Development and Evaluation of a Hydrogel containing *Momordica cochinchinensis* Spreng Extract for Topical Applications

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The purpose of this research was to develop a hydrogel containing the extract of Gac fruit (*Momordica cochinchinensis* Spreng) with appropriate physicochemical properties and good dermatological efficacy. The Gac aril fruit was extracted by maceration in dichloromethane, and its antioxidant activity was determined through a DPPH assay. The very low water-solubility of the Gac extract is responsible for its incompatibility with the hydrogel. To overcome this drawback, Labrafree™ PG and Tween 60 were used to develop the hydrogel due to their potent potential for solubilizing the Gac extract. The prepared hydrogels displayed good physical properties, a homogeneous orange gel, appropriate pH, and viscosity. After storage in an accelerated condition for six months, the hydrogels of the Gac extract had physical stability and high remaining amounts of beta-carotene and lycopene within the range of 90.25 - 94.61%. The skin efficacy of hydrogel containing the Gac fruit extract was found using 14 healthy female volunteers over a 30-day period of daily application. Topical application of the hydrogel containing the Gac fruit extract, which contains antioxidants, significantly moisturizes the skin and enhanced its elasticity ($p \leq 0.05$; ANOVA). This makes it suitable for use as a skin care product.

**Keywords**: *Momordica cochinchinensis* Spreng Extract. Antioxidants. Hydrogel. Skin efficacy.

INTRODUCTION

Gac (*Momordica cochinchinensis* Spreng) belongs to the Cucurbitaceae family and is indigenous to Southeast Asia. Due to highly color intense of Gac aril, it has been used as a natural colorant in traditional Asian cuisines. Gac red aril is also the most nutritious part of the Gac fruit, with a high amount of oil and extremely high levels of bioactive compounds, such as carotenoids, polyphenolics, and fatty acids (Ishida *et al.*, 2004; Kubola, Meeso, Siriamornpun, 2013). Gac aril has health benefits, including anticancer, anti-inflammatory, and particularly antioxidant effects due to its very high concentration of carotenoids, such as lycopene and beta-carotene (Chuyen *et al.*, 2015; Lacatusu *et al.*, 2014; Mai *et al.*, 2013). Lycopene content in Gac aril is eight-fold greater than the levels found in tomatoes. In addition, its beta-carotene content is five-fold greater than the levels in carrots (Aoki *et al.*, 2002; Singh, Kawatra, Sehgal, 2001). Gac fruit is safe for human and animal consumption and is applied in beverage, food, pharmaceutical, and cosmetic products (Vuong, 2013). Commercial Gac products, such as Gac oil, Gac aril, and dried Gac aril powder have been recently introduced into the market.

The human organism has a defense system against the destructive action of free radicals in the form of antioxidants. The human antioxidative defense system can neutralize free radicals before they can damage the tissue. Nevertheless, the endogenous antioxidant defense systems are incomplete without exogenous antioxidants, such as carotenoids, vitamin E, vitamin C, and polyphenols, playing an essential role in antioxidant
mechanisms (Bouayed, Bohn, 2010). Antioxidants appear to have protective effects against cardiovascular diseases, immunosuppression, skin cancer, and skin aging (Chuyen et al., 2015; Leeuvinum, Krisadaphong, Petsom, 2015). Scavenging of reactive oxygen species is one of the mechanisms of action underlying the protective effects exhibited by antioxidants on the skin. Antioxidants induced the differentiation of keratinocytes resulting in improved skin parameters (Lorencini et al., 2014). Carotenoids are one of the most important antioxidants for human skin. In addition, they serve as marker substances for the complete antioxidant status of the human epidermis (Haag et al., 2011; Lademann et al., 2011).

Nowadays, there is a great tendency to use natural products for cosmetics instead of synthetic chemicals because of their safety when applied topically. Skin care products are normally formulated in semisolid preparations, such as creams, emulsions, and gels. Hydrogels are cross-linked polymer networks swollen in a liquid medium (Croisfelt et al., 2019). As hydrogels display a water swollen three-dimensional (3D) viscoelastic network, they are expected to enhance the hydration of the stratum corneum (Chirani et al., 2015). Hydrogels have promising applications in the pharmaceutical and cosmetic industries due to several favorable properties, including transparent, emollient, moisturizing effect, cooling effect, non-oily touch, water-washable, easy application, ease of spreading, highly biocompatible with a low risk of inflammation, and compatibility with several excipients (Khan et al., 2020; Croisfelt et al., 2019; Chirani et al., 2015).

Inspired by the antioxidant activities of abundant carotenoids found in Gac aril, in this research, Gac extract was developed in hydrogel products to improve skin conditions and boost healthy skin. However, there are two challenges in the development of hydrogels containing the Gac extract. Firstly, because of the high lipophilicity of lycopene and beta-carotene in the Gac fruit, they are insoluble in water (Paz et al., 2014; Peng et al., 2018). Formulating hydrophilic gels containing water-insoluble compounds may be complicated. Non-ionic surfactants are used in this system to improve the solubilization of lycopene and beta-carotene to obtain a homogeneous hydrogel. Secondly, although high amounts of lycopene and beta-carotene are found in Gac aril, they are able to degrade by environmental and storage conditions, such as oxygen, light, and temperature (Brito-oliveira et al., 2017; Chuyen et al., 2015; Mai et al., 2013). Thus, one of the important issues for Gac formulation is the prevention of carotenoid loss.

The aims of the present work were to: (1) develop and evaluate the physical properties of hydrogel containing the Gac extract, and (2) assess the dermatological effects of the hydrogel of Gac extract, including skin irritation and skin analysis by examining 3 parameters (skin water content, trans-epidermal water loss, and skin elasticity) over 30 days.

**MATERIAL AND METHODS**

**Material**

Fresh Gac fruits were purchased from a local market in Nakhon Si Thammarat, Thailand. Standard beta-carotene (Type II, ≥95% purity, batch no. LRAA4126), lycopene analytical standard (94.3% purity, batch no. BCBT4006), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were supplied by Sigma-Aldrich (Missouri, USA). Labrafac® PG (propylene glycol dicaprylocaprate) was obtained from Gattefosse (Leon, France). Labrasol (Caprylocapropyl macrogol-8 glycerides) was purchased from Gattefossé (Saint-Priest, France). Poloxamer 407 (poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)) was purchased from Chocques, France. Cremophor EL (polyoxyethylene castor oil derivatives) was procured from BASF (Ludwigshafen, Germany). Isopropyl palmitate was obtained from Musim Mas (Sumatera Utara, Indonesia). Tween 60 was purchased from Croda (Singapore). Phenoxyethanol was procured from Schülke (Norderstedt, Germany). Carbopol® 940 was obtained from Lubrizol (Tanjong Penjuru, Singapore). Tween 20 was purchased from Nof Corporation (Tokyo, Japan). Dichloromethane, acetonitrile, and methanol (HPLC grade) were obtained from RCL Labscan (Bangkok, Thailand). All other chemicals were of analytical grade.
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Preparation of a hydrogel containing the Gac fruit extract

Gac fruit extraction

Red aril was scooped from the fresh Gac fruit, and the seeds were removed from the aril part. Gac paste was dried under hot air oven at 50 °C for 24 h. Approximately 25 g of dried sample was macerated with 750 mL of dichloromethane for 3 days at room temperature (adapted from Kubola et al., 2013; Sun et al., 2011). The solvents were removed by rotary evaporator. Gac extracts were gradually dried under vacuum oven and stored in a light-resistant container at -20 °C.

Quantification of beta-carotene and lycopene in the Gac fruit extract

The Gac extract (4 mg) was diluted with dichloromethane (1.4 mL), mixed using a vortex mixer for 20 minutes, and then filtered through a 0.22-µm membrane. The amounts of beta-carotene and lycopene in the Gac extract were analyzed by an HPLC system (Dionex) (Kha et al., 2013). A C18 column (VertiSep™ UPS C18 column 4.6 x 250 mm, 5 µm, Ligand Scientific, Bangkok, Thailand) was used. The mobile phase consisted of acetonitrile, dichloromethane, and methanol in the ratio, 50:40:10. The flow rate of mobile phase was 1 mL min⁻¹, and the injection volume was 20 µL. The UV detector was set at a wavelength of 450 nm. The retention times of lycopene and beta-carotene were 6.5 and 8.8 min, respectively. The mean peak areas for each concentration were calculated from three determinations. The standard curve was constructed by plotting concentrations against the peak areas. Both concentration ranges of 10-50 lycopene and 2-30 µg mL⁻¹ beta-carotene showed good linearity with a correlation coefficient of 0.9999. The intraday precision of the method was determined by repeated three replicates for each concentration of the sample to exhibit the percentage relative standard deviation (%RSD) of 0.26-2.15, and the %RSD of the interday precision acquired in the range of 2.70-3.21. The recovery percentage of the method was between 94.78 ± 0.85 and 103.14 ± 0.59.

Determination of the antioxidant activity in the Gac fruit extract through a DPPH assay

The free radical scavenging activity of the Gac fruit extract was determined using the 2,2-diphenyl-1-picryl-hydrayl-hydrate (DPPH) assay (Moraes-de-Souza et al., 2008). The Gac fruit extract was dissolved in 95 % ethanol to various concentrations and mixed with the DPPH solution in ethanol (24 µg mL⁻¹) to provide the final concentrations of the tested antioxidant in the range of 125 - 625 µg mL⁻¹. The mixed solutions were incubated in the dark at room temperature for 30 min. The decrease in absorbance at 515 nm was then detected with a UV-Visible spectrophotometer. Ascorbic acid was used as the positive control. The percentage of inhibition (% inhibition) was calculated using following Eq. 1:

\[\text{% inhibition} = \left[1 - \frac{\text{Abs}_{515} \text{ sample}}{\text{Abs}_{515} \text{ blank}}\right] \times 100 \quad (1)\]

Where \(\text{Abs}_{515} \text{ sample}\) and \(\text{Abs}_{515} \text{ blank}\) are the absorbances determined at 515 nm for the incubation of the sample and blank (95% ethanol), respectively.

A calibration curve between the percentage of inhibition and sample concentration was constructed. The \(IC_{50}\) value represents the concentration required to inhibit 50% of the oxidation used for comparing the DPPH scavenging activity.

Determination of beta-carotene and lycopene solubility from the Gac fruit extract in various surfactants

The ability of seven non-ionic surfactants (Tween 20, Tween 60, Tween 80, Span 80, Labrasol, LabraFac™ PG, and Chemophor EL) to dissolve the Gac extract was studied. The extract (200 mg) was added to each surfactant (2 mL) and mixed well for 20 min by using a vortex. Thereafter, the mixture was shaken at room temperature for 72 h and then centrifuged at 6000 rpm for 10 min at 25 °C to separate undissolved extract. The supernatant was filtered through a 0.45-µm membrane and diluted with dichloromethane. The amounts of beta-carotene and lycopene were determined by HPLC, similar to the previous condition.
**Preparation of hydrogel of the Gac fruit extract**

The composition of the hydrogel of the Gac fruit extract is presented in Table I. The Gac fruit extract (1%) was dissolved in a mixture containing Labrafil<sup>TM</sup> PG, Tween 60, BHT, and EDTA. Carbopol<sup>®</sup> 940 gel was prepared by dispersing polymer in propylene glycol and purified water while poloxamer 407 was slowly dispersed in cold water (5 °C) with constant stirring, and then kept at room temperature for gel formation. Phenoxyethanol and isopropyl palmitate were added to the gel. The pH of the carboxomer gel was adjusted to 5-6 using triethanolamine. Finally, the mixture containing the Gac fruit extract was added slowly with continuous blending until a homogenous hydrogel was obtained. The prepared hydrogel was packed in a wide-mouth glass jar covered with a screw-capped plastic lid and stored in the dark at room temperature.

| Ingredients          | F1  | F2  | F3  | F4  | F5  | F6  |
|----------------------|-----|-----|-----|-----|-----|-----|
| Gac fruit extract    | 1   | 1   | 1   | 1   | 1   | 1   |
| Carbopol<sup>®</sup> 940 | 1   | 1   | -   | -   | 1   | 1   |
| Poloxamer 407        | -   | -   | 20  | 20  | 5   | 5   |
| Tween 60             | -   | 5   | -   | 5   | -   | 5   |
| Labrafil<sup>TM</sup> PG | 10  | 5   | 10  | 5   | 10  | 5   |
| Propylene glycol     | 15  | 15  | 15  | 15  | 15  | 15  |
| Phenoxyethanol       | 1   | 1   | 1   | 1   | 1   | 1   |
| Isopropyl palmitate  | 3   | 3   | 3   | 3   | 3   | 3   |
| Triethanolamine      | qs  | qs  | -   | -   | qs  | qs  |
| EDTA                 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | qs  |
| BHT                  | 0.05| 0.05| 0.05| 0.05| 0.05| 0.05|
| Purified water qs to | 100 | 100 | 100 | 100 | 100 | 100 |

**Physical evaluation of the prepared hydrogel of the Gac fruit extract**

*Visual examination*

All developed hydrogels were inspected for color, syneresis, and homogeneity by visual observation after hydrogels were set in the container.

*pH determination*

The pH of the prepared hydrogels was determined using a digital pH meter (Jenway<sup>®</sup>, model 3510, UK), which was calibrated before use with the standard buffer solution at pH 4.0, 7.0, and 9.0. Measurements of pH were carried out in triplicate and average values were calculated.

*Spreadability test*

The spreadability of hydrogels was studied by measuring the spreading diameter for 0.5 g of hydrogel between two horizontal plates (20 cm x 20 cm). The standard weight (500 g) was applied on the upper plate and left for 5 min. Diameters of the spread circles were measured in cm. The obtained results were an average of three determinations (Helal et al., 2012).
Viscosity test

The viscosity of the different hydrogel formulae was determined at 25 °C using a rheometer (Thermo Scientific®, model MARS60, plate rotor P35Ti and lower plate TMP35, Germany) with a 3.5-cm parallel plate, a plate gap of 1.0 mm, and a shear rate in the range, 0.100-100.0 s⁻¹. Samples were applied to the lower plate using a spatula to ensure that formulation shearing did not occur. The reading was taken at a shear rate of 5.5-6 s⁻¹. The evaluation was conducted in triplicate and average values were calculated.

Quantification of beta-carotene and lycopene in prepared hydrogels

The hydrogel formulation (0.6 g) was diluted with dichloromethane (2 mL) in the tube covered with aluminum foil for protection from light. The compounds were mixed using a vortex mixer for 40 min. The tube was centrifuged at 6,000 rpm for 20 min at 25 °C and the supernatant was filtered through a 0.22-µm membrane. The quantities of beta-carotene and lycopene in the prepared hydrogels were analyzed by the HPLC method described above.

Stability test

Formulations F1-F6 were stored in airtight, light-resistant containers. Accelerated stability testing was used to observe the possible physicochemical changes (physical properties, pH, spreadability, viscosity, as well as beta-carotene and lycopene contents) that could occur during storage of the hydrogel. The study was performed at constant controlled temperature (40 ± 2 °C) and humidity (75±5% relative humidity (RH)), for 6 months, according to ICH procedure (Note for guidance on stability testing, 2019).

Evaluation of efficacy of the hydrogel containing the Gac extract

Study protocol

The research was designed as a randomized (the side of the forearms) and placebo-controlled trial. The study received ethical approval from the appropriate Ethical Committee of Walailak University, WUEC-16-100-01. Fourteen healthy female volunteers (age, 20-40 years old) living in Nakhon Si Thammarat, Thailand, and having skin Fitzpatrick types II–IV were enrolled in these studies after providing informed consent. The exclusion criteria were: the presence of any skin allergic diseases, pregnancy, nursing mother or those undergoing treatment with oral contraceptives or undergoing intense exposure to ultraviolet radiation. Volunteers were instructed to not apply any topical products including moisturizers, creams, or skin care products to the inner forearms for 2 weeks before and during the study. During the test period, the volunteers were allowed to wash normally, but could not use any other skin care products on their arms. Prior to all measurements, subjects were left in the room at least 20 min to allow the skin to adapt fully to room conditions, including room temperature (25 ± 2 °C) and humidity (45-60%). The inner forearm was used in this research. The test area (4 cm²) was 13 cm from the wrist fold. Each test was performed with the subject seated, both forearms lying horizontally on the arm rests, and the ventral aspects facing up (Farboud, Nasrollahi, Tabbakhi, 2011).

Skin irritation test

Skin irritation test allows the identification of substances with significant skin irritation potential. The protocol is designed to avoid the production of irritant reactions and meets the high ethical standards. Two hydrogels were applied on the forearms of each volunteer using Finn chambers (0.5 cm²) as occlusive patch test devices, for 48 h. One hydrogel contained Gac extract (the formulation) while the other hydrogel did not (control). Skin irritation responses were graded 30 min and 24 h after patch removal by visual assessment on a 4-point scale (0, no reaction; +, weakly positive reaction (mild erythema or dryness); ++, moderately positive reaction (distinct erythema possibly spreading beyond the test area); ++++, strongly positive reaction (strong, often spreading erythema with edema)) (Basketter et al., 2004). A volunteer with any positive reaction, such as erythema, dryness, or edema across the treatment site
was considered to have demonstrated a positive irritant reaction. As such, no further tests were performed with that individual.

*Skin analysis tests*

The volunteers who displayed no reaction in the skin irritation test were included in these studies. Each volunteer was administered two hydrogels on the forearms. One hydrogel contained the Gac extract (formulation) while the other hydrogel did not (control). Each hydrogel was marked “right” or “left”, indicating application of the hydrogel to the right or left forearm, respectively. The hydrogel (approximately 0.25 g) was gently applied by the volunteers themselves once every night before bed for 30 days. Skin assessments were measured on day 0 (basic value, prior to application of the hydrogel) and after 15 and 30 days with multiple probes for skin assessments made by Courage-Khazaka Electronic GmbH in Germany.

*Measurement of skin moisture content and trans-epidermal water loss*

The stratum corneum water content was measured with a skin capacitance meter (Corneometer® CM825, Courage-Khazaka, Electronic GmbH, Germany); the device measures the electrical capacitance of the skin as a reflection of the hydration state of the horny layer. The average value of five measurements was used. The amount of trans-epidermal water loss was determined from dry areas of the test areas using an evaporimeter (Tewameter® TM300, Courage-Khazaka, Electronic GmbH, Germany). The device was registered in g·m⁻²·h⁻¹ after probe equilibration on the skin for approximately 90 s. Three measurements were taken at each time. Lower average values indicated better skin conditions (Constantin *et al.*, 2014; Kim, Cho, Lee, 2015).

*Measurement of skin elasticity*

Skin elasticity was determined using Cutometer® MPA580 (Courage-Khazaka, Electronic GmbH, Germany). A 350-mbar suction was transmitted to the skin for 18 s. This was followed by a relaxation time of 2 s and then two repetitions. The resulting curve for each measurement represents the elastic qualities of the skin. The following parameters of the skin elasticity were analyzed: Ue, immediate extensibility; Ur, immediate refraction; R5 or Ur/Ue, pure elasticity of the skin without viscous deformation or cutaneous elasticity (Kapoor, Saraf, 2009; Saraf *et al.*, 2011).

*Mathematical analysis*

Percentage changes for individual values of different parameters, taken at 0, 15, and 30 days for each volunteer, were calculated using the following Eq. 2:

\[
\text{Percentage change} = \left| \frac{A - B}{B} \right| \times 100
\]

(2)

Where A is individual value of any parameter on days 15 and 30, and B is 0 h (baseline) value for that parameter.

*Statistical analysis*

All experiments were performed in triplicate to validate the statistical analysis. Results are expressed as mean ± SD. ANOVA and Student’s t-test were performed on the data sets. For all tests, significance was achieved at p-values ≤ 0.05.

**RESULTS AND DISCUSSION**

**Preparation of a hydrogel containing the Gac fruit extract**

*Extraction of the Gac fruit extract*

The extract from dried Gac fruit aril was dark orange semi-solid substance with a yield of 23.80 ± 0.008 %w/w. The crude extract consisted of beta-carotene content of 2.89 ± 0.01 and lycopene content of 23.42 ± 0.23 mg/g of crude extract.

The DPPH assay has been generally used to determine the antioxidant capacity of food or plant extracts. The electron or hydrogen atom donating abilities of the tested compounds led to the bleaching of a purple-
colored alcoholic solution for the stable DPPH radical at an absorbance of 515 nm (Moraes-de-Souza et al., 2008). The scavenging activity of the Gac fruit extract \( (IC_{50} = 471.31 \pm 0.26 \mu g mL^{-1}) \) was lower than that of ascorbic acid, positive control \( (IC_{50} = 3.63 \pm 0.28 \mu g mL^{-1}) \). The low activity of the Gac fruit extract could be ascribed to small amounts of antioxidant compounds in the crude extract. However, Gac fruit extract has more advantage than ascorbic acid in term of stability. Ascorbic acid is highly soluble in aqueous, and it is easily oxidized to an inactive form (Oyetade et al., 2012).

**Solubilization of the Gac extract**

The ability of various surfactants to solubilize the Gac extract was evaluated by determining the amount of beta-carotene and lycopene that dissolved in each surfactant. Non-ionic surfactants should be employed owing to their lower toxicity than ionic surfactants. The concentration of beta-carotene and lycopene solubilized in various surfactants is shown in Figure 1. The absence of surfactant, the amounts beta-carotene and lycopene were not detected because of their insolubility in water (Paz et al., 2014). Labrafac™ PG (propylene glycol dicaprylocaprate) could maximize Gac extract’s solubility with beta-carotene \( (11.86 \pm 0.43 \mu g mL^{-1}) \) and lycopene \( (100.97 \pm 1.56 \mu g mL^{-1}) \). Tween 60 (Polyethylene glycol sorbitan monostearate) increased the Gac extract’s solubility with beta-carotene \( (8.56 \pm 0.45 \mu g mL^{-1}) \) and lycopene \( (39.17 \pm 1.44 \mu g mL^{-1}) \). Thus, Labrafac™ PG and Tween 60 were selected as surfactants for further development of the hydrogel.

**FIGURE 1** - Concentration of beta-carotene and lycopene solubilized in various surfactants (mean ± SD, n = 3).

**Physical properties of the prepared hydrogels of the Gac fruit extract**

Hydrogels of the Gac fruit extract were successfully prepared by using Carbopol® 940 and Poloxamer 407 as gelling agents. Carbopol® 940 was chosen due to its excellent characteristics, high viscosity at low concentration, and its capability to retain water, leading to the formation of a cross-linked polymeric network that reduces the loss of moisture from the skin surface,
impeding water evaporation (Lee *et al*., 2014). Poloxamer 407 was selected because of its low toxicity and is approved by the U.S. Food and Drug Administration for preparations. It is also attractive as a delivery system through the skin for local pain and inflammation (Mallandrich *et al*., 2017).

The prepared hydrogel formulae were inspected visually for their color and syneresis. All developed formulations appeared as yellowish-orange hydrogels, with good homogeneity and no lumps or syneresis. The pH of all formulations was in the range of 5-6, which is considered acceptable for avoiding the risk of irritation upon application to the skin (Schmid-Wendtner, Korting, 2006). Viscosity is an important parameter that is interrelated with many properties of a formulation, such as pourability of the product from the container, spreadability, etc. Data presented in Table II revealed that increasing the viscosity always resulted in a decrease in spreadability as demonstrated by the lower diameter of the circle that was spread. The F6 formulation had an appropriate viscosity and spreadability, which indicated that it could be easily applied without the possibility of a runoff. This finding assured that F6 maintained a good wet contact time when applied to the site of application Therefore, F6 was selected for further skin efficacy evaluation. The results are shown in Table II.

### Table II - Physical properties of the prepared hydrogels of the Gac fruit extract

| Formulations | Appearance   | Color          | Homogeneity | pH          | Spreadability (cm) | Viscosity (cp) |
|--------------|--------------|----------------|-------------|-------------|--------------------|----------------|
| F1           | yellowish-orange | no             | Good        | 5.63 ± 0.02 | 5.50 ± 0.10        | 39,663.33 ± 135.03 |
| F2           | yellowish-orange | no             | Good        | 5.53 ± 0.01 | 5.43 ± 0.06        | 39,880.00 ± 122.61 |
| F3           | yellowish-orange | no             | Good        | 5.23 ± 0.03 | 5.77 ± 0.06        | 35,690.00 ± 131.65 |
| F4           | yellowish-orange | no             | Good        | 5.16 ± 0.01 | 3.97 ± 0.06        | 65,810.00 ± 143.91 |
| F5           | yellowish-orange | no             | Good        | 5.35 ± 0.02 | 4.23 ± 0.06        | 57,796.67 ± 128.64 |
| F6           | yellowish-orange | no             | Good        | 5.29 ± 0.03 | 4.83 ± 0.21        | 46,526.67 ± 122.37 |

Values are expressed as mean ± SD of triplicate measurements.

Quantification of beta-carotene and lycopene in the prepared hydrogels

Results of beta-carotene and lycopene contents in the prepared hydrogels are shown in Figure 2. The amounts of beta-carotene and lycopene in all prepared hydrogels ranged from 29.34 - 32.18 and 430.61 - 456.33 µg g⁻¹ of the hydrogel, respectively. Such data indicated that beta-carotene and lycopene were uniformly distributed throughout the hydrogel.
Stability test

The prepared hydrogels were found to be stable following 6 months (180 days) of storage at 40 °C ± 2 °C/75% RH ± 5% RH. No significant change was detected in the parameters evaluated, including physical appearance, spreadability, pH, and viscosity. After storage in an accelerated condition, all prepared formulae had remaining amounts of carotenoids, with beta-carotene in the range, 91.64 - 94.61% and lycopene in the range, 90.25 - 93.67% (Figure 2).

Carotenoid molecules contain a series of conjugated double bonds, which cause them to be very susceptible to ultraviolet irradiation, ions, heating, and aerobic environment (Mao et al., 2018). In this research, four strategies were provided for prevention the carotenoid loss which is the important issue for Gac formulation. Firstly, the preparation process of the Gac fruit hydrogel

**FIGURE 2** - The amount of carotenoids in the prepared hydrogel formulations on days 0 and 180 (after storage at 40 °C/75% RH) (A): beta-carotene and (B): lycopene (mean ± SD, n = 3).
did not require the use of high temperature. Secondly, the compact three dimensional network structure of hydrogel had good physical stability that might act as physical barrier against adverse stresses (e.g., light, and heat), offering good protection for the sensitive substances incorporated within the system. Thirdly, butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid (EDTA) were used as an antioxidant and chelating agent, respectively. Fourthly, the formulations were stored in airtight, light-resistant containers resulting in high amounts of carotenoids remaining in the formulation.

Evaluation of the efficacy of the prepared hydrogel

Skin irritation test

To ascertain the skin irritation potential of the prepared hydrogel, its application onto human forearms was conducted. A patch irritation test for both hydrogels containing the Gac extract and those without the extract was performed. After the test, there was no positive reaction, such as erythema, dryness, or edema across the treatment site of all volunteers. Therefore, the formulation and control were classified as negligible irritants for human skin application.

Measurement of skin moisture content and trans-epidermal water loss

The stratum corneum is the first barrier of the human body. Indeed, it serves as the body’s defense against the external environment and prevents water loss from the skin. Therefore, moisture in the stratum corneum is a key indicator in dermatology and cosmetics (Li et al., 2011). The results of the percentage of increase in skin water content for control (hydrogel without Gac extract) and a formulation containing the Gac extract after the 15- and 30-day application period are shown in Figure 3. For the formulation, water content significantly increased by 16.57% after 15 days of use and increased up to 31.36% after 30 days compared to the values before formulation use \((p \leq 0.05)\). Skin water content slightly increased by 1.79% and 4.81% after 15 and 30 days of use of the control, respectively.

The amount of trans-epidermal water loss was studied, and the results are presented in Figure 4. After 15 days of use, the formulation group showed a statistical decrease of 5.25% in trans-epidermal water loss while the control group had an insignificant decrease of 2.56% compared to values recorded before each treatment \((p \leq 0.05)\). After 30 days of use, the formulation group had a statistical decrease of 7.99% in the amount of trans-epidermal water loss while the control group had a lower decrease of 2.98% compared to values before each treatment \((p \leq 0.05)\).

The above results indicated that the formulation was more effective than the control in increasing the stratum corneum water content and preventing excessive loss of water from the skin. Antioxidants have been reported to stimulate dermal fibroblasts for the synthesis of collagens. As collagen intensity increased, the hydration level was also enhanced (Waqas et al., 2017). The antioxidant abilities of the Gac fruit extract are attributable to its beta-carotene and lycopene content. Thus, the formulation containing the Gac fruit extract could cause a significant increase in the hydration level of the stratum corneum and was suitable for use as a skin care product.
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**FIGURE 3** - Percentage increase in skin water content for the control (hydrogel without the Gac extract) and the formulation containing the Gac extract after the 15- and 30-day application period compared to the values before treatment (mean ± SD). *Significantly different from day zero of each treatment (p ≤ 0.05).

**FIGURE 4** - Percentage decrease in trans-epidermal water loss for the control (hydrogel without the Gac extract) and the formulation containing the Gac extract after the 15- and 30-day application period compared to values before treatment (mean ± SD). *Significantly different from day zero of each treatment (p ≤ 0.05).
Measurement of skin elasticity

The ability of the skin to stretch and then retreat to its normal state is called elasticity. The changes in cutaneous elasticity (R5-parameter) following the application of control (hydrogel without Gac extract) and a formulation containing Gac extract are presented in Figure 5. After applying the control for 15 and 30 days, cutaneous elasticity significantly increased relative to values before treatment at 8.81% and 15.93%, respectively (p ≤ 0.05). Furthermore, after applying the formulation for 15 and 30 days, cutaneous elasticity significantly increased to 19.40% and 30.43%, respectively (p ≤ 0.05). Interestingly, the percent changes in cutaneous elasticity of the formulation group were dramatically greater than those of the control group at 15 and 30 days (p ≤ 0.05). An increase in cutaneous elasticity (R5-parameter) indicates the enhancement of skin elasticity. The improvement in skin elasticity of the control and formulation groups may be due to the moisturizing effect of the isopropyl palmitate contained in the product, and it may be a good characteristic of the product with water-saturated hydrogel. The emollient formed a hydrogel film on the skin surface, restoring the barrier function of the skin. Besides the appropriate properties of the product, the presence of the antioxidant agents, beta-carotene, and lycopene, in the Gac extract promoted the increase in skin elasticity following the application of the formulation. Antioxidants protect the cell membrane by neutralizing free radicals, and block oxidative stress to the tissues of the body. Antioxidants provide skin elasticity by actively countering free radical attacks. Antioxidants were found to promote the differentiation of keratinocytes, resulting in improved skin parameters (Lorencini et al., 2014). Skin elasticity is particularly influenced by dermal collagen. Antioxidants protect the skin by inhibiting the breakdown of collagen and promoting new synthesis, resulting in the improvement of skin elasticity in the formulation containing the Gac extract.

![Percentage increase in cutaneous elasticity](image)

**FIGURE 5** - Percentage increase in cutaneous elasticity for the control (hydrogel without the Gac extract) and the formulation containing the Gac extract after the 15- and 30-day application period compared to values before treatment (mean ± SD). * Significantly different from day zero of treatment (p ≤ 0.05).

CONCLUSION
Herein, the Gac aril fruit was extracted by maceration using dichloromethane as a solvent. The non-ionic surfactants, Labraflo™ PG and Tween 60, were used to improve the solubilization of the Gac extract to prepare the homogenous hydrogel. The hydrogels prepared using Carbopol® 940 and Poloxamer 407 as gelling agents had stable characteristics under the accelerated condition for six months, with good physical properties, including the appearance of a homogenous orange gel, appropriate pH, and viscosity. The beta-carotene and lycopene contents also remained high. Data from skin efficacy evaluation showed that the hydrogel containing the Gac fruit extract promoted skin water content and decreased trans-epidermal water loss, resulting in increased skin hydration. Furthermore, the formulation enhanced skin elasticity due to the emollient effect of water-saturated hydrogel and the potent antioxidant activities of the Gac extract.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICS APPROVAL

All procedures performed in human participants were in accordance with the principles of the Declaration of Helsinki and approved by Human Research Ethics Committee of Walailak University (15 September 2017/ WUEC-16-100-01).

CONSENT TO PARTICIPATE

Informed consent was obtained from all individual participants.

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