CLINICAL EFFICACY OF TOPICAL MANGOSTEEN EXTRACT NANOPARTICLE LOADED GEL COMPARED WITH 1% CLINDAMYCIN GEL IN MILD TO MODERATE ACNE VULGARIS

BY

MISS KARUNA SRIVIRIYAKUL

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
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ABSTRACT

Acne vulgaris is the most common sebaceous gland disorder in puberty and young adults. For developing new medication for acne vulgaris must be targeted on the mechanism involving in acne pathogenesis. 0.5%(w/w) mangosteen nanoparticle loaded gel, a xanthone in *Garcinia mangostana* extract from mangosteen fruit rind, is well known for its anti-oxidant and anti-microbial properties including inhibitory effect on *Propionibacterium acnes.* A new innovation of the encapsulation of α-mangostin into nanoparticle facilitates the active ingredients directly into the sebaceous gland via transfollicular route leading to the interestingly alternative treatment for acne and post acne erythema.

This study aims to investigate the clinical efficacy of 0.5% alpha-mangostin in nanoparticle loaded gel in the reduction of acne vulgaris and post acne erythema**.** Twenty patients with mild to moderate acne vulgaris with average (SD) age of 24 (6)

years old were included into double-blind, spit-face, randomized, control study. The 0.5% (w/w) mangosteen nanoparticle loaded gel was applied randomly to one side of the face and 1% clindamycin gel was applied to the other side twice daily for 12 weeks. The efficacies were evaluated using lesion count, digital photography and biometric measurement (Antera 3D) for acne erythema percentage reduction. Alpha-mangostin treatment group showed significant acne comedone reduction from the baseline in the week 12 with the mean of 6.07 (5.5) to 2.24 (1.9) $P = 0.001$. The pustule, papule and nodule median lesion counts from baseline to week 12 showed statistically significant change from 1.5 to 0 ($P = 0.001$), 2 to 1 ($P = 0.001$) and 1 to 0 ($P = 0.013$), respectively. Interestingly, post-acne erythema (PAE) significantly decreased in from 1.51 to 1.44 at week 12 ($P < 0.001$).

The result showed that 0.5% (w/w) mangosteen nanoparticle loaded gel significantly effective results in the treatment of acne in improvement of comedone and post-acne erythema. Moreover, there was no statistically significant difference between 0.5% (w/w) mangosteen nanoparticle loaded gel and 1% clindamycin gel in the reduction of the number of acne lesions and the presence of adverse effects. Hence 0.5% (w/w) mangosteen nanoparticle loaded gel could be the natural product options in the treatment of acne that could decrease the overuse of topical antibiotic and prevent the development of the drug resistant bacteria in acne patients.

Keywords: *Garcinia mangostana,* alpha-mangostin, Mangostin fruit rind, Nanoparticle, Acne vulgaris, Clinical trial.

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Miss Karuna Sriviriyakul

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont)

TABLE OF CONTENTS (Cont)

TABLE OF CONTENTS (Cont)

LIST OF TABLES

LIST OF TABLES (Cont)

LIST OF FIGURES

LIST OF FIGURES (Cont)

LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
AMPs	Antimicrobial peptides
AP-1	Activator protein-1
BP	Benzyl peroxide
DHT	Dihydrotestosterone
FFA	Free fatty acid
FOX O1	Forkhead box protein-O1
FPT	Fitzpatrick skin phototype
GAGs	Global Acne Grading System
HPLC	High performance liquid chromatography
IL	Interleukin
IGF-1	Insulin-like growth factor-1
IQR	Interquartile range
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
MMP	Metrix metalloproteinase
MNLG	0.5% (w/w) mangosteen nanoparticle loaded gel
MRSA	Methicillin-resistant *Staphylococcus aureus*
NF-kB	The nuclear factor kappa B
P.acnes	*Propionibacterium acnes*
PAE	Post acne erythema
PAR-2	Protease-activated receptor-2
PGE2	Prostaglandin E2

Symbols/Abbreviations	Terms
PIH	Post inflammatory hyperpigmentation
PPAR	Peroxisome proliferator activated receptor
ROS	Reactive oxygen species
TLR	Toll-like receptors
TNF-α	Tumor necrosis factor – α
TG	Triglyceride
UV	Ultraviolet

CHAPTER 1
REVIEW OF ACNE VULGARIS

1.1 Epidermiology

Propiobacterium acnes is a gram positive rod, anaerobic bacteria that plays an important role in pathogenesis of comedogenic and inflammatory acne. *P.acne* produces numerous enzyme such as lipase, hyaluronidase and acid phosphatase to spread of the acne lesions.

In a recent years, there is an increasing in the antibiotic resistance strain of *Propiobacterium acnes* due to widely use of antibiotics, biofilm formation from *P.acnes*.

Prevalence of acne occurs in middle to late teenager period with 88% of adolescents and may persist through the third decade of life.

Acne vulgaris is the multifactorial factors including hormones (dihyrotestosterone, dehydroepiandrosterone sulfate (DHEA-S), acne after menstrual cycle, genetics factor, high glycemic diet food (1).

Latest guideline from Journal of American Academic of Dermatology 2016 stated that benzyl peroxide and topical retinoid is the recommended treatment for mild acne vulgaris or a combination of topical antibiotics and topical is recommended for mild-to-moderate acne lesion. Topical antibiotics also recommended as a first line therapy but should be used in combination with benzyl peroxide or topical retinoid to limit the development of *P.acnes* resistance strains. Oral antibiotics may be added in moderate acne vulgaris. In severe type, Topical antibiotics with benzyl peroxide with topical retinoid may be used with or without oral isotretinoin.

1.2 Pathogenesis

The pathogenesis of acne is multifactors. There are 4 key steps in the acne pathogenesis (2).

1.2.1 Follicular epidermal hyperproliferation

Microcomedones caused by increase keraionocyte adhesion in infundibulum leads to “plug” in the follicular opening then accumulation of sebum, keratin, bacteria with more inflammatory cells results in comedones formation.

Hyperkeratinization in the follicular infundibulum and sebaceous gland resulting in microcomedones. The increasing of number of keratohyalin granules accumulation of lipid droplets, and follicular hyperkeratinization result in the pressure effect (see Figure 1.1) (3).

IL-1α induces hyperkeratinization in follicular infrainfundibulum. IL-1α can activate basal keratinocytes and increase in keratin synthesis. The increasing of DHT may act on infundibular keratinocytes leading to abnormal hyperkeratinization and the formation of microcomedone. Androgens play an important role in acne pathogenesis. Acne severity does not correlate with serum androgen levels. It is postulated that androgens may play only a permissive sole in initiating acne development or the higher responsiveness of androgen receptors can determine the formation of acne vulgaris (4). Sebaceous glands express all the necessary enzyme for the biosynthesis of testosterone form de novo cholesterol, ingested in dairy products and from circulating dehydroepiandrosterone. Androgen influences acne pathogenesis in term of the proliferation and differentiation of sebocytes and infrainfundibular keratinocytes.

Testosterone and DHT are effective on sebocyte proliferation and induction of lipogenesis in vitro in concentrations above the physiological level through the upregulation of sterol-response-element binding protein (SREBP).

Microcomedones are caused by hyperproliferation of the keratinocyte of the infrainfundibulum of the follicular canal. It is shown that higher activity of 5α-reductase detected in infrainfundibulum is related to abnormal differentiation of keratinocytes and comedogenesis (4, 5).

Insulin-like growth factor (IGF-1) like drinking milk can rise in IGF-1 through disproportionate elevation in blood sugar and serum insulin levels. Hyperglycemic food also mediated elevation of DHT (3). Plugging of keratinocyte by hormone-induced keratinocyte hyperproliferation can increase the pressure of the follicular wall until the pilosebaceous duct can no further expand can the cause hypoxic condition in the ductal environment, developing intraductal *P.acnes* colonies.

1.2.2 Excess sebum production

Patients with acne produce more sebum and the components of sebum including triglycerides and lipoperoxides- both of which play a role in acne pathogenesis.

Triglycerides are broken down into free fatty acid byP.acnes enzyme lipase. Free fatty acid is comedogenic and is the potent chemotactic factors that recruit more inflammatory cells into the acne.

Lipoperoxides produce proinflammatory cytokines and active peroxisome peroxidase, resulting in increased sebum production. Lipoperoxides can induce the alteration in keratinocyte proliferation and differentiation, and peroxides can induce the production of pro-inflammatory cytokines and activation of Peroxisome proliferator-activated receptors (PPAR).

PPARs (Peroxisome proliferator activated receptor) are also increased by the increase level of IGF1/insulin receptor (lipid and glucose homeostasis) and can be blocked by Fox O1 nuclear receptor. Without Fox O1 nuclear receptor, PPARs will co-regulate sebaceous gland lipogenesis and increase sebum production.

Oral isotretinoin can upregulate Fox O1 nuclear transcription factor by suppress androgen receptor expression, so isotretinoin can interfere with sebocyte cell cycle, promote sebocyte apoptosis, decrease sebum and decrease comedogenesis.

Seborrhea is not sufficient for the development of acne. Increased sebum secretion occurs in all adolescent, but only who have incorrect regulation of lipid metabolism can develop acne such as decrease amount of linoleic acid (3).

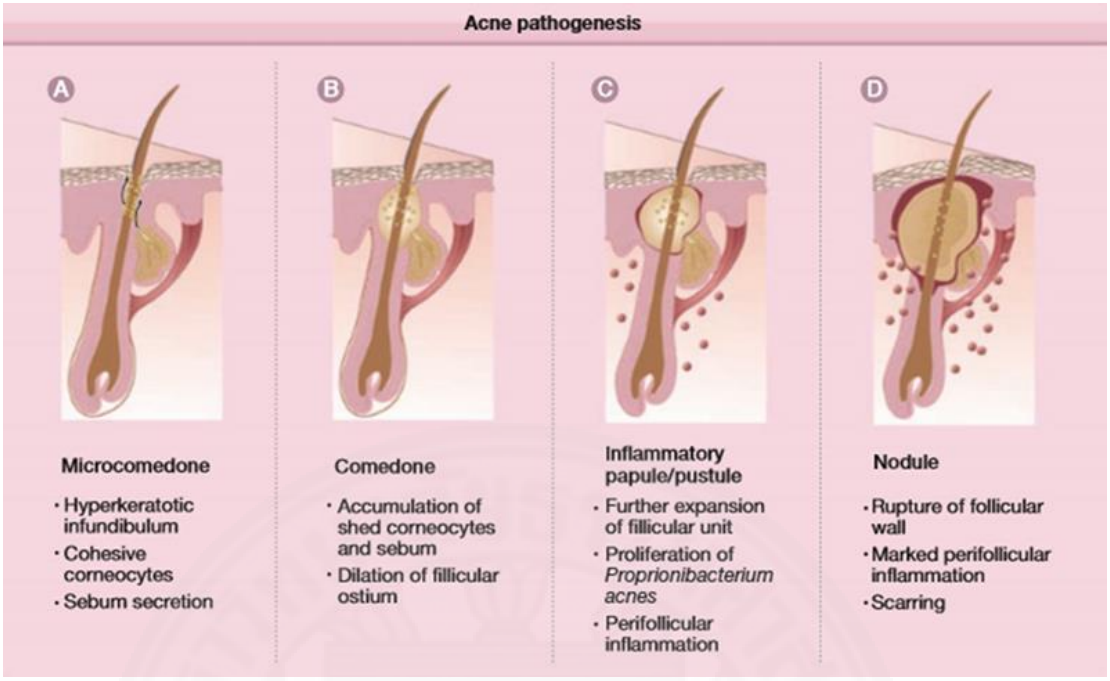


Figure 1.1 Pathophysiology of inflammatory acne (6)

1.2.3 *Propiobacterium acnes*

P.acnes is a gram positive, an anaerobic bacteria that found in the sebaceous follicle. The patients with acne vulgaris will have higher density *of P.acne. P.acnes* stimulates expression of cytokines by binding to TLR-2 on keratinocytes.

P.acnes is still controversial in the acne pathogenesis because it belongs to the resident microbiota. The pahtogenic strains of *P.acnes* can be the cause of pathogenesis of acne (3).

P.acnes induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines from various cell types. Certain *P.acnes* strains may cause an opportunistic infection worsening acne lesions.

The different *of P.acnes* strains can be differently induce the expression of antimicrobial peptides. *P.acnes* strains that solely induce antimicrobial peptide and cannot express proinflammatory cytokines can lead to resistance to *P.acnes.*

P.acnes also releases exogeneous protease to activate PAR2 receptors on human keratinocytes. PAR2 will make abnormal epidermal barrier homeostasis and increase cutaneous inflammation by upregulation of IL-8.

Activation of PAR2 will increase intracellular calcium release by keratinocytes, thus Tetracycline can block PAR2 expression by chelating of calcium downstream signaling.

Biofilm is a glycocalyx polymers acts as a protective exoskeleton as a physical barrier. Not every *Propiobacterium acnes* can produce biofilm, there is the only some pathologic strains of *P.acnes* have biofilm formation. Biofilm increase a chance of antibiotic resistance as much as 50-500 times more resistance. Biofilm helps bacteria adhere to the environmental surface such as dental plaque, implanted prostatic devices, urinary cathetors and also pilosebaceous lining (3, 7).

Biofilm can alter the external environment in which *P.acnes* lives and affect P.acnes enzymes by regulating pH, oxygen tension suitable for anaerobic bacteria.

There are many types of antibiotics acts as inhibitors of biofilm, such as erythromycin, clindamycin, minocycline in combination with benzyl peroxide to form free radical with defective in cell wall protein synthesis and susceptible for benzyl peroxide.

Figure 1.2 *P.acnes* with biofilm formation (2)

P.acnes lipase plays an important role in acne pathogenesis by hydrolyzes triglyceride into free fatty acid. Free fatty acid acts as chemotactic for attact neutrophils. Neutrophils which are attracted will release hydrolytic enzyme such as lipase, acid phosphatase, hyaluronidase, and protease and increase the spreading of acne, follicular rupture and causing scar formation.

Free fatty acid can regulate TLRs signalling endogenously and play a roles in regulate proinflammory cytokine formation (8).

Lipase (Produced by *P.acnes*) change

TG → LIPASE → FFA

FFA is a chemotactic factors to induce inflammatory cells infiltration

There are many lipase inhibitors such as erythromycin, tetracycline, clindamycin, and macrolides. Herbal medicine such as *Terminalia chebula* has the lipase inhibitor properties.

1.2.4 Inflammation

P.acnes and Lipopolysaccharide can significantly upregulate the expression of proinflammtory cytokines. LPS induce the production of IL-8, TNF-α, and IL-1α. In acne-involved skin, there is a strong expression of IL-8 than those of healthy skin (4). IL-1 plays a central role in regulation of inflammation and immune response, control cutaneous homeostasis. IL-1 dramatically increases during the early stages of acne development and promote hypercornification and comedo formation.

IL-8 cannot be detected in normal skin, but increase significantly in acne patients. IL-8 increase follicular inflammation by recruitment of neutrophils and promote neutrophils release of lysosomal enzyme resulted in rupture of the follicular epithelium. Human b-defensin increases in the early acne lesion. B-defensin plays a role in protecting pilosebaceous unit form microbial invasion and markedly increase in comedome.

TLRs are transmembrane proteins that play a role in innate immunity. TLRs mainly express on monocytes, macrophages, dendritic cells and granulocytes. TLRs induce the production of inflammatory cytokines, prostaglandins, leukotrienes and chemokines. TLRs can send signal to T and B cell receptor mediated signaling pathways for both innate and adaptive immune response. TLRs can be a new target for inflammatory diseases including acne vulgaris (5).

TLR2 on macrophage can recognize gram positive bacteria (lipoteichonic acid) and TLR4 can recognize lipopolysaccharide in gram negative bacteria. Sebaceous glands establish a link between sebum, lipid metabolism,

antimicrobial peptides and innate immunity in the pathogenesis of acne. Topical retinoids (adapalene) are effective in early inflammatory acne by decrease TLR2 and TLR 4 expression in macrophage.

1.3 Guideline Management of Acne Vulgaris

In Journal of American Academic of Dermatology 2016, they classified acne vulgaris into mild moderate and severe acne. The treatment of acne vulgaris is due to severity and the pathogenesis of acne is due to *P.acnes* or gram negative bacteria or Pityrosporum folliculitis. These different pathogens also have different management of *P.acnes* (9).

Topical therapy commonly used in acne vulgaris include benzyl peroxide, salicylic acid, antibiotics, combination of antibiotics with BP, or retinoid with BP, or retinoid with antibiotics.

Benzyl peroxide is an antibacterial agent that kills *P.acnes* through the release of free radicals and is mildly comedolytics. No resistance to this agent has been reported. The additional of BP to antibiotics enhances the results and reduces resistance development.

Topical antibiotics for acne are working through anti-inflammatory mechanisms, and via antibacterial effects. Antibiotics should not be used as monotherapy because of the development of antibiotic resistance strains. Antibiotics should be used in combination with BP for increasing efficacy and decreasing in the development of resistant bacterial strains. 1% clindamycin solution or gel is preferred as topical antibiotics for acne therapy. (clindamycin alone is pregnancy category B).

Oral antibioitics Tetracycline is typically used in the management of acne due to anti-inflammatory actions (independent of antibactierial) and the efficacy to block biofilm, and inhibit MMPs, PAR2, modulating chemotaxis of neutrophils and also Calcium chelating agents that can block the release of serine protease form lamella bodies and also have as antibacterial properties.

Patients must be evaluated for gram negative folliculitis (always uniform and disruptive pustules, rarely nodules) in the perioral and perinasal regions, with the “clue” of prolonged tetrycycline used. *Klebsialla* and *Serratia are* the common cause

of gram negative folliculitis but it is generally treated with isotretinoin and antibiotics with sensitive to gram negative bacteria.

Table 1.1 Guideline management of acne vulgaris (9)

	Mild	Moderate	Severe
1st line treatment	Benzyl Peroxide Topical Retinoid Topical antibiotics	Topical combination therapy (BP + ATB + Retinoid) with or without oral antibiotics	Oral antibiotics + Topical combination therapy or Isotretinoin
Alternative	Topical Retinoid or BP Consider Topical dapsone	Add combined oral contraceptive or spironolactone (female) or consider oral isotretinoin)	Consider change in oral antibiotic or add combined oral contraceptive or oral spironolactone

Topical retinoids are vitamin A derivatives that are effective in the treatment of acne vulgaris

Three types of these active agents are available

1. Tretinoin (0.025%, 0.1% in cream or gel)
2. Adapalene (0.1%, 0.3% in cream of 0.1% lotion)
3. Tazarotene (0.05%, 0.1% cream or gel or foam)

Each retinoid binds to different set of retinoic acid receptors. Tretinoin binds to α, β, ϒ retinoic acid receptor. Tazarotene and adapalene selectively binds to β, ϒ receptors. Therefore, there are slightly different in the efficacy of retinoid derivatives.

Retinoids are the core of topical therapy for acne vulgaris because they are comedolytic, resolving the microcomedone lesion and are anti-inflammatory. Retinoids may be limited by side effects including dryness, peeling, erythema and irritation which can be managed by reducing frequency of application.

Adapalene 0.1% and BP 2.5% approved for use in patient > 9 years old and fixed combination of 1.2% clindamycin phosphate/ tretinoin 0.025% gel approved for patients > 12 years of age.

1.4 Clindamycin and the role of anti-inflammatory effects

Clindamycin is the antibiotics in the class of lincosamide that exhibits antibacterial mechanism of action by binding ribosome 50S of microorganism including *P.acnes* to prevent protein synthesis and inhibit cell wall synthesis, so bacterial will susceptible to be phagocytosed (10, 11).

Clinadamycin also indirectly inhibit pro-inflammatory enzyme such as lipase, protease, hyaluronidase and neuramidase which are produced by *P.acnes*. *P.acnes* lipase can hydrolyse triglyceride to free fatty acid. This catalytic enzyme will damage the follicular wall leads to leakage of the content into the surrounding dermis leads to further inflammation. *P.acnes* release pro-inflammatory factors to enter the dermis surrounding the follicle and inflammatory cells such as neutrophils, monocyte and macrophages to the "perifollicular region".

Free Fatty acid hydrolysed from triglycerides is comedoginic. Clindamycin will be indirectly inhibiting comedone stage of acne by this mechanism. Clindamycin can also suppress leukocyte chemotaxis especially neutrophils.

Neutrophils can release proinflammatory cytokines such as IL-8, IL-1α, TNF-α to activate phagocytosis. TNF-α is the cytokine that can upregulate prostaglandin and collagenase to the tissue and produce more inflammatory effects in response to *P.acnes.*

Clindamycin can also reduce induced nitric oxide synthase (iNOS) which stimulated inflammatory cells to secrete Nitric oxide. Nitric oxide is the mediator of inflammatory and vascular response.

Even *P.acnes* strains still receive the benefits from topical clindamycin not only antimicrobial activity but also anti-inflammatory properties for inflammatory acne lesions.

CHAPTER 2
REVIEW OF LITERATURE
Garcinia Mangostana

2.1 Introduction

Mangosteen (*Garcinia mangostana Linn*.) is a tropical famous fruit that found in the Southeast Asia such as Thailand, India, Myanmar and Malaysia. Mangosteen is in a family Clustaceae. It has been used in the traditional medicine for anti-diarrheal drug, skin infection, anti-inflammation such as eczema, dermatitis and also used as anti-bacterial for *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella thypimurium, Bacillus subililis, methicillin-resistant Staphylococcus aureus* (MRSA) and 13 species of *Enterococcus spp.* and enhances the ability of phagocytic cells to kill *Salmonella enteritidis in vitro.*

Xanthones including α-mangostin, β-mangostin, γ-mangostin, gartanin and 8-deoxygartanin are the major secondary metabolites found in mangosteen fruits (12).

Figure 2.1 Physical characteristics of mangosteen fruit (12)

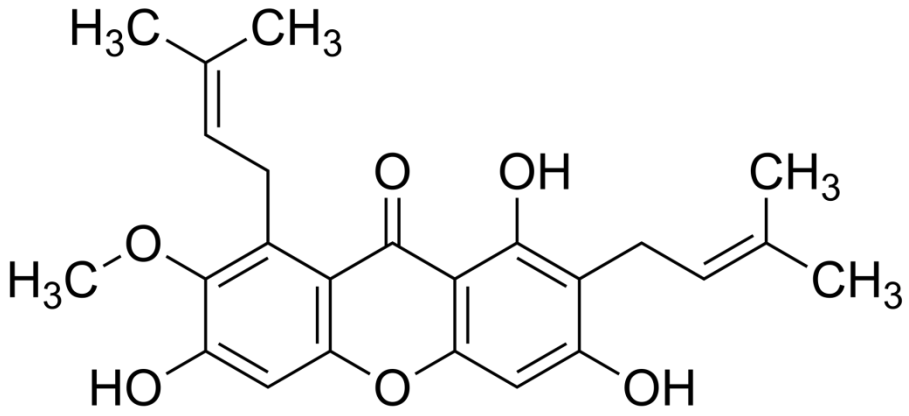


Figure 2.2 Chemical structure of alpha-mangostin (13).

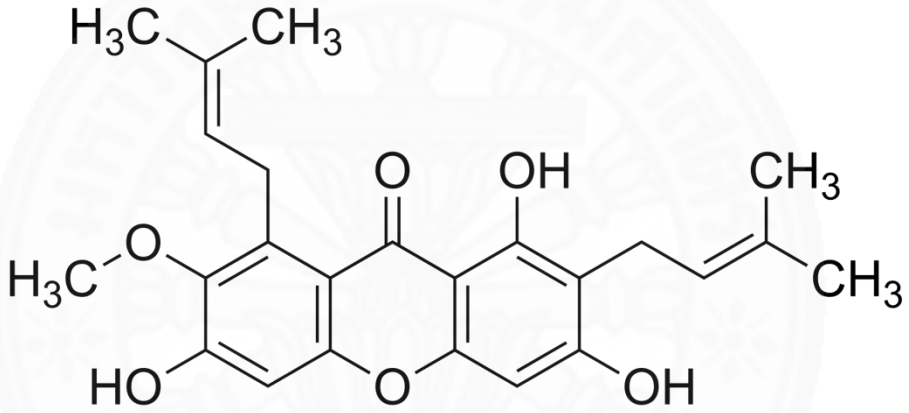


Figure 2.3 Chemical structure of gamma-mangostin (13).

2.2 Pharmacological properties of xanthones from mangosteen

2.2.1 Anti-tumoral activity

Xanthones isolated from mangosteen-fruit pericarp have anticancer activities. Six xanthones (α, β, γ-mangostins, mangostinone, garcinone E and 2-isopronyl-1,7-dihydrowy-3-methoxy xanthone) isolated from mangosteen-fruit pericarp exhibited cell growth inhibition affected to leukemia cell lines. Alpha-mangostin promoted the highest inhibitory activity (12).

2.2.2 Anti-fungal, anti-viral and anti-bacterial activities

Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium and *Bacillus subtilis* were highly susceptible to xanthones. γ-mangostin, 1-isomangostin and 3-isomangostin from *Garcinia mangostana* extract have anti microbial activities against *S.aureus* in both penicillin susceptible and resestant strains.

For anti fungal properties, *Epidermophyton floccosum*, *Alternaria solania*, *Mucor* sp., *Rhizupus* sp. and *Cunninghamella echinulata* were also highly susceptible to xanthones.

Furthermore , alpha- and beta-mangostins and garcinone B exhibited antituberculosis potential against *Mycobacterium tuberculosis* with an MIC of 6.25 μg/ml (12).

Table 2.1 Subceptability of *Garcinia mangostana* extract against *Propiobacterium acnes* and *Staphylococcus epidermidis* (14).

	MIC (mg/ml)	MBC (mg/ml)
P.acnes	0.039	0.039
S.epidermidis	0.039	0.156

2.2.3 Anti-oxidant properties

Propiobacterium acnes can acts as an immunostimulator and produce a variety of enzymes through TLR-2 and TLR-4 induced proinflammatory cytokines such as IL-1α, IL-8, and tumor necrosis factor-alpha (TNF-α) by mononuclear cell such as T-helper cells in acne patients (15).

Propiobactirium acne can also activate local inflammation by chemotactic factors to recruit neutrophil to the site of lesions by *P.acnes* lipase that hydrolyse triglyceride to free fatty acid that is chemotactic factors and inflammatory mediators also produces reactive oxygen species (ROS).

ROS will send the secondary messenger to induce to production of transcription factors such as NF-kB, AP-1 to generate cytokines production and produce more inflammatory acne lesions. Reducing the ROS is very important to control inflammatory acnes.

Chomnawang et al. (15) measured anti-oxidant properties of *Garcinia mangostana* extract to stimulated venous blood from healthy volunteers, peripheral blood added with *P.acnes* extract, positive control and negative control without extracts. It showed that *Garcinia mangostana* extract can reduce ROS production from neutrophils. Anti-inflammatory effects of *Garcinia mangostana* extract are free radical scavenging by scavenging ROSs produced from neutrophils and inflammatory cells.

Garcinia mangostana extract (50 µg/ml) showed inhibitory activity against TNF-α production (more than 70% inhibition from peripheral blood mononuclear cells by *P.acnes* stimulation)(15).

2.3 Pharmacological properties of xanthones from mangosteen

2.3.1 Anti-baccterial properties

There is some of the experiment about the effects of *Garcinia mangostana* on acne vulgaris as followed

Chomnawang et al (14), explained about the results from disc diffusion method of active compound mangostin (xanthone derivative) of mangosteen pericarp extract, had strong inhibitory effects against *Propiobacterium acnes*, with the greatest. MIC and MBC of *Garcinia mangostana* suggested a bactericidal effects against *P.acnes* and *S.epidermidis* of magnosteen pericarp extract (Table 2.1).

Chomnawang et al (15), studied the effect of *Garcinia mangostana* on inflammation caused by *Propiobacterium acnes* . It was found that *Garcinia mangostana* can effectively reduce pro inflammatory cytokines TNF-α (stimulated from *P. acnes*) production in dose-dependent manner determined by ELISA method and can reduce reactive oxygen species production up to 77.8-1.28%. This anti inflammatory property is useful in the treatment of inflammatory acne lesions (15).

Pothitirat et al. (16), studied anti-bacterial propertie of 95% ethanolic extract mangosteen pericarp as a result from the strong anti-bacterial effect of α-mangostin in xanthone derivatives from mangosteen extract. α-mangostin from *Garcinia mangostana* extract is a good souce of anti-acne compound because α-mangostin which promotes strong anti-bacterial activity. This compound could be prepared from dichloromethane extract with the yield around 46.21% w/w. MIC and

MBC values to *P.acnes* of *Garcinia mangostana* extract is 3.91 µg/ml respectively and MIC and MBC values to *S.epidermidis* are 3.91 and 15.63 µg/ml.

Table 2.2 Yields of crude extracts, contents of α-mangostin in *G. mangostana* fruit rind extracts prepared using different solvents and their MIC and MBC values against *P. acnes* and *S. epidermidis* (16).

Solvents	**%Yeild of drude extract (%dry weight)**	**% α-magnostin (w/w)**	***P.acnes* MIC (ug/ml)**	***P.acnes* MBC (ug/ml)**
Hexane	0.97(7.54)	17.21	7.81	7.81
DCM	8.01(1.39)	46.21	3.91	3.91
EtOH	20.20(0.34)	18.03	7.81	15.63
H2O	27.5(0.02)	0.54	500	>500

Porntip-pan in *et al.*(17) had a pilot study in 10 healthy volunteers with mild to moderate acne, 4 weeks spilt-face study compared with gel base in division of Dermatology, Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. 1.2% α-mangostin nanoparticle was applied twice daily for 4 weeks with 2.5% benzyl peroxide gel as standard treatment. The result showed significant improvement of inflammatory acne lesions in both Acne Severity Index Score and Inflammatory acne counts in between mangosteen nanoparticle and gel base control and significant improvement between the nanoparticle side 1 month before and after treatment.

However, there is the lack of the study of mangosteen extract to reduce comedone, inflammatory acne, post inflammatory hyperpigmentation and post acne erythema lesions. In order to find an alternative treatment of acne by using herbal extract instead of antibiotics to treat acne vulgaris.

2.3.2 Anti-allergy activities

From Nakatani *et al.*(18) showed that mangosteen extracts inhibited histamine release and prostaglandin E2 synthesis in rat glioma cells and has a potent

inhibitory potentials to IgE-mediated histamine release in rat glioma cells. Both α- and γ-mangostin can also inhibit both COX1 and COX2 enzymes in *in vitro* test.

2.3.3 Anti-inflammatory activities

Chomnawang et al (15, 19) studied the effect of *Garcinia mangostana* extract on inflammation caused by *P.acnes* showed that *G.mangostana* extract reduced TNF-α levels by ELISA method and also have free radical scavenging properties that can suppress the production of inflammatory cytokines which can be useful in the treatment of acne vulgaris lesions.

Alpha- and Gamma-mangostins inhibited NO and PGE2 from LPS-stimulated macrophage cells and promoted antagonism effects to histamine H1 receptor (19). PGE2 can induce inflammatory cells such as neutrophils, monocytes, fibroblasts, endothelial cells to the site of infection which is rapidly induced inflammatory stage in acne lesions.

Table 2.3 Compare the properties of α, β, and ϒ mangostin.

Properties	**α-mangostin**	**β-mangostin**	**ϒ-mangostin**
Histaminergic and serotonergic receptor blocking agents (24).	alpha-mangostin can inhibit H1 receptor in guinea-pig in dose-dependent manner.	-	Can inhibit tryptamine (5-HT) in the rabbit aorta smooth muscle in concentration dependent manners.
Antifungal properties (25).	Show anti fungal properties against *Epidermophyton floccosum, Alternria solan, Mucor sp, Antifungal properties Rhizopus sp.*	-	Show effective inhibiting effect against fungi. *Fusarium oxysporum vasinfectum, Alternaria tenuis* and *Dreschlera oryzae* and show moderate fungicidal against *Trichophyton mentagraphytes and Microsporum gypseum*
Antibacterial properties (14).	Against *P.acnes, S.aureus,* both penicillin susceptible and resistant strains. *S.epidermidis*	-	Show anti bacterial affect to *S.aureus. P. aeruginosa, Salmonella tyhimarium*, and *Bacillus subtilis*
Anti-*microbacterium (13)*	Anti *Mycobacterium tuberculosis* effect with MIC of 6.25 µg/ml	Anti *mycobacterium tuberculosis* effect with MIC of 6.25 µg/ml	

CHAPTER 3
MATERIALS AND METHOD OF 0.5% (w/w) MANGOSTEEN NANOPARTICLE LOADED GEL PREPARATION

3.1 Physical property of 0.5% (w/w) mangosteen nanoparticle loaded gel

Mangosteen nanoparticle loaded gel contains gel base and 0.5% (w/w) mangosteen nanoparticle loaded gel. Gel base comprises of xanthan gum, propylene glycol, glycerin, allantoin, phenoxythanol, methylisothiozolinone, Chlorphenesin and water.

Gel base characteristic is transparent with some viscosity, colorless and no smell. When gel base mixes with 0.5% (w/w) of mangosteen extract in nanoparticle loaded gel that contain alpha-mangostin 94% (HPLC), it can blend together in one layer without separation into layers. alpha-mangostin nanoparticle can disperse equally in gel base and have soft yellow color at the concentration of 0.5% (w/w) mangosteen nanoparticle loaded gel.

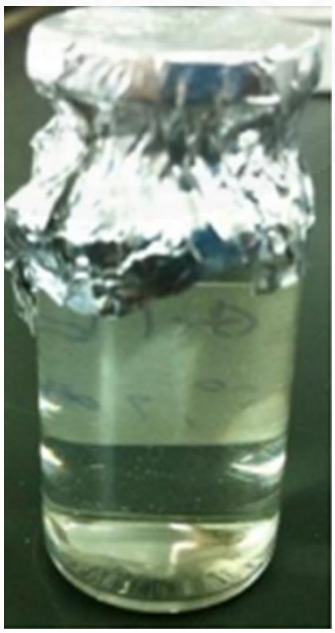


Figure 3.1 the physical characteristic of gel base

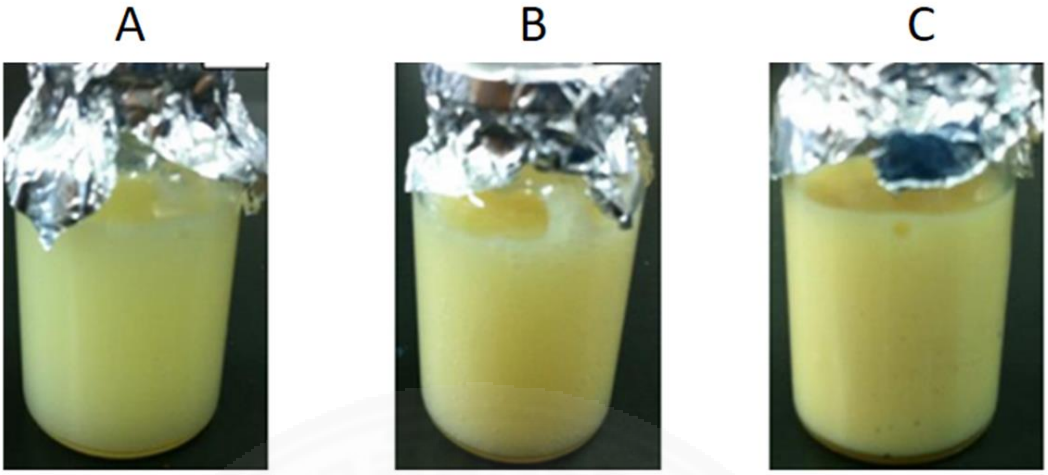


Figure 3.2 Physical characteristics of mangosteen nanoparticle loaded gel at the concentration of 0.3% (A), 0.6% (B) and 1.2% (C) (w/w) mangosteen nanoparticle loaded gel**.**

The physical property of mangosteen extract nanoparticle gel is yellow color with mild smell of mangosteen. The more concentration of alpha-mangostin yield more yellow color of the gel. After leave on the room temperature for 120 hours, there is no separation into layer or no sediment.

pH of α-mangostin is 5.5 equally in all concentration which is suitable for human skin even in the children

Mangosteen nanoparticle loaded gel contain 0.5%(w/w) of natural extract of *Garcinia mangostanta Linn*. in 100 ml of nanoparticle loaded gel. Mangosteen extract has 94% alpha-mangostin (HPLC). (Welltech Biotechnology Co., Ltd., Bangkok, Thailand) solution. (Product code WAEX002-P). The physical characteristic of the mangosteen nanoparticle loaded gel has pale yellow appearance and mild odor of mangosteen characteristic. The average of alpha-mangostin size is 453.7 nm. measured by Zetasizer (Malvern instruments Ltd., Malvern, United Kingdom). Serial number of the mangosteen nanoparticle loaded gel extract (MNLG) is MAL 1076669.

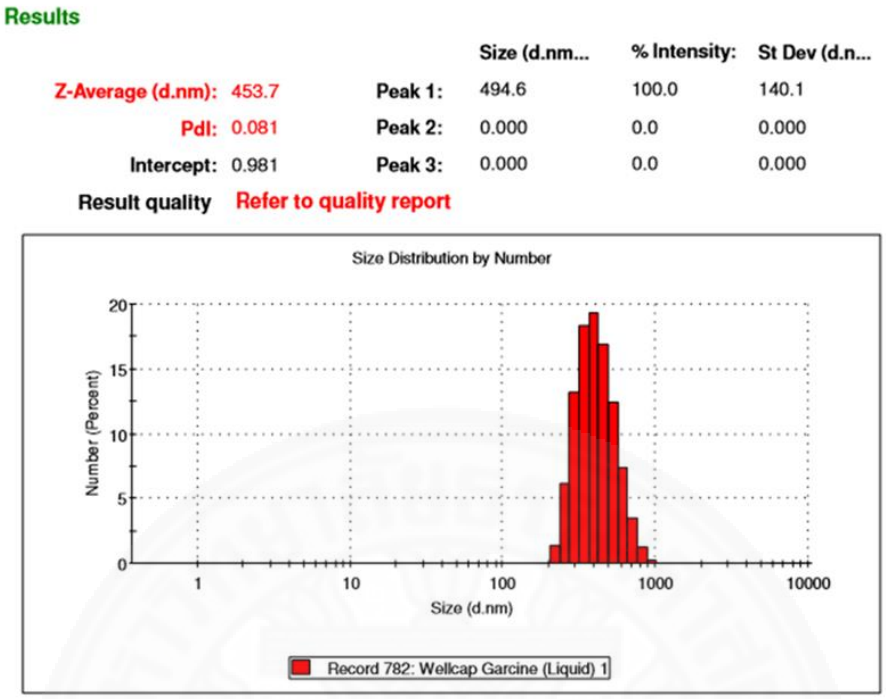


Figure 3.3 Size distribution of Mangosteen nanoparticle loaded gel (MNLG) reported by Zetasizer (Malvern Instrument Ltd., United Kingdom)

Table 3.1 Physical characteristics of 0.3%, 0.6%, and 1.2% (w/w) mangosteen nanoparticle loaded gel

α-mangostin (w/w)	**Physical characteristics**				
	pH	**color**	**odor**	**viscosity**	**Separation and sediment**
Gel base 0%	5.5	colorless	No smell	Optimal for usage	No separation no sediment
0.3%	5.5	Pale yellow	Mild smell	Optimal for usage	No separation no sediment
0.6%	5.5	Pale yellow	Mild smell	Optimal for usage	No separation no sediment
1.2%	5.5	Pale yellow	Mild smell	Optimal for usage	No separation no sediment

3.2 Stability test of 0.5% (w/w) mangosteen nanoparticle loaded gel

3.2.1 Test in fixed temperature

After leaving mangosteen nanoparticle gel in 45 degree Celsius for 7 days and then leaves in room temperature, the general appearance of mangosteen nanoparticle gel did not change in their viscosity, no separation of the gel into layers, and keep the same pH 5.5.

Table 3.2 Physical characteristics of 0.3%, 0.6%, and 1.2% (w/w) mangosteen nanoparticle loaded gel after stability test at 45 degree Celsius for 7 days.

α-mangostin (w/w)	**Physical characteristics**				
	pH	**color**	**odor**	**viscosity**	**Separation and sediment**
Gel base 0%	5.5	Colorless	No smell	Optimal for usage	No separation no sediment
0.3%	5.5	Pale yellow	Mild smell	Optimal for usage	No separation no sediment
0.6%	5.5	Pale yellow	Mild smell	Optimal for usage	No separation no sediment
1.2%	5.5	Pale yellow	Mild smell	Optimal for usage	No separation no sediment

3.2.2 Test with changing in temperature

After leaving the mangosteen nanoparticle gel in 4 degree Celsius for 24 hours and then change to keep in 30 degree Celsius for 24 hours and repeat switching for 4 cycles. The result showed that no change in general appearance of mangosteen nanoparticle gel and no separation of the solvents into layers. pH 5.5 does not change from baseline pH.

Table 3.3 Physical characteristics of 0.3%, 0.6%, and 1.2% (w/w) mangosteen nanoparticle loaded gel after stability test at 4 and 30 degree Celsius for 4 cycles.

α-mangostin (w/w)	**Physical characteristics**				
	pH	**color**	**odor**	**viscosity**	**Separation and sediment**
Gel base 0%	5.5	colorless	No smell	Optimal for usage	No separation no sediment
0.3%	5.5	Pale yellow	Mild smell	Optimal for usage	No separation no sediment
0.6%	5.5	Pale yellow	Mild smell	Optimal for usage	No separation no sediment
1.2%	5.5	Pale yellow	Mild smell	Optimal for usage	No separation no sediment

The results of the test can summarize that mangosteen nanoparticle gel has good stability and mangosteen extract nanoparticle can well dissolve into the gel base without separation into layers.

3.2.3 Irritation test

Mangosteen nanoparticle loaded gel were tested for skin irritation by 2 methods which are single closed patch test and repeated open test (use test) . The detail of each test is as following;

3.2.3.1 Single closed patch test

Mangosteen nanoparticle gels in different concentrations were used at the concentration of 0.3% (w/w), 0.6% (w/w) and 1.2% (w/w) were test on volunteer's back skin and have 2.5% SDS was used as a positive control and water was used as negative control and pure gel base was also tested to differentiate the irritation caused by gel base in 20 volunteers with 4 males (20%) and 16 females (80%). The irritation chamber filled with the 0.3% (w/w), 0.6% (w/w) and 1.2% (w/w) alpha-mangosteen pericarp extract, positive and negative controlled was shown in the Figure 3.3

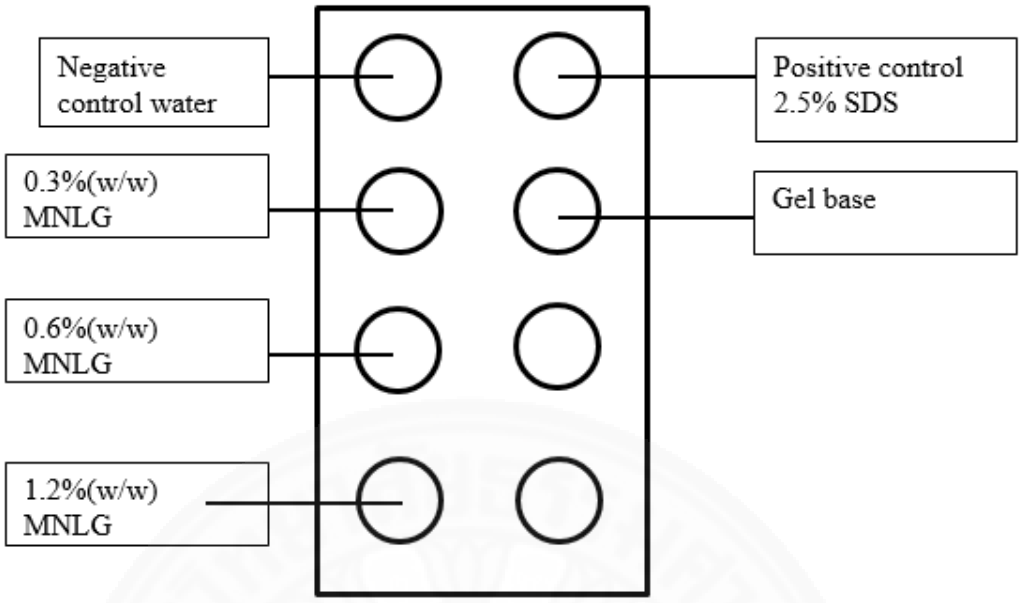


Figure 3.3 Closed patch test

The closed patch test was attached to back skin of the volunteers for 24 hours and observe the sign of irritation at 48 hours after start the test. The results showed as the following scales.

Table 3.4 Grading scale for skin irritancy test (17).

Scale	Response
0	No reaction
1	Doubtful reaction, barely perceptible erythema
2	Weak reaction, Slight erythema and dryness across of treatment site
3	Moderate reaction, moderate erythema and possibly spreading with barely edema at the margin; papules may be present
4	Strong reaction: moderate erythema with generalized edema
5	Strong reaction: severe erythema with severe edema, with or without vesicles, pustules or ulcer

Closed patch test on the subjects using the aluminium patch test (Finn cahmber) and closed on top with adhesive tape (3M Thailand Ltd. Bangkok, Thatiland) for 48 hours and reopen after 24 hours and 48 hours after application. Blinded dermatologist will assess the reaction on the patient's skin and took a picture of the reaction on the patient's skin.

Figure 3.4 Fin chamber were covered by sticky tape on volunteer's backs

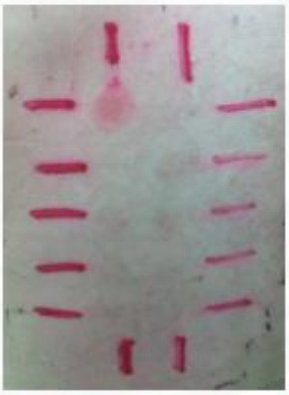


Figure 3.5 The sample of volunteer's back after uncover the Finn chamber

Table 3.5 The number and % of skin irritation test in volunteers

Sample	Time	N (%) Grading of skin reaction n=20				
		Negative	Positive	1+	2+	3+
2.5%SDS	24 hours	15	85	40	20	25
	48 hours	20	80	20	20	20
water	24 hours	100	-	-	-	-
	48 hours	100	-	-	-	-
0.3%(w/w) α-mangostin nanoparticle loaded gel	24 hours	95	5	5	-	-
	48 hours	100	-	-	-	-
0.6%(w/w) α-mangostin nanoparticle loaded gel	24 hours	95	5	5	-	-
	48 hours	100	-	-	-	-
1.2%(w/w) α-mangostin nanoparticle loaded gel	24 hours	90	10	10	-	-
	48 hours	95	5	5	-	-
Gel base	24 hours	100	-	-	-	-
	48 hours	100	-	-	-	-

Table 3.6 24 hours after closed patch test with 48 hours open for observation

Samples	24 hours	2.5% SDS		
		Irritation	No irritation	P-value
0.3%w/w mangosteen extract nanoparticle gel	Irritation	1	0	0.000
	No irritation	16	3	
0.6%w/w mangosteen extract nanoparticle gel	Irritation	1	0	0.000
	No irritation	16	3	
1.2%w/w mangosteen extract nanoparticle gel	Irritation	2	0	0.000
	No irritation	15	3	
Gel base	Irritation	0	0	0.000
	No irritation	17	3	

From the table 3.6, 20 participants who applied 0.3% (w/w) mangosteen nanoparticle loaded gel and positive control with 2.5% SDS had 1 irritation on both sides, 16 had no irritation on 0.3% (w/w) mangosteen nanoparticle loaded gel but irritated to 2.5% SDS and 3 participants had no irritation on both sides , 0.6% (w/w), 1.2% (w/w) of mangosteen nanoparticle loaded gal had significant different in the irritation test.

20 participants who applied 0.6% (w/w) mangosteen extract nanoparticle gel had 1 irritation on both side, 16 participants had no irrititaion on 0.6% (w/w) mangosteen nanoparticle loaded gel side but irritate on positive control side. 3 participants had no irritation on both sides.

20 participants who applied 1.2% (w/w) mangosteen nanoparticle loaded gel had 0 participant who had irritation form 1.2% (w/w) mangosteen nanoparticle loaded gel, 17 participants had irritation to positive control 2.5% SDS but no irritation side effect on 1.2% (w/w) magnosteen nanoparticle loaded gel and 3 participants who had no irritation on both sides.

3.2.3.2 Repeated open application test (use test)

Repeated open application test (use test) can test for the application to the skin with expose to the air, environment in the same condition as real application of the mangosteen extract nanoparticle gel. By using 0.5% (w/w) mangosteen nanoparticle loaded gel applied to the forearm of the same 20 participants twice daily for 14 days and follow up for observing sign of skin irritation at day 1,2,3,7, and 14 by dermatologists. The result is shown in the following table;

Table 3.7 Showing the side effects in the volunteers

Side effects	**Numbers(N)**	
	Yes	**No**
Erythema	-	20
Itching	-	20
Edema	-	20
Burning	-	20
Vesicle/Pustule	-	20

The side effects were evaluated by dermatologist and the result found that there was only 1 volunteers that irritated from the open application test at day 7 after application with only doubtful result and other volunteers did not have any irritation after the application of 0.5% (w/w) mangosteen nanoparticle loaded gel in the forearm twice daily for 14 days which mimic the real everyday use of the 0.5%(w/w) mangosteen nanoparticle loaded gel in the real situation.

CHAPTER 4
RESEARCH METHODOLOGY

4.1 Study design

Double-blinded, randomized controlled trial, split-face study.

4.2 Study population

Thai males and females subjects in the age of 18-40 years old with mild to moderate acne vulgaris (from GAGS) who wants to treat their acne condition at Lion Supannahong Foundation Clinic, Mahathun Building, Plenjit, Bangkok.

4.3 Sample Size

4.3.1 Sample size determination

The sample size was calculated from the formula of within individual sample.

By repeated ANOVA measurement, within factors analysis.

Effect size = 0.025

Power = 95%

Sample Size = 32 + drop out 20% = 40

4.3.2 Data Collection

1. baseline characteristics
2. sex; male, female
3. age
4. lesion counts total, non-inflammatory and inflammatory acne
5. GAG score mild = 1, moderate = 2, severe =3, very severe = 4
6. Porphyrin reduction (%)

4.4 Selection Criteria

4.4.1 Inclusion criteria

4.4.1.1 Healthy females and males in the age of 18-40 years.

4.4.1.2 Inform consent was done by read and signed with all patients before enrolling the study.

4.4.1.3 Volunteers who have mild to moderate acne vulgaris calculated the scores from the global acne grading system.

4.4.2 Exclusion criteria

4.4.2.1 Pregnancy

4.4.2.2 Breast feeding

4.4.2.3 Subject undertaken topical and systemic treatment for acne within 2 weeks of enrollment.

4.4.2.4 Subject undertaken topical corticosteroids, laser and light therapy, peeling, dermabrasion within 4 weeks.

4.4.2.5 Subjects who use systemic retinoids within 6 months.

4.4.2.6 Prohibited enrollment of patients with severe acne requiring isotretinoin or other conditions that requiring interfering treatment.

4.4.2.7 Severe systemic disease or diseases of the facial skin other than acne.

4.4.2.8 History of photosensitivity

4.4.3 Discontinuation criteria

4.4.3.1 Dramatically change in acne symptoms such as severe acne, acne fulminans that requiring isotretinoin or other treatments assessed by dermatologists.

4.4.3.2 Subjects who are unwilling to unable to comply with the study requirements.

4.4.3.3 Subjects who are currently enrolling in any other clinical study.

4.4.3.4 Subjects who take oral contraceptive pills before entry to the trial are allowed to continue their oral contraceptive pills without changing the drugs and dosage.

4.5 Study location

Lion Suphannahong Foundation Clinic, Mahathun Building, Plenjit, Bangkok.

4.6 Study Procedures

4.6.1 Application methods

Patients were applied with 2.5% benzyl peroxide on the entire face before facial washing in the morning for 5 minutes once daily (estimated 1 gram of 2.5% benzyl peroxide per entire face per day). Patients washed their face with cleanser in the morning (Cleanser and sunscreen from Pan Rajdhevee Group Co., Ltd., Bangkok, Thailand). Then apply 0.3% (w/w) alpha-mangosteen extract nanopartical loaded gel one side of the face and 1% topical clindamycin gel on another side twice daily.

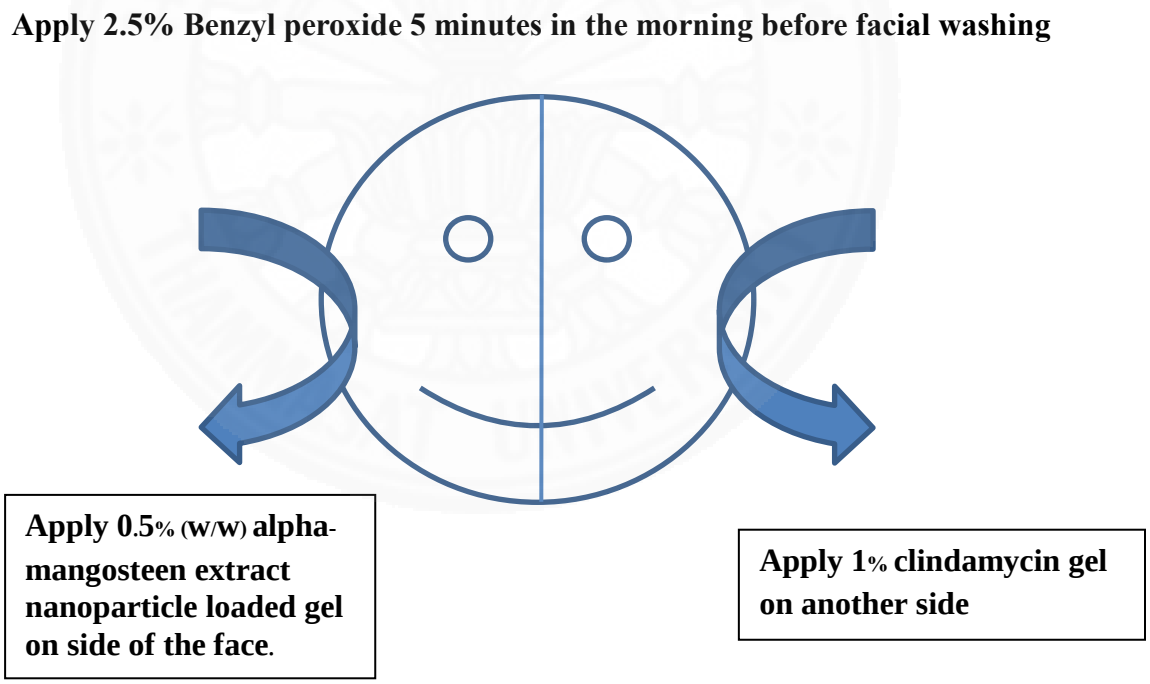


Figure 4.1 Research methodology

4.6.2 Time schedule of research subjects

The experiment was conducted for 12 weeks with evaluation at week 0, 2, 4, 8, and 12. At each visit, the tolerability, adverse effects, and quality of life evaluation form will be assessed by subjects and investigators.

On the third day and 1 week after starting the experiment, volunteers were asked for irritation via telephone.

Table 4.1 The global acne grading system

The global acne grading system	
Location	Factor
Forehead	2
Right cheek	2
Left cheek	2
Nose	1
Chin	1
Chest and upper back	3

No acne lesion = 0, Comedones = 1, Papules = 2, Pustules = 3, Nodules = 4

Global Acne Grading System

Scores = Factor * Grading (0-4)

Mild acne = 1-18

Moderate acne = 19-30

Severe acne = 31-38

Very severe = > 39

4.7 Outcome Measurement

4.7.1 Wood's lamp examination

Wood's lamp test is the tool for diagnosis of skin disease such as dermatophyte, tinea capitis, erythrasma, vitiligo or melasma. Wood's lamp will emit black light in ultraviolet A spectrum (320-450 nm. peak 365 nm.) Porphyrins are producted by Propionibacterium acnes and can be seen indirectly by Wood's lamp fluorescence light as orange-red dots on patiens skin. Porphyrins will absorp fluorescent light from Wood's lamp light ,absorb the invisible light and emit the visible light to the examinar's eyes.

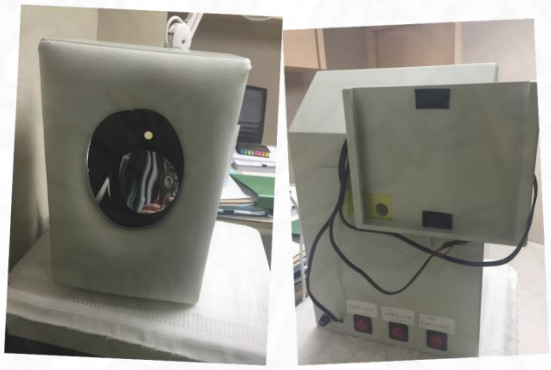


Figure 4.2 Wood's lamp examination instrument

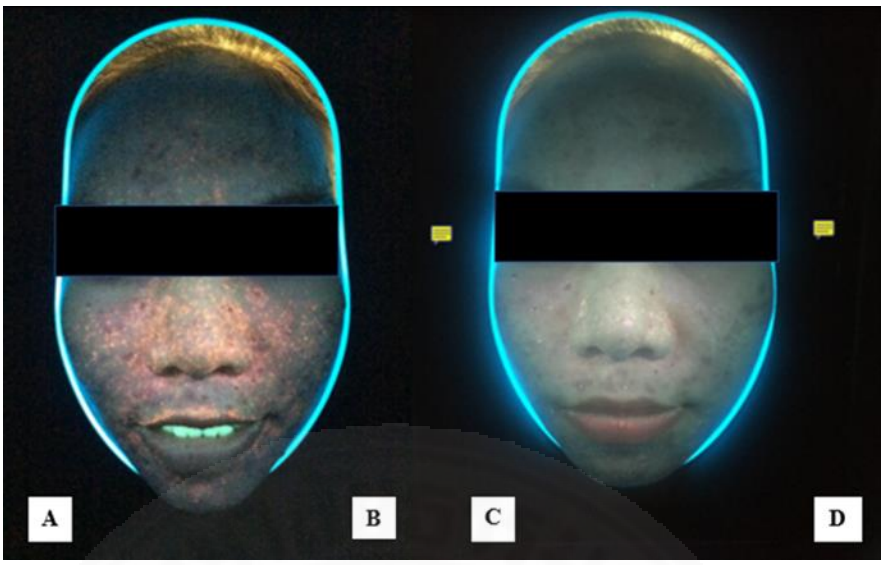


Figure 4.3 Wood's lamp examination of patient 2 showing porphyrin reduction in both sides after 12 weeks. (A-C) 1% clindamycin-treated side from baseline to week 12. (B–D) 0.5% (w/w) mangosteen nanoparticle loaded gel treated side from baseline to week 12.

4.7.2 Acne lesion counts

Acne lesion counts is the record of a number of acne lesions on both side of the face of the patients by dermatologists. Blinded-dermatologist counted the amount of acne lesion on each side in every follow up visit at week 0, week 2, week 4, week 8 and week 12 and record in the chart record. Patients were asked about the adverse effects in everytime they came in the clinic and were recorded on the adverse effects form.

4.7.3 Photograph and Antera 3D

Patients were taken photograph in every follow up visit in 3 positions. Straight face, left 45 degree and right 45 degree. (Canon EOM M10). Subjects were taken Antera 3D photograph on both of their cheeks at week 0, week 2, week 4 and week 12 of the follow up visit at the Lion Supannahongsa Clinic.

4.7.4 Paitents satisfaction scores

Patients were asked on their satisfaction scores in both sides after using 0.5% (w/w) mnagosteen nanoparticle loaded gel and 1% clindamycin in the last

time of the follow up at week 12. Patients were asked “Considering the facial area treated, have you notice any improvement of acne lesions on the mangosteen nanoparticle loaded gel side.” “Considering the facial area treated, have you notice any improvement of acne lesions on the 1% clindamycin side.” 0-2 equal little or no improvement, 3-4 equal some improvement, 5-6 equal fair improvement, 7-8 equal good improvement and 9-10 equal exellent improvement.

4.8 Follow up plan

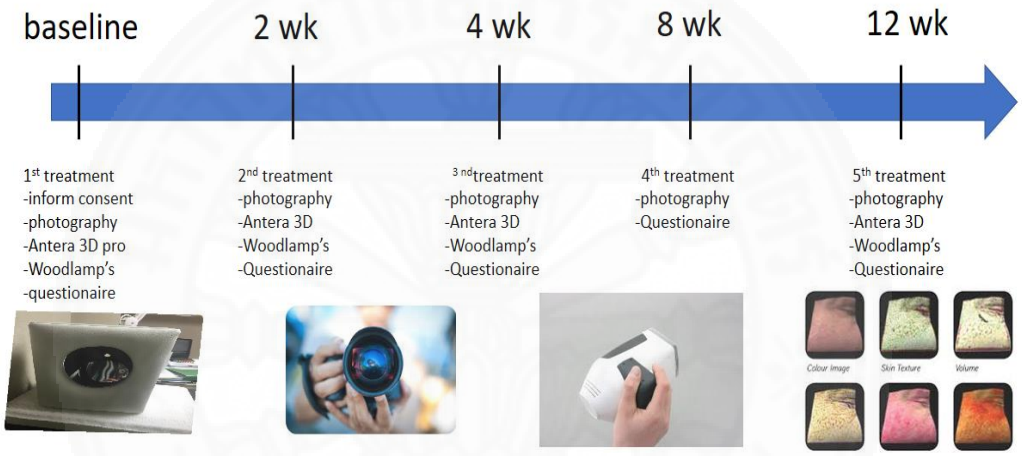
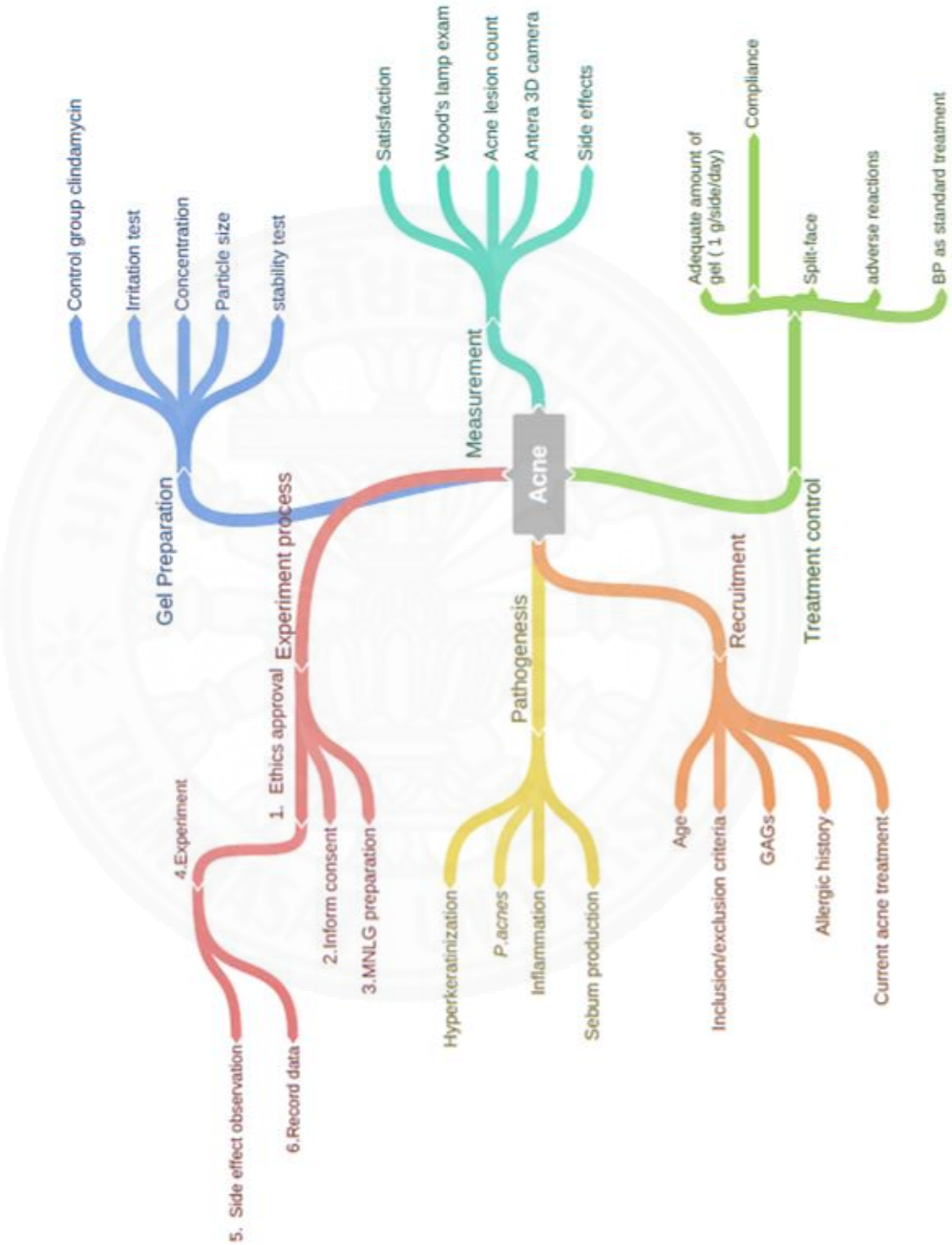


Figure 4.4 Follow up plan

4.9 Conceptual framework



CHAPTER 5
RESULTS

5.1 Dermographic data

Demographic information: twenty-eight, 24 females (85.7%) and 4 males (14.3%), with mild to moderate acne vulgaris according to guildline management from table 1.1 with global acne grading system scored between 1-30 were enrolled in the study. Patients randomly applied 0.5% alpha-mangosteen nanoparticle loaded gel and 1% clindamycin gel to one side of the face randomly for 12 weeks

There were 4 missing patients from this clinical study during 12 weeks. The details of the demographic data were revealed in table 5.1

Table 5.1 Demographic data

Variable	Statistic
Age (years)	
mean ± SD.	25.14 ± 5.86
range	[18 - 39]
Gender, n(%)	
Female	24 (85.7%)
Male	4 (14.3%)

Variables	Baseline		Week 12		p-value[(w)]
	n	Mean ± SD	n	Mean ± SD	
GAGs score	28	15.43 ± 5.96	27	15.33 ± 6.19	0.103
Fitzpatrick skin type	28	3.79 ± 0.63	27	3.78 ± 0.64	1

Values presented as mean ± SD. P-value corresponds to Paired t-test. [(w)] Comparison with in group.

According to table 5.1 that demonstrated the demographic data of the subjects, most of the subjects were female (85.7%) more than male (15%). The mean age was 25.14 ± 5.86 years old (Range between 18-40 years). All subjects were

evaluated for the severity of the global acne grading system. The means of the Global acne grading system scores in participants is 15.43 ± 5.96 at baseline and 15.33 ± 6.19 in the end of the study. According to global acne grading system scores 1-18 is considered mild acne and 19-30 is considered moderate acne in severity. The mean score of Fitzpatrick skin type in this clinical study is 3.79 ± 0.63 at baseline and 3.78 ± 0.64 in the end of the study.

5.2 Clinical evaluation for acne lesion count

All enrolled subjected had been taken photograph at straight face, and 45 degree left and right. Subjects were evaluated for non-inflammatory and inflammatory acne by acne lesion counts in each follow-up visit by dermatologist (not by photograph). Clinical visit was scheduled at week 0, week 2, week 4, week 8 and week 12. Subjects were also evaluated for porphyrin reduction by Wood's lamp examination and were grading by three blinded physicians and were evaluated for post acne erythema and post inflammatory hyperpigmentation by Antera 3D.

Antera 3D is the three dimension analysis of the skin texture, wrinkles, scar, pore size and also multianalysis of the skin color by analyzing of hemoglobin and melanin index of the individual patients.

According to Table 5.2 demonstrates the comedone lesion counts of the participants at each follow up visit. Clinical visits were scheduled at the baseline, week 2, week 4, week 8 and week 12. The collected datas of comedone lesions were counted by physicians at Lion Supannahongsa Clinic at each visit as shown in table 5.1 and the statistical analysis was evaluated by Wilcoxon signed ranks test comparison with in groups, and comparison between 0.5% MNLG side and 1% clindamycin side.

From appendix F table 5, total comedone lesion in 0.5% MNLG side at baseline equal 570 comedone lesion and decrease down to 170 after application of 0.5% MNLF for 12 weeks. In 1% clindamycin gel side at baseline had 450 comedone lesions and decrease down to 220 after application of 1% clindamycin for 12 weeks.

513-170/513 * 100 = 55.33% decreasing in comedone lesion in MNLF side. 450-170/450 * 100 = 62.22% decreasing in comedone lesion in 1% clindamycin side.

Table 5.2 Comedone reductions at baseline, week 2, week 4, week 8, and week 12 compared with 1% clindamycin for the treatment of mild to moderate acne vulgaris

Variables	Mangosteen	Clindamycin	p-value[(b)]
	Mean ± SD.	Mean ± SD.	
Comedone			
Baseline	6.07 ± 5.52	6.18 ± 4.7	0.877
Week 2	3.68 ± 3.51*	3.82 ± 3.4*	0.691
Week 4	4.12 ± 3.09*	3.73 ± 3.4*	0.462
Week 8	3.04 ± 2.2*	3.13 ± 2.94*	0.846
Week 12	2.24 ± 1.92*	2.48 ± 1.96*	0.513

Values presented as mean ± SD. P-value corresponds to Paired t-test. [(w)] Comparison with in group, [(b)] Comparison between groups.

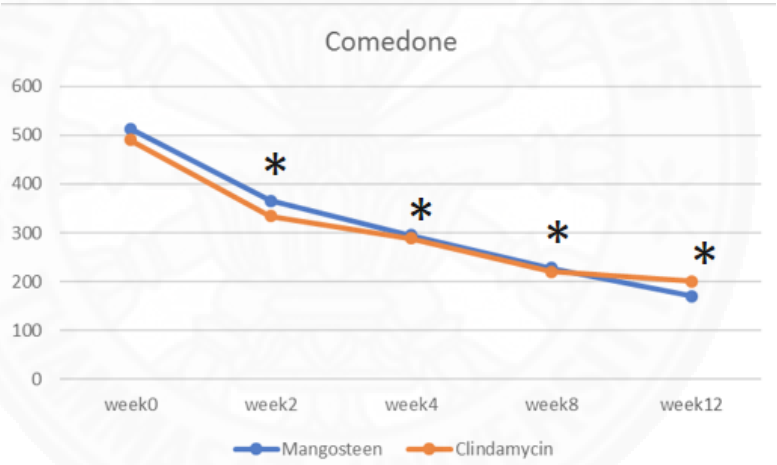


Figure 5.1 Chart showing the reduction of comedone lesions.

From the figure 5.1 showing the graph of the reduction of comedone lesions.

The blue line represent the comedone reduction in the 0.5% (w/w) mangosteen nanoparticle loaded gel side and the orange line represent the comedone reduction in 1% clindamycin side. Both sides showing significant reduction in comedone lesion after week 2 of the application and continuously significant until the end of the study.

The median counts of the facial comedone for mangosteen group on different weeks are outlined in Table 5.2 the mean reduction of comedone in mangosteen side from baseline to week 12 is from 6.07 ± 5.52 to 2.24 ± 1.92 (P = 0.001). Whereas the mean reduction of comedone in 1% clindamycin side from baseline to week 12 is from 6.18 ± 4.7 to 2.48 ± 1.96 ($P < 0.001$).

Between group analysis 0.5% (w/w) alpha-mangosteen nanoparticle loaded gel side and 1% clindamycin side were no significant differences between the two groups with regard to the changes in the mean number of both comedone acne lesions (P = 0.843). (see Table 5.2)

Table 5.3 Pustule reduction at baseline, week 2, week 4, week 8, and week 12 compared with 1% clindamycin for the treatment of mild to moderate acne vulgaris.

Variables	Mangosteen	Clindamycin	p-value[(b)]
	Mean± SD.	Mean ± SD.	
Baseline	1.82 ± 1.96	2.18 ± 1.98	0.331
Week 2	1.04 ± 1.82	1.04 ± 1.4*	1.000
Week 4	1.27 ± 1.34	0.69 ± 1.01*	0.070
Week 8	0.63 ± 1.1*	0.58 ± 0.97*	0.883
Week 12	0.2 ± 0.41*	0.33 ± 0.76*	0.417

Values presented as mean ± SD. P-value corresponds to Paired t-test.[(w)] Comparison with in group, [(b)] Comparison between groups.

The mean reduction of pustules in 0.5% (w/w) mangosteen nanoparticle loaded gel side is form baseline to week 12 is from 1.82 ± 1.96 to 0.2 ± 0.41 (P = 0.001). Whereas in 1% clindamycin is from baseline to week 12 is from 2.18 ± 1.98 to 0.33 ± 0.76 ($P < 0.001$). Between group analysis 0.5% (w/w) alpha-mangosteen nanoparticle loaded gel side and 1% clindamycin gel side were not statisticaly significant differences between the two groups with regard to the changes in the mean reduction of both pustule acne lesions (P = 0.48) at week 12 of the study. (see Table 5.3)

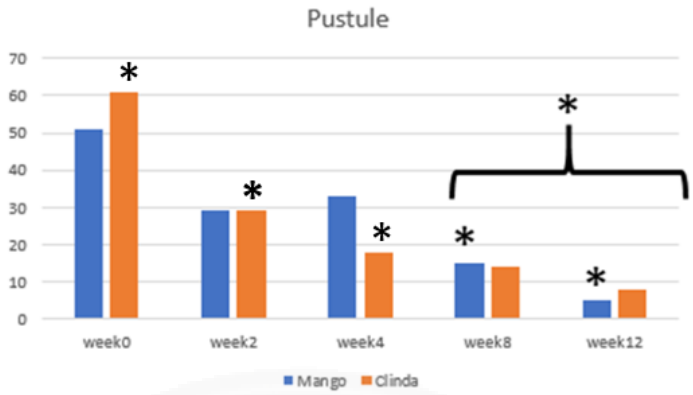


Figure 5.2 Chart showing the reduction of pustule lesions.

From figure 5.2 showing the bar chart of the reduction of pustule lesions. The blue color represent 0.5% (w/w) mangosteen nanoparticle loaded gel. The orange color represent 1% clindamycin side. The result showed that 0.5% (w/w) mangosteen nanoparticle loaded gel side significantly decrease in pustule reduction at week 8 and week 12 of the study. Whereas, 1% clindamycin gel side has statistically significant different in the reduction of pustule lesion since week 2 and continuously decrease until week 12 of the study.

Table 5.4 Median scores of papule lesion counts

Variables	Mangosteen	Clindamycin	p-value[(b)]
	Mean± SD.	Mean ± SD.	
Baseline	2.39 ± 2.7	2.61 ± 2.48	0.541
Week 2	1.86 ± 1.67	1.75 ± 1.69*	0.656
Week 4	1.92 ± 1.26	1.73 ± 1.66	0.346
Week 8	1.08 ± 1.02*	1 ± 0.93*	0.679
Week 12	1.12 ± 1.3	1.36 ± 1.44*	0.471

Values presented as mean ± SD. P-value corresponds to Paired t-test.[(W)] Comparison with in group, [(b)] Comparison between groups.

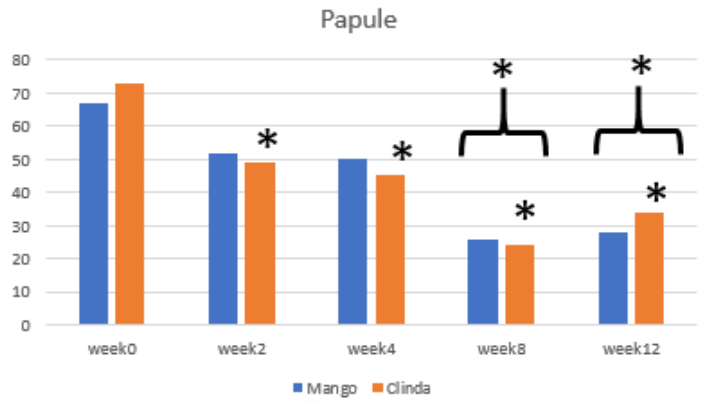


Figure 5.3 Chart showing the reduction of the papule lesions.

From figure 5.3 showing the bar chart of the reduction of papule lesions. The blue color represent 0.5% (w/w) mangosteen nanoparticle loaded gel. The orange color represent 1% clindamycin side. The result showed that 0.5% (w/w) mangosteen nanoparticle loaded gel side significantly decrease in papule reduction at week 8 and week 12 of the study. Whereas, 1% clindamycin gel side has statistically significant different in the reduction of pustule lesion since week 2 and continuously decrease until week 12 of the study.

The mean count of the facial papules for 0.5% (w/w) mangosteen nanoparticle loaded gel group on different weeks are outlined in Table 5.4. Within group analysis of papule reduction in 0.5%(w/w) mangosteen nanoparticle loaded gel group from baseline to the end of week 12 is from the mean of 2.39 ± 2.7 to 1.12 ± 1.3 at week 12. Unfortunately there is not significant different in 0.5% (w/w) mangosteen nanoparticle loaded gel side in the mean reduction (P = 0.069). Whereas, in 1% clindamycin gel side shows statistical significant in papule lesion count at week 8 and week 12. The mean reduction of pustules form baseline to week 12 in the 1% clindamycin gel side is from 2.61 ± 2.48 to 1.36 ± 1.44 at week 12 (P = 0.005).

Between group analysis 0.5% (w/w) mangosteen nanoparticle loaded gel side and 1% clindamycin gel side were no significant differences between the two groups with regard to the changes in the mean number of both papule acne lesions (P = 0.597) at 12 weeks of the study. (see Table 5.4)

Table 5.5 Median scores of nodule lesion counts

Variables	Mangosteen	Clindamycin	p-value[(b)]
	Mean± SD.	Mean ± SD.	
Baseline	1.79 ± 2.56	1.39 ± 1.42	0.367
Week 2	0.89 ± 1.5	1.14 ± 1.56	0.336
Week 4	1.08 ± 1.41	1.38 ± 1.96	0.381
Week 8	1.46 ± 1.72	1.54 ± 2.3	0.759
Week 12	0.76 ± 1.2*	0.8 ± 1.22*	0.908

Values presented as mean ± SD. P-value corresponds to Paired t-test.[(w)] Comparison with in group, [(b)] Comparison between groups.

According to Table 5.5 demonstrated the nodule lesion counts of the participants at each follow up visit. Clinical visits were scheduled at the baseline, week 2, week 4, week 8 and week 12. The collected data of nodule lesions were counted by physicians at Lion Supannahongsa Clinic at each visit as shown in table 5.5 and the statistical analysis was evaluated by Wilcoxon signed ranks test comparison with in groups, and comparison between 0.5% MNLG and 1% clindamycin.

The mean counts of the nodules for 0.5% MNLG on different weeks are outlined in Table 5.5. Within group analysis of nodule reduction in 0.5% mangosteen nanoparticle loaded gel group from baseline to the end of week 12 is from the mean of 1.79 ± 2.56 to 0.76 ± 1.2 in the mean reduction (P = 0.013). The mean reduction of nodule was not statistically significant different in the mean reduction in 0.5% (w/w) mangosteen nanoparticle loaded gel side at 2, 4 and 8 week but at week 12 the mean reduction of nodule was statistically significant in the reduction of nodules in 0.5% (w/w) mangosteen nanoparticle loaded gel side (P = 0.013).

The mean reduction of nodules form baseline to week 12 is from 1.39 ± 1.42 to 0.8 ± 1.22 at week 12 of the study (P = 0.021). Acne reduction in 1% clindamycin gel side was not statistically significant different in the nodule reduction at week 2, 4 and 8. However, 1% clindamycin gel side showed statistical significant different in nodule lesion only at week 12 of the study.

Between group analysis of 0.5% (w/w) mangosteen nanoparticle loaded gel side and 1% clindamycin gel side were not statistical significant differences between the two groups with regard to the changes in the mean number of both nodule acne lesions at 12 weeks of the study(P = 0.949). (see Table 5.5)

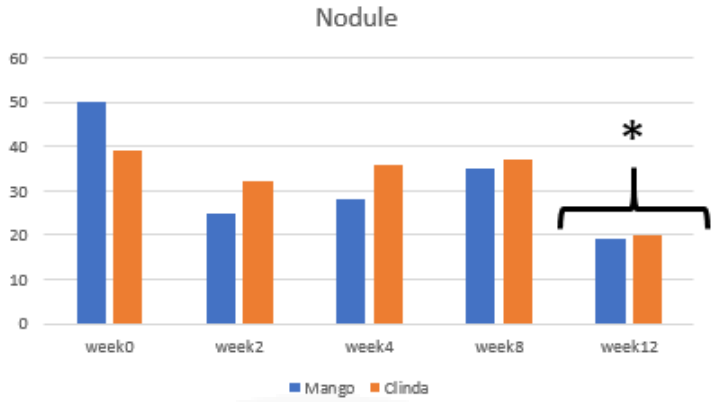


Figure 5.4 Chart showing the reduction of the nodule lesions.

From figure 5.4 showing the bar chart of the reduction of nodule lesion. The blue color represent 0.5% (w/w) mangosteen nanoparticle loaded gel. The orange color represent 1% clindamycin side. The result showed that 0.5% (w/w) mangosteen nanoparticle loaded gel side significantly decrease in nodule reduction at week 12 of the study. Likewise, 1% clindamycin gel side had statistically significant different in the reduction of nodule lesion only at week 12 of the study.

Table 5.6 Mean scores of inflammatory acne lesion counts

Variables	Mangosteen	Clindamycin	p-value[(b)]
	Mean ± SD.	Mean ± SD.	
Inflammatory			
Baseline	18.32 ± 13.45	17.5 ± 12.87	0.429
Week 2	13.07 ± 9.33*	11.93 ± 8.77*	0.113
Week 4	11.35 ± 10.45*	11.12 ± 7.94*	0.821
Week 8	9.5 ± 8.57*	9.17 ± 8.14*	0.716
Week 12	6.8 ± 6.34*	8.04 ± 9.52*	0.401

Values presented as median (IQR). P-value corresponds to Wilcoxon signed ranks test. (w) Comparison with in group, (b) Comparison between groups.

According to Table 5.6 demonstrated the total inflammatory acne lesion of the participants at each follow up visit. Clinical visits were scheduled at the baseline, week 2, week 4, week 8 and week 12. The collected data of inflammatory lesions were counted by physicians at Lion Supannahongsa Clinic at each visit as shown in table 5.6 and the statistical analysis was evaluated by Wilcoxon signed ranks test comparison

with in groups, and comparison between 0.5% mangosteen nanoparticle loaded gel group and 1% clindamycin gel group.

The percent reduction of the inflammatory acne lesion count in the mangosteen side from baseline to 12 week was 67.05% where as the inflammatory acne lesion count in the clindamycin side from baseline to 12 week was 64.16%.

From appendix F table 10, total inflammatory lesion count in MNLF side at the baseline equal 170 and after 12 week of the study total inflammatory count decrease down to 56 inflammatory lesion. In 1% clindamycin side at the baseline equal 173 inflammatory lesion and decrease down to 62 after application of 1% clindamycin for 12 weeks.

170-56/170 * 100 = 67.05% decreasing in inflammatory lesion in MNLF side. 173-62/173 * 100 = 64.16% decreasing in inflammatory lesion in 1% clindamycin side.

The mean lesion count of the facial total inflammatory acne lesions for 0.5% (w/w) mangosteen nanoparticle loaded gel group on different weeks are outlined in Table 5.6, within group analysis of inflammatory acne lesion in 0.5% (w/w) mangosteen nanoparticle loaded gel group from baseline to the end of week 12 is from the mean of 18.32 ± 13.45 to 6.8 ± 6.34 in the mean reduction ($P < 0.001$).

Acne reduction in 1% clindamycin gel side showed statistical significant in reduction of the inflammatory acne in every visit from week 2 to week 12. The mean reduction of inflammatory acne in 1% clindamycin gel side is form 17.5 ± 12.87 at week 0 to 8.04 ± 9.52 at week 12 ($P < 0.001$).

Between group analysis 0.5% MNLG and 1% clindamycin side were no significant differences between the two groups with regard to the changes in the median number of both inflammatory acne lesions ($P = 0.466$) at 12 weeks of the study. (see Table 5.6)

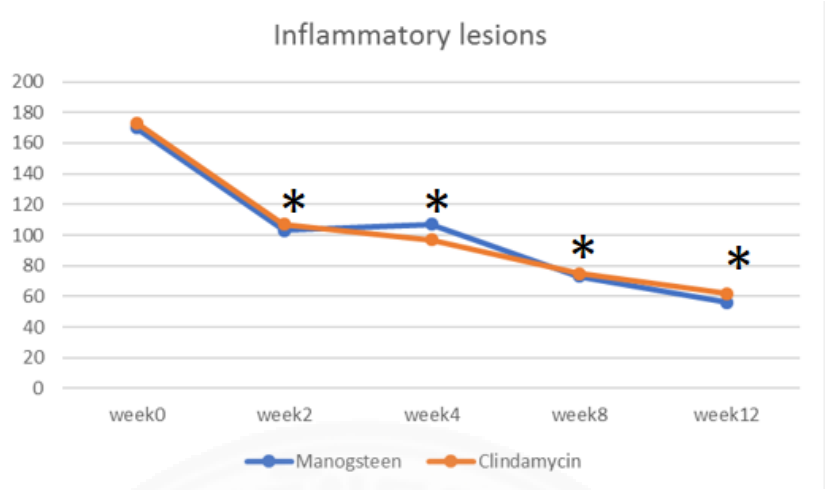


Figure 5.5 Chart of inflammatory acne lesion counts

From figure 5.5 showing the bar chart of the reducion of nodule lesion. The blue color represent 0.5% (w/w) mangosteen nanoparticle loaded gel. The orange color represent 1% clindamycin side. The result showed that 0.5% (w/w) mangosteen nanoparticle loaded gel side significantly decrease in total inflammatory acne lesion significantly starting at week 2 and continue to week 12 of the study. In the 1% clindamycin gel side also had significant reduction in total inflammatory acne from week 2 to week 12 of the study. Both sides had similar in the slope of the graph which were not statistically significant differences.

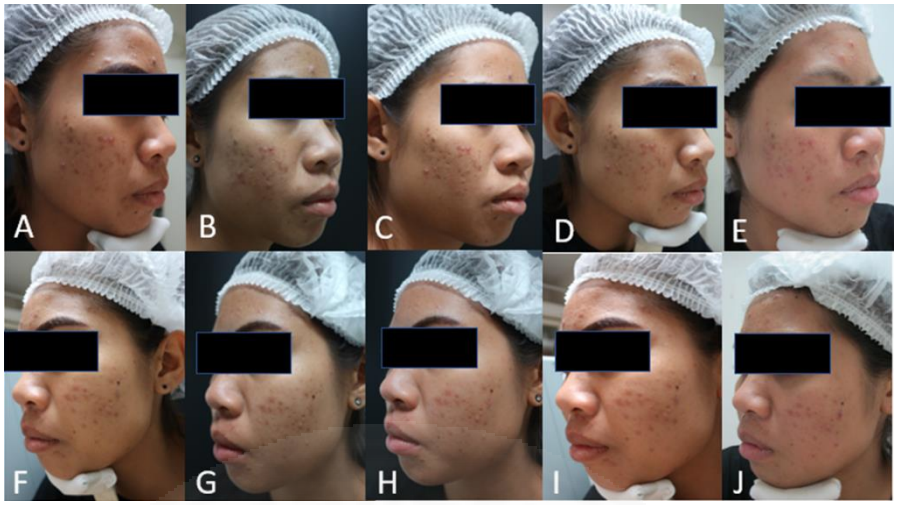


Figure 5.6 Evaluation of 0.5% (w/w) mangosteen nanoparticle loaded gel effect on acne patient 1 using the split face study: (A-E) 1% clindamycin-treated side from baseline, week 2, week 4, week 8 to week 12. (F – J) 0.5% MNLG treated side from baseline, week 2, week 4, week 8 to week 12.

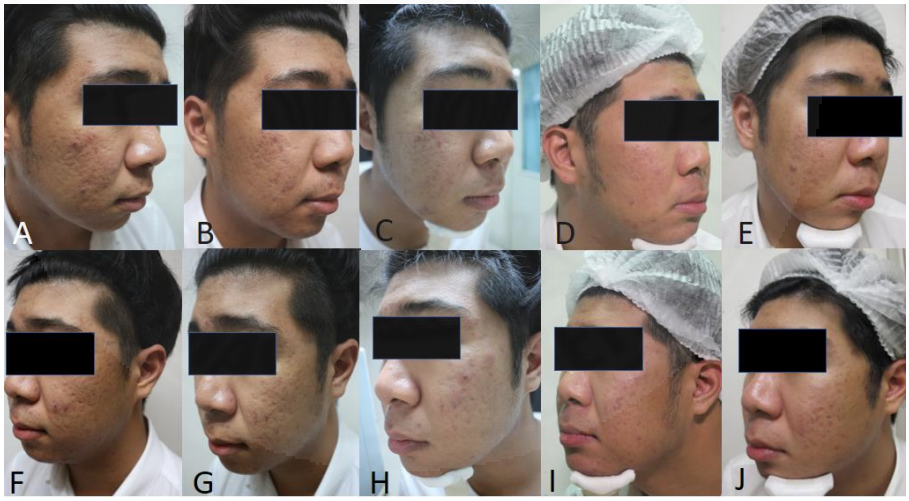


Figure 5.7 Evaluation of 0.5% (w/w) mangosteen nanoparticle loaded gel effect on acne patient 2 using the split face study: (A-E) 1% clindamycin-treated side from baseline, week 2, week 4, week 8 to week 12. (F – J) 0.5% MNLG side from baseline, week 2, week 4, week 8 to week 12.

Table 5.7 The median scores of post-acne erythema from Antera 3D

Variables	Mangosteen	Clindamycin	p-value[(b)]
	Median (IQR)	Median (IQR)	
PAE			
Baseline	1.51 (1.39, 1.77)	1.55 (1.33, 1.71)	0.206
Week 4	1.71 (1.35, 1.9)	1.66 (1.34, 1.77)	0.023*
Week 12	1.44 (1.23, 1.53)*	1.46 (1.25, 1.6)*	0.587

According to Table 5.7 demonstrated that post acne erythema index of the participants at each follow up visit evaluated from Antera 3D biometric instruction. Clinical visits were scheduled at the baseline, week 2, week 4, and week 12. Clinical study of post acne erythema index scores at each visit were shown in table 5.7 and the statistical analysis was evaluated by Wilcoxon signed ranks test comparison within group, and comparison between 0.5%(w/w) mangosteen extract nanoparticle loaded gel group and 1% clindamycin group analysis.

Figures 5.3 showing the post acne erythema on 0.5% (w/w) mangosteen nanoparticle loaded gel group and 1% clindamycin group. Both sides were not statistically significant in decreasing post acne erythema at forth week but after application for 12 week both 0.5% (w/w) mangosteen nanoparticle loaded gel and 1% clindamycin group showing statistically significant decreased in post acne erythema.

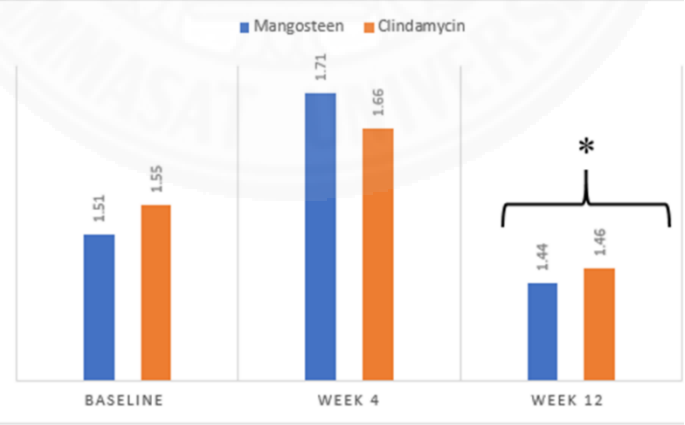


Figure 5.8 Bar chart of post acne erythema showing significant result at week 12 after application on both sides.

According to table 5.7, within group analysis of 0.5% (w/w) mangosteen nanoparticle loaded gel group and 1% clindamycin gel group at week 4 and week 12. Both MNLG and 1% clincamycin group at week 4 did not show improvement in acne

redness reduction compared with baseline. However, both group showed statistically significant reduction in the post acne erythema at week 12. The mean erythema index scores of the post acne erythema for 0.5% (w/w) mangosteen nanoparticle loaded gel group on different weeks are outlined in Table 5.7. Within group analysis of post acne erythema in the MNLG group from baseline to the end of week 12 is from the median of 1.51 to 1.44 in the median reduction ($P < 0.001$). The median reduction of post acne erythema is statistically significant in the reduction in MNLG side at week 12 of the experimental study.

Acne reduction in 1% clindamycin side showed statistical significant in post acne erythema reduction at week 12 but not statistically significant in the median reduction at week 4. The median reduction of post acne erythema index scores from Antera 3D biometric assessment form baseline to week 12 is from 1.55 to 1.46 at week 12 ($P < 0.027$).

Between group analysis MNLG side and 1% clindamycin side were no significant difference between the two groups with regard to the changes in the median number of both inflammatory acne lesions ($P = 0.587$) at 12 weeks of the study. (see Table 5.7)

Figure 5.4 showing Antera 3D biometric assessment picture of the patient's cheek. Acne erythema index was calculated by the hemoblobin index level. Picture in the left side showing the baseline post acne erythema of the patient and the picture on the right side showing decrease in post acne erythema of the same patient at week 12 after application of 0.5% (w/w) mangosteen nanoparticle loaded gel.

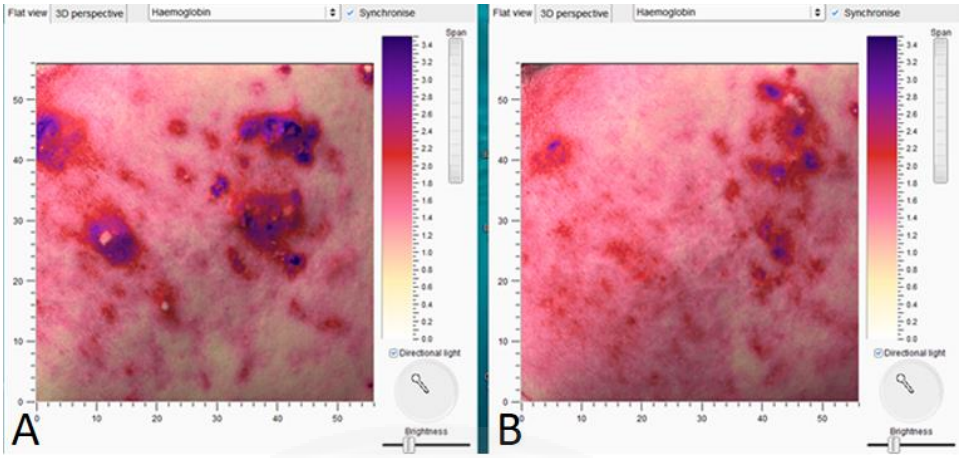


Figure 5.9 Post acne erythema evaluated from Antera 3D biometric scores system in 0.5% (w/w) magnosteen nanoparticle loaded gel side at baseline and 12 weeks after application.

Figure 5.4 showing Antera 3D biometric assessment picture of the patient's cheek. Acne erythema index was calculated by the hemoblobin index level. Picture A showing the baseline post acne erythema of the patient and picture B showing decrease in post acne erythema of the same patient at week 12 after application of 0.5% (w/w) mangosteen nanoparticle loaded gel.

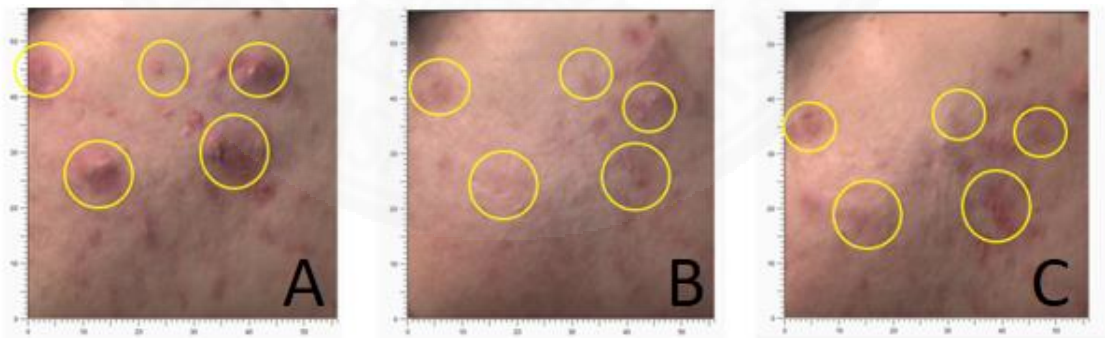


Figure 5.10 Acne leson matching at baseline and after 4 weeks of application of 0.5% (w/w) magnosteen nanoparticle loaded gel at baseline, 2 week and 4 week respectively evaluated by Antera 3D biometric camera. The yellow circle represented the inflammatory acne lesions on the cheek of the patients that decreased in their inflammation and redness after application 0.5% (w/w) magnosteen nanoparticle loaded gel for 4 weeks. (A) Patient's cheek that

applied 0.5% (w/w) mangosteen nanoparticle loaded gel side at baseline. (B) Patient's cheek that applied 0.5% (w/w) mangosteen nanoparticle loaded gel side at 2 week. (C) Patient's cheek that applied 0.5% (w/w) mangosteen nanoparticle loaded gel side at 4 week.

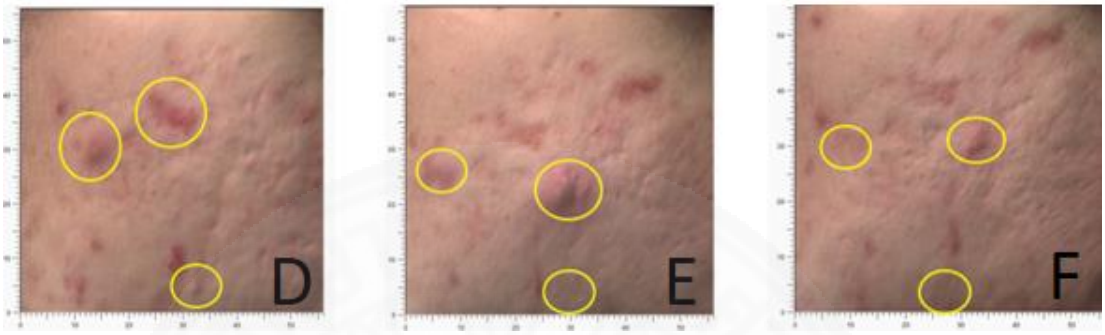


Figure 5.11 Acne leson matching at baseline and after 4 weeks of application of 1% clindamycin gel evaluated by Antera 3D biometric camera at baseline, 2 week, and 4 week respectively. The yellow circle represent inflmmatory acne lesions on the cheek of the patients on the 1% clindamycin side that decreased in their inflammation and redness after application of 1% clindamycin gel for 4 weeks. (D) Patient's cheek that applied 1% clindamycin at baseline. (E) Patient's cheek that applied 1% clindamycin side at 2 week. (F) Patient's cheek that applied 1% clindamycin side at 4 week.

Table 5.8 Median scores of post-inflammatory hyperpigmentation from biometic Antera 3D

Variables	Mangosteen (L)	Clindamycin (R)	p-value[(b)]
	Median (IQR)	Median (IQR)	
PIH			
Baseline	0.51 (0.47, 0.56)	0.51 (0.46, 0.56)	0.565
Week 4	0.52 (0.48, 0.57)	0.52 (0.49, 0.55)	0.618
Week 12	0.51 (0.45, 0.55)	0.52 (0.47, 0.55)	0.684

According to Table 5.8 demonstrated post inflammatory hyperpigmentation index scores of the participants at each follow up visit evaluated from biometric Antera

3D. Clinical visits were scheduled at the baseline, week4 and week 12. Clinical study of post inflammatory hyperpigmentation index scores at each visit were shown in table 5.8 and the statistical analysis was evaluated by Wilcoxon signed ranks test comparison within group, and comparison between 0.5% (w/w) magnosteen nanoparticle loaded gel and 1% clindamycin gel group analysis.

According to table 5.8, within group analysis of both groups did not show improvement in post inflammatory hyperpigmentation reduction at 4 week and 12 week compared with baseline. Between group analysis of both groups was also not show statistically significant improvement in post inflammatory hyperpigmentation at 4 week and 12 week compared with baseline.

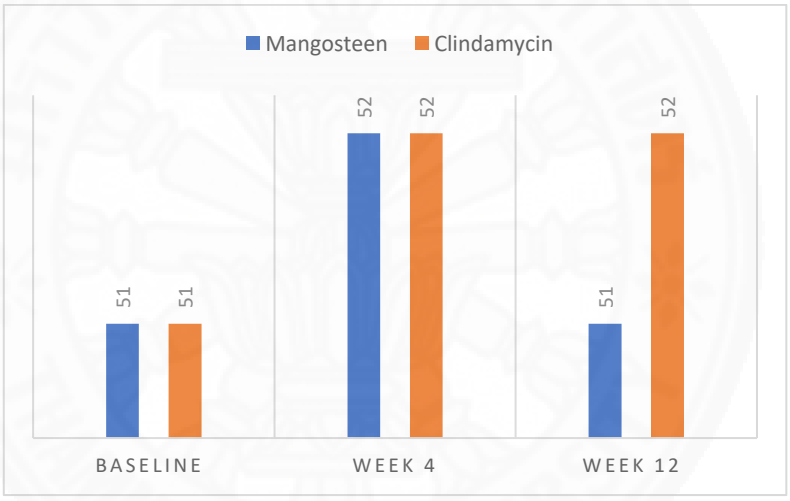


Figure 5.12 Bar chart of post inflammatory hyperpigmentation showed no statistical significant diference in the decresing the melanin pigment on patient's cheek in both 0.5% (w/w) mangosteen nanoparticle loaded gel side and 1% clindamycin gel side after application for 12 weeks.

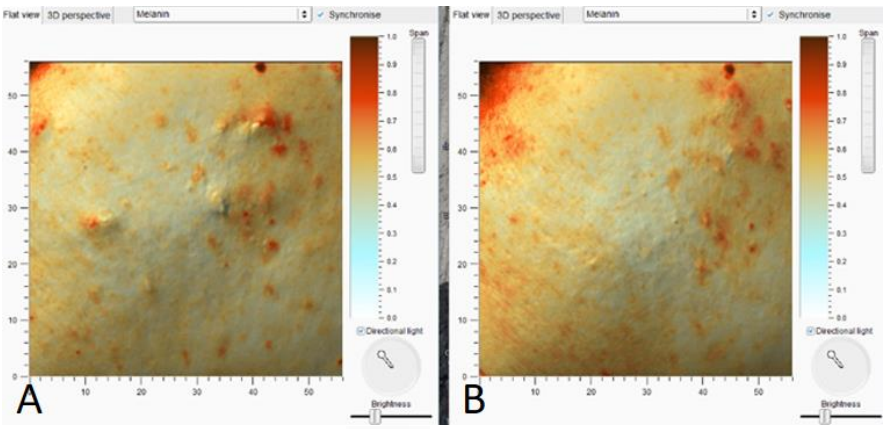


Figure 5.13 Post inflammaotry hyperpigmentation evaluated from Antera 3D biometric scores system in (A) 0.5% (w/w) mangosteen nanoparticle loaded gel side showing melanin index of post inflammatory hyperpigmentation at baseline on the patient's cheek. (B) 0.5% (w/w) mangosteen nanoparticle loaded gel side at 12 weeks after application on the same area and the same patient.

According to figure 5.8, this picture showed post inflammatory hyperpigmentation of the acne patients at baseline and at 12 weeks after applied 0.5% (w/w) mangosteen nanoparticle loaded gel. There is not statistically significant different in the decrease of the melanin index before and 12 weeks after application of 0.5% (w/w) mangosteen nanoparticle loaded gel.

Table 5.9 Wood's lamp examination grading by 3 blinded-dermatologists.

Variables	Mangosteen	Clindamycin	p-value[(b)]
	Median (IQR)	Median (IQR)	
Average Expert panel assessment			
Baseline	2.61 ± 0.58	2.55 ± 0.6	0.249
Week 12	1.27 ± 0.35*	1.25 ± 0.39*	0.649

Values presented as median (IQR). P-value corresponds to Wilcoxon signed ranks test. (w) Comparison with in group, (b) Comparison between groups.

The median count of the woodlamp's examination grading scores by 3 blinded-dermatologists for 0.5% (w/w) mangosteen nanoparticle loaded gel group on different weeks is outlined in Table 5.9. Within group analysis of the woodlamp's examination grading score reduction in 0.5% (w/w) mangosteen nanoparticle loaded gel group from baseline to the end of week 12 is from the median of 2.61 ± 0.58 to 1.27 ± 0.35 in the median reduction ($P < 0.001$). The median reduction of porphyrin grading scores is statistically significant in the reduction in 0.5% (w/w) mangosteen nanoparticle loaded gel side in every visit from week 2 to week 12.

Acne reduction in 1% clindamycin side showed statistical significant in porphyrin reduction in every visit from week 2 to week 12. The median reduction of porphyrin grading scores is form baseline to week 12 is from 2.55 ± 0.6 to 1.25 ± 0.39 at week 12 ($P < 0.001$).

Between group analysis 0.5% (w/w) mangosteen nanoparticle loaded gel side and 1% clindamycin side were not statistically significant difference between the two groups with regard to the changes in the median number of grading scores ($P = 0.649$) at 12 weeks of the study.

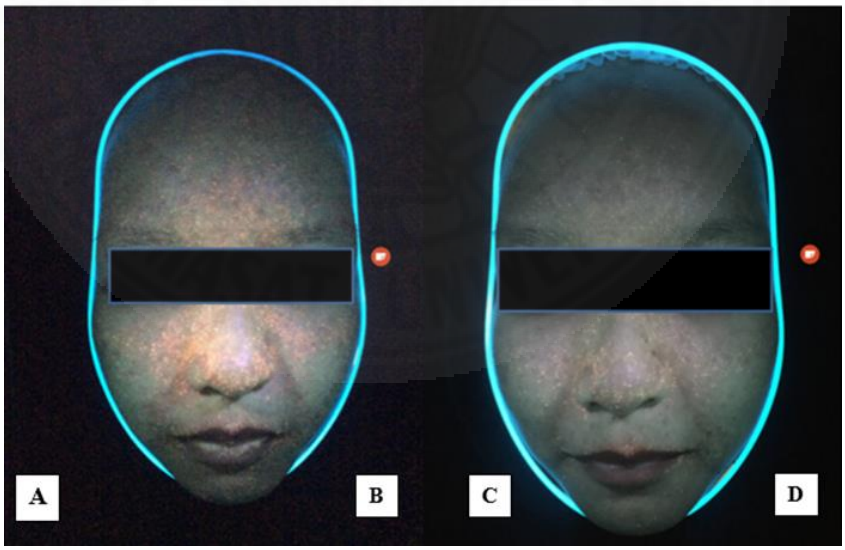


Figure 5.14 Wood's lamp examination of patient 1 showing porphyrin reduction in both sides after 12 weeks. (A-C) 0.5% MNLG-treated side from baseline to week 12. (B – D) 1% Clindamycin-treated side from baseline to week 12.

Table 5.10 The median of clinical grading by 3 blinded-dermatologists.

Variables	Mangosteen	Clindamycin	p-value[(b)]
	Median (IQR)	Median (IQR)	
Clinical grading			
Baseline	2.33 (2, 2.83)	2.33 (2, 2.67)	0.819
Week 12	1.33 (1, 1.67)*	1.67 (1.17, 2)*	0.004*

Values presented as median (IQR) and mean ± SD. P-value corresponds to Wilcoxon signed ranks test and Paired t-test. [(w)] Comparison with in group, [(b)] Comparison between groups.

The median count of the clinical examination grading scores by 3 blinded-dermatologists for 0.5% (w/w) mangosteen nanoparticle loaded gel on different weeks is outlined in Table 5.10. Within group analysis of the clinical examination grading score reduction in 0.5% (w/w) mangosteen nanoparticle loaded gel group from baseline to the end of week 12 is from the median of 2.33 to 1.33 in the median reduction ($P < 0.001$). The median reduction of clinical grading scores is statistically significant in the reduction in 0.5% (w/w) mangosteen nanoparticle loaded gel side at week 12.

Acne reduction in 1% clindamycin gel side showed statistical significant decreasing in clinical grading examination of the severity of acne at week 12. The median reduction of clinical grading scores form baseline to week 12 is from 2.33 to 1.67 at week 12 ($P < 0.001$).

Between group analysis, 0.5% (w/w) mangosteen nanoparticle loaded gel side and 1% clindamycin side were not statistically significant difference at baseline, however; at week 12 between the two groups there was statistically significant differences in clinical grading system which mangosteen side received better outcome (P=0.004).

5.3 Side effects from the clinical trial

Patient clinical side effects were evaluated by phone at day 0, day 7 after starting the experiment and every follow up visit. Patients were observed the signs of irritation such as itching, burning, versicle, edema, erythema and scaling and were noted in the patient record form.

Table 5.11 Statistical analysis of adverse reactions between 2 groups at baseline, week 2, week 4, week 8 and week 12 of the study.

Variables	Mangosteen	Clindamycin	p-value[(b)]
	n (%)	n (%)	
Baseline			
1 = mild irritation	0 (0%)	0 (0%)	1
2 = moderate irritation	0 (0%)	0 (0%)	
Negative	28 (100%)	28 (100%)	
Week 2			
1 = mild irritation	2 (7.1%)	2 (7.1%)	0.157
2 = moderate irritation	1 (3.6%)	0 (0%)	
Negative	25 (89.3%)	26 (92.9%)	
Week 4			
1 = mild irritation	2 (7.7%)	0 (0%)	0.157
2 = moderate irritation	0 (0%)	0 (0%)	
Negative	24 (92.3%)	26 (100%)	
Week 8			
1 = mild irritation	0 (0%)	0 (0%)	1
2 = moderate irritation	0 (0%)	0 (0%)	
Negative	25 (100%)	25 (100%)	
Week 12			
1 = mild irritation	0 (0%)	0 (0%)	1
2 = moderate irritation	0 (0%)	0 (0%)	
Negative	25 (100%)	25 (100%)	
p-value[(w)]			
Week 2	0.102	0.157	
Week 4	0.157	1	
Week 8	1	1	
Week 12	1	1	

Values presented as frequency (%). P-value corresponds to Wilcoxon signed ranks test. [(w)] Comparison with in group, [(b)] Comparison between groups.

The statistical analysis of the adverse reactions will be assessed in every follow up visit and the statistical analysis showed that in both 0.5% (w/w) mangosteen nanoparticle loaded gel side and 1% clindamycin gel side had no statistically significatn in their adverse reaction.

According to table 5.14 demonstrated the adverse effects of the gel. 1 = mild irritation and 2 = moderate irritation. Mild irritation was equal to slight erythema and dryness across of treatment site and moderate irritation was equal to moderate erythema and possibly spreading with barely edema at the margin papules may be present at the 0.5% (w/w) mangosteen nanoparticle loaded gel treatment side.

At week 2, in 0.5% (w/w) magnosteen nanoparticle loaded gel side had 2 mild irritation (7.1%) and 1 moderate irritation (3.6%) compared with 1% clindamycin side which had 2 mild irritation. No statistically significant different in the adverse reactions between both group during the second week ($P = 0.157$)

At week 4, in 0.5% (w/w) mangosteen nanoparticle loaded gel side had 2 mild irritation (7.7%) compared with 1% clindamycin group (0%). No statistically significant different in the adverse reactions between both sides during 12 weeks of the study. ($P = 1$).

5.4 Patient satisfaction scores

According to table 5.15 demonstrated all participant satisfaction in each follow-up visit. Afterwards, the researcher conducted to find out and compared subsequent median scores between two groups.

Table 5.12 Statistical analysis of patient satisfaction scores at each visit between the sides of application in both 0.5% (w/w) mangosteen nanoparicle loaded gel and 1% clindamycin gel side.

Variables	Mangosteen	Clindamycin	p-value[(b)]
	Median (IQR)	Median (IQR)	
Week 2	6.43 ± 0.84	6.46 ± 0.84	0.573
Week 4	6.69 ± 0.79*	6.73 ± 0.78*	0.746
Week 8	7.04 ± 0.89*	6.92 ± 0.95*	0.417
Week 12	7.21 ± 0.83*	7.17 ± 0.76*	0.714

Values presented as frequency (%). P-value corresponds to Wilcoxon signed ranks test. (w) Comparison with in group, (b) Comparison between groups.

Based on table 5.15, the median of patient satisfaction scores of both groups had significantly increased after one month of application. (Median scores 6.69 ± 0.79 in 0.5% (w/w) mangosteen nanoparticle loaded gel side after one month and median score 6.73 ± 0.78 in 1% clindamycin side after one month of application.) The highest values were at week 12 (Median scores 7.21 ± 0.83 in 0.5% (w/w) mangosteen nanoparticle loaded gel side and 7.17 ± 0.76 in 1% clindamycin side) without statistically significant difference. The median of patient satisfaction scores inclined gradually at each follow-up visit until reach the highest at the end of the follow-up visit.

The patient satisfaction scores were asked patients by dermatologists range from 1-10. Less than 3 equal little or no improvement, 4-5 equal some improvement, 5-6 equal fair improvement, 7-8 equal good improvement, 9-10 equal exellent improvement

Comparison between two groups demonstrated that 0.5% (w/w) mangosteen nanoparticle loaded gel side had higher score than 1% clindamycin-treated side at week 8 and week 12. However, there was no statistically significant different in patient's satisfaction of the use between 0.5% (w/w) mangosteen nanoparticle loaded gel and 1% clindamycin gel at the end of the study ($P = 0.714$).

CHAPTER 6
DISCUSSION AND CONCLUSION

6.1 Discussion

The study was conducted in 28 mild to moderate acne vulgaris patients during 12 weeks to evaluate acne vulgaris patients in both the clinical study by acne lesion count and clinical grading by expert panel assessment and also evaluate with biometric assessment for post acne erythema and post inflammatory hyperpigmentation by Antera 3D camera analysis and porphyrin reduction by Woodlamp's examination which can explain more information about the clinical study of 0.5% (w/w) mangosteen nanoparticle loaded gel for the treatment of acne vulgaris

For comedone reduction, porphyrin expert panel assessment showed significant reduction in the porphyrin grading which can indirectly showed the amount of *Propionibacterium acnes* as pathogenic flora inside the sebaceous follicles. It was found that (1) 0.5% (w/w) mangosteen nanoparticle loaded gel significantly reduced porphyrin red spots from the pictures of patients and also correlated with statistical significant for comedone reduction within 14 days, but no statistically significant difference when compared with clindamycin on the other side of the face as standard treatment, (2) 0.5% (w/w) mangosteen nanoparticle loaded gel significantly lessened pustule, nodule acne lesions, and post acne erythema after 3 months of application. This can explain by anti-inflammatory properties of active ingredients alpha-mangostin in 0.5% (w/w) mangosteen nanoparticle loaded gel in reducing PGE2 and TNF-α, the potent inflammatory cytokines to recruit more inflammatory cells into the lesions, and (3) from *in vitro* study of 0.5% (w/w) mangosteen nanoparticle loaded gel, the anti-oxidant properties in alpha-mangostin nanogel can reduce reactive oxygen species from inflammatory cells which can resolve inflammatory lesions faster (15).

Inflammatory lesions could be divided into 3 major types including pustular lesion, papular lesion, and nodular lesion. In the pustular lesions, 0.5% (w/w) mangosteen nanoparticle loaded gel significantly reduced the lesions at week 8 of the clinical study, which was non-inferior to the standard treatment of clindamycin. In

nodular lesion also showed statistical significant different in nodule lesion reduction at week 12. However, only papule lesions that showed no statistically significant different in the number of papule reduction at week 12 from baseline, whereas clindamycin gel demonstrated statistical significant different in the reduction of the number of papule lesion count from baseline at week 12.

0.5% (w/w) mangosteen nanoparticle loaded gel is the xanthone derivatives from mangosteen pericarp extract. For anti-microbial properties of the final concentration of 0.5% (w/w) mangosteen nanoparticle loaded gel maintains the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of 15.625 mg/ml and >250 mg/ml, respectively for *Propionibacterium acnes(17)*. In particular, the sustained release properties of liposome in nanoparticle gel can help the penetration of 0.5% (w/w) mangosteen nanoparticle loaded gel deep into the sebaceous follicles which are the homing of *Propionibacterium acnes*, and the release of active ingredient alpha-mangostin slowly from the encapsulation to reduce side effects due to irritation.

For comedone lesions, the lower number of comedones was importantly due to the reduction in the accumulation of *Propionibacterium acnes* within sebaceous follicles, indirectly by less porphyrin in Wood's lamp examination. In the meantime, 0.5% (w/w) mangosteen nanoparticle loaded gel decreased TNF-α and inflammatory cytokines that induced more recruitment of inflammatory cells into hair follicles. Whilst, 0.5% (w/w) mangosteen nanoparticle loaded gel effectively reduced inflammatory lesions at the second week compared with clindamycin gel as a standard treatment and showed no statistical significant different in the reduction of total acne lesions compared with 1% clindamycin gel.

The anti-inflammatory properties of 0.5% (w/w) mangosteen nanoparticle loaded gel in the treatment of acne vulgaris significantly improve non-inflammatory acne lesions, such as comedone and inflammatory acne lesions; namely, microcomedone stages, papulopustular lesions, nodulocystic acne lesions, and post acne erythema. For the inflammatory lesions within sebaceous glands, macrophages produce significant amounts of TNF-α and IL-1 into the lesions to recruit more neutrophils and lymphocytes, with TNF-α acting as chemoattractant. Following the anti-TNF-α properties, 0.5% (w/w) mangosteen nanoparticle loaded gel lowers the

recruitment of inflammatory cells and inflammatory cytokines, with the inhabitation of inducible nitric oxide (NO) synthase produced by macrophages (26). NO is an enzyme that has vasodilatation property to make redness in the inflammatory lesions which are the primary cause of post acne erythema. Whereas, PGE2 is inhibited by 0.5% (w/w) mangosteen nanoparticle loaded gel in vitro in *glioma cells* of the rat.(18) Consequently, alpha-mangostin from *Garcinia mangostana* pericarp extract inhibits PGE2 from inflammatory cells stimulated by LPS of *Propionibacterium acnes.* Hence, alpha-mangostin has anti-inflammatory effects from PGE2 inhibitor.

With the free radical scavenging properties, 0.5% (w/w) mangosteen nanoparticle loaded gel also decrease ROS produced by inflammatory cells. ROS sends the secondary messenger to induce the production of transcription factors such as NF-kB, AP-1 in the generating of cytokines that cause more inflammatory acnes. NF-kB is the nuclear transcription factor which in inactive state stays in the cytosol. During the active state, NF-kB is in the nucleus and acts as the transcriptional factor to produce more inflammatory cytokines and hydrolytic enzymes. However, controlling ROS reduction is very crucial in the inflammatory stages of acne lesions.

Moreover, we also evaluate post acne erythema and post inflammatory hyperpigmentation in both clinical grading by expert panel assessment from 3 evaluators and also evaluated from Antera 3D biometric camera to evaluate the hemoglobin index and melanin index reduction. Post acne erythema showed statistically significant in post-acne erythema reduction from after applying 0.5% (w/w) mangosteen nanoparticle loaded gel for 12 weeks. During the end of clinical study at week 12, both the 0.5% (w/w) mangosteen nanoparticle loaded gel and the 1% clindamycin gel side revealed a statistically significant reduction of post-acne erythema, but no statistically significant different when compared between the two groups ($P = 0.587$). Post acne erythema evaluation scores demonstrated the reduction which significantly improved from baseline to week 12 ($P < 0.001$), and post acne erythema also significantly reduce from baseline to week 12 on the side with applied clindamycin gel as standard treatment ($P = 0.027$).

The results in this study were compatible with the study by *Porntip Pan-In* et al (17). In our clinical study, 0.5% (w/w) mangosteen nanoparticle loaded gel could reduce up to 67.05% of the inflammatory acne lesions from baseline after application

for 12 weeks. Whereas, the study by *Porntip Pan-In* et al demonstrated that 1.2% (w/w) mangosteen nanoparticle loaded gel improved more than 50% of inflammatory acne lesions without serious side effects when compared with placebo. Our study yielded a newly interesting information about post acne erythema and post inflammatory hyperpigmentation treatment of acnes assessed by the biometric Antera 3D. The results also revealed that 0.5% (w/w) alpha-mangostin nanoparticle loaded gel was effective to reduce the number of comedogenic acnes and inflammatory acne lesions at the end the second week after treatment.

From the multi-center study of *Jose Alexandre de Souza Sittart* evaluated for the efficacy and safety properties of a fixed-dose combination gel with 0.1% adapalene plus 2.5% benzyl peroxide (Epiduo, Galderma laboratories, Switzerland) which is the standard treatment in mild to moderate acne vulgaris showed that 2.5% benzyl peroxide and 0.1% adapalene could decrease in the number of acne lesions and progressively reduced over the 12 weeks, reaching 73.9% reduction for inflammatory acne lesions, 73% reduction for non-inflammatory and 68.9% for total lesions in 12 weeks. (28).

0.5% (w/w) mangosteen nanoparticle loaded gel has an as anti-microbial, anti-inflammatory, and anti-oxidant agents could be another treatment of choice for physicians in patients with mild to moderate acne vulgaris. 0.5% (w/w) mangosteen nanoparticle loaded gel could also improve acne lesions in all stages and reduce the lesions in the post acne erythema by lowering the number of inflammatory cells and reducing reactive oxygen species and inflammatory. Our study showed the fascinating result of 0.5% (w/w) mangosteen nanoparticle loaded gel for post-acne erythema reduction from both clinical evaluation by the experts and biometric assessment by Antera 3D biometric camera evaluation. This will bring an initiating discovery for using of alpha-mangostin for the treatment of some erythematous inflammatory lesions.

0.5% (w/w) mangosteen nanoparticle loaded gel has an as anti-microbial, anti-inflammatory, and anti-oxidant agents could be another treatment of choice for physicians in patients with mild to moderate acne vulgaris. 0.5% (w/w) mangosteen nanoparticle loaded gel could also improve acne lesions in all stages and reduce the lesions in the post acne erythema by lowering the number of inflammatory cells and reducing reactive oxygen species and inflammatory. Our study showed the fascinating

result of 0.5% (w/w) mangosteen nanoparticle loaded gel for post-acne erythema reduction from both clinical evaluation by the experts and biometric assessment by Antera 3D biometric camera evaluation. This will bring an initiating discovery for using of alpha-mangostin for the treatment of some erythematous inflammatory lesions.

Table 6.1 Compare percent reduction between 2.5% Benzyl peroxide + 0.5% mangosteen gel, 2.5% benzyl peroxide + clindamycin, and 2.5%benzyl peroxide + 0.1% adapalene

	Comedone reduction	**Inflammatory reduction**	**Sample Size**	**Duration**	**Study design**
2.5% BP + 0.5% Mangosteen gel	66.86%	67.05%	28	12 weeks	with clindamycin
2.5% BP + Clindamycin gel	55.33%	64.16%	28	12 weeks	with mangosteen
2.5%BP + 0.1% Adapalene (28).	73.00%	73.90%	79	12 weeks	Open label study
2.5% BP +mangosteen gel (17).		>50%	10	4 weeks	placebo

However, 0.5% (w/w) mangosteen nanoparticle loaded gel did not show significant result in reduction of post-inflammatory hyperpigmentation. Post inflammatory hyperpigmentation is the late stage of inflammation resulting from the accumulation of melanin from the increased melanin synthesis by melanocytes. Nonetheless, 0.5% (w/w) mangosteen nanoparticle loaded gel could not improve the post inflammatory hyperpigmentation may be because not having tyrosinase inhibition efficacy as potent as other blenching agents such as kojic acid. In the meantime, tyrosinase inhibition effect is antioxidant independent. Anti-oxidant properties did not involve in the tyrosine pathway. (27)

For safety and tolerability assessment, after applied 0.5% (w/w) mangosteen nanoparticle loaded gel for 2 weeks, most of the patients (89.3%) had no adverse

reactions. 2 patients (7.1%) had mild irritation and only 1 moderate irritation (3.6%) on alpha-mangostin side. Whereas 1% clindamycin had 2 mild irritation (7.1%).

At 4 week, 0.5% (w/w) mangosteen nanoparticle loaded gel side decreased in the number of patient who received adverse reactions. 2 mild irritation (7.7%) with resolve spontaneously. Compared with no patients had adverse effects from clindamycin side after 4 weeks of treatment. At 8 week until the end of the study, there is no patients had erythema, burning, scaling and dryness from the application form both sides.

Moreover, there is no statistically significant difference between the 0.5% (w/w) mangosteen nanoparticle loaded gel and the 1% clindamycin gel in the presence of adverse effects after application since week 1 until week 12 (P=0.157).

Considering the patient satisfaction questionaires evaluation on every follow-up visit from the patients. Most of them reports no discomfort and being satisfied with the results of acne reduction. A few had mild dryness of the face with only little discomfort but can resolve spontaneously. Comparing combination therapy between standard treatment 2.5% benzyl peroxide with 0.1% adapalene and 2.5% benzyl peroxide and 0.5% (w/w) mangosteen nanoparticle loaded gel showed no statistically significant different in the results of inflammatory acne reduction.

Hence, 0.5% (w/w) mangosteen nanoparticle loaded gel could be the optional herbal medication in the treatment of acnes to prevent the overuse of topical antibiotics in acne patients. Despite the concerns relating to the antibiotics resistance strains of *P. acnes*, the limited use of antibiotics in both topical and oral forms is recommended together with additional medications that have the efficacious properties of anti-inflammation and anti-microbial agents for *Propionibacterium acnes* as a new alternative treatment of acne vulgaris.

Nevertheless, the limitations of this study are the small sample size and some loss follow-up patients.

6.2 Conclusion

0.5% (w/w) mangosteen nanoparticle loaded gel can significantly improve comedones, inflammatory acne lesion and post-acne erythema within 12 weeks of

treatment. Interestingly, there is not statistically significant difference between 0.5% (w/w) mangosteen nanoparticle loaded gel and 1% clindamycin gels in the reduction of comedones, inflammatory acne lesions, and post-acne erythema during 12 weeks of the study. No serious side effects from clinical application of 0.5% (w/w) mangosteen nanoparticle loaded gel and 1% clindamycin and mild to moderate acne vulgaris patients seemed to be satisfied with the improvement of their acne lesions after 3 months of treatment in both sides.

6.3 Recommendations

Further studies of 0.5% (w/w) mangosteen nanoparticle loaded gel are suggested in larger sample size to verify the effectiveness in acne vulgaris treatment. Nonetheless, the prescription of herbal medication can be an adjunctive treatment in acne vulgaris patients to reduce the problem of overuse antibiotics and prevent the drug resistant bacteria in these patients.

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APPENDICES

APPENDIX A

LETTER OF ACCEPTANCE

The JSPS-NRCT Follow-Up Seminar 2017 and 33rd International Annual Meeting in Pharmaceutical Sciences (JSPS-NRCT 2017 and IAMPS33)
Faculty of Pharmaceutical Sciences, Chulalongkorn University
254 Phayathai Road, Patumwan, Bangkok 10330 THAILAND
Tel: +66 2218 8261 Fax: +66 2255 8227

Date: 26 January 2017

Abstract Title:
Clinical efficacy of topical mangosteen extract in nanoparticle loaded gel compared with 1% clindamycin gel in treatment of mild to moderate acne vulgaris

Authors:
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Pornlert Trithossadech, Suparuj Lueangarun

Dear Ms. Karuna Sriviriyakul,

We have the pleasure to inform you that your abstract has been accepted for **poster presentation** in the JSPS-NRCT Follow-Up Seminar 2017 and 33rd International Annual Meeting in Pharmaceutical Sciences (JSPS-NRCT 2017 AND IAMPS 33), which will be held on 2-3 March 2017 at The Berkeley Hotel Pratunam, Bangkok, Thailand.

For further information regarding the Guidelines for presentation, please visit the following website: http://www.pharm.chula.ac.th/am2017/

Should you have any inquiries, please kindly contact Assoc. Prof. Dr. Boonchoo Sritularak at boonchoo.sr@chula.ac.th.

Yours truly,

Pornchai R.

Assoc. Prof. Pornchai Rojsitthisak, Ph.D.

Chair of Scientific Program Committee

APPENDIX B

ABSTACT FOR PROCEEDING

PP-36

Clinical efficacy of topical mangosteen extract in nanoparticle loaded gel compared with 1% clindamycin gel in treatment of mild to moderate *Acne vulgaris*

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Keywords: *Garcinia mangostana*, Alpha-mangostin, Mangostin fruit rind, Nanoparticle, *Acne vulgaris*

Acne vulgaris is the most common sebaceous gland disorder in young adults. In a bid to develop new medication for acne vulgaris, the mechanism involving in acne pathogenesis must be targeted. Alpha-mangostin, is well known for anti-oxidant and anti-microbial properties. This study aimed to investigate the clinical efficacy of 0.5% α-mangostin in nanoparticle gel for the reduction of acne vulgaris and post acne erythema. Twenty patients with mild to moderate acne vulgaris and mean (SD) age of 24 (6) years were enrolled in this double-blind, spit-face, randomized, control study. The α-mangostin gel was applied randomly to one side of the face, and 1% clindamycin gel was applied to the other side twice daily for 28 days. The efficacies were evaluated using lesion count, digital photography, and biometric measurement (Antera 3D) for acne erythema percentage reduction. In the α-mangostin treatment group, a significant acne comedone reduction was noted from baseline to the week 4, with the median of 17.5 to 7 (P = 0.001). The pustule, papule and nodule lesion counts from baseline to week 4 showed a non-statistically significant change from 1.5 to 1 (P = 0.187), 1.5 to 2 (P = 0.947), and 1 to 0 (P = 0.303), respectively Interestingly, PAE significantly decreased from 1.69 to 1.47 at week 4 (P =0.009). The α-mangostin nanoparticle gel was found significantly effective in the treatment of acne with comedone and post-acne erythema improvement. Moreover, there was no statistically significant difference between α-mangostin and clindamycin for the reduction in the number of acne lesions and the presence of adverse effects.

The JSPS-NRCT Follow-Up Seminar 2017 and 33rd International Annual Meeting in Pharmaceutical Sciences (JSPS-NRCT 2017 and IAMPS 33)

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APPENDIX C

MANUSCRIPT FOR PUBLICATION

TJPS Vol.41 (Supplement Issue) 2017

Thai Journal of Pharmaceutical Sciences (TJPS)
The JSPS-NRCT Follow-Up Seminar 2017 and
33rd International Annual Meeting in Pharmaceutical Sciences

Clinical efficacy of topical mangosteen extract nanoparticle loaded gel compared with 1% clindamycin gel in the treatment of mild to moderate acne vulgaris

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Keywords: *Garcinia mangostana*, Alpha-mangostin, mangostin fruit rind, nanoparticle, acne vulgaris, treatment

Introduction

Acne vulgaris is the most common sebaceous gland disorder in puberty and young adults. There are 4 major pathogenetic factors of acne vulgaris, including follicular hyperkeratinization, excessive sebum production, *Propionibacterium acnes (P. acnes)* and follicular inflammation.[1,2,3] Recent studies have proven that inflammation occurs in all stages of acne lesions, including microcomedone.[4] The sequelae of the acne, such as post acne erythema, post inflammatory hyperpigmentation and acne scarring, are the result of inflammatory cytokines such as lipase, hyaluronidase and matrix metalloproteinase from inflammatory cells.[2,5,6,7] Mangosteen (*Garcinia mangostana*) is a famous tropical fruit in Thailand. It has been used traditionally in medicine for the treatment of diarrhea, skin infections, skin inflammation such as eczema, and dermatitis.[8,9,10,11] Xanthones in mangosteen fruit rind includes α,β,γ-mangostin. α-mangostin has the strongest effect for anti- *P. acnes* property.[8,12] This study will identify the reduction properties in non-inflammatory and inflammatory acne lesions as well as the reduction in post acne erythema and post inflammatory hyperpigmentation using 0.5% α-mangostin in nanoparticle gel.

Methods

From November to December 2016, a double-blind, randomized control, spit-face pilot study was conducted for 4 weeks to evaluate the efficacy and safety profile of 0.5 % α-mangostin nanoparticle gel and 1% clindamycin gel. Acne patients were randomly given a tube containing gel labeled either A or B. For symptom control, 2.5% benzyl peroxide was applied by all patients for 10 minutes once daily. Patients were assessed by dermatologists at follow-up visits after week 2 and 4. Dermatologic examination included acne lesion count using digital photograph record (Canon EOS M10). Acne redness was evaluated by Antera 3D (Miravex limited, Dublin, Ireland).

Inclusion and exclusion criteria

Male and female patients aging in range from 18 to 40 years in healthy condition with mild to moderate acne vulgaris were evaluated using the Global Acne Grading System. Subjects were excluded if they were pregnant or breast feeding, had severe acne conditions, or were receiving topical or oral antibiotics within 2 weeks. They were also excluded if they had undergone acne procedures such as dermabrasion or laser within the previous 1 month, taken systemic or topical steroid medications within 4 weeks or oral isotretinoin within 6 months of observation. All patients signed a consent form prior to participation.

Materials

α-mangostin nanoparticle gel and 1% clindamycin was formulated by Welltech Biotechnology Co., Ltd., Thailand (94% α-mangostin soluble in dichloromethane) into a gel base at a final concentration of 0.5%. The gel base was composed of water, xanthan gum, propylene glycol, glycerin, allantoin, and preservatives. A stability test was done by storing mangosteen gel at 45 degrees Celsius for 7 days prior to observation. The gel did not change in color or viscosity, while pH did not change from baseline. An irritation test was conducted with 20 volunteers using a closed patch test for 24 hours to observe for signs of erythema at 48 hours post application. Testing showed that 0.5% α-mangostin resulted in only 1 patient (5%) presenting with mild irritation, with 19 patients showing no irritation (95%). Comparison between groups for irritation was not statistically significant different ($P = 0.16$) using McNemar statistical analysis.

Statistical analysis

The Wilcoxon Signed Rank Test for statistical analysis was used for the comparison of the within group as well as comparison between treatments. *P.value* < 0.05 was considered statistically significant.

Results

This study consisted of 20 males and 4 females with 4 lost to follow-up patients. The mean (SD) age was 24 (6) years with skin types III, IV and V of 25%, 62.5% and 12.5%, respectively. The mean (SD) of Global Acne Grading System scores (GAGS) [13] was 16 (6) .

In α-mangostin treatment group, significant comedone reduction was shown in the group from baseline to the end of Week 4 from the median of 17.5 to 7 ($P = 0.001$). However, inflammatory lesions such as pustules, papules and nodules showed a reduction without statistical significance ($P > 0.05$). The pustule, papule and nodule lesion counts from baseline to Week 4 showed minimal, non-statistically significant change from 1.5 to 1 ($P = 0.187$), 1.5 to 2 ($P = 0.947$) and 1 to 0 ($P = 0.303$), respectively. Interestingly, Post-acne erythema (PAE) decreased significantly from 1.69 to 1.47 at Week 4 ($P = 0.009$).

The clindamycin treatment group revealed statistically significant improvement in both comedone and inflammatory acne lesions. The median reduction of comedone, inflammatory pustule, papule and nodule from baseline to Week 4 was 15.5 to 10 ($P = 0.003$), 2 to 0 ($P = 0.009$), 2.5 to 2 ($P = 0.056$) and 1 to 1 ($P = 0.48$), respectively. Moreover, PAE also significantly reduced from a baseline of 1.66 to 1.45 ($P = 0.033$) at Week 4. However, there was no statistically significant difference between α-mangostin and clindamycin for improvement of comedone, pustule, papule, nodule, and the reduction of PAE (Table 1-2 and Figure 1)

Minor adverse effects were present in both groups without significant difference ($P = 0.157$) at Week 4, including 2 cases of mild irritation and 1 case of moderate irritation in the mangosteen gel group and 1 case of mild irritation in the clindamycin gel group. All side effects resolved spontaneously within 2 days.

Table 1. Acne lesion count in α-mangostin and clindamycin at baseline, 2 and 4 weeks.

	Mangostine			Clindamycin			p-value[b]		
	Baseline (n=20)	Week 2 (n=21)	Week 4 (n=20)	Baseline (n=20)	Week 2 (n=21)	Week 4 (n=20)	Baseline	Week 2	Week 4
Comedone	17.5 (10.5, 24.5)	13 (6, 17)	7 (5.5, 17)	15.5 (10, 24)	12 (6, 18)	10 (6, 17.5)	0.397	0.585	0.300
p-value[w]	Reference	0.001*	0.001*	Reference	0.002*	0.003*			
Pustule	1.5 (0, 2.5)	0 (0, 2)	1 (0, 2.5)	2 (1, 4)	0 (0, 2)	0 (0, 2)	0.268	0.822	0.279
p-value[w]	Reference	0.279	0.187	Reference	0.025*	0.009*			
Papule	1.5 (0, 4.5)	2 (0, 3)	2 (1, 3)	2.5 (1, 4.5)	2 (0, 3)	2 (1, 2.5)	0.101	0.857	0.807
p-value[w]	Reference	0.452	0.947	Reference	0.045*	0.056			
Nodules	1 (0, 3.5)	0 (0, 1)	0 (0, 2.5)	1 (1, 3)	1 (0, 2)	1 (0, 2)	0.888	0.534	0.604
p-value[w]	Reference	0.088	0.303	Reference	0.150	0.498			

Table 2. Inflammatory acne count, PAE and post inflammatory hyperpigmentation (PIH) in α-mangostin and clindamycin treatment group at baseline, 2 and 4 weeks evaluated from Antera 3D biometric camera.

	Mangostine		Clindamycin		p-value[b]	
	Baseline (n=20)	Week 4 (n=20)	Baseline (n=20)	Week 4 (n=20)	Baseline	Week 4
PAE	1.69 (1.35, 1.9)	1.47 (1.26, 1.72)	1.66 (1.34, 1.77)	1.45 (1.27, 1.64)	0.036*	0.409
p-value[w]	Reference	0.009*	Reference	0.033*		
PIH	0.52 (0.48, 0.57)	0.5 (0.47, 0.54)	0.52 (0.49, 0.55)	0.51 (0.48, 0.56)	0.618	0.727
p-value[w]	Reference	0.192	Reference	0.584		

Values presented as median (IQR). The p-value corresponds to Wilcoxon signed ranks test. (w) Comparison with in group, (b) Comparison with in treatments.

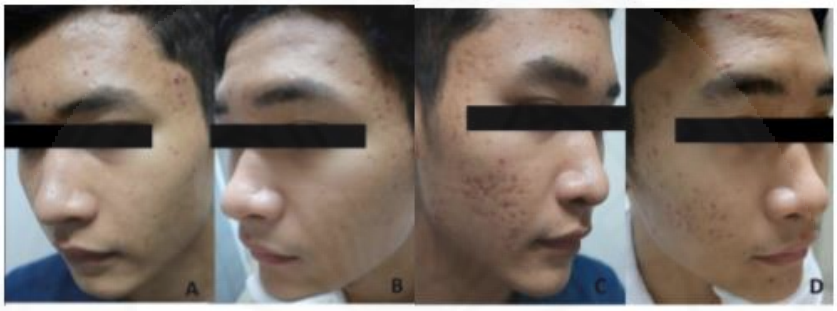

Figure 1. Photographs of subject showing (A) Clindamycin-treated side at baseline (B) Clindamycin-treated side at 4 weeks, (C) α-mangostin nanoparticle-treated side at baseline, (D) α-mangostin nanoparticle-treated side after 4 weeks of treatment

Discussion

This study showed the efficacies of α-mangostin for the treatment of acne vulgaris, which showed significant improvement in non-inflammatory acne lesions and PAE. However, clindamycin showed additional efficacies on acne treatments, such as the reduction of non-inflammatory, inflammatory acne and post-acne erythema. Meanwhile, α-mangostin gel did not significantly improve inflammatory acne. However, there was no significant difference between topical α-mangostin nanogel and 1% clindamycin in the efficacies of acne treatment and adverse effects. The mechanisms of action associated with α-mangostin properties can be summarized as follows: α-mangostin is a well-known, potent anti-inflammatory and anti-oxidant agent expected to improve inflammatory skin diseases such as dermatitis, skin infection and acne vulgaris. *In vitro* study of α-mangostin showed that it can reduce inflammatory cytokines such as PGE2, TNF-α and IL-1α. In addition, α-mangostin has free-radical scavenging properties that reduce ROS. ROS will send a secondary messenger to induce the production of transcription factors such as NF-kB, AP-1 to generate cytokine production and produce more inflammatory acne.[13] ROS reduction is very important for control of inflammatory acne. Despite the presence of concerns relating to antibiotic-resistant strains of *P. acnes,* the limited use of antibiotics in both topical and oral form and finding additional medication that has efficacious anti-microbial properties for *P.acnes* and anti-inflammation can help physicians find new solutions for the treatment of acne vulgaris.

Conclusion

Alpha-mangostin nanoparticle gel showed significant efficacy in the treatment of acne for improvement of comedone and post-acne erythema after application of α-mangostin nanoparticle gel for only 28 days. Moreover, there was no statistically significant difference between α-mangostin nanoparticle gel and clindamycin gel for reduction in the number of acne lesions and the presence of adverse effects. Hence, α-mangostin could be an alternative option as herbal medication for the treatment of acne that would prevent the overuse of topical antibiotics by acne patients.

Conflict of interest

All authors have none to declare.

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APPENDIX D

BEST POSTER PRESENTATION AWARDS

PP-36

Certificate of a Presentation Award

This is to certify that

Karuna Sriviriyakul

has received an outstanding poster presentation award entitled

Clinical efficacy of topical mangosteen extract in nanoparticle loaded gel compared with 1% clindamycin gel in treatment of mild to moderate *Acne vulgaris*

at The JSPS-NRCT Follow-Up Seminar 2017 and
33rd International Annual Meeting in Pharmaceutical Sciences
Faculty of Pharmaceutical Sciences, Chulalongkorn University
March 2-3, 2017
The Berkeley Hotel Pratunam, Bangkok, Thailand

Associate Professor Pornchai Rojsitthisak, Ph.D.
Chair of Scientific Committee
Associate Dean, Faculty of Pharmaceutical Sciences
Chulalongkorn University

Assistant Professor Rungpetch Sakulbumrungsil, Ph.D.
Chair of Organizing Committee
Dean, Faculty of Pharmaceutical Sciences
Chulalongkorn University

APPENDIX E
CASE RECORD FORM

No._____

Name_____Surname_____Age _____ Sex_____

Address _____

_____

_____

Telephone _____

Emergency contact _____

Name_____ Related_____ _____

Address _____

_____

Telephone _____

Case record form 1 – inclusion and exclusion criteria

THE EFFECACY OF TOPICAL MANGOSTEEN EXTRACT IN THE TREATMENT OF ACNE VULGARIS COMPARED WITH 1%CLINDAMYCIN GEL	Subject Initials: Subject ID:	
Inclusion criteria (Any no answer excludes subject from the study)		
Healthy males or females older than 18 years of age	☐ Yes	☐ No
Clinical diagnosis of mild to moderate acne vulgaris	☐ Yes	☐ No
Investigator's global assessment grade 1-3 (No nodulocystic acne lesion)	☐ Yes	☐ No
Willing to follow the protocol, including good compliance to apply the drugs, using provided facial wash and sunscreen	☐ Yes	☐ No
Informed consent agreement signed by the subject Dated signed __/__/__ (dd/mm/yy)	☐ Yes	☐ No
Exclusion criteria (Any "Yes" answer excludes subject from study)		
Pregnancy or planning to become pregnant, or breast feeding	☐ Yes	☐ No
Severe acne vulgaris that require oral isotretinoin	☐ Yes	☐ No
Having received systemic isotretinoin within 6 months	☐ Yes	☐ No

Having topical or systemic antibiotics, benzyl peroxide, retinoids or any other drugs that will interfere with the result within 4 weeks	☐ Yes	☐ No
Having steroid, immunosuppressive drugs within 4 weeks	☐ Yes	☐ No
Having a history of skin cancer	☐ Yes	☐ No
Having a known history of photosensitivity	☐ Yes	☐ No
Having dramatically change in acne symptoms	☐ Yes	☐ No
Subject is currently enrolled in a clinical study of any other unapproved investigational drug of devices	☐ Yes	☐ No

Case Record form 2 – dermographic data

THE EFFECACY OF TOPICAL MANGOSTEEN EXTRACT IN THE TREATMENT OF ACNE VULGARIS COMPARED WITH 1%CLINDAMYCIN GEL	Subject Initials:
	Subject ID:

1. Age: _____ years
2. Sex

   ☐ Male ☐ Female

3. Skin type I II III IV V VI
4. History of drug allergy _____
5. Smoking ☐ Yes (heavy, occasionally) ☐ No
6. Drinking ☐ Yes (heavy, occasionally) ☐ No
7. Age of onset of acne _____ months/years
8. Duration of acne _____ months/years
9. History of acne treatment (if yes go to question 10)
10. Past medical treatment of acne
    a. Topical antibiotics (please specify_____)
    b. Topical benzyl peroxide (please specify _____)
    c. Oral antibiotics (please specify _____)
    d. Cleansings (please specify _____)
    e. Others (please specify_____)

11. Current medical treatment of acne
    a. Topical antibiotics (please specify_____)
    b. Topical benzyl peroxide (please specify _____)
    c. Oral antibiotics (please specify _____)
    d. Cleansings (please specify _____)
    e. Others (please specify_____)

12. Past procedural treatment of acne
    a. Comedone extraction (please specify_____)
    b. Chemical peeling (please specify_____)
    c. Medical injection (please specify_____)
    d. Others (please specify_____)

13. Past laser treatment of acne (please specify_____)

Case record form 3 – Acne lesion count

Time	Date	Acne lesions	Lt	Rt	GAGS
At base line		1.non comedone			
		2.comedone			
		3.Porphyrin			
Week 2		1.non comedone			
		2.comedone			
		3.Porphyrin			
Week 4		1.non comedone			
		2.comedone			
		3.Porphyrin			
Week 8		1.non comedone			
		2.comedone			
		3.Porphyrin			
Week 12		1.non comedone			
		2.comedone			
		3.Porphyrin			

Case record form 4 – patient evaluation form

THE EFFECACY OF TOPICAL MANGOSTEEN EXTRACT IN THE TREATMENT OF ACNE VULGARIS COMPARED WITH 1%CLINDAMYCIN GEL	Subject Initials:
	Subject ID:

1. Considering the facial area treated, have you notice any improvement of acne lesions on the mangosteen nanoparticle loaded gel side.
    1. Little or no improvement (0-2)
    2. Some improvement (3-4)
    3. Fair improvement (5-6)
    4. Good improvement (7-8)
    5. Excellent improvement (9-10)

2. Considering the facial area treated, have you notice any improvement of acne lesions on the 1% clindamycin side.
    1. Little or no improvement (0-2)
    2. Some improvement (3-4)
    3. Fair improvement (5-6)
    4. Good improvement (7-8)
    5. Excellent improvement (9-10)

3. Overall satisfaction score
    1. Very satisfied
    2. Satisfied
    3. Dissatisfied
    4. Very dissatisfied

3. Adverse effect evaluation

Inching	☐ Yes	☐ No
Burning	☐ Yes	☐ No
Crusting	☐ Yes	☐ No
Greasy scale	☐ Yes	☐ No
Dermatitis	☐ Yes	☐ No

Appendix G

Table 1. Demographic data collection

Subject No.	Age	Gender	GAGs	FPT
1	22	F	19	4
2	27	F	7	4
3	19	M	15	3
4	28	F	22	5
5	28	F	12	4
6	23	F	27	4
7	24	F	12	3
8	28	F	12	3
9	18	F	14	5
10	19	F	23	4
11	18	F	14	4
12	36	F	24	4
13	27	F	10	4
14	33	F	14	3
15	28	F	30	4
16	19	M	16	5
17	26	M	13	4
18	25	F	11	4
19	18	F	10	3
20	22	F	26	4
21	19	F	8	4
22	20	M	16	4
23	39	F	12	3
24	19	F	16	4
25	28	F	13	3
26	28	F	12	3
27	28	F	10	3
28	35	F	14	4

Table 2 Comedone lesion counts

Comedone lesion counts												
			Mangosteen					Clindamycin				
Subject No.	Age	Gender	Week 0	Week 2	Week 4	Week 8	week 12	Week 0	Week 2	Week 4	Week 8	Week 12
1	22	F	26	18	6	8	6	25	20	17	5	4
2	27	F	5	4	1	2	6	8	4	2	2	4
3	19	M	18	13	24	14	4	17	18	14	24	6
4	28	F	18	13	21	6	11	23	13	20	7	6
5	28	F	8	4	5	2	2	6	4	7	4	2
6	23	F	57	33	38	30	6	44	24	25	19	8
7	24	F	13	8	5	3	3	10	6	5	5	5
8	28	F	10	16	15	10	12	7	9	18	8	11
9	18	F	12	12	-	-	-	13	10	-	-	-
10	19	F	23	20	19	14	14	13	12	12	8	6
11	18	F	15	6	13	14		18	5	10	8	
12	36	F	18	5	6	4	5	11	4	11	4	4
13	27	F	11	14	7	8	5	10	18	6	8	4
19	18	F	57	47	42	36	33	60	46	36	34	33
20	22	F	40	18	19	13	9	41	16	22	23	39
21	19	F	18	10	7	-	1	14	12	6	-	2
22	20	M	25	20	8	5	5	18	16	9	4	4
23	39	F	3	4	3	1	4	6	4	4	0	2
24	19	F	5	13	2	8	5	7	9	5	7	7
25	28	F	12	6	2	3	3	10	7	5	6	5
26	28	F	11	8	4	4	4	21	7	3	2	8
27	28	F	23	12	12	6	9	11	10	4	4	24
28	35	F	7	10	-	-	-	5	7	-	-	-
Total			513	366	295	228	170	450	334	289	220	201

Table 3 Pustule lesions counts

Pustule lesion counts												
			Mangosteen					Clindamycin				
Subject No.	Age	Gender	week0	week2	week4	week8	week12	week0	week2	week4	week8	week12
1	22	F	2	6	0	0	1	1	3	0	1	2
2	27	F	0	0	0	0	0	0	0	0	0	0
3	19	M	0	6	0	2	1	2	4	2	0	0
4	28	F	1	0	2	4	0	5	4	3	0	0
5	28	F	0	0	1	1	0	1	0	2	0	0
6	23	F	0	2	4	2	1	2	3	0	4	1
7	24	F	2	0	1	0	0	0	0	0	0	0
8	28	F	2	0	3	0	0	2	1	0	0	0
9	18	F	2	0	-	-	-	4	0	-	-	-
10	19	F	3	2	2	0	0	5	1	0	1	3
11	18	F	6	3	2	0	-	4	1	1	1	-
12	36	F	2	3	2	0	0	3	2	0	0	0
13	27	F	1	0	1	0	0	5	0	0	0	0
14	33	F	4	0	0	0	0	1	0	0	0	0
15	28	F	4	0	2	0	0	4	0	0	0	0
16	19	M	2	0	0	0	0	0	0	3	0	1
17	26	M	3	0	0	-	1	2	2	0	-	0
18	25	F	0	0	3	3	0	3	0	1	0	0
19	18	F	7	0	4	1	1	6	2	2	1	1
20	22	F	5	0	3	0	0	5	0	2	2	-
21	19	F	1	1	0	-	0	1	0	0	-	0
22	20	M	1	4	0	0	0	4	1	0	0	0
23	39	F	0	0	2	0	0	0	0	0	1	0
24	19	F	0	2	0	1	0	0	4	1	2	0
25	28	F	0	0	1	0	0	1	0	1	0	0
26	28	F	0	0	0	1	0	0	0	0	1	0
27	28	F	3	0	0	0	0	0	0	0	0	0
28	35	F	0	0	-	-	-	0	1	-	-	-

Table 4. Papule lesion counts

Papule lesion counts												
			Mangosteen					Clindamycin				
Subject No.	Age	Gender	week 0	week 2	week 4	week 8	week 12	week 0	week 2	week 4	week 8	week 12
1	22	F	1	2	4	3	2	2	0	4	1	0
2	27	F	0	2	1	0	1	1	2	0	1	2
3	19	M	3	3	2	2	4	4	6	2	2	3
4	28	F	0	6	3	0	2	3	5	2	2	1
5	28	F	2	2	2	1	0	0	0	0	0	0
6	23	F	5	6	3	0	4	6	5	3	0	2
7	24	F	0	0	2	0	0	2	2	1	0	2
8	28	F	0	0	2	1	1	0	0	1	1	0
9	18	F	4	2	-	-	-	1	2	-	-	-
10	19	F	4	2	4	2	1	2	2	5	2	1
11	18	F	4	3	2	2	-	2	2	2	2	-
12	36	F	1	0	4	2	0	0	0	4	3	0
13	27	F	1	2	2	1	1	4	2	2	2	0
14	33	F	0	0	1	0	0	2	0	3	0	0
15	28	F	5	2	4	2	0	6	2	6	0	3
16	19	M	4	2	2	2	3	3	3	2	1	4
17	26	M	0	0	2	-	0	5	1	2	-	4
18	25	F	6	3	0	0	0	6	2	0	1	0
19	18	F	5	0	0	1	2	4	0	2	0	2
20	22	F	12	2	2	3	0	11	4	1	2	2
21	19	F	2	2	1	-	1	1	2	0	-	0
22	20	M	3	4	3	1	3	2	3	2	2	2
23	39	F	1	0	1	0	0	1	1	0	0	0
24	19	F	2	3	1	0	2	2	2	0	0	2
25	28	F	0	0	0	1	0	0	0	0	1	0
26	28	F	0	0	0	0	0	1	1	0	0	4
27	28	F	0	2	2	2	1	2	0	1	1	0
28	35	F	2	2	-	-	-	0	0	-	-	-

Table 5 Nodule lesions counts

Nodule lesion counts												
			Mangosteen					Clindamycin				
Subject No.	Age	Gender	Week 0	Week 2	Week 4	Week 8	Week 12	Week 0	Week 2	Week 4	Week 8	Week 12
1	22	F	0	0	3	2	2	3	2	1	1	1
2	27	F	1	0	0	0	1	0	0	1	0	0
3	19	M	1	1	0	3	0	4	1	4	0	1
4	28	F	0	1	2	0	0	0	1	0	1	0
5	28	F	1	1	0	0	0	3	2	2	1	3
6	23	F	3	4	5	8	1	4	2	6	9	3
7	24	F	1	0	0	2	2	1	0	0	1	1
8	28	F	0	0	0	0	0	1	0	0	1	0
9	18	F	1	1	-	-	-	0	0	-	-	-
10	19	F	4	1	1	1	1	3	4	6	0	0
11	18	F	0	0	1	1	-	0	0	0	0	-
12	36	F	4	4	3	1	2	1	1	1	2	2
13	27	F	0	0	0	0	0	0	0	0	0	0
14	33	F	1	2	3	3	0	1	2	4	5	3
15	28	F	12	4	2	1	0	3	6	5	2	4
16	19	M	4	0	2	2	1	2	0	2	2	0
17	26	M	4	0	0	-	0	2	1	2	-	0
18	25	F	1	0	0	0	0	3	1	0	0	0
19	18	F	0	0	0	0	0	1	0	0	0	0
20	22	F	4	5	3	2	4	4	3	1	4	0
21	19	F	0	0	0	-	0	0	0	0	-	0
22	20	M	3	1	2	2	0	1	4	1	2	0
23	39	F	1	0	0	1	0	1	0	0	0	0
24	19	F	4	0	0	3	4	1	2	0	6	1
25	28	F	0	0	0	1	1	0	0	0	0	0
26	28	F	0	0	0	1	0	0	0	0	0	0
27	28	F	0	0	1	1	0	0	0	0	0	1
28	28	F	0	0	-	-	-	0	0	-	-	-

Table 6 Inflammatory lesion counts

Inflammatory lesion counts												
			Mangosteen					Clindamycin				
Subject No.	Age	Gender	Week 0	Week 2	Week 4	Week 8	Week 12	Week 0	Week 2	Week 4	Week 8	Week 12
1	22	F	3	8	7	5	5	6	5	5	3	3
2	27	F	1	2	1	0	2	1	2	1	1	2
3	19	M	4	10	2	7	5	10	11	8	2	4
4	28	F	1	7	7	4	2	8	10	5	3	1
5	28	F	3	3	3	2	0	4	2	4	1	3
6	23	F	8	12	12	10	6	12	10	9	13	6
7	24	F	3	0	3	2	1	3	2	1	1	3
8	28	F	2	0	5	1	1	3	0	1	2	0
9	18	F	7	3	-	-	-	5	2	-	-	-
10	19	F	11	5	7	0	2	10	7	11	3	4
11	18	F	10	6	5	3	-	6	3	3	3	-
12	36	F	8	7	9	3	2	4	3	5	5	2
13	27	F	2	2	3	1	1	9	2	2	2	0
14	33	F	5	2	4	3	0	4	2	7	5	3
15	28	F	22	6	8	3	4	13	8	11	2	7
16	19	M	10	2	4	4	4	5	3	7	3	5
17	26	M	7	0	2	-	2	9	4	4	-	4
18	25	F	7	3	3	3	0	12	3	1	1	0
19	18	F	12	0	4	2	3	11	2	4	1	3
20	22	F	21	7	8	5	4	20	7	4	8	2
21	19	F	3	3	1	-	1	2	2	0	-	0
22	20	M	7	9	5	3	3	7	8	3	4	2
23	39	F	2	0	3	1	0	2	1	0	1	0
24	19	F	6	5	0	4	6	3	8	0	8	3
25	28	F	0	0	1	2	1	1	0	1	1	0
26	28	F	3	1	0	3	1	2	0	0	1	1
27	28	F	2	0	-	-	-	0	0	-	-	-
Total			170	103	107	73	56	173	107	97	75	62

Table 7 Post-acne erythema scores from biometric Antera 3D

Post acne erythema								
			Mangosteen			Clindamycin		
Subject No.	Age	Gender	week0	week4	week12	week0	week4	week12
1	22	F	1.77	1.77	1.67	1.73	1.73	1.69
2	27	F	1.28	1.28	1.23	1.15	1.15	1.18
3	19	M	1.39	1.74	1.18	1.29	1.7	1.32
4	28	F	1.34	1.33	1.23	1.27	1.24	1.25
5	28	F	1.39	1.36	1.14	1.45	1.34	1.07
6	23	F	1.46	1.83	1.42	1.53	1.8	1.46
7	24	F	1.35	1.31	1.19	1.33	1.3	1.24
8	28	F	1.24	1.27	1.22	1.18	1.21	1.21
9	18	F	-	-	-	-	-	-
10	19	F	1.51	-	1.46	1.87	-	2.16
11	18	F	-	-	-	-	-	-
12	36	F	1.92	2.4	1.5	1.34	1.65	1.15
13	27	F	1.61	1.74	1.44	1.62	1.66	1.49
14	33	F	1.1	1.15	1.06	1.09	1.33	0.94
15	28	F	1.84	1.95	1.74	1.71	1.66	1.6
16	19	M	1.91	2.02	1.51	1.72	1.8	1.75
17	26	M	1.68	1.6	1.61	1.67	1.73	1.49
18	25	F	1.82	1.84	1.81	1.55	1.66	1.46
19	18	F	1.46	1.61	1.57	1.8	1.89	1.57
20	22	F	1.78	2.01	1.52	1.67	1.86	1.38
21	19	F	1.59	1.64	1.32	1.56	1.43	1.46
22	20	M	2.03	2	1.63	1.81	1.91	1.61
23	39	F	1.45	1.67	1.12	1.57	1.63	1.37
24	19	F	1.58	-	1.44	1.52	-	1.37
25	28	F	1.44	-	1.53	1.4	-	1.63
26	28	F	1.52	-	1.33	1.78	-	1.67
27	28	F	1.25	-	1.38	1.29	-	1.58
28	35	F	-	-	-	-	-	-

Table 8 Post inflammatory hyperpigmentation scores from biometric Antera 3D assessment index scores

Post inflammatory hyperpigmentation								
			Mangosteen			Clindamycin		
Subject No.	Age	Gender	Week0	Week4	Week12	Week0	Week4	Week12
1	22	F	0.51	0.51	0.49	0.56	0.56	0.55
2	27	F	0.42	0.42	0.41	0.53	0.53	0.47
3	19	M	0.68	0.68	0.55	0.58	0.55	0.54
4	28	F	0.55	0.56	0.55	0.53	0.54	0.56
5	28	F	0.47	0.48	0.54	0.46	0.46	0.55
6	23	F	0.47	0.49	0.56	0.46	0.49	0.57
7	24	F	0.48	0.5	0.47	0.5	0.49	0.49
8	28	F	0.45	0.48	0.41	0.46	0.48	0.45
9	18	F	-	-	-	-	-	-
10	19	F	0.63	-	0.61	0.6	-	0.58
11	18	F	-	-	-	-	-	-
12	36	F	0.6	0.65	0.56	0.5	0.51	0.47
13	27	F	0.49	0.47	0.45	0.5	0.5	0.45
14	33	F	0.43	0.4	0.43	0.38	0.38	0.39
15	28	F	0.55	0.55	0.56	0.53	0.54	0.52
16	19	M	0.56	0.56	0.6	0.57	0.6	0.58
17	26	M	0.47	0.47	0.51	0.5	0.52	0.5
18	25	F	0.57	0.6	0.53	0.55	0.5	0.53
19	18	F	0.5	0.56	0.58	0.57	0.58	0.58
20	22	F	0.51	0.61	0.49	0.51	0.52	0.52
21	19	F	0.57	0.58	0.52	0.56	0.53	0.53
22	20	M	0.52	0.52	0.46	0.57	0.57	0.53
23	39	F	0.47	0.46	0.45	0.46	0.46	0.47
24	19	F	0.57	-	0.5	0.59	-	0.53
25	28	F	0.53	-	0.52	0.48	-	0.46
26	28	F	0.46	-	0.44	0.45	-	0.47
27	28	F	0.39	-	0.44	0.33	-	0.4
28	28	F	-	-	-	-	-	-

Table 9 Wood's lamp examination grading scores at baseline

	Mangosteen				Clindamycin			
	Week 0				Week 0			
Subject No.	Evaluator1	Evaluator2	Evaluator 3	Mean	Evaluator 1	Evaluator2	Evaluator3	Mean
1	3	3	3	3	3	3	3	3
2	2	3	4	3	2	3	4	3
3	4	3	3	3.33	4	3	3	3.33
4	2	4	2	2.67	2	4	2	2.67
5	2	3	2	2.33	2	3	2	2.33
6	4	4	3	3.67	4	4	2	3.33
7	1.5	2	2	1.83	1.5	2	2	1.83
8	1.5	2	3	2.17	1.5	2	2	1.83
9	-	-	-	-	-	-	-	-
10	2.5	3	3	2.83	3	3.5	3	3.17
11	-	-	-	-	-	-	-	-
12	2	3	1	2	2	3	1	2
13	1	2	4	2.33	1	2	2	1.67
14	1	2	3	2	1	2	3	2
15	2.5	3	3	2.83	2.5	3	3	2.83
16	3	3	2	2.67	4	3	2	3
17	2	2	2	2	2	2	2	2
18	3	2	3	2.67	3	2	3	2.67
19	4	3	4	3.67	4	3	4	3.67
20	3	3.5	2	2.83	3	3	1	2.33
21	2	4	4	3.33	2	4	4	3.33
22	1	2	2	1.67	1	2	2	1.67
23	2	2	2	2	2	2	2	2
24	2	4	2	2.67	2.5	4	2	2.83
25	2.5	2	3	2.5	2	2	2	2
26	3	4	3	3.33	3	4	2	3
27	2	2	2	2	2	2	3	2.33

Table 10 Wood's lamp examination grading scores at 12 week.

	Mangosteen				Clindamycin			
	Week 12				Week 12			
Subject No.	Evaluator1	Evaluator2	Evaluator3	Mean	Evaluator1	Evaluator2	Evaluator3	Mean
1	2	2	2	2	2	1	2	1.66
2	2	2	2	2	2	2	2	2
3	1	1	1	1	1	2	1	1.33
4	1	2	1	1.33	1	1	1	1
5	0.5	1	1	0.83	0.5	1	1	0.83
6	1	2	1	1.33	1	1	1	0
7	1.5	1	1	1.17	1	1	1	0
8	1.5	1	1	1.17	1.5	1	1	1.17
9	-	-	-	-	-	-	-	-
10	1	2	1	1.33	2	2.5	1	1.83
11	-	-	-	-	-	-	-	-
12	1	2	1	1.33	1	2	1	1.33
13	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1
15	1	2	1	1.33	1	1	1	1
16	3	1.5	1	1.83	3	1	1	1.67
17	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1.5	1	1.17
19	2	2	2	2	3	2	2	2.33
20	1	2	1	1.33	1.5	2	1	1.5
21	1	2	1	1.33	1	1	1	1
22	1	1	1	1	1	1	1	1
23	0.5	1	1	0.83	0.5	1	1	0.83
24	1	2	1	1.33	1	2	1	1.33
25	1	1	1	1	1	1	1	1
26	2	1	1	1.33	2	1	1	1.33
27	1	1	1	1	1	1	1	1

Table 11 Clinical grading scores at baseline by 3 blinded-dermatologists at baseline.

	Mangosteen				Clindamycin			
Subject No.	Evaluator1	Evaluator2	Evaluator3	Mean	Evaluator1	Evaluator2	Evaluator3	Mean
1	2	3	3	2.67	1	3	2	2
2	0	3	3	2	1	3	4	2.67
3	2	3	2	2.33	2	3	2	2.33
4	1.5	4	5	3.5	1.5	4	4	3.17
5	2	3	1	2	2	3	2	3
6	2.5	4	4	3.5	2.5	4	4	3.5
7	1	2	2	1.67	1	2	2	1.67
8	2	2	3	2.33	1	2	3	2
9	-	-	-	-	-	-	-	-
10	1.5	3	3	2.5	2	3.5	4	3.17
11	-	-	-	-	-	-	-	-
12	1	3	5	3	0	3	5	2.67
13	2.5	2	4	2.83	3.5	2	5	3.5
14	3.5	2	2	2.5	3	2	2	2.33
15	0	3	2	1.67	1	3	2	2
16	0	3	3	2	0	3	2	1.67
17	2	2	1	1.67	2	2	2	2
18	2.5	2	4	2.83	2	2	3	2.33
19	2	3	2	2.33	1	3	1	1.67
20	3	3.5	1	2.5	2	3	1	2
21	2	4	1	2.33	2	4	4	3.33
22	0	2	3	1.67	1	2	2	1.67
23	2	2	3	2.33	2.5	2	4	2.83
24	1.5	4	3	2.83	1.5	4	2	2.5
25	2	2	2	2	2	2	3	2.33
26	3	4	3	3.33	2	4	2	2.67
27	2	2	2	2	2	2	3	2.33
28	-	-	-	-	-	-	-	-

Table 12 Clinical grading scores at week 12 by 3 blinded-dermatologists at 12 week.

	Mangosteen				Clindamycin			
Subject No.	Evaluator1	Evaluator2	Evaluator3	Mean	Evaluator1	Evaluator2	Evaluator3	Mean
1	1.5	2	3	2.17	1	1	3	1.67
2	1	2	1	1.33	1	2	2	1.67
3	1	1	1	1	1	2	2	1.67
4	2	2	3	2.33	2	1	4	2.33
5	0.5	1	1	0.83	0.5	1	2	1.17
6	1	2	3	2	1	1	4	2
7	1	1	1	1	1	1	2	1.33
8	1	1	1	1	1	1	2	1.33
9	-	-	-	-	-	-	-	-
10	2	2	3	2.33	2	2.5	4	2.83
11	-	-	-	-	-	-	-	-
12	1	2	2	1.67	2	2	3	2.33
13	1	1	2	1.33	1.5	1	2	1.5
14	1	1	2	1.33	2	1	2	1.67
15	0	2	1	1	0	1	1	0.67
16	0	1.5	1	0.83	0	1	1	0.67
17	1	1	1	1	1	1	2	1.33
18	2	1	3	2	2	1.5	3	2.17
19	1	2	1	1.33	1	2	1	1.33
20	2	2	2	2	2	2	3	2.33
21	1	2	1	1.33	1	1	4	2
22	0	1	1	0.67	1	1	3	1.67
23	2	1	2	1.67	3	1	5	3
24	0.5	2	1	1.17	0.5	2	1	1.17
25	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1	1
28	-	-	-	-	-	-	-	-

Table 13 Irritation of the mangosteen gel and clindamycin gel at baseline, week 2, week 4, week 8 and week 12 of the study.

	Mangosteen					Clindamycin				
Subject No.	week0	week2	week4	week8	week12	week0	week2	week4	week8	week12
1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
3	Neg	2	1	Neg	Neg	Neg	1	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
9	Neg	Neg	-	-	-	Neg	Neg	Neg	-	-
10	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
11	Neg	1	Neg	Neg	-	Neg	Neg	1	Neg	Neg
12	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
13	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
14	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
15	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
16	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
17	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
18	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
19	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
20	Neg	1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
21	Neg	Neg	1	-	-	Neg	Neg	Neg	Neg	-
22	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
23	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
24	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
25	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
26	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
27	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
28	Neg	Neg	-	-	--	Neg	Neg	Neg	-	-

Table 14 Patient satisfaction scores of improvement of acne vulgaris in the side of mangosteen and clindamycin

Patient Satisfaction Scores								
	Mangosteen				Clindamycin			
Subject No.	Week 2	Week 4	Week 8	Week 12	Week 2	Week 4	Week 8	Week 12
1	6	6	6	7	6	6	6	7
2	7	7	7	6	7	7	7	7
3	8	7	7	8	8	8	8	8
4	7	7	7	7	7	6	7	7
5	7	7	7	7	7	6	5	7
6	7	7	7	7	7	7	7	7
7	6	6	7	7	6	7	8	8
8	7	8	8	7	7	7	7	7
9	7	-	-	-	7	-	-	-
10	6	6	7	7	6	6	7	7
11	6	6	5	-	5	6	6	-
12	7	7	8	8	7	7	8	8
13	6	7	7	7	7	7	7	8
14	5	6	7	7	6	6	7	7
15	7	8	8	8	7	8	8	8
16	6	7	8	8	6	7	8	8
17	6	8	8	8	6	7	7	7
18	5	5	6	5	5	6	5	5
19	6	6	6	6	6	6	6	6
20	7	7	8	8	7	7	7	7
21	6	7	-	-	6	7	-	-
22	7	7	7	8	7	8	8	8
23	7	7	8	8	7	7	7	7
24	5	6	7	8	5	6	7	7
25	6	7	7	7	6	7	7	7
26	5	5	5	6	5	5	5	6
27	7	7	8	8	7	8	8	8
28	8	-	-	-	8	-	-	-

BIOGRAPHY

Name	Miss Karuna Sriviriyakul
Date of Birth	August 6, 1988
Educational Attainment	2008. Doctor of Medicine, Srinakharinwirot University, Thailand
Work Position	Physicians
Good Clinical Practice	2016

Publications

Karuna Sriviriyakul, Pongtip Sitthisarn, Clinical efficacy of topical mangosteen extract nanoparticle loaded gel compared with 1% clindamycin gel in the treatment of mild to moderate acne vulgaris. Thai Journal of Pharmaceutical sciences. 2016; 41:121-124.

Work Experiences

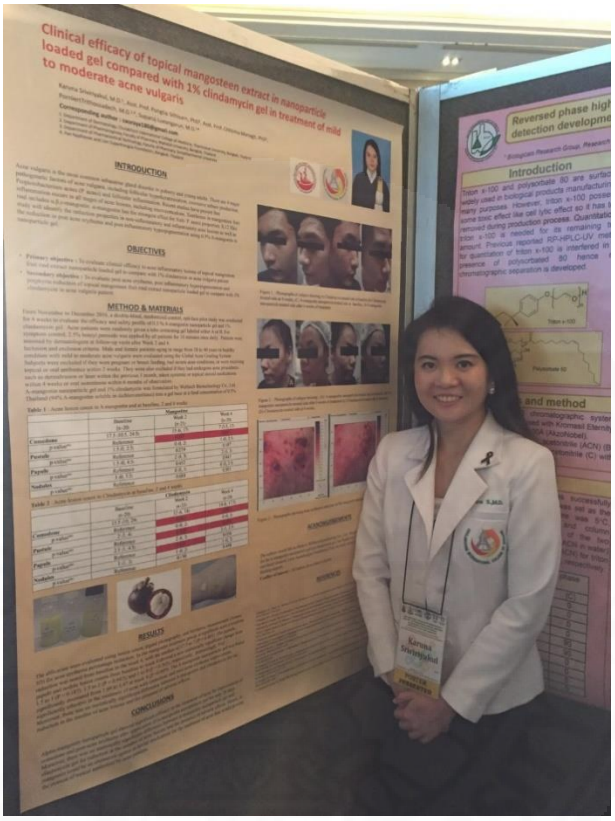
2014 General Physician, Department of Family Medicine, Ramathibodi Hospital, Bangkok, Thailand.

2013-2014 Internship at HRH Princess Maha Chakri Sirindhorn Medical Center, Nakhonayok, Thailand.

Awards Best poster presentation awards in "Clinical efficacy of topical mangosteen extract nanoparticle loaded gel compared with 1% clindamycin gel in mild to moderate acne vulgaris" from 33rd INTERNATIONAL ANNUAL MEETING IN PHARMACEUTICAL SCIENCES (JSPS-NRCT 2017 AND IAMPS 33).

BEST POSTER PRESENTATION AWARDS

FROM 33rd INTERNATIONAL ANNUAL MEETING IN PHARMACEUTICAL SCIENCES (JSPS-NRCT 2017 AND IAMPS 33) AT BERKERLY HOTEL DURING MARCH 2-3, 2017.



ETHICS APPROVAL

Certificate of Approval

Human Research Ethics Committee of Thammasat University No.1 (Faculty of Medicine)

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Number of COA	016/60
Title of Project	CLINICAL EFFICACY OF TOPICAL MANGOSTEEN EXTRACT NANOPARTICLE LOADED GEL COMPARED WITH 1% CLINDAMYCIN GEL IN MILD TO MODERATE ACNE VULGARIS
Project No	MTU-EC-OO-2-120/59
Principal Investigator	Karuna Sriviriyakul (M.D.)
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Document Reviewed

1. Protocol No. 3 : dated January 12 ,2017
2. Information Sheet No. 3 : dated January 12 ,2017
3. Consent Form No. 3 : dated January 12 ,2017

The Human Ethics Committee of Thammasat University No.1 (Faculty of Medicine) is in full compliance with international such as Declaration of Helsinki, The Belmont Report, CIOMS Guidelines and the International Practice (ICH-GCP)

This document is a record of review and approval / acceptance of a clinical study protocol. The Human Research Ethics Committee of Thammasat University No.1 (Faculty of Medicine) has approved the above study and the following documents for use in the study at the Ethics Committee meeting on August 9, 2016. (15/2016)

Approval period 1 year.

Progress report deadline : 26 July , 2017

Signed: .....

(Assistant Professor Sumalee Kondo)

Assistant Secretary of the Human Research Ethics Committee of Thammasat University No.I

(Faculty of Medicine)

Signed:.....

(Associate Professor Waipoj Chanvimalueng (M.D.))

Chairman of the Human Research Ethics Committee of Thammasat University No.I

(Faculty of Medicine)

Date of Approval : **January 27, 2017**

Date of Expire : **January 26, 2018**