

# Cleansing lotion containing tamarind fruit pulp extract.

## III. Study of lightening efficacy and skin irritation on Asian skin type

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**ABSTRACT:** Cleansing lotion containing 8% w/w tamarind pulp extract was prepared and tested for its clinical effects on skin properties. The study was performed using 38 healthy Thai female volunteers, and consisted of a home-use, double-blind, randomized side of face and placebo controlled trial. The test product (emulsion with the extract) and the placebo product (emulsion without the extract) were applied and gently massaged on each side of facial skin for 2 minutes twice daily for 8 weeks and the difference in skin colour between test and placebo side of face was measured. In addition, redness, moisture content, pH and transepidermal water loss (TEWL) of skin, adverse events, subject's satisfaction, and compliance of volunteers were assessed. We found that the melanin value on the side where the test product was applied was significantly less than that of the other side at week 4 ( $p < 0.015$ ). The mean differences between the test and the placebo side of all skin parameters including melanin value, pH and moisture content elasticity were not significant ( $p > 0.05$ ) at the end of study (week 8). Erythema and TEWL of the skin were not different between the side on which the test was applied and the placebo side indicating that the product was safe. In addition, most volunteers were satisfied with the cleansing effect of the product containing the extract.

**KEYWORDS:** alpha-hydroxyl acids, skin properties

### INTRODUCTION

Facial cleansing lotion is a skin care product used to remove dirty materials such as oily residues from cosmetics. Oil-in-water (o/w) emulsion cleans the skin satisfactorily because the water phase can wash the dirt without leaving any oily feeling. Facial skin cleanser containing alpha hydroxyl acids (AHAs) are now frequently used or prescribed by dermatologists and are also present in a wide range of heavily promoted cosmetic products. A low concentration (less than 4%) of AHAs has been suggested for daily application<sup>1</sup>. The primary action of AHAs is to exfoliate dead skin cells by weakening the bonds that hold dead skin cells together, thus resulting in a skin which looks bright<sup>2,3</sup>. AHAs range from simple

aliphatic compounds to complex molecules. Many of these substances originate from natural sources and are often referred to as "fruit acids." However, a number of synthetic sources provide access to compounds with analogous structures. The AHAs now used in cosmetics are usually produced by chemical synthesis because the synthetic products offer higher purity and consistent quality.

The debate over natural versus synthetic AHAs has been ongoing for several years. The acidifying property of AHAs have the potential to irritate the skin. However, this is also coupled with their ability to stimulate cell renewal. It has been found that the therapeutic index (the ratio of stimulation to irritation) of the natural AHAs surpasses that of the synthetic AHAs. It is likely that the natural AHAs contain

natural soothing agents which can reduce the irritation they cause, and not interfere with the stimulatory activity<sup>4</sup>. Hence, from a clinical viewpoint, using naturally derived AHAs in cosmetics will be much more beneficial than using synthetic AHAs.

Botanical secrets have been passed down through generations as herbal folklore, and nowadays botanical extracts are playing an increasingly important role in cosmetics. For the cosmetics industry, isolation and purification of the active ingredient within the crude extract are sometimes not needed because such isolation and purification may lead to a loss in the biological activity<sup>5,6</sup>. Tamarind (*Tamarindus indica* L., Leguminosae) is a common tree occurring in humid tropical areas including SE Asia. Its fruit pulp has an acidic taste and has been used for centuries as a skin-scrubbing material to promote a smoother and lighter skin. The visible improvement in the skin raises the question about the components in the tamarind pulp and actions on the skin of those components. It has been shown that the fruit pulp of tamarind contains AHAs including tartaric acid (8–23.8%), lactic acid (2%), citric acid, and malic acid<sup>7</sup>. Pectin and inverted sugar were also found in the fruit pulp<sup>7</sup>, both of which are hygroscopic and can improve the skin appearance through a moisturizing action. These findings led to the interest in using the extract derived from the fruit pulp of tamarind for cosmetics.

Recently, we have prepared a facial cleansing formula (oil-in-water, o/w emulsion) containing tamarind pulp extract. Due to the concern over the possibility of irritation from AHAs after prolonged application, a preliminary study assessing the irritation effects of the product after cumulative exposure on the inner forearm was conducted. The results obtained from the visual scoring scale indicated no irritation signs or adverse symptoms from using the test product. Mean differences of TEWL and erythema values between test product and de-ionized water, and between test and placebo products were not statistically significant ( $p > 0.05$ )<sup>8</sup>. The present study therefore aimed to determine the effects of the product containing the extract from tamarind fruit pulp when used as facial cleansing lotion. The efficacy of the product was tested by measuring the skin moisture, colour and pH after application of the product containing the extract and comparing with the results from a placebo. Also, skin irritation was determined from erythema and transepidermal water loss values, and by dermatological evaluation.

## PRODUCT PREPARATION

### Plant material and preparation of the extract

The fresh brownish fruit pulp of *Tamarindus indica* was collected from Phetchaboon province, Thailand. A voucher specimen of a stem including leaves and flowers has been deposited in the herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University (Collection No. Viyoch 001). This specimen was essentially identical to the voucher specimens (Serial No. PBM 04737) already preserved there.

The extraction process was performed in the same way as in our previous studies<sup>9,10</sup>. Briefly, 1 kg of fresh brownish tamarind fruit pulp without seeds was extracted with 4.5 l of water overnight at room temperature. The resultant paste was then filtered through a cloth in order to remove coarse particles. The aqueous solution of the fruit pulp was lyophilized using a freeze dryer (FTS system Dura dry type FD 95C12, FTS Systems Inc.).

### Quantification of tartaric acid

The content of tartaric acid, a major AHA found in the extract of tamarind fruit pulp, was determined by using high performance liquid chromatography (HPLC). Under isocratic conditions, the Econosphere C8 column with 5  $\mu\text{m}$  and 250 mm  $\times$  4.60 mm diameter (Alltech Associates Inc.) was eluted with 0.05 M potassium dihydrogen phosphate (pH 3). Quantification of the tartaric acid in the powder extract or the emulsion was calculated from the standard curve constructed using the peak area at 215 nm versus various concentrations of tartaric acid (analytical grade, Riedel deHaen). All experiments were performed in triplicate.

### Oil-in-water (o/w) emulsion preparation

All materials were used as received and were cosmetics grade. Disodium ethylenediamine-tetraacetic acid (disodium EDTA), glycerin, isopropyl myristate, liquid paraffin (viscosity of 70), stearic acid, stearyl alcohol, sorbitan monooleate (Spans 80), polyoxyethylene sorbitan monostearate (Tweens 60), propylene glycol, propyl paraben, and methyl paraben were supplied by Srichand United Dispensary Co., Ltd., Bangkok.

The o/w emulsion containing the tamarind pulp (the test product) was prepared according to the formula from our previous study<sup>11</sup>. The preparation procedure is as follows. Carbopol aqua SF (0.2% w/w, Hong Huat Co., Ltd.) was dispersed in deionized water. Other water phase ingredients (5.5% w/w Tween 60, 5% w/w propylene glycol, 5% w/w

glycerin, 0.15% w/w Disodium EDTA, 0.7% w/w triethanolamine (Riedel deHaen), 0.2% w/w methyl paraben, 0.02% w/w propyl paraben, and deionized water) were added to Carbopol dispersing solution. The water phase solution mixture was heated to 75 °C while the oil phase ingredients (1.5% w/w Span 60, 5% w/w isopropyl myristate, 1% w/w stearyl alcohol, 1.5% w/w stearic acid, and 3% w/w liquid paraffin) were heated to 70 °C. The water phase was constantly added to the oil phase with rapid agitation. Agitation was continued until the emulsion cooled to 40–45 °C. Then 8% w/w of the lyophilized powder of the tamarind pulp extract (corresponding to 2% w/w of tartaric acid) was dissolved in deionized water and added to the emulsion. The pH and viscosity of the final product was then tested. For the placebo product, the preparation process was the same as the above except for addition of the extract solution. Also, a small amount of colouring agent (< 0.001% w/w) was added to adjust its colour so as to match that of the test product.

## CLINICAL STUDY

### Study design

The study was designed as a home-use, double-blind, randomized side of face and placebo controlled trial. The allocation assignments of the side of face were generated using a random table. The allocation code was placed in an opaque envelope. The study was conducted in accordance with the ethics principles of the Declaration of Helsinki and was consistent with Good Clinical Practice guidelines. The protocol was approved by the institutional review board of Naresuan University with the permission number 48 02 0010 on 20 September 2006. Written informed consent was obtained from all participating volunteers.

### Subjects

Eligible volunteers were healthy Thai females aged 18–40. Exclusion criteria included exposure to topical steroid, alpha-hydroxyl acids (AHAs), and salicylic acid on the face 1 week prior to the study. Other exclusion criteria included treatment with oral systemic steroids, retinoids, or other systemic photosensitizing drugs within 1 month prior to the study. In addition, volunteers who had skin disease, or wounds on the face, a history of atopic dermatitis, skin hypersensitivity reaction, or a history of allergic reactions to cleansing product ingredients or AHAs products were excluded.

### Application of the products

Each volunteer received two products (test and placebo) in identical packages and the appearance of products was identical. They were asked to apply one type of product on one side of their face and the other on another side of face twice daily for 8 weeks. Volunteers were asked to use approximately 1 g (i.e., 0.5–1.5 g) of each product, gently massaging it in for 2 min, and then wipe it with cotton, and rinse it off with water. During the study period they were allowed to continue using their personal cosmetic products and were instructed to use sunscreen products of at least SPF 15.

### Assessments

All measurements were conducted at the Cosmetics and Natural Product Research Centre, Naresuan University Hospital, Naresuan University. Before the measurements, all volunteers had to rest in a room, under constant environmental conditions of  $22 \pm 2$  °C and  $55 \pm 5\%$  relative humidity, for at least 20 min<sup>12,14</sup>. The evaluation began at the same time (at some time between 8:00 and 12:00) for all visits. The baseline values of colour, moisture content, transepidermal water loss (TEWL), pH, and erythema (redness) of the skin on both sides of the face were obtained by measuring these properties at week 0 before application. These parameters were then measured again every 2 weeks during the 8-week study period except for colour and erythema which were measured every week. Each time, the measurements were done before the application of the products that day.

Colour, redness, and moisture content were measured in 3 places on each cheek – at the centre of each cheek and two lateral points 2.5 cm either side of the centre. TEWL and pH were only measured at the centre so as to minimize the skin stress resulting from these types of measurement. The colour and redness, moisture content, pH, and TEWL were measured (noninvasively) using, respectively, a Mexameter MX 18, a Corneometer CM 825, a Skin-pH-Meter PH 900, and a Tewameter TM 300 (all from Courage + Khazaka Electronic GmbH)<sup>12,14,15</sup>.

Clinical signs and symptoms of skin irritation were used to evaluate the mildness of product. Signs and symptoms of itching, burning, stinging, skin redness, or erythema, eczema, rash, scaling, and oedema were assessed by volunteers themselves using a validated questionnaire every week after application. In addition, all skin signs and symptoms which occurred during the study period were recorded in a diary twice a day by the volunteers themselves. If symptoms

appeared, clinical evaluation of skin irritation by a dermatologist was registered using the visual scoring scales of Frosch & Kligman and COLIPA<sup>12,13,16</sup> based on the three main types of skin lesions (erythema, scaling, and oedema domains).

Finally, the efficacy of the product was also evaluated by measuring the volunteers' satisfaction with the product by means of a validated questionnaire at the end of the study.

### Quality control of clinical study

The quality of clinical study was assessed by measuring the compliance of volunteers (weighing the products, assessment frequency, and checking the procedure of product use), and determining the degree of blindness (checking the volunteers' answers about product at the end of study).

### Statistical analysis

The differences of each skin property between the areas where the test and placebo products were applied were tested for significance using a paired *t*-test. If the data did not show a normal distribution, the log transfer and non-parametric statistics were used to adjust and to analyse all data, respectively. Statistical significance was set at  $p < 0.05$ . According to our preliminary study, the estimated difference in skin colour, as measured by the Mexameter MX 18, between the test and the placebo products was 30 units with a standard deviation of 62.1 units. A minimum sample size of 34 patients was required to have 80% power to detect 30 units of the difference of skin colour, with a type I error of 5%. All analyses were undertaken with an intention-to-treat approach. All results were reported using descriptive statistics.

## RESULTS

### Quantification of tartaric acid in the extract

The aqueous extract of tamarind pulp gave a light-brown powder after lyophilization. The content of tartaric acid in the extract powder averaged  $24.7 \pm 0.2\%$  w/w, as determined by HPLC. This batch of the extract was kept in tight container at 4 °C and  $70 \pm 10\%$  relative humidity and used throughout the study.

### Product characteristics

The preparation containing the tartaric acid (the test product) was light brown in colour and had the appearance of a liquid o/w emulsion. Its viscosity was about 2700 centipoises as measured by a Brookfield digital rheometer (Model DV-III). The product was pH 4. Using HPLC we found that the quantity of tartaric acid averaged  $15.9 \pm 0.6$  mg/g of the liquid

**Table 1** Skin properties of measurement area at week 0.

Skin property	Value (mean $\pm$ SD) before application of	
	Test	Placebo
colour	228.60 $\pm$ 48.54	230.47 $\pm$ 50.74
moisture	47.97 $\pm$ 7.89	48.65 $\pm$ 8.37
TEWL	12.11 $\pm$ 3.27	11.59 $\pm$ 2.78
redness	220.22 $\pm$ 40.35	222.00 $\pm$ 42.93
pH	4.63 $\pm$ 0.09	4.75 $\pm$ 0.70

emulsion). Before the addition of the colouring agent, the placebo was creamy in colour with a pH of 6.

### Characteristics of volunteers

After the 3 month of enrolment procedure, 38 Thai healthy females had enrolled in this study. The average age of the volunteers was 25.6 with a standard deviation of 4.3 years. The skin types of volunteers (as classified by themselves) was dry (55%), normal (21%), mixed (19%), or oily (5%). Volunteers had little exposure to direct sunlight because all used sunscreen and 79% of them had indoor lifestyles during the day (student and office worker). None of them changed their lifestyle or their use of other cosmetics during the study period. Moisturizing products were used as concomitant cosmetics by 92% of the volunteers.

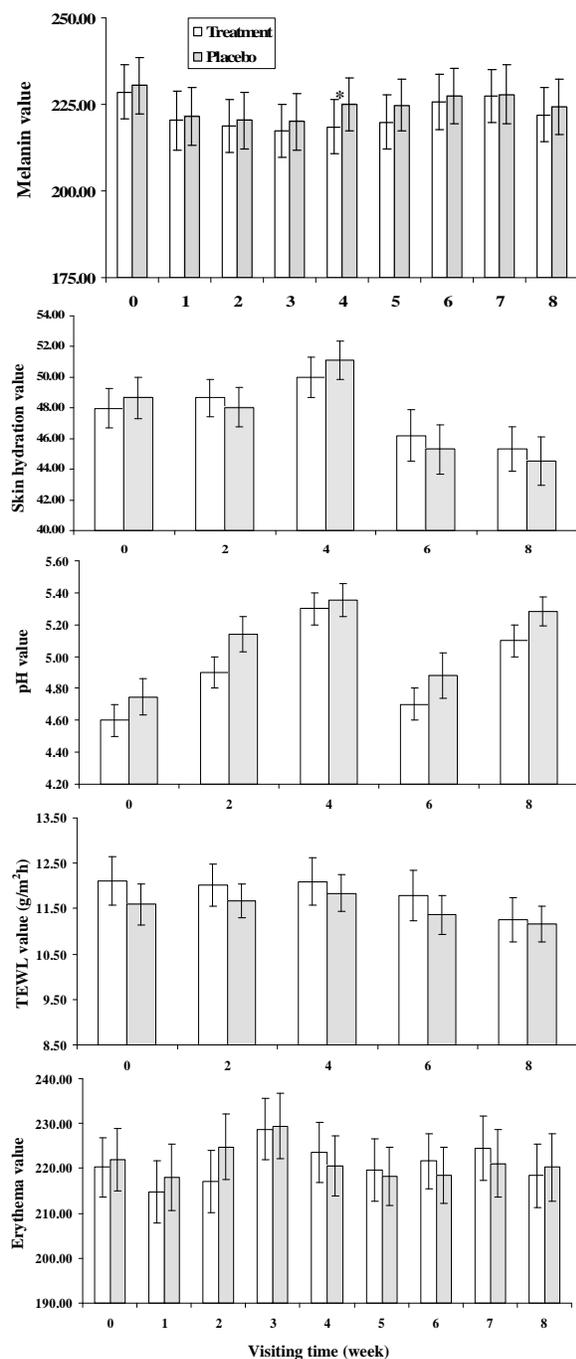
### Skin properties

Baseline values of all skin properties obtained from both sides of the face are shown in Table 1. The skin properties of the volunteers after application of the test and placebo products are presented in Fig. 1. At week 4, the average melanin value of the test product facial area was significantly less than that of the placebo ( $p < 0.015$ ). However, it was not statistically significant at week 8. The mean differences between test and placebo of the other parameters were not significant at any time ( $p > 0.05$ ).

### Satisfaction of volunteers and clinical signs and symptoms of skin irritation

Approximately two-thirds (67.6%) of the volunteers were preferred the test product while the rest (32.4%) preferred the placebo product. Volunteers' satisfaction scores of the cleansing effect, lightening effect, overall effects, and overall liking of the test product were higher than those of the placebo product ( $p < 0.05$ ).

Clinical signs and symptoms of skin irritation as recorded in the questionnaires and diaries were similar for the test and the placebo products. Clinical evaluation by a dermatologist indicated that one volunteer



**Fig. 1** Skin melanin values (a.u.), moisture content (1 unit  $\equiv$  a water content of stratum corneum of 0.02 mg/cm<sup>2</sup>), pH values, transepidermal water loss (TEWL), and erythema (a.u.) during study. Each bar is the mean value and error bars are the standard error of the mean. \* = significant difference between test and placebo ( $p < 0.05$  using paired  $t$ -test).

had mild erythema on both sides of the face at week 2.

### Quality control of clinical study

On average, 102.8 g of the test product and 102.5 g of the placebo product were used. This was consistent with all volunteers using the products twice daily at the appropriate dose. Only one volunteer did not follow the protocol (at week 1). The result of the assessment blindness using the questionnaire showed that 37.8% of the volunteers could identify which one was the test product.

### DISCUSSION

The concentration of AHAs generally used in commercial facial skin cleansing products is 0.5–2% by weight of formula. The concentration of AHAs used in our study was within this range. The pH value of our test product (pH 4) is close to the normal pH range of the skin (4.5 to 5.5)<sup>17</sup>. Although pH 4 is sufficient to maintain activity of AHAs, generally a pH of 2–3 is required for maximal AHA activity<sup>18</sup>. However, it has been recommended that the pH of skin formulations should not be below 4 to avoid irritation<sup>4</sup>. We therefore believe that the test formula should be capable of exerting its cosmetic benefits with minimal skin irritation.

Our results suggest that the active ingredient in our test product did not affect skin moisture or pH. The significant difference in melanin value after 4 weeks suggests that the tamarind had an actual skin lightening effect. However, this appeared to be temporary as there was no significant difference by the end of the study. This might be due to skin adjustment resulting in a decrease in skin response to the fruit acid. This result corresponds with a previous study which demonstrated that the ability of glycolic and lactic acids to promote cell renewal decreased by 30–40% after 10 weeks of application<sup>18</sup>.

Generally, both instrumental measurement and subject self-assessment are used to determine the efficacy of cosmetic products<sup>19–22</sup>. Instrumental methods are ideal at providing objective data on individual aspects of what the product does to the skin. Instruments can be highly sensitive to small differences suggesting skin benefits which the consumer may find irrelevant or imperceptible. However, the data from the instrumental method can lead to an incomplete view of product performance. For this reason, the instrument method must be relevant to the objectives and should be aligned with subject self-assessment. In our study, regarding the satisfaction scored by the volunteers, the scores given for cleansing effect, lightening effect, and overall effects of the test product were higher than those for the placebo product ( $p < 0.05$ ). This

suggests that the measured melanin value changes and other benefits were perceivable to the user.

The major concerns with daily use of AHA-based products are the possibility of disruption of skin barrier function and skin irritation, which are indicated by higher TEWL and higher redness, respectively. The absence of skin irritation signs and symptoms over the study period (a result which coincided with our previous study<sup>8</sup>) implies that the test product is mild to the skin. This was at least partly due to the low concentration of AHAs and the pH value not being below 3.5. In addition, it is possible that the pulp extract contains natural soothing agents which can reduce the irritation potential and not interfere with the stimulatory activity of the tartaric acid. Furthermore, tartaric acid itself has an antioxidant effect which can reduce disruption of skin barrier function<sup>23</sup>.

The adverse reaction experienced by one of the subjects was unlikely to be due to the pulp extract since the subject had mild skin redness on both sides of face. The cause of the irritation was presumably sensitivity to one or more of the ingredients contained in the both products.

The results of this study possess high internal validity because volunteers had high compliance and there was no loss to follow up. In addition, several procedures were performed to ensure the quality of the study including the randomization, blinding of evaluators and volunteers, calibrating the instrument, and training subjects and evaluators prior to the beginning of the study. Furthermore, our findings can be applied to the real usage because no volunteers changed their cosmetic use, and all skin types were included in our study.

A limitation of this study is the population recruited. As the study was performed in healthy volunteers, the obtained results may not apply to the excluded population, such as volunteers who have skin disease, a history of skin hypersensitivity, or a history of allergic reactions to cleansing products.

## CONCLUSIONS

To our knowledge, this is the first study assessing the effects of on skin of a facial cleansing product containing extract of tamarind fruit pulp. Our findings indicate that the product is mild and has some the potential to cause skin lightening. However, a further clinical study using a larger number of volunteers would provide stronger evidence on its efficacy and safety.

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