the test for major infringements of the protocol, for administrative reasons or any other motive.

Nevertheless, premature removal of a high percentage of subjects from the test could have made the test difficult or impossible to interpret. Consequently, any premature exit without valid motives should have been avoided as much as possible.

Every premature exit must have been classified under one of the following headings:

- Adverse Event occurrence,
- Serious Adverse Event occurrence,
- Withdrawal of consent,
- Untraceable panelist,
- Appearance of exclusion criteria,
- Non-adherence to the protocol,
- Other reason.

Replacement conditions are described as followed;

* If the premature exit was not related to the test treatment, the subject was replaced. Any replacement must have been previously discussed with the trial conductor.

3.5.5 Subject selection

3.5.5.1 Inclusion criteria

General criteria

- Healthy subjects.
- Subjects having given their informed, written consent.
- Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by the clinical trial center could have been expected.

Specific criteria

- Age: 18-35 years old.

- Subjects with greasy facial skin and acneic lesions.
- Type: Asian female.
- Phototype: III, IV or V.

3.5.5.2 Non-inclusion criteria

- Pregnant or nursing women.
- Cutaneous pathology on face, other than acne (eczema, etc).
- Serious or progressive diseases that the investigator judges may interfere with the study.
- Volunteers undergoing a topical or systemic treatment:
  - anti-inflammatories and/or anti-histamines during the previous week,
  - cough suppresants and/or corticoids during the four previous weeks,
  - retinoids and/or immuno-suppressors during the six previous months.
- Any acne treatment within the previous month by oral or local route.
- Unstable weight.
- Excessive exposure to sunlight or UV rays during study within previous month.
- Excessive use of alcohol or tobacco.

3.5.5.3 Associated treatment during the study

No systemic treatment likely to modify the skin condition was authorized during the test. No use of dermopharmaceutical or cosmetic products other than cleansing products was authorized on the test zones the previous evening or during the study.

3.5.5.4 Number of subjects

The study is carried out on 22 subjects.

3.5.5.5 Tested product

Before the beginning of the study, each product was kept at room temperature in a dedicated air-conditioned room. "5% Mangosteen gel" is the clear colorless gel.

3.5.6 Statistical method

Data from this test were analyzed with a paired signed ranks test. This method is based on the calculation of the differences observed between values obtained before and after use of the product, on a same volunteer, the ranks of these differences not taking into account their signs and the calculation of the sum of the ranks of the positive differences (Y+) and the sum of the ranks of the negative differences (Y-). If the treatment is not effective, Y+ and Y- should be equal.

For all non-parametric tests, if a difference is of 0, the corresponding paired comparisons are discarded from the analysis and the n value is consequently reduced. The null hypothesis (H0) is that the treatment has no efficacy. Under (H0), the probability of obtaining a difference between before and after treatment at least as big as the one observed is calculated.

If this probability is less than or equal to 5%, the null hypothesis (H0) is rejected. The alternative hypothesis (H1) that there is a significant difference between before and after treatment is accepted. Probability p characterizes the signification level of this conclusion.

On the other hand, if p is greater than 5%, there is no reason for rejecting the null hypothesis (H0). From these data, no differences between before and after treatment could be evidenced.

Before carrying out a test, a type I error of 5% is chosen (which corresponds to the risk of rejecting a true null hypothesis).

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Quantitative analysis

In this study, α-mangostin, one of the major and interesting active compounds from *G. mangostana* was identified and quantitated with HPLC chromatography to standardize the quantity and quality of the extract. α-Mangostin was present in our GME at 32.56 ± 0.35 g/100 g of GME.

4.2 Antimicrobial activities

Antimicrobial activities of the ethanolic extracts of *Garcinia mangostana* (Guttiferae) against microorganisms (*Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis, Escherichia coli* and *Propionibacterium acnes*), examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter, and MIC values. The results were given in **Tables 4-1 – 4-2**.

Table 4-1 Antimicrobial activity of *Garcinia mangostana* extracts against the bacterial strains tested based on disc diffusion method.

Bacterial species	Inhibition zone in diameter (mm)	
	Garcinia mangostana extracts	Negative control
Staphylococcus aureus	18	-
Salmonella albany	10	-
Bacillus subtilis	14	-
Escherichia coli	8	-
Propionibacterium acnes	1	-

The maximal inhibition zones for bacteria strains; *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis*, *Escherichia coli* and *Propionibacterium acnes* which were sensitive to *Garcinia mangostana* ethanol extracts, were 18, 10,14, 8 and 1 mm, respectively.

Table 4-2 The MIC values of *Garcinia mangostana* extracts against the bacterial strains tested based on agar dilution assay.

Bacterial species	*Garcinia mangostana* extracts
Gram positive	
Staphylococcus aureus	0.96 μg/ml
Bacillus subtilis	15.00 μg/ml
Gram negative	
Salmonella albany	3.87 μg/ml
Escherichia coli	1.93 μg/ml
Gram positive anaerobe	
Propionibacterium acnes	2.5 mg/ml

Mangosteen extracts had a minimum inhibitory concentration (MIC) of 0.96 μg/ml against strains of *S. aureus*, which was greater than that of the antibiotic vancomycin (MIC = 3.13-6.25 μg/ml).[66] Extracts of mangosteen were also examined to determine the inhibitory effect against *S. albany*, *B. subtilis*, *E. coli and P. acne* with the minimum inhibitory concentration of 3.87 μg/ml, 15 μg/ml, 1.93 μg/ml and 2.5 mg/ml, respectively.

4.3 Clinical examination for anti-acne effect

The test was done on 22 female and 100% of subjects finished the study. The experimental conditions were conformed to the protocol.

4.3.1 Subject characteristics

Table 4-3, below, presents the observations concerning all the volunteers included.

Table 4-3 Volunteer characteristics in clinical study.

No. of Subject	Name (the 3 first letters)	First name (the 2 first letters)	Age	Sex	Phototype	Previous medical or surgical events or medical treatment	Current medical events or treatment
1	TAN	AR	20	F	III	NO	NO
2	WON	CH	20	F	III	NO	NO
3	LEO	NU	24	F	IV	NO	NO
4	TEA	AU	21	F	III	NO	NO
5	JUN	NU	23	F	IV	NO	NO
6	WOR	OB	25	F	III	NO	NO
7	TUP	NU	29	F	IV	NO	NO
8	BOO	PI	20	F	IV	NO	NO
9	NIR	AN	20	F	III	NO	NO
10	JUN	KA	26	F	III	NO	NO
11	SEA	SU	22	F	IV	NO	NO
12	TAN	KA	20	F	IV	NO	NO
13	KOS	SU	21	F	IV	NO	NO
14	BUA	TI	27	F	IV	NO	NO
15	TAM	MO	27	F	IV	NO	NO
16	BUA	WA	27	F	IV	NO	NO
17	BUA	WA	24	F	IV	NO	NO
18	PAK	WE	20	F	IV	NO	NO
19	PUM	PU	28	F	III	NO	NO
20	JIN	CH	20	F	IV	NO	NO
21	JUN	JU	27	F	IV	NO	NO
22	CHI	SU	22	F	IV	NO	NO

Mean	**23**	22	**F**	0	**I**
Median	23	0	**M**	0	**II**
Minimum	*20*			7	**III**
Maximum	*29*			15	**IV**
SEM	**1**			0	**V**
				0	**VI**

4.3.2 Efficacy on acne lesions

The efficacy on acne lesions were carried out by dermatologist responsible in this study under the same conditions, counted the retentional and inflammatory lesions on the entire face (except the nasal pyramid), at D0 and D28 after used product.

The variations (Δ) of the number of acne were calculated according to the following formula:

$$\Delta = Ti - T0$$

with:

Ti: the number of acne at each time of kinetics.

T0: the number of acne at starting time after used blotter

A variation in percentage is then calculated according to the following formula:

$$\frac{\text{Average No on D28 - Average No on D0}}{\text{Average No on D0}} \times 100$$

Table 4-4 below expresses the average numbers obtained for each type of acne lesion at the beginning and end of the study as well as the total number of these lesions.

Table 4-4 The average numbers and the total number obtained for each type of acne lesion at the beginning and end of the study.

Vol	Comedones			Microcysts			Papules			Pustules			Total no of lesions		
	D0	D28	ΔD28	D0	D28	ΔD28	D0	D28	ΔD28	D0	D28	ΔD28	D0	D28	ΔD28
1	0	0	0	1	0	-1	8	4	-4	2	0	-2	11	4	-7
2	3	3	0	0	0	0	5	2	-3	0	0	0	8	5	-3
3	3	4	1	3	3	0	50	18	-32	4	3	-1	60	28	-32
4	0	0	0	0	0	0	15	9	-6	4	1	-3	19	10	-9
5	8	0	-8	1	0	-1	2	2	0	4	0	-4	15	2	-13
6	25	7	-18	0	0	0	15	5	-10	0	0	0	40	12	-28
7	1	0	-1	5	2	-3	14	10	-4	2	0	-2	22	12	-10
8	0	2	2	0	0	0	12	9	-3	3	3	0	15	14	-1
9	3	1	-2	3	0	-3	0	0	0	0	0	0	6	1	-5
10	1	1	0	6	2	-4	0	8	8	4	0	-4	11	10	-1
11	0	2	2	5	0	-5	10	6	-4	2	8	6	17	16	-1
12	4	3	-1	0	0	0	30	25	-5	3	3	0	37	31	-6
13	5	0	-5	5	0	-5	7	8	1	0	0	0	17	8	-9
14	3	2	-1	3	0	-3	25	9	-16	5	2	-3	36	13	-23
15	3	0	-3	2	1	-1	25	8	-17	6	1	-5	36	10	-26
16	3	1	-2	2	0	-2	11	3	-8	2	0	-2	18	4	-14
17	25	9	-16	0	1	1	6	9	3	0	0	0	31	19	-12
18	3	3	0	3	0	-3	50	33	-17	7	5	-2	63	41	-22
19	0	0	0	3	0	-3	7	7	0	3	1	-2	13	8	-5
20	7	0	-7	3	1	-2	15	9	-6	0	2	2	25	12	-13
21	0	0	0	3	0	-3	0	5	5	2	1	-1	5	6	1
22	3	2	-1	3	0	-3	4	3	-1	0	0	0	10	5	-5
Mean	**4.5**	**1.8**	***-2.7***	**2.3**	**0.5**	***-1.9***	**14.1**	**8.7**	***-5.4***	**2.4**	**1.4**	***-1.0***	**23.4**	**12.3**	***-11.1***
Median	3.0	1.0	*-1.0*	3.0	0.0	*-2.0*	10.5	8.0	*-4.0*	2.0	0.5	*-1.0*	17.5	10.0	*-9.0*
Minimum	*0.0*	*0.0*	*-18.0*	*0.0*	*0.0*	*-5.0*	*0.0*	*0.0*	*-32.0*	*0.0*	*0.0*	*-5.0*	*5.0*	*1.0*	*-32.0*
Maximum	*25.0*	*9.0*	*2.0*	*6.0*	*3.0*	*1.0*	*50.0*	*33.0*	*8.0*	*7.0*	*8.0*	*6.0*	*63.0*	*41.0*	*1.0*
SEM	**1.5**	**0.5**	***1.1***	**0.4**	**0.2**	***0.4***	**3.0**	**1.6**	***1.9***	**0.4**	**0.4**	***0.5***	**3.4**	**2.1**	***2.0***
P*	0.016			0.001			0.007			0.037			0.000		

* Test of Wilcoxon: P>0.05 non significant P<0.05 significant

The counting of acne lesions at the beginning of the study before any application and at the end of the study after 28 days of twice-daily use showed a statistically significant decrease of these lesions (P<0.05), particularly with the number of papules. **Figure 4-1** shows the graph expressing the evaluation of variation of acne lesion after use 28 days.

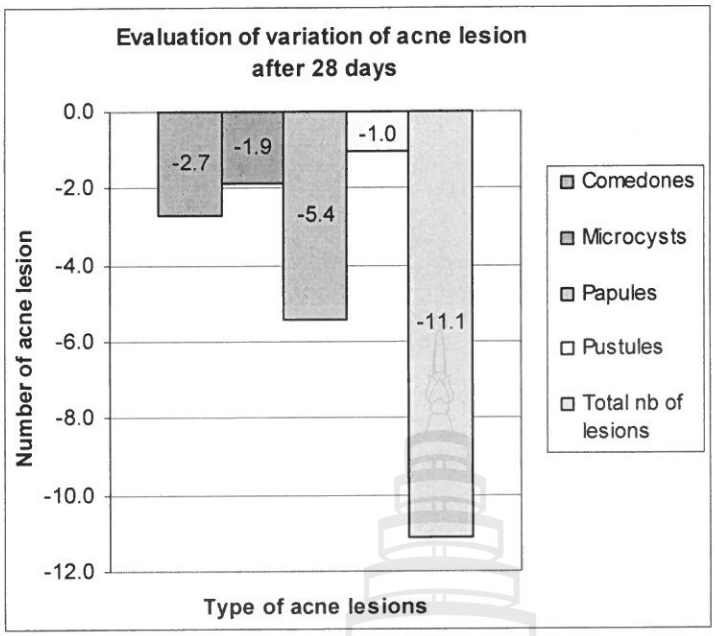


Fig. 4-1 The evaluation of variation of acne lesion after 28 days

4.3.3 Cutaneous tolerance

The clinical examination of the cutaneous state of the face done by the dermatologist as well as the sensations reported by the volunteers were expressed in **Table 4-5** below.

Table 4-5 The clinical examination of the cutaneous state of the face done by the dermatologist as well as the sensations reported by the volunteers.

Vol.	Signs reported by the volunteers		Clinical signs observed by the dermatologist on D28
	Functional signs	Physical signs	
1	NO	NO	NO
2	NO	NO	NO
3	NO	NO	NO
4	NO	NO	NO
5	NO	NO	NO
6	NO	NO	NO
7	NO	NO	NO
8	NO	NO	NO
9	NO	NO	NO
10	slightly burning sensation and itching for 3 days after start apply product	slightly redness and skin slightly dryness for 2-3 days after start to apply product.	NO
11	NO	NO	NO
12	NO	NO	NO
13	NO	NO	NO
14	NO	NO	NO
15	NO	NO	NO
16	NO	NO	NO
17	NO	NO	NO
18	NO	NO	NO
19	NO	NO	NO
20	NO	NO	NO
21	NO	NO	NO
22	NO	NO	NO

Table 4-6 The synthesis of the results from clinical examination of the cutaneous state.

	Number of volunteers	Percentage
Total reported signs	1	4.5%
Pertinent reported signs	0	0%
Total observed clinical signs	0	0%
Pertinent observed clinical signs	0	0%

Under these study conditions, the product could be considered very well tolerated on the cutaneous level as shown in **Table 6**: the reported signs did not last more than five minutes after application and are normal with this kind of product.

4.3.4 Subjective evaluation questionnaire

The synthesis of the answers that were given by the volunteers to the subjective evaluation questionnaire is presented below.

GLOBAL APPRECIATION OF THE PRODUCTS AND THEIR PROPERTIES

Q1 – Do you usually use a product to treat acne?

Yes	87%
No	13%

Q2 – What is your global opinion about the products?

Very pleasant	9%
Pleasant	61%
Neither unpleasant nor pleasant	30%
Unpleasant	0%
Very pleasant	0%

Q3 to Q5 – What do you think of their aspect, texture and fragrance?

	Very pleasant	Pleasant	Neither pleasant nor unpleasant	Unpleasant	Very unpleasant
Q3 - Aspect	0%	52%	39%	9%	0%
Q4 - Texture	0%	52%	30%	17%	0%
Q5 – Fragrance	0%	48%	22%	26%	4%

Q6 to Q8 – Immediately after application, did these products leave your skin:

	Yes	No
Q6 - Sticky	43%	57%
Q7 - Oily	43%	57%
Q8 - Shiny	39%	61%

Q9- Did the product spread easily?

Very easily	18%
Fairly easily	73%
Fairly difficultly	9%
Very difficultly	0%

Q10 – Did the product penetrate quickly ?

Very quickly	10%
Fairly quickly	62%
Fairly slowly	29%
Very slowly	0%

PRODUCT EFFICACY

Q11 – Did you notice an improvement on your skin state or aspect after 28 days of treatment?

Important improvement	22%
Moderate improvement	26%
Slight improvement	35%
No change	17%

Q12 – Regarding blackheads, did you notice a variation in their number after 28 days of treatment?

number of *blackheads*:

much less	17%
less	70%
no change	13%
more	0%
much more	0%

Q13 to Q15 – Did you notice a variation in the number, size and inflammation of pimples after this treatment ?

Q13 – number of pimples:

much less	0%
less	91%
no change	9%
more	0%
much more	0%

Q14 – average size of these pimples:

much smaller	17%
smaller	74%
no change	9%
bigger	0%
much bigger	0%

Q15 – pimples redness:

much less red	17%
less red	61%
no change	22%
more red	0%
much more red	0%

TOLERANCE

Q16 – Did you feel any intolerance sensations when using this product?

Yes	9%
No	91%

Q17 – Did you stop using the product?

Yes	0%
No	100%

Q18 – if yes, time of interruption :

If it was interrupted, was it :

Q26 • because of an intolerance reaction?

Yes	0%
No	0%

Q27- • for other reasons?

Yes	0%
No	0%

if yes, specify:

FUTURE USE OF THE PRODUCT

Q19 At the end of this test, would you like to continue using the product?

Yes	87%
No	13%

Q20- Would you like to buy this product?

Certainly	13%
Probably	70%
Maybe	13%
Probably not	4%
Certainly not	0%

70%, 91%, 74% and 61% of volunteers noticed an improvement effect on their acne in particular for the variation in the number of blackhead and a variation in the number, size and inflammation of pimples after this treatment, respectively, as shown in **Figure 4-2 to Figure 4-5**.

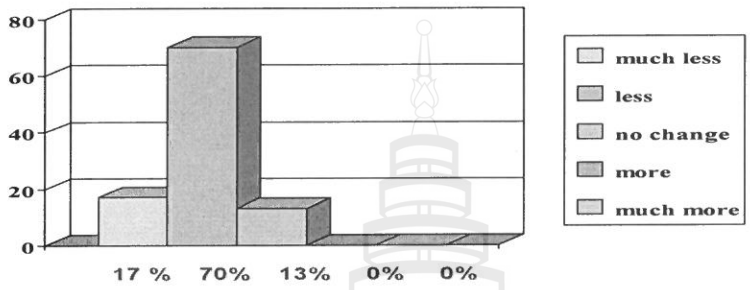


Fig. 4-2 The improvement effect on the number of blackhead

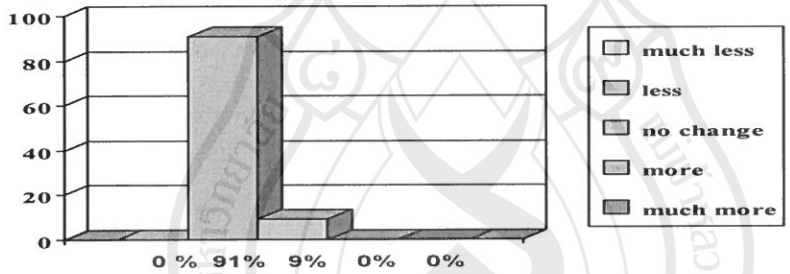


Fig. 4-3 The improvement effect on the number of pimples

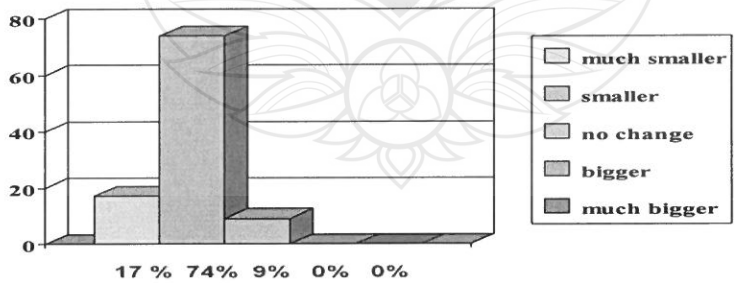


Fig. 4-4 The improvement effect on the size of pimples

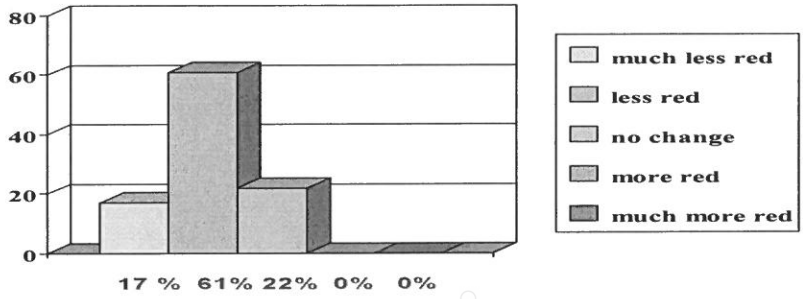


Fig. 4-5 The improvement effect on the inflammation of pimples

CHAPTER V
CONCLUSION

In the present study, *Garcinia mangostana* extracts were examined for antimicrobial activity against *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis*, *Escherichia coli* and *Propionibacterium acnes*. The results showed that *Garcinia mangostana* extracts could effectively inhibit the growth of *Staphylococcus aureus*, *Salmonella albany*, *Bacillus subtilis*, *Escherichia coli* and *Propionibacterium acnes*. Among those, the extracts of *Garcinia mangostana* showed strong inhibitory effects against *Staphylococcus aureus* (zone of inhibition ≥15 mm).

Subsequent experiments were conducted to determine inhibitory concentrations of *Garcinia mangostana* extracts. The MIC values against; Gram-positive: *Bacillus subtilis*, Gram-negative: *Salmonella albany*, *Escherichia coli* and Gram-positive anaerobe *Propionibacterium acnes* were 15 μg/ml, 3.87 μg/ml, 1.93 μg/ml and 2.5 mg/ml, respectively. The highest activity was shown against the Gram-positive bacterium *Staphylococcus aureus*. These results are very interesting since this microorganism can be commonly involved in skin infections. In particular, *Garcinia mangostana* extracts showed an interesting anti-bacterial activity against *Staphylococcus aureus* with a MIC 0.96 μg/ml for the ethanol extract of the fruit hulls. This plant species is one of the most popular herbal remedies of Thailand and it is used in traditional medicine for the treatment of abdominal pain, dysentery, diarrhoea, suppuration, infected wound, leucorrhoea and chronic ulcer and gonorrhoea.

Therefore, this result may suggest that *Garcinia mangostana* extracts possess compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases in human.

The efficacy on acne lesions of mangosteen extracts was also investigated. The efficacy on acne lesions was carried out by dermatologist responsible in this study under the same conditions, counted the retentional and inflammatory lesions on the entire face (except the nasal pyramid), at D0 and D28 after twice-daily used with 22 female volunteers at the age of 23±1. After used the " 5 % Mangosteen gel " on acne lesions for 28 days, the number of acne lesions include of comedones, microcysts, papules and

pustules showed a significant decreased from the beginning and for the cutaneous tolerance, the "5 % Mangoteen gel " could be considered very well tolerated on the cutaneous level. Globally, the volunteers appreciate the tested product according to evaluation questionnaires. A Majority of volunteers noticed an improvement effect on their acne in particular for the variation in the number of blackhead and a variation in the number, size and inflammation of pimples after this treatment.

In conclusion, the obtained results confirm the presence of anti-bacterial principles in Mangosteen extract and demonstrate that these plant could represent a new source of anti-microbial agents, less expensive than the imported drugs. Mangosteen extracts are the novel compounds for cosmetic and pharmaceutical use in acne treatment according to their strong *in vitro* antimicrobial activity and their efficacy on acne lesions.

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BIOGRAPHY

NAME	Miss Nattakan Panyachariwat
DATE OF BIRTH	October 23, 1980.
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Kasetsart University, 1998–2002: Bachelor of Science (Chemistry) Mahidol University, 2002–2005: Master of Science (Organic Chemistry)
EDUCATION GRANTS	The Higher Education Development Project: Post-graduate Education and Research Program in Chemistry (PERCH) The Development and Promotion of the gifted in Science and Technology Project (DPST)
HOME ADDRESS	135 Moo 4 Nang-Lae, Muang, Ching Rai, 57100 Thailand E-mail:pri2100m@hotmail.com