



***Garcinia mangostana* hydrogel patch: bactericidal activity and clinical safety for acne vulgaris treatment**

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Abstract

Background and purpose: *Garcinia mangostana*, simply known as mangosteen, has long been used by Thai traditional medicine because of its reported antibacterial and anti-inflammatory activities for the treatment of skin infections. In this study, mangosteen pericarps were developed into a hydrogel patch to eradicate acne-inducing bacteria.

Experimental procedure: The *G. mangostana* extract was investigated for bactericidal activity. A hydrogel patch containing the extract was examined for mechanical properties, antibacterial activity, *in vitro* release, skin permeation, and a phase I clinical study of skin irritation and allergic testing by a closed patch test.

Finding/Results: The *G. mangostana* hydrogel patch made from carrageenan and locust bean gum powders was yellow in color, smooth, durable, and flexible. This *G. mangostana* hydrogel patch was effective against *Cutibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. The active ingredient, α -mangostin, was released and permeated from the *G. mangostana* hydrogel patch within the first 30 min at $33.16 \pm 0.81\%$ and $32.96 \pm 0.97\%$, respectively. The *G. mangostana* hydrogel patch showed no irritation in 30 healthy volunteers. However, two volunteers had delayed allergic contact dermatitis to 0.5% (w/w) *G. mangostana* hydrogel patch.

Conclusion and implication: This hydrogel patch containing *G. mangostana* ethanolic extract is not recommended for patients who have any reaction to mangosteen but has utility as an anti-acne facial mask.

Keywords: Anti-acne; Closed patch test; *Garcinia mangostana*; Hydrogel patch.

INTRODUCTION

Garcinia mangostana Linn. (GM) or mangosteen is a tropical tree with widespread distribution throughout Thailand, India, Sri Lanka, and Myanmar (1). In Thai traditional medicine, the pericarp of GM, mangosteen rind, has an astringent taste and has been used as an anti-diarrheal for over a hundred years (2). Folk

medicine uses GM for wound healing, eczema, and skin infection (3). At present, there are many commercial preparations containing herbal extracts, such as witch hazel, tree tea, tomato, or cucumber that are claimed to be anti-acne products without any scientific evidence.

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DOI: 10.4103/1735-5362.355195

However, there are several studies to support the use of GM. Previous studies have shown that GM has a strong antimicrobial effect against *Cutibacterium acnes* (formerly *Propionibacterium acnes*) and *Staphylococcus epidermidis* (4-6), anti-inflammatory activity via nitric oxide production inhibition, as well as prostaglandin E₂, interleukin-4, tumor necrosis factor- α production inhibition (7-9) and antioxidant properties (10-11). The major constituent from the pericarp of GM was α -mangostin, which has antibacterial activity against acne-causing bacteria (5,12), antioxidant, anticancer, anti-inflammatory, anti-allergy, analgesic, anti-fungal, and antiviral properties (12). Therefore, the pericarp of GM may have potential utility as an anti-acne agent.

There are several treatment options for acne vulgaris, starting with topical therapies, including retinoids, benzoyl peroxide, and antibiotics, however, most acne products can cause dryness, redness, peeling, and skin irritation. Dryness or skin irritation can deprive the stratum corneum of water and lead to inflammation (13). Transdermal products represent an attractive alternative to other dosage forms. A hydrogel patch is usually used for sensitive skin due to its cooling, hydrating, and soothing effects (14). Furthermore, hydrogel contains high amounts of water, which could increase the skin moisture level when applied as a facial mask (15) and could deliver active ingredients into the skin (16-17). For these reasons, a GM hydrogel was developed for use as an anti-acne hydrogel patch.

In the development of herbal cosmetic products, regulatory bodies require skin patch testing for potential adverse effects. Therefore, this study aimed to investigate the effectiveness of the GM hydrogel patch on bacteria-induced acne, physicochemical properties, *in vitro* release, *in vitro* skin permeability, and contact dermatitis testing by closed patch test in a clinical phase I study on human skin.

MATERIALS AND METHODS

Plant material and extraction

The ripe fruits of GM were purchased from a local market, originally collected from

Chanthaburi province, Thailand. The voucher specimen, SKP 214 09 13 01, was identified by the herbarium of the Southern Center of Thai Medicinal Plants at the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand. The pericarps were separated, washed, and dried in a hot air oven at 50 °C for 3 days. The dried powdered plant material was macerated with 95% ethanol for 72 h and evaporated to dryness with a rotary evaporator (Rotavapor R-205, Germany) at 45 °C. The maceration was repeated twice and the dried extracts were combined. The yield of extract was calculated as percentage yield (yield%). The extract was kept at -20 °C until used.

Determination of marker content

The content of α -mangostin in GM ethanolic extract was analyzed by high-performance liquid chromatography (HPLC). The HPLC system (Agilent® 1200; Agilent Technologies, USA) is equipped with a photodiode array detector (G1315D) with a C18 column (Agilent®, 4.6 × 250 mm, 5 μ). The sample volume of 10 μ L was injected into the HPLC system. The gradient elution was performed A:B by 0.1% ortho-phosphoric acid (A) and acetonitrile (B) as follows: 0 min, 95:5; 30 min, 5:95; 35 min, 95:5 at a flow rate of 1.0 mL/min. The diode array detector was set at a wavelength of 316 nm. The content of α -mangostin in the samples was calculated using a linear equation from a standard curve.

Determination of antimicrobial activity

Minimal inhibitory and bactericidal concentrations

The three organisms used in this study were *C. acnes* (DMST 14916), *S. aureus* (ATCC 25923), and *S. epidermidis* (ATCC 12228). *C. acnes* was grown on brain heart infusion (BHI) agar under anaerobic conditions using GasPak systems (AnaeroPack®-MicroAero, Japan) at 37 °C for 72 h. *S. aureus* and *S. epidermidis* were grown on nutrient agar under aerobic conditions at 37 °C for 18-24 h.

A two-fold serial microdilution assay determined the minimal inhibitory concentration (MIC). The two-fold dilutions ranging from 256 to 2 μ g/mL of GM ethanolic

extract and 16 to 0.125 µg/mL of α-mangostin were prepared, and 50 µL of each dilution was added to a 96-well microtiter plate. Then, 50 µL of broth culture containing *C. acnes*, *S. aureus*, and *S. epidermidis* with 0.5 McFarland turbidity was added to each well. The final concentration of GM extract and α-mangostin in 96-well plates were 128 to 1 µg/mL and 8 to 0.0625 µg/mL, respectively. Clindamycin (Sigma-Aldrich, USA) was used as the positive control. The plates were incubated at 37 °C for 72 h under anaerobic conditions for *C. acnes*, 18-24 h under aerobic conditions for *S. aureus* and *S. epidermidis*. After the specified time period, resazurin (Sigma-Aldrich, USA) was added to each well as an oxidation-reduction indicator. Resazurin can be reduced by viable cells to resorufin. As a result, a color change from blue to pink is representative of the presence of metabolically active bacteria cells. Conversely, a well that remains blue indicates a compound that inhibits the growth of bacteria (18-19). Therefore, the MIC value was calculated by the lowest concentration that demonstrated a blue color. The aliquot from the wells that inhibited the growth of tested strains was applied onto BHI agar for *C. acnes* and Mueller Hinton agar (MHA) for *S. aureus* and *S. epidermidis*. The minimal bactericidal concentration (MBC) was calculated by the lowest concentration that showed no growth of the tested strain.

Preparations of GM hydrogel patch

For the hydrogel preparation, carrageenan (1.5% w/w) and locust bean gum (1.5% w/w) powders in the ratio of 1:1 were homogeneously mixed and dissolved in distilled water at 75 °C using a mixer homogenizer. The KCl (0.1% w/w) was slowly added into the mixture for increasing gel strength, followed by 0.5% w/w GM ethanolic extract. The stirring was continued until the homogeneous gel was obtained. The gel was then poured onto a glass plate with controlled thickness at 3 mm by a micrometer film applicator. Then a polyvinyl chloride (PVC) sheet was placed on top of the hydrogel patch and pulled off to release the patch from the glass plate. The PVC sheet with hydrogel patch was kept in an aluminium foil sealed bag at 4 °C until used.

Physicochemical properties of GM hydrogel patch

Tensile strength and percentage elongation break test

The hydrogel patch was cut in a rectangle shape (1 × 4 cm²) and evaluated by a tensile tester (TA.XT plus, Stable Micro Systems, UK). The cross-section of the hydrogel patch was fixed by adhesive tape between the cell grips. The test conditions were the test speed of 1 mm/s and the trigger force of 5 g. Force was gradually applied until the hydrogel patch broke.

Thickness

The thickness at five different positions on the GM hydrogel patch was measured using an analog thickness gauge.

Physical characteristics

The color of the GM hydrogel patch was measured using Chroma Meter CR-400 (Konica Minolta, Japan). The CIE L* a* b* (L, lightness; a, redness; and b, yellowness) were determined.

Surface morphology

The surface morphology of the GM hydrogel patch and blank-hydrogel patch were studied. All samples were incubated at 40 °C for 72 h to remove the moisture before testing. The dried samples were coated with gold (QUORUM Q150R ES, UK) and evaluated with a field emission scanning electron microscope (FE-SEM; JEOL JSM7800F, Japan) and imaged at an acceleration voltage of 5 kV.

Antibacterial activity of hydrogel patch

The broth culture of *C. acnes*, *S. aureus*, and *S. epidermidis* were adjusted in turbidity to 0.5 McFarland standard. *C. acnes* was plated on BHI agar. *S. aureus* and *S. epidermidis* were plated on MHA agar. The GM hydrogel patch, blank-hydrogel patch (without GM extract; negative control), and 0.05% clindamycin hydrogel patch (positive control) in 8 mm diameter were put on the inoculated plates. The inhibition zone was measured across the disc (mm).

***In vitro* release and skin permeation of GM hydrogel patch**

The release of α -mangostin from the GM hydrogel patch was determined through a cellulose dialysis membrane (12000-14000 Da molecular weight cut off, CelluSep[®], USA) using a modified Franz cell diffusion system. The dialysis membrane was placed between a donor compartment and receptor chamber. Deionized water was circulated in the receptor cell to give 37 ± 0.5 °C. The receptor chamber capacity was 6 mL with an effective diffusion area of 1.77 cm^2 and filled with 1% Tween[®] 80 in phosphate-buffered saline (PBS) pH 7.4 (receptor medium) (20) under continuous stirring by a magnetic stirrer. A GM hydrogel patch was cut into $2 \times 2 \text{ cm}^2$ and placed onto the dialysis membrane between the donor and receptor units.

The permeation of α -mangostin from the GM hydrogel patch was determined through newborn pig skin. The newborn pigs weighing 1.1-1.7 kg that died naturally after birth were obtained fresh from Rajamangala University of Technology Suvarnabhumi farm (Phra Nakhon Si Ayutthaya, Thailand). GM hydrogel patches were placed on the pig skin in the donor unit. At 0.5, 1, 2, 4, 6, and 8 h, 800 μL of receiving solution in the receptor chamber was withdrawn and replaced with the same volume of fresh receptor medium. The α -mangostin content was analyzed by HPLC.

Skin irritation and allergic testing in healthy volunteers

Skin irritation and allergic testing were obtained by a closed patch test. The study protocol was approved by the Medical Ethics Committee of the Faculty of Medicine, Thammasat University (Ethics No. MTU-EC-TM-4-065/59). The data was collected at the skin center, Thammasat University Hospital. During testing, the atmospheric temperature was between a low temperature of 23-26 °C to a high temperature of 30-34 °C from November 19 to December 9, 2020. An open-label study was conducted on 30 healthy volunteers aged between 18 to 60 years who did not receive antihistamine, corticosteroids, and immunosuppressive therapy in the previous 2 weeks. Volunteers were excluded if they were

pregnant, breastfeeding, planning a pregnancy, had an underlying skin disease, or had a skin lesion on the test area. The test samples included (a) 0.25, 0.5, 1, 2, 3, 4, and 5% (w/w) GM ethanolic extracts in white petrolatum, (b) 0.5% GM hydrogel patch, (c) blank-hydrogel patch, and (d) white petrolatum as negative control were loaded into the chamber (size $8 \times 8 \text{ mm} \times 10$ chambers/patch, allergEAZE clear patch test chamber, Canada) and the patch was applied onto the back of volunteer for 48 h. The patch was covered with the patch protector (3M Health Care, USA) and micropore tape (3M Health Care, USA) to secure the tested patch. Skin allergy and irritation were observed at 30 min and 24 h after patch removal by a dermatologist using the International Contact Dermatitis Research Group scale (21) and the Cosmetic, Toiletry, and Fragrance Association (22). Skin responses were calculated according to the following formula and determined a safe zone using a human primary irritation index (22).

Statistical analysis

MIC and MBC values were investigated in triplicate independent experiments. Other *in vitro* data collected in triplicate were presented as mean \pm standard deviation (SD) using GraphPad Prism software, version 8.0.1 (San Diego, CA, USA). Differences were considered statistically significant for *P* values lower than 0.05.

RESULTS

The extract of G. mangostana

The GM ethanolic extract was reddish-brown in color. The percentage of yield was 21.38% (w/w). α -Mangostin was the standard marker as shown in HPLC chromatograms (Fig. 1), with a retention time of 30.3 min (Fig. 1A). The content of α -mangostin was 125.14 mg/g extract (12.51% w/w of extract) as calculated from $y = 49.746x - 541.79$ ($R^2 = 0.9995$). GM ethanolic extract showed significant activity against *C. acnes*, *S. aureus*, and *S. epidermidis* with the ratio MBC/MIC of 1-2. However, the MIC and MBC values of GM extract were lower than those of clindamycin, reference standard Table 1.

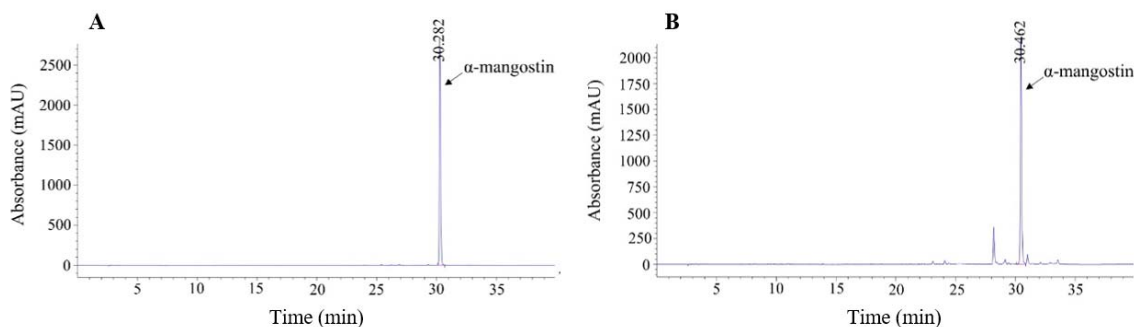


Fig. 1. HPLC Chromatograms of (A) α-mangostin (400 µg/mL) at 316 nm and (B) *Garcinia mangostana* Linn. ethanolic extract (2.5 mg/mL) at 316 nm. HPLC, High-performance liquid chromatography.

Table 1. Antimicrobial activity against *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* of GM ethanolic extract and α-mangostin.

Samples	<i>Cutibacterium acnes</i>			<i>Staphylococcus aureus</i>			<i>Staphylococcus epidermidis</i>		
	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC
GM extract	4	8	2	8	16	2	8	16	2
α-Mangostin	1	4	4	1	2	2	1	2	2
Clindamycin	0.031	0.063	2	0.125	0.5	4	0.125	0.25	2

GM, *Garcinia mangostana* Linn; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration

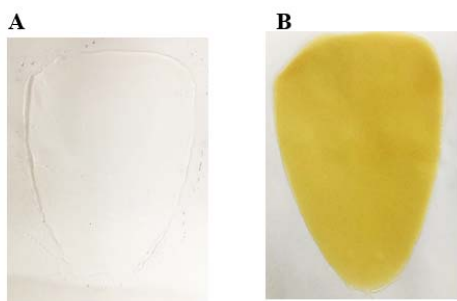


Fig. 2. (A) Blank-hydrogel patch without GM ethanolic extract and (B) GM hydrogel patch. GM, *Garcinia mangostana* Linn.

GM hydrogel patch

The blank-hydrogel patch was transparent and colorless, while the GM hydrogel patch was yellow in color with 70.90, 6.26, and 65.60 for L*, a*, and b*, respectively (Fig. 2). The surface morphology of the dried GM hydrogel patch is demonstrated in Fig. 3. The GM hydrogel patch made from carrageenan and locust bean gum dried up after 2 min with a thickness of 0.66 ± 0.02 mm and could be easily pulled off from the glass plate. Tensile strength

and percentage elongation in Table 2 revealed that the GM hydrogel patch was durable and flexible. There was no difference in tensile strength and percentage elongation between the GM hydrogel and blank-hydrogel patches. The antibacterial activity of the GM hydrogel patch by the disc diffusion method showed activity against all tested microorganisms, especially *C. acnes*, while the blank-hydrogel patch had no activity (Table 2).

In vitro release and skin permeation of GM hydrogel patch

The release profile of α-mangostin from the GM hydrogel patch is shown in Fig. 4A. The releasing rate of α-mangostin from the GM hydrogel patch had a burst release at the first 30 min and continuously increased from 30 min to 3 h. Then after 3 h, it increased at a constant rate. Approximately 30% of α-mangostin was detected after the first 30 min. The percentage cumulative release was 46.96 ± 3.10% after 8 h with a flux of 6.71 µg/cm²/h. Considering its drug release after 30 min to 8 h, the cumulative drug release was fitted to a first-order model (R² = 0.9908).

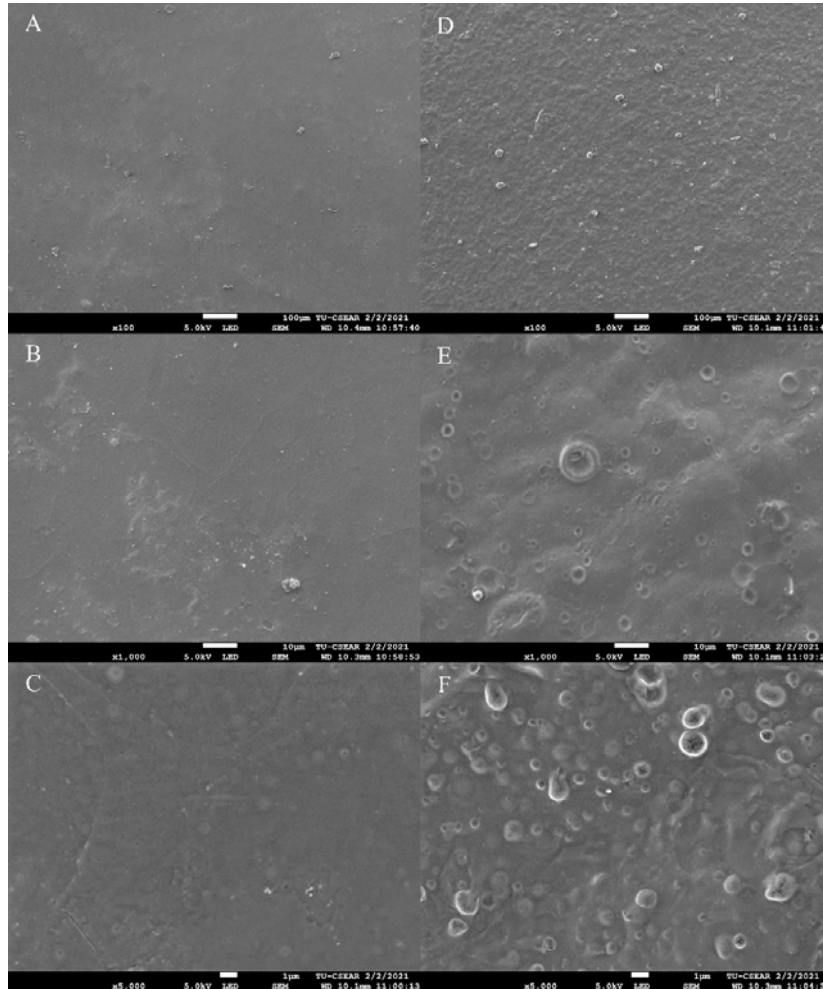


Fig. 3. FE-SEM microphotographs of (A-C) blank-hydrogel patch without GM extract and (D-F) 0.5% (w/w) GM hydrogel patch. The magnifications of (A) and (D) were $\times 100$, (B) and (E) were $\times 1000$, and (C) and (F) were $\times 5000$. FE-SEM, field emission scanning electron microscope; GM, *Garcinia mangostana* Linn.

Table 2. The mechanical properties and antibacterial activities against *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* of hydrogel patch.

Samples	Thickness (mm)	Tensile strength (kg/cm ²)	Elongation break test (%)	Inhibition zone (mm; mean \pm SD, n = 3)		
				<i>Cutibacterium acnes</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
GM hydrogel patch 0.5%	0.66 \pm 0.02	1.41 \pm 0.15	262.15 \pm 12.99	13.88 \pm 0.25	9.00 \pm 0.00	11.00 \pm 0.00
Blank-hydrogel patch	0.70 \pm 0.03	1.43 \pm 0.32	242.05 \pm 16.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Clindamycin hydrogel patch 0.05%	0.32 \pm 0.03 ^{a,b}	1.34 \pm 0.26	196.51 \pm 13.72 ^{a,b}	57.17 \pm 0.29	31.33 \pm 0.58	30.67 \pm 0.58

^a $P < 0.05$ Indicates a significant difference between clindamycin hydrogel patch and GM hydrogel patch; ^b $P < 0.05$ significant difference between clindamycin hydrogel patch and blank-hydrogel patch; GM, *Garcinia mangostana* Linn.

In vitro skin permeation was performed to evaluate the α -mangostin content from a GM hydrogel patch permeating into the skin. The percentage of cumulative skin permeation was $32.96 \pm 0.97\%$ and $38.50 \pm 1.90\%$ after 30 min and 8 h, respectively (Fig. 4B). It showed continuous skin permeation at a slightly constant rate with a flux of $5.50 \mu\text{g}/\text{cm}^2/\text{h}$. Moreover, skin permeation after 30 min to 8 h was fitted to a zero-order model according to the highest cumulation of permeation determination ($R^2 = 0.9885$).

Skin irritation and allergic testing

For skin patch testing, 30 healthy volunteers (4 males and 26 females) aged

32.37 ± 8.29 years were recruited. None of the volunteers dropped out or missed the follow-up visits throughout the study period. No volunteers had any reaction to the test materials at 30 min and 24 h after patch removal (Fig. 5).

However, at 11 days post patch removal, two volunteers (6.67%) developed delayed allergic reactions with palpable erythema to 2, 3, 4, and 5% (w/w) of GM ethanolic extracts in white petrolatum (Fig. 5D) and 0.5% (w/w) GM hydrogel patch whereas blank-hydrogel patch and white petrolatum did not induce an allergic reaction.

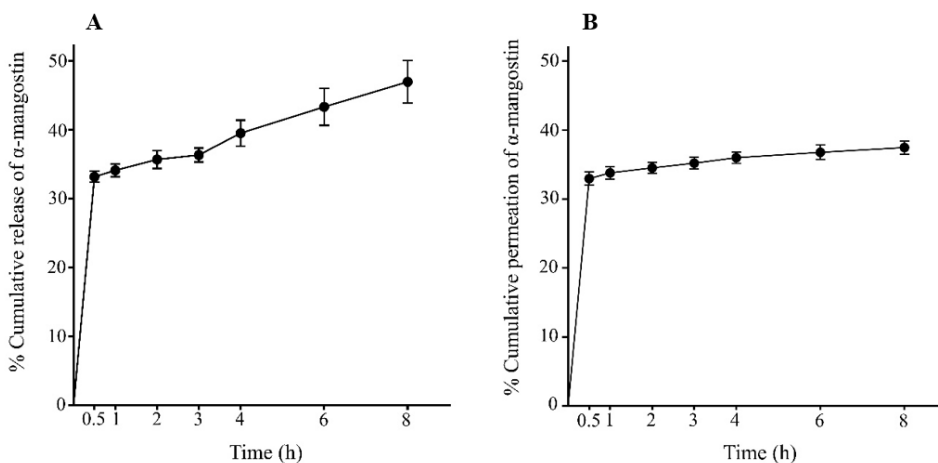


Fig. 4. (A) *In vitro* release and (B) skin permeation of α -mangostin from *Garcinia mangostana* Linn. hydrogel patch

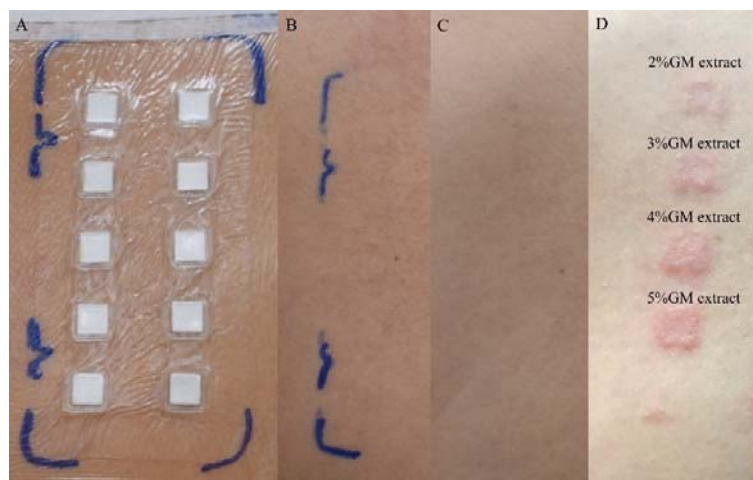


Fig. 5. Photographs of a patient volunteer who developed a delayed allergic reaction. (A) The patch was applied on the upper back for 48 h, (B) at 30 min after patch removal showed a negative reaction, (C) at 24 h after patch removal showed a negative reaction, and (D) at 11 days after patch removal showed palpable erythema.

DISCUSSION

GM pericarp has been utilized for skin infection in Thai traditional medicine for centuries. Moreover, the National List of Essential medicine uses 10% (w/v) GM pericarp ethanolic extract in solution for wounds (23). The crude ethanolic extract of ripe GM fruit rind in this study showed comparable α -mangostin content to literature values of Pothitirat *et al.* (5), that found the mature fruit of GM had 13.63% w/w of α -mangostin content and supported the potent antibacterial activity against pathogenic bacteria that cause acne with a strong anti-*C. acnes* and *S. epidermidis*. The GM ethanolic extract and α -mangostin-reduced DNA replication, DNA repair, and the fatty acid production pathway, potentially enhancing bactericidal activity against microbes (24). The results in Table 1 demonstrate that GM ethanolic extract inhibited the growth of *C. acnes*, *S. aureus*, and *S. epidermidis* and eliminated them with a bactericidal effect. Antibacterial agents are considered bactericidal when the ratio of the MBC to MIC is ≤ 4 (25). Therefore, the crude ethanolic extract was developed into an anti-acne product due to the demonstrated biological activity.

In this study, a hydrogel patch was made from carrageenan and locust bean gum. Carrageenan and locust bean gum biopolymers have been widely used in cosmetics, pharmaceuticals, and food (26,27). This GM hydrogel patch was durable and flexible due to the carrageenan-locust bean gum mixture with KCl as an enhancer of gel strength (28). Tensile strength and percentage elongation of the GM hydrogel patch was no different when compared with the blank-hydrogel patch. This indicated that the GM ethanolic extract did not affect the strength and flexibility of the hydrogel patch. SEM images indicated that a blank-hydrogel patch had a smooth surface, while a GM hydrogel patch showed a bulged surface and some cavities. These findings suggest that a GM extract was completely loaded in hydrogel-based carrageenan and locust bean gum. Moreover, the antibacterial activity of the hydrogel patch revealed that GM ethanolic extract added to the hydrogel patch at a concentration of 5 mg/g was sufficient to inhibit *C. acnes*, *S. aureus*, and *S. epidermidis*.

Although the 0.05% clindamycin hydrogel patch demonstrated greater inhibition than the GM hydrogel patch, the disadvantage of topical clindamycin monotherapy was that it could increase *C. acnes* resistant strains (29). Topical clindamycin is usually used to treat mild to moderate acne. However, Cunliffe *et al.* (30) reported that topical clindamycin monotherapy increased resistant *C. acnes* count to more than 1600% from baseline to week 16. In addition, another study found that 18.8% and 51.7% of *C. acnes* and *S. epidermidis* strains isolated from patients with acne were resistant to clindamycin (31).

In the *in vitro* release and permeation studies, α -mangostin demonstrates low water solubility. Therefore, Tween[®] 80 was added to the receptor medium to increase its solubility (32). The GM hydrogel patch not only diffused in inoculum agar with antibacterial activity but also permeated through the membrane and pig skin as shown in the release and permeation profiles. The burst release in the first 30 min is advantageous in mask patch products. Moreover, the GM hydrogel patch showed prolonged drug release, resulting in reduced water loss from the epidermis and delivering α -mangostin into the skin. A previous study of the α -mangostin-loaded film-forming gel also indicated the same results as a biphasic profile with a fast permeation within the first 30 min and followed by slow permeation (32). Considering drug release of GM hydrogel patch after 30 min to 8 h, the cumulative drug release was fitted to a first-order model and is consistent with the release rate being directly proportional to the concentration of the drug undergoing reaction (33). *In vitro* skin permeation was fitted to a zero-order model, which suggests that the drug release remained constant throughout the delivery and also that drug absorption is a mass transfer or diffusion-limited process through the skin (33).

A human closed patch test was used to evaluate both allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD). ACD is an individual immunological response to an allergen (34), whereas ICD is a non-immunological response that occurs when in contact with an agent (35). A positive reaction at 48 h after patch application, which is subsequently negative at 72 h, indicates

irritation is due to the tested materials (36). A positive reaction at 48 and 72 or 96 h after patch application indicates an allergic reaction is occurring (37). The skin response of GM extract and GM hydrogel patch was 0.00. An *et al.* reported a skin response of less than 1.40, which equates to a z-score < 1 and was defined as a safety zone tested sample (22). Therefore, GM ethanolic extracts at concentrations of 0.5-5% (w/w) and GM hydrogel patch were determined to be non-irritating and can be used on human skin without irritation. A study by Asatjarit *et al.* on *in vitro* skin irritation test of α -mangostin-rich extract-loaded film-forming solution also supported these results due to its being non-toxic to normal human foreskin fibroblast cells which suggest a lack of potential to develop skin irritation (20). However, Lueangarun *et al.* obtained different results and reported that 0.3, 0.6, and 1.2% mangosteen nanoparticle gel caused a mild irritation reaction while the gel base did not (38).

Interestingly, this is the first report that observed a delayed allergic reaction to mangosteen. An allergic reaction was considered to be due to individual susceptibility that involved the interference of allergen with the host immune response (34). ACD starts when exogenous allergens come into contact with the skin. Dermal dendritic cells and Langerhans cells are mainly involved in antigen presentation and sensitization of these haptens to T-lymphocytes (39). Cytokines produced by keratinocytes, antigen-presenting cells, and T cells recruit antigen-nonspecific T cells and macrophages to participate in a local inflammatory reaction, resulting in skin inflammation with swelling, itchiness, and pain (40). α -Mangostin is a hydrophobic compound that strongly interacts with the membrane (32). Tanngoen *et al.* reported that α -mangostin-loaded film-forming gel accumulated a high amount of α -mangostin in the epidermis of the skin (32). In addition, a previous study has demonstrated that 38.3% of α -mangostin could be detected in the applied area after a single application of 1.2% mangostin nanoparticle gel on day 7 (41). Referring to earlier studies reported by Tanngoen *et al.*, α -mangostin could remain in the skin for a long period of time and therefore may induce an allergic reaction. However, α -mangostin in the study conducted

by Pan-In *et al.* was loaded in a nanoparticulate delivery system, which provided a sustained release; in contrast, the GM ethanolic extract in this study was directly loaded into the hydrogel patch. Presently, there are many anti-acne products available including both traditional and natural options. Clindamycin is a commonly prescribed topical medication to treat acne-reported skin irritation with dryness and itching (38). Moreover, tea tree oil products, one of the popular topical anti-acne, also reported ACD (42) and side effects of treatment with minimal pruritus, burning, and scaling (43). Although these treatments show benefits for anti-acne, their potential side effects remain.

In summary, a GM hydrogel patch has the potential to be used as an anti-acne product. It is recommended that the GM hydrogel patch should be further evaluated for successful treatment in acne patients to verify its effectiveness and should be included in follow-up evaluations for at least 2 weeks after concluding treatments to evaluate subjects for assessment of delayed allergic contact dermatitis. A late safety evaluation is highly recommended for the clinical study of the formulation that consists of GM.

CONCLUSION

This study revealed that a GM ethanolic extract had potent activity against *C. acnes*. The developed GM ethanolic extract hydrogel patch demonstrated excellent physical appearance and mechanical properties. The release and skin permeation rate of a GM hydrogel patch was constant throughout the delivery. A concentration of 0.5% (w/w) GM extract in the hydrogel patches demonstrated a lack of irritation on human skin, but a possibly delayed allergic reaction was found in two volunteers. However, a phase II clinical trial study will be necessary to evaluate and determine its efficacy and safety in patients with acne vulgaris.

Acknowledgments

This research was financially supported by the Thailand Science Research and Innovation Fundamental Fund through Grant No. TUFF 28/2565. The authors are grateful to the Department of Biotechnology, the Korea National University of Transportation for

providing the facilities to develop and study the hydrogel patch.

Conflict of interest statement

The authors declared no conflicts of interest in this study.

Authors' contribution

A. Itharat conceived and supervised the project. K. Phumlek carried out the experiments and wrote the manuscript with support from A. Itharat and N.M. Davies. P. Pongcharoen and P. Chakkavitumrong evaluated skin reaction in a clinical study. H.Y. Lee provided the technical method for the hydrogel patch preparation. G.S. Moon and S. Panthong provided the technical method for antibacterial activity. M.H. Han performed antibacterial activity of hydrogel patch. W. Ketjinda verified the analytical methods. N.M. Davies provided grammatical revisions to the manuscript. All authors read and approved the final version of the manuscript.

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