J. Cosmet. Sci., **68,** 257–269 (July/August 2017)

Formulation of chitosan patch incorporating *Artocarpus altilis* heartwood extract for improving hyperpigmentation

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Accepted for publication June 24, 2017.

Synopsis

Artocarpus altilis heartwood extract contains the bioactive compound artocarpin which exhibits melanogenesis inhibitory activity. However, the extract has poor solubility which affects the skin permeability of the compound. A chitosan hydrogel patch incorporating *A. altilis* heartwood extract was formulated to enhance the delivery of an amount of artocarpin sufficient for depigmenting the skin. The extract was prepared as an o/w microemulsion before blending with an aqueous solution of chitosan. The hydrogel patch was formulated by blending in a 1:1 ratio by weight of 4% w/w chitosan solution and 0.04% w/w extract microemulsion which provides optimal values of the mechanical properties of the patch. The release of artocarpin from the formulated patch (artocarpin content, 0.07 mg/cm^2) exhibited two phases; the rapid rate (0–15 min) averaged 0.73 μg/min/mm^2, and the slow rate (15–240 min) averaged 0.02 μg/min/mm^2. The formulated patches significantly improved the hyperpigmented area of the subjects after 3 weeks of application. No adverse events were observed. The results indicate that the formulated chitosan hydrogel patch delivers an effective amount of incorporated artocarpin depigmenting action.

INTRODUCTION

Artocarpus altilis belongs to the Moraceae family. This evergreen tree, called Sa-Kae in Thai, is found throughout the tropical areas of Southeast Asia and has long been used in traditional folk medicines. Several studies have shown that *A. altilis* heartwood extract contains phenolic compounds with the ability to inhibit the activity of tyrosinase (1–3), a key enzyme for melanin synthesis. Recent studies have identified artocarpin as a major compound in *A. altilis* heartwood extract (4–8). The artocarpin in the extract decreases melanin production of B16F1 melanocytes (4,5) and exhibits skin depigmenting effects on the ultraviolet B (UVB)-induced hyperpigmented dorsal skin of C57BL/6 mice (4).

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This suggests the potential of the extract for application in the depigmentation process. However, the incorporation of this extract in cosmetic formulations such as an oil-in-water emulsion is limited because of its low solubility in an aqueous phase, thus affecting its permeability through skin and its efficacy at the action site. Artocarpin has poor ability to permeate through skin because of its large molecular weight (MW > 400) and lipophilicity (log partition coefficient > 4). To improve this limitation, we formulated the extract into a form that can promote skin penetration of the compound, leading to enhanced depigmenting efficacy.

The hydrogel patch is a dosage form that is designed particularly for the delivery of the active compounds into the skin for fast activation of the desired amount which results from hydration of the stratum corneum. The hydrogel patch that we developed required an aqueous polymer to act as a rate-controlling matrix. Chitosan, a natural aqueous polymer, attracted our interest because of its biodegradability and nontoxicity. Previous studies had shown that a patch prepared from chitosan exhibits good bioadhesion to the skin because of its net positive charge properties (9,10).

In the present study, to incorporate the extract into the chitosan hydrogel patch, the extract was initially formulated into an oil-in-water (o/w) microemulsion, which was then blended with an aqueous solution of chitosan polymer (9). The resulting solution was then cast into a mold to shape the hydrogel patch. We were then able to determine the release characteristics of the artocarpin, the major bioactive component of the formulated patch, and test and clinically observe any resulting skin irritation as well as the efficacy on skin depigmentation of the formulated patch, and to evaluate its potential for skin depigmentation.

MATERIALS AND METHODS

PREPARATION OF THE EXTRACT

The heartwood of *A. altilis* was collected on July (raining season) from Phitsanulok Province, Thailand. The heartwood portion was chipped and dried at 50°C by using a hot-air oven. The dried chipped heartwood (1 kg) was then macerated at room temperature for 2 cycles (2 days/cycle) with sufficient diethyl ether (analytical grade, Labscan Asia, Co., Ltd., Bangkok, Thailand), according to our previous studies (6,7). The extract from each cycle was pooled and filtered through a filter paper to remove unwanted residue and concentrated to dryness using a rotary evaporator. The extract was further dried in a desiccator, and kept in a tight amber glass bottle at 4°C for further studies.

QUANTIFICATION OF ARTOCARPIN IN THE EXTRACT

The content of artocarpin in the extract was determined by using isocratic high performance liquid chromatography (HPLC). The HPLC instrument consisted of an SPD-20A UV detector and an LC-20AP pump (Shimadzu Co., Ltd., Kyoto, Japan). A Phenomenex Gemini C18 column with 5 µm and 250 × 4.60 mm diameter was applied as the stationary phase. The mobile phase consisted of a mixture of methanol (analytical grade, Labscan Asia, Co., Ltd.) : water (80:20). The flow rate of mobile phase was set at 1 ml/min, and the injection volume was 20 µl. The quantification of artocarpin was based on the peak area

at 282 nm using a calibration curve of a standard artocarpin. The study was performed in triplicates. The standard artocarpin used in this study was isolated from a diethyl ether extract of *A. altilis* heartwood, according to previous studies (7,11).

FORMULATION OF CHITOSAN HYDROGEL PATCH INCORPORATING THE EXTRACT

Preparation of the extract into the oil-in-water (o/w) microemulsion. The o/w microemulsion system consisted of 0.04% w/w extract powder, 1% w/w isopropyl myristate (IPM; Nikko Chemicals Pte. Ltd., Jurong Island, Singapore), 12.8% w/w polyoxyethylene sorbitan monosterate (Tween® 80; Nof Corporation, Tokyo, Japan), 6.4% w/w glycerin (Namsiang Trading Co., Ltd., Bangkok, Thailand), and 79.4% w/w of deionized (DI) water. The extract powder was dissolved in IPM to obtain the internal oil phase of the microemulsion system. The external water phase consisted of Tween® 80, glycerin, and DI water. The water phase was continuously added to the oil phase with slightly mixing. The obtained microemulsion was transparent, and the mean hydrodynamic diameter of its internal oil phase was 31.8 ± 1.2 nm as measured thrice by photon correlation spectroscopy employing a Zetasizer (Model ZetaPALS; Brookhaven Instruments Coporation, Holtsville, NY).

Preparation of chitosan solution. The chitosan used had a molecular weight in the range of 100,000–1,000,000 Dalton and more than 95% degree of deacetylation (Aqua Premier Co., Ltd, Chonburi, Thailand). The total heavy metal and ash contents in chitosan were less than 10 ppm and 2%, respectively. Chitosan with a specified amount at 4% w/w was dispersed in a part of DI water. Then, lactic acid (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was added to dissolve the chitosan and to control the pH of the chitosan solution in a range of 3–4. The chitosan–lactic acid mixture was stirred at room temperature until a clear yellowish solution was obtained.

Formulation of a chitosan hydrogel patch incorporating the extract. The o/w microemulsion containing 0.04% w/w extract was blended with the 4% w/w chitosan solution in a ratio of 1 to 1 by weight. NaCl (Ajax Finechem Pty. Ltd., New South Wales, Australia) at an amount of 1% w/w was then added in the blended mixture. The blended mixture was further agitated for 1 h and left standing until free from air bubbles. The 21 grams of the resultant solution was cast on a clear petri dish with a diameter of 9 cm and kept on a level surface at 35°–40°C in a hot-air oven. After 2–3 d, the casted patch was peeled off from the petri dish and kept for further determinations. The amount of artocarpin in the patch was 0.07 mg/cm^2, according to analysis by using HPLC. The thickness of an individual patch was controlled to 500 ±10 μm.

DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF THE FORMULATED PATCH INCORPORATING THE EXTRACT

Mechanical properties. The tensile tester (Instron® Model 4411 S/N H2082; Instron Ltd., Buckinghamshire, UK) was used to determine the tensile strength and percent elongation at break of the formulated patches. The patch specimens were cut out in a rectangle with a length of 70.0 mm and a width of 10.0 mm. The thickness of each specimen was calculated as the average value of three separate measurements taken along the middle of 20 mm. The cross-section area of the tested patch was calculated by multiplying the mean thickness with gauge width. The tested patch was clamped by upper and lower grips. The rate of grip separation was 12.5 mm/min and loading weight was 200 N. The testing

room condition was controlled at 23 ± 2°C with 40–70% relative humidity. The ultimate tensile strength and percent elongation at break value were calculated by these formulas:

$$\text{Ultimate tensile strength (kg/mm}^2\text{)} = \frac{\text{breaking load}}{\text{cross-section area of the tested specimen}}$$

$$\text{Percent elongation at break} = \frac{\text{Different in the length at breaking point} \times 100}{\text{original length of the tested specimen}}$$

MOISTURE CONTENT

Moisture analyzer (Sartorius Model MA30, DKSH GmbH, Hamburg, Germany) was used to measure the percentage of moisture content of the formulated patches. The tested patches were cut in 0.2 × 0.2 cm^2 and approximately 5 g was placed in a preweighted aluminum dish. The dishes and contents were put in an oven at 105°C for 12 min and then placed in a **desiccator** to cool down before weighing. The percentage of moisture content was calculated from the below formula:

$$\%\ \text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight} \times 100}{\text{Initial weight}}$$

The experiment was performed in triplicate.

Surface morphology. The surface morphology of the formulated patch was determined by using a scanning electron microscope (SEM Model 1455VP; LEO, Cambridge, UK). The tested patches were dried in a hot-air oven at 40°C and kept in a desiccator to constant weight before testing. Double-sided tape was used to keep the dried patch attached on the stub. All tested patches on stub were coated with gold. All images were manifested at 350 times.

IN VITRO RELEASE STUDY OF ARTOCARPIN FROM THE FORMULATED PATCH INCORPORATING THE EXTRACT

In vitro release of artocarpin from the formulated patches was evaluated by using the vertical Franz diffusion cell (Model V6A-02/90824; PermeGear Inc., Hellertown, PA). The area of the tested patch exposed to the phosphate buffer (pH 7.4), the receptor medium, was 2.27 cm^2. The volume of the phosphate buffer in the receptor chamber was 12 mL with the temperature set at 37°C. This whole assembly was kept on a magnetic stirrer, and the receptor medium was stirred continuously using a magnetic bead. The receptor medium were collected at various time intervals (5, 10, 15, 30, 60,120, and 240 min) and replaced with an equal volume of fresh medium. The amount of artocarpin that diffused through the patch and accumulated in the receptor medium was determined by HPLC. The study was run in triplicate.

EFFICACY AND TOLERANCE STUDY OF THE FORMULATED PATCH INCORPORATING THE EXTRACT

The study protocol was approved by Human Ethical Committee, Naresuan University, Phitsanulok, Thailand with the permission number 54 03 03 0004. All procedures were

performed at Cosmetics and Natural Products Research Center, Naresuan University, Phitsanulok, Thailand.

Selection criteria for subjects. Healthy Thai male or female with age 20–45 year and having a melanin value, in a hyperpigmented area on facial skin, in a range of 290–490 AU were included. Subjects were excluded from the study if they had been smoking and alcoholic. Female subjects were excluded if they were pregnant or lactating. Subjects were to have discontinued topical application of any medicines, steroids, photosensitizing, or cosmetics with depigmenting agents such as tretinoin, α-hydroxy acids, β-hydroxy acids, hydroquinone, kojic acid, and arbutin on facial skin for at least 2 weeks before study. In addition, subjects were not to have used any systemic steroids, hormones, antibiotics, and antihistamines for at least 4 weeks before the study. Other exclusion criteria included subjects who had a scar or burn with a diameter of more than 2 cm per each area on face or forearm, or who had history of atopic skin reaction, eczema, psoriasis, or recurrent or active herpes simplex on face or forearm. Excessive exposure to sunlight was to be avoided.

Irritation test. Subjects were screened according to inclusion and exclusion criteria. Only subjects who met the criteria entered the skin irritation test (4-h patch test), which was designed to assess the skin tolerance to the formulated patch. All subjects were asked to sign an informed consent before screening into the study.

To assess the irritant contact dermatitis, the chitosan hydrogel patch incorporating the extract (the tested patch, 4 cm^2 of patch containing 0.29 mg of artocarpin), 20% sodium lauryl sulfate (SLS; BASF (Thai) Ltd., Bangkok, Thailand) (positive control, 0.2 ml), and distilled water (negative control, 0.2 ml) were tested using the 4-h human patch test. The testing samples were applied to the three areas on subject's forearm. Each area was separated by 3 cm^2 from each other, then covered with a Webril pad for up to 4 h. The test patch and substances were removed or wiped off from the skin. Treatment sites were assessed for the presence of irritation at 0.5, 24, 48, and 72 h after patch test removal. The evaluation of skin irritation was composed of objective assessment using Mexameter® (Model MX 18; Courage and Khazaka Electronic GmbH, Cologne, Germany) for skin redness measurement (erythema index) and visual assessment by a dermatologist in three domains including erythema, scaling, and oedema, according to the scale of Frosch & Kligman and COLIPA (9,12,13).

Efficacy test. The study design was a randomized, double-blind and parallel study. The site of testing was the facial skin. The subjects were randomly assigned to either receive the test patch (size of 4 cm^2 of the formulated patch containing 0.29 mg of artocarpin, test group) or the control patch (size of 4 cm^2 of the formulated patch without the extract, control group) applying it to the hyperpigmented area. The control patch had components similar to the test patch but without the extract. To determine the balance in skin properties of the two groups, their skin properties at the hyperpigmented area were evaluated before (baseline, week 0) and after patch application. Duration of the study was 8 weeks.

At week 0 of the study, subjects arrived at the testing room at 8.00 am. They were asked to wash their non–make up face with clean water, pat the face dry with a towel, and wait for 30 min before proceeding to the next procedure of measuring the skin properties. The temperature and humidity of the test room were controlled to 25 ± 2°C and 50–70% RH, respectively. Skin properties including skin colors (melanin and erythema values), moisture content and skin pH of the hyperpigmented area were measured by using Mexameter®, Corneometer® (Model CM285; Courage and Khazaka Electronic GmbH) and

Skin-pH meter (Courage and Khazaka Electronic GmbH), respectively. After completing baseline measurements, color photographs of subjects were taken. After that, all subjects received the test or control patch (three pieces/1 week usage) for using at home and were asked to record in their diary any abnormal sensation and/or feeling from the application of the patches. Subjects were instructed to apply the received patches every two days (Monday, Wednesday, and Friday) by slightly pressing them on the hyperpigmented area at night after facial cleansing. The duration of application for each night was 30 min. Any facial product except products containing steroids, photosensitizing, and depigmenting agents were allowed to be used at the patch-applied area after 20 min of the patch application. At week 1, 3, 6, and 8 of study period, subjects arrived at the test room around 8.00 am and waited for 30 min before evaluation of their skin properties. The dermatologist also assessed the irritation score on the applied area. Subjects were asked to return the package of used products while receiving the new one at each visit. In addition, the application time recorded in the personal diary was also examined.

STATISTICAL ANALYSIS

All data were expressed as the mean ± standard deviation (SD). Student's *t*-test was used for comparison the skin parameters between two independent groups, and analysis of variance (ANOVA) was used for multiple comparisons. $p < 0.05$ was considered statistically significant.

RESULTS

CONTENT OF ARTOCARPIN IN THE ARTOCARPIN-ENRICHED EXTRACT

The extract was a dry yellow powder. The artocarpin content was 89.5 ± 6.5% w/w, according to an HPLC assay.

CHARACTERISTICS OF THE FORMULATED CHITOSAN HYDROGEL PATCH INCORPORATING THE EXTRACT

When the chitosan solution was blended with the microemulsion in the ratio of 1:1 by weight, it was observed that they were compatible. The formulated patches were yellowish, transparent, and glossy in appearance (Figure 1). The ultimate tensile strength of the patches was 5.07 ± 0.08 N/mm^2, the percentage of elongation at the break point was 35.04 ± 0.14%, and the moisture content was 51.4 ± 0.9%. The surface morphology of the formulated patches was rough and porous as can be seen in the photomicrographs (Figure 2).

RELEASE OF ARTOCARPIN FROM THE FORMULATED PATCH INCORPORATING THE EXTRACT

Figure 3 illustrates the cumulative amount of artocarpin released from the formulated patch after 30 min (67%) and after 240 min (86%). The release pattern exhibited two

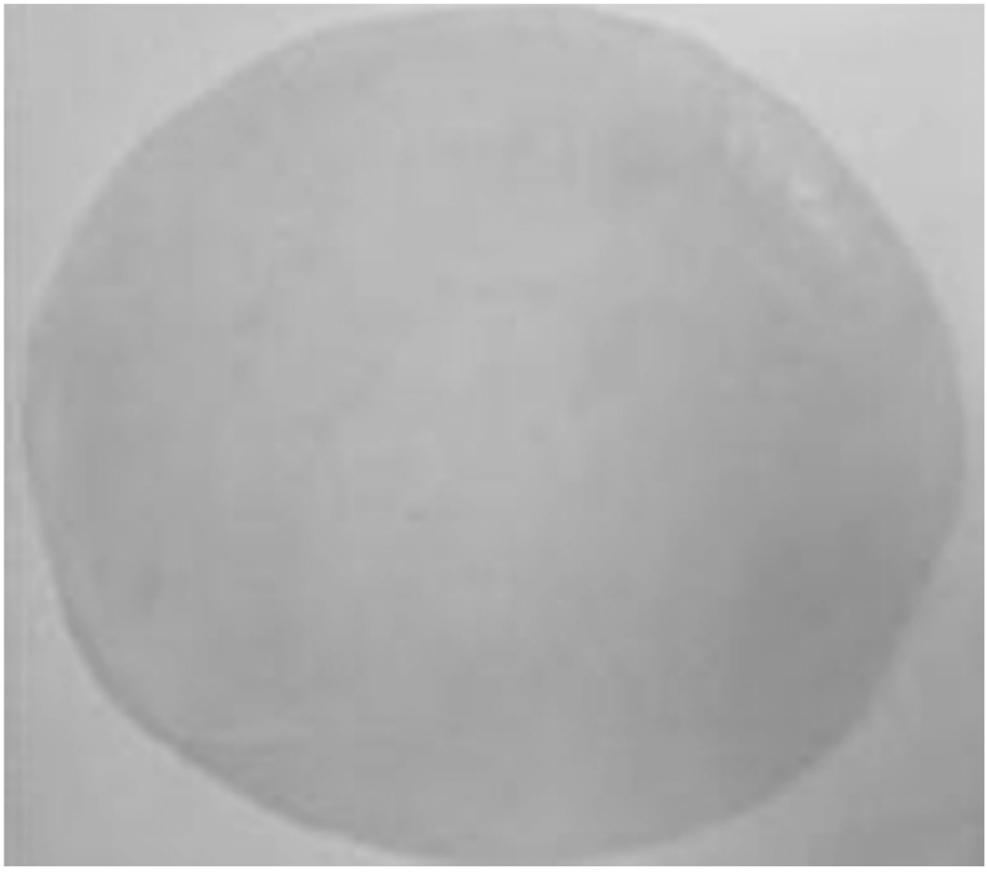

Figure 1. An appearance of the formulated chitosan hydrogel patch incorporating the extract.

distinct phases; a the rapid rate in the 0–15 min period, with an average release rate of 0.73 μg/min/mm^2, and a slow rate from 15 min to 240 min, with the average release rate of 0.02 μg/min/mm^2.

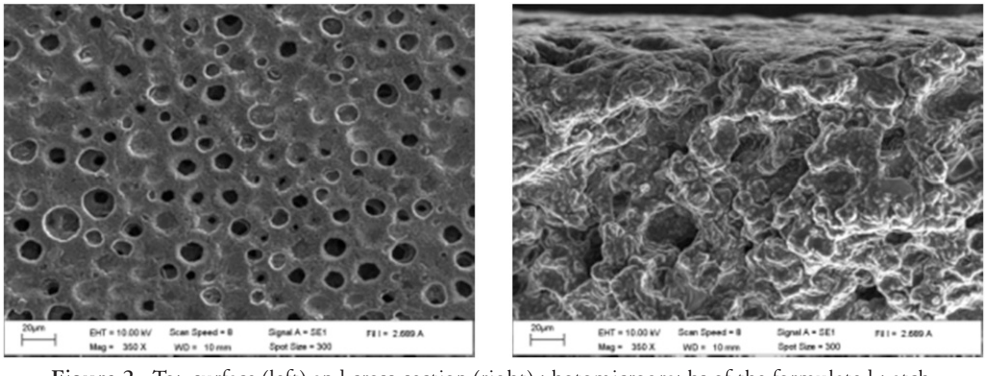

Figure 2. Top surface (left) and cross-section (right) photomicrographs of the formulated patch.

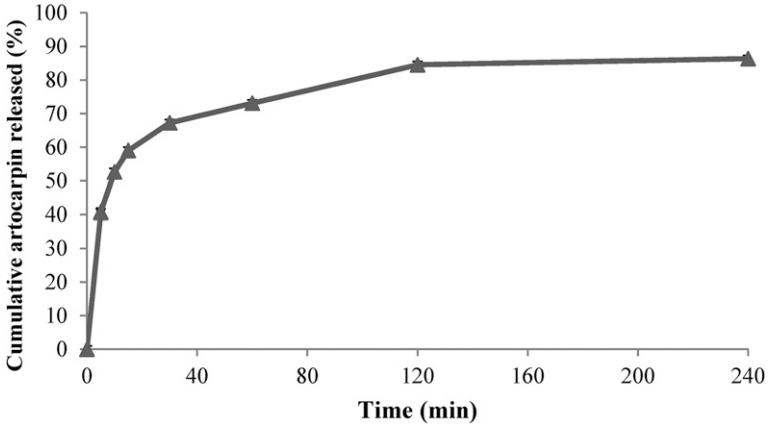

Figure 3. The cumulative content of artocarpin released from the formulated patch. Each point represents mean ± SD of percentage of artocarpin released ($n = 3$).

EFFICACY AND TOLERANCE EVALUATION

Ninety-five Thai subjects were initially screened for recruitment according to the specified inclusion criteria, with 62 subjects successfully enrolled for the skin irritation test. Three of these subjects withdrew from the study because of personal reasons, leaving a sample set of 59 subjects. Figure 4A shows an example photo of the response to the 20% SLS and the tested patch after the 4-h patch test. The erythema value (using a Mexameter®) of the skin applied with SLS significantly increased compared against the value at baseline (before irritation test) (Figure 4B). Irritated skin returned to normal at 24 h after pad removal. The visually observed results coincided with the results from erythema measurement.

All 59 subjects tolerated the tested patches and were subsequently enrolled and randomized into two groups for the efficacy study. The test group had 30 subjects, and 29 subjects were placed in the control group. All subjects completed the 8 weeks of the study. Table I shows the demographic and mean baseline characteristics of the subjects. The subjects ranged in age from 24 to 51 year (mean ± SD, 42.2 ± 6.0 year) and were predominantly female. All subjects in both groups had a melanin value in the range of 290–490 AU (test group, 370.8 ± 2.8 AU; control group, 369.1 ± 4.1 AU). There was no significant difference in skin properties between the groups. Subjects in both groups were assessed by a dermatologist who reported none as having erythema, scaling, or oedema on their faces.

Mean ± SD values of the measured parameters in the test and control groups are shown in Table II. A significant difference in the mean melanin value between the test group (mean ± SD, 346.4 ± 4.4 AU) and the control group (mean ± SD, 364.5 ± 5.1 AU) was first observed at week 3 ($p < 0.001$). There was a statistically significant percentage change in melanin value as compared to the baseline in the within-group results for the test patch. By the eighth week of treatment, a decrease in the melanin value from baseline was observed in 27 of the 30 subjects of the test group and 12 of the 20 subjects of the control group. The decrease observed in the control group was not statistically significant. In both the test and control groups, moisture content was significantly increased after 8 wk when compared with the baseline values of each group.

We also assessed the mildness of the tested patch on the facial skin by measuring skin pH and redness. We found that no subject in either group showed a significant change in skin pH

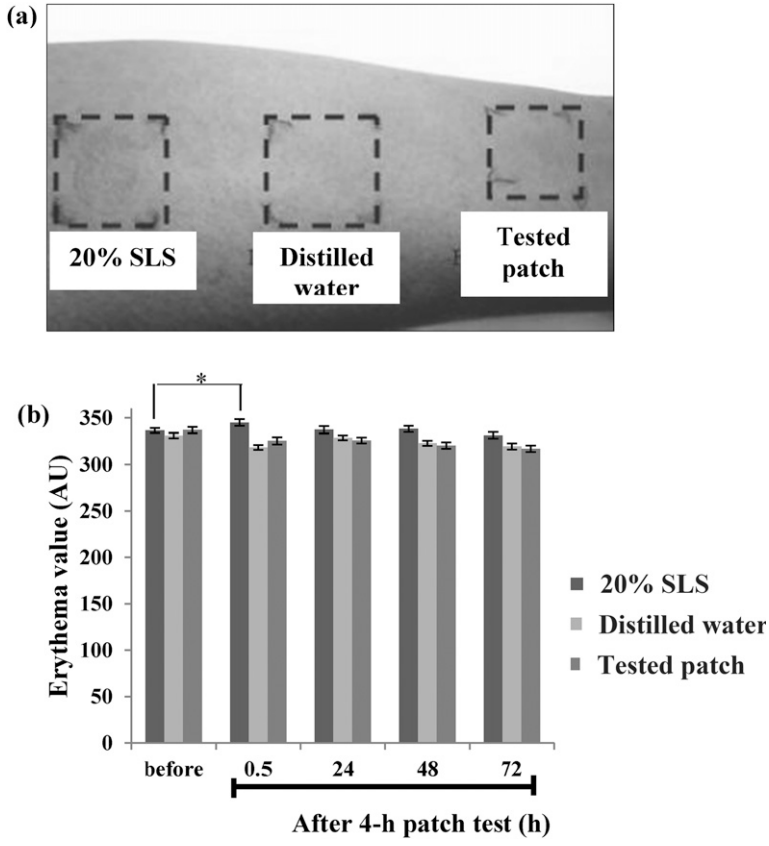

Figure 4. SLS-irritated skin of volunteers forearm after 4-h treatment of 20% SLS (a) and erythema values of subjects forearm treated with 20% SLS, distilled water and the tested patch for 4 h (b). Each bare represents mean ± SD of erythema value in arbitrary unit (AU). (n = 59; *p < 0.05 by unpaired student's t-test).

or redness when compared with baseline. Based on the dermatologist's reports and the self-reporting by the subjects, no sign of skin irritation was observed throughout the study. By interviewing the subjects and assessing their diaries, we found that all subjects followed the instructions of product application. They also avoided sun exposure by wearing protective facial covering or hat.

DISCUSSION

Our previous studies reported that *A. altilis* heartwood extract contains artocarpin as a major constituent and has a potent inhibitory activity on melanin production in B16F1 melanoma cells and UVB-induced hyperpigmented dorsal skin of brownish guinea pigs (4,5). There are three important mechanisms of the extract, variously discussed, which inhibits melanogenesis: (a) inhibition of tyrosinase activity, (b) inhibiting transportation of tyrosine, and (c) inhibiting melanin production in the melanosome (4,12). In the present study, the extract from *A. altilis* heartwood was isolated, formulated into o/w microemulsion and then incorporated in the aqueous polymer, chitosan, which acted as a polymeric

Table I
Demographic and baseline characteristics of subjects enrolled in the efficacy study ($N = 59$)

Characteristics	Test group ($n = 30$)	Control group ($n = 29$)	p*
Age, mean (SD), year	41.3 (6.9)	42.2 (6.9)	0.618
Sex, no. (%)			
Male	2 (6.7)	1 (3.5)	
Female	28 (93.3)	28 (96.5)	
Race, no. (%)			
Thai	30 (100)	29 (100)	
Education, no. (%)			
Primary school	20 (66.7)	18 (62.1)	
High school	10 (33.3)	8 (27.6)	
Bachelor's degree or equivalent	-	3 (10.3)	
Occupation, no (%)			
Agriculturalist	3 (10)	3 (10.3)	
Freelance/personal business	5 (16.7)	6 (20.7)	
Contingent worker	22 (73.3)	20 (69.0)	
Skin properties, mean (SD)			
Melanin value, AU	370.8(2.8),	369.1 (4.1)	0.812
Moisture content, AU[a]	45.7 (3.3)	46.9 (4.3)	0.233
Skin pH	5.6 (0.5)	5.5 (0.5)	0.446
Erythema value, AU	417.7 (3.4)	418.1 (3.4)	0.653

AU: arbitrary unit.
*2-Group t-test with a 2-sided significance level of 0.05.
[a]One unit represents a water content of stratum corneum of 0.02 mg/cm^2.

device for delivering the bioactive compound to the skin. We observed that the bioactive components of the *A. altilis* heartwood extract were released from the formulated patch at the effective amount, resulting in the depigmenting efficacy in the clinical study.

Our experimental process began with the preparation of the *A. altilis* heartwood extract. We then analyzed the extract by using HPLC. Our analysis showed a high content of artocarpin ($89.5 \pm 6.5\%$ w/w) in the prepared extract, which coincides with the $90.6 \pm 5.1\%$ w/w of artocarpin reported in our previous study (7). These results clearly indicate that artocarpin is a major compound of the *A. altilis* heartwood extract.

For the formulation of the chitosan hydrogel patch, lactic acid was used to dissolve chitosan (9,10,13), and the chitosan solution was then blended with the extract microemulsion by stirring. The blended solution did not show incompatibility because of the external phase of the microemulsion being water which is miscible with the aqueous solution of the polymer. The formulated patches showed proper flexibility with tensile strength in the range of 5–10 N/mm^2 and percentage elongation at break point in the range of 20–50%. These values allow the patches to be handled and to adhere to the skin (9,10,14). The flexibility of the formulated patches is possibly caused by the plasticizer activity of the incorporated ingredients, such as lactic acid (9,10,15), NaCl (16,17), and glycerin (18,19) which together interrupt the compactness of the polymeric network. As well, the hygroscopic behavior of these plasticizers kept the patch in a hydrogel state and maintained a high moisture content. Water remaining in the hydrogel patch supported the incorporated microemulsion surfactant (Tween® 80) by solubilizing the IPM oil which is a dissolving solvent in the extract.

Table II
Measured parameters in the test and control groups. Each value is mean ± SD

Parameter	Test group (n = 30)	Control group (n = 29)	P_a	% change (test group)	% change (control group)	P_b
Melanin value, AU						
Week 0	370.8 (2.8)	369.1 (4.1)	0.067	0	0	0
Week 1	367.3 (9.2)	365.3 (3.2)	0.273	2.7 (7.1)	1.5 (4.0)	0.410
Week 3	346.4 (4.4)	364.5 (5.1)	<0.001	8.3 (8.9)	1.3 (5.5)	0.001
Week 6	335.8 (8.8)	364.4 (5.9)	<0.001	12.5 (12.2)	1.4 (5.2)	<0.001
Week 8	317.7 (9.9)	362.8 (2.7)	<0.001	19.0 (13.4)	2.3 (4.6)	<0.001
Moisture content, AU[a]						
Week 0	45.7 (3.3)	46.9 (4.3)	0.233	0	0	0
Week 1	51.3 (1.5)	49.6 (0.9)	<0.001	13.2 (18.5)	6.9 (19.4)	0.207
Week 3	51.8 (1.5)	52.3 (1.4)	0.191	20.1 (31.1)	14.7 (28.9)	0.493
Week 6	58.5 (3.1)	53.2 (2.2)	<0.001	36.2 (43.8)	16.8 (29.9)	0.053
Week 8	56.4 (2.2)	57.9 (1.7)	0.005	32.2 (45.5)	29.4 (43.6)	0.810
Skin pH						
Week 0	5.56 (0.04)	5.45 (0.05)	<0.001	0	0	0
Week 6	5.45 (0.04)	5.47 (0.04)	0.060	1.7 (4.4)	0.7 (4.7)	0.402
Week 8	5.54 (0.09)	5.32 (0.04)	< 0.001	0.3 (10.4)	1.7 (10.6)	0.611
Erythema value, AU						
Week 0	417.7 (3.4)	418.1 (3.4)	0.653	0	0	0
Week 1	421.2 (4.6)	409.3 (4.4)	0.067	1.7 (16.9)	1.0 (11.3)	0.853
Week 3	419.8 (3.3)	408.8 (4.7)	0.059	2.3 (22.4)	1.1 (13.4)	0.805
Week 6	416.3 (3.2)	402.3 (5.1)	0.023	1.4 (17.2)	2.5 (13.8)	0.788
Week 8	414.3 (5.7)	400.4 (4.0)	0.052	0.4 (18.6)	3.7 (12.2)	0.425

P_a: one-way ANOVA compare two mean of each parameter ($p < 0.05$). P_b: one-way ANOVA compare two mean of % change ($p < 0.05$).
[a]One unit represents a water content of stratum corneum of 0.02 mg/cm^2.

Our previous studies revealed that the surface morphology of the chitosan patch was compact (10,13). However, in the present study, we observed porous structures throughout the formulated patch. It is possible that the presence of other ingredients, such as NaCl in the casting solution interrupted the compactness of the polymeric network of chitosan, resulting in the formation of porosity (17,20). These porous structures influenced the patch flexibility and the release of the incorporated bioactive components through the patch. We found a high rate and amount of artocarpin release (about 70% of the initial content of artocarpin in the casted patch) in the first 30 min. As the formulated patch was composed of high porous structures, the receptor medium was able to quickly diffuse into the patch. This led to the fast release rate of the artocarpin to the receptor cell (9,21), after which a low amount of artocarpin was released through the patch, because of the low amount of artocarpin remaining in the patch.

A 4-h human patch test is an alternative method of determining the acute skin irritation potential of a tested substance (22). When the substance being tested was applied to the skin and then covered with cotton bandage material, such as a Webril pad, the stratum corneum was hydrated. This consequently increased the penetration of the substances through the skin and thereby accelerated the incidence of skin redness and irritation. In the present study, irritation on the volunteers' forearms was not observed after applying the test patch, whereas skin irritation was clearly observed after applying 20% SLS, which was the positive control for identifying substances or preparations that were classified as

irritants (23). When the test patches were applied on subjects' faces for 8 weeks (three times/week, 30 min for each time), erythema, scaling, and oedema on applied area were not observed, and the skin pH at the applied area was maintained in the normal range (5.5–6.5). These results demonstrate the safe and beneficial use of the tested patches for use in cosmetics.

The outermost layer of the skin is the stratum corneum which plays a role as a barrier layer relying on its lipid and keratin composition and organization. The skin permeation of compounds such as artocarpin, which has a large size (MW > 400 dalton) and lipophilic nature (log partition coefficient, log P > 4) is generally poor (24,25). Reversible alteration of the organization of keratinized protein and/or fluidization of the lipids by using permeation enhancers is widely implemented to improve the skin permeation of poor permeation compounds. In the present study, the extract enriched with the bioactive compound, artocarpin, was formulated into the hydrogel patch to improve skin permeability of artocarpin. Water molecules accumulated in the stratum corneum during patch application which contributed to skin moisturization and acted as a permeation enhancer by changing the structure of the keratinized protein in the stratum corneum (26). The microemulsion nonionic surfactant (Tween® 80) present in the formulated patch also facilitated the permeability of bioactive components by reversely changing the conformation and/or fluidization of the lipid bilayer (26). We found that the formulated patch containing the extract (0.07 mg of extract/cm^2 of patch) significantly improved the hyperpigmented area after 3 weeks of application. This implies that the amount and rate of release of artocarpin from the formulated patch achieved an effective topical delivery, and in addition, a significant increase in the skin moisture content was found after patch application.

In conclusion, the artocarpin-enriched *A. altilis* heartwood extract was formulated into a hydrogel patch by using a microemulaion technique to solubilize the lipophilic extract and by using polymeric chitosan to control the delivery of the artocarpin. The formulated patch was effective in skin depigmentation and was mild on the skin causing no skin redness or irritation. However, the small number of subjects in this study did not include the variety of skin types which may be found in studies with a larger more diverse sample. Future studies with a larger number of subjects and a longer duration should be performed to confirm the beneficial effects of the formulated patch for improving hyperpigmentation.

ACKNOWLEDGEMENTS

Financial supports from the Commission on Higher Education, Ministry of Education, Thailand, and International Laboratories Corp. Ltd. We also thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC), the S&T Postgraduate Education and Research Development Office (PERDO), the Commission on Higher Education for facility support. Many thanks to Mr. Roy Morien of the Naresuan University Language Centre for his editing assistance and advice on the English expression in this document.

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