CHAPTER III

RESEARCH METHODOLOGY

This chapter describes the methodologies of this study including overview of design, setting, recruitment of the participants, participant eligibility criteria, subject enrollment procedure, interventions, study procedures, outcome measurement, sample size determination and data analysis, quality assurance of study, data processing and management. The detail of each topic is given below.

1. Overview of design

The design of this study was randomized, double blinded, placebo-controlled trial. The unit of randomization was the side of face (Left or right), where each side was applied either tamarind-containing lotion or placebo. The number of expected participants enrolled was 40 subjects aged 18 to 40 years, as calculated to have 80% power to detect the significant difference of color skin measured by Mexameter. All subjects were followed-up for 8 weeks to measure color skin (primary outcome measure), elasticity, pH, moisture, transepidermal water loss (TEWL) of the skin. All participants were asked to sign informed consent before being recruited in this study.

2. Setting

The randomized controlled trial (RCT) was conducted in Cosmetics and Natural Products Research Center (COSNAT), at Naresuan University Hospital, Naresuan University, Phitsanulok, Thailand. The measurement room was controlled temperature and air humidity, as 23 $\pm$ 2 °C and 40-60% of air humidity.

3. Recruitment of the participants

Participants were selected and randomized the side of face in each participant. Each side of participant's face applied either test or placebo products. The sampling

universe was female aged 18 to 40 years old in Phitsanulok. Each study participant was selected using baseline eligibility described below.

4. Participant eligibility criteria

4.1 Inclusion criteria

Healthy female with age 18-40 years old

4.2 Exclusion criteria

4.2.1 Subject who was allergic to cleansing product ingredients or AHA product

4.2.2 Subject who had history of skin hyperallergic reaction

4.2.3 Subject who had history of extreme sun sensitivity

4.2.4 Subject who had history of recurrent or active herpes simplex on face

4.2.5 Subject who had facial warts

4.2.6 Subject who had history of atopic skin reaction or eczema or psoriasis on face

4.2.7 Subject who used oral isotretinoin within 1 month prior to the study

4.2.8 Subject who used topical steroid, tretinoin, benzoyl peroxide, AHA, BHA (salicylic acid), hydroquinone, arbutin, kojic acid, or other substances which induced photosensitivity on skin face within 1 week prior to start the study

4.2.9 Subject who had wound, ulcer or scar

4.2.10 Subject who had a large scar on face

4.2.11 Subjects who had extensive pigmentary disorders such as malasma, post-inflammation

4.2.12 Subject who smoking, alcoholism or any drug abuse

4.2.13 Pregnancy or nursing women

Target population of our study was the healthy female with normal skin. In addition, we used melanin values of preliminary of healthy female to determine the sample size of this study. Therefore, only healthy female with normal skin was included in our study. We selected only the participants who had 18 to 40 years old because of several reasons. First, because the process of product used in this study was quite

difficult, we prefered to include only subjects capable of following the protocol. Second, we included only young subjects in the study because they can show the significant difference of skin color in this study period. In general, skin color will be able to improve in all age groups if they use appropriate cosmetics. However, in old-age individuals, the rate of improvement may slow and cannot show the significant difference during the short period.

We excluded the subjects who had a high risk of develop side effect such as, subject who was allergic to cleansing product ingredient or AHA product, subject who had history of skin hyperallergic reaction etc., and who had small chance to show the difference of primary outcomes such as subjects who had extensive pigment disorders such as melasma. In addition, we excluded the pregnancy or nursing women due to the reason of ethic.

4.3 Discontinuation criteria

4.3.1 The subjects voluntarily withdraw from the study themselves.

4.3.2 The subjects had allergic reaction or developed the adverse effects from study products during the study, that were confirmed by the dermatologist.

4.3.3 The skin color of one side of face is noticeably different from the other.

5. Procedure to ensure the safety of participants

We performed several procedures to ensure the safety of participants. There were the inclusion-exclusion criteria, discontinuation criteria, safety information for subjects, safety data sheet of all ingredients, and assessment procedure to monitor adverse events by subjects themselves and by the dermatologist.

6. Subject enrollment procedures

The enrollment procedures took place in the setting room approximately 3 months prior to start the study. We identified eligible subjects based on their characteristics (Appendix F). The investigator introduced the study information to eligible subjects (Appendix B). All subjects were asked to sign informed consent before the

study began (Appendix D). The investigator continued to recruit subjects into the study until 40 subjects had been enrolled.

7. Interventions

7.1 Description of interventions (Study products)

Interventions or study products were two different products used to clean facial skin. The first one was test product while the other was placebo product. The formula of test product consisted of 5.9 %w/w of Tween®60, 1.5 %w/w of Span®80 , 0.2 %w/w of Carbopol®aqua SF, 5.0 % w/w of glycerin, isopropyl myristate and propylene glycol, 0.15 %w/w of disodium EDTA, 0.7 %w/w of triethanolamine, 1 %w/w of stearyl alcohol, 1.5 %w/w of stearic acid, 3.0 %w/w of liquid paraffin, 0.2 %w/w of methyl paraben, 0.02 %w/w of propyl paraben and 2.0 %w/w of tartaric acid (Table2). The formula of placebo was similary to test product except tartaric acid. All ingredients were supplied by Namsiang International Co., Ltd. (Table2), except tartaric acid was prepared by investigator nurse.

7.2 The direction for use the product

One subject used both the test and the placebo products. One product was applied on one side of face while another was applied on the other side of their face. The direction for use the product was provided below.

- Wet face with clean water, then dispense product about 5-baht coin into the palm
- Spread the product over the half face and massage for 1 minute, then leave it for 1 minute and wipe it off with cotton
- Then, rinse thoroughly half face with clear water

7.3 Randomization and allocation concealment of interventions

We randomized each side of participant's face to receive the interventions at the start date of study (Week 0). A simple randomization method was used in this study. Its code was prepared using a random table and concealed in sequentially numbered, sealed, opaque envelops, and kept by the independent staff.

8. The procedure of study

8.1 The method of study

8.1.1 Participants arrived at the study room around 8.00 a.m. Then they waited for 30 minutes before proceeding to step of measuring skin properties including skin color, skin erythema (redness), transepidermal water loss (TEWL) of skin, skin moisture, skin pH, and skin elasticity. All properties were measured 3 times in each side of face except the skin elasticity, TEWL, and skin pH that were measured only one time to reduce the stress of skin (Table6).

8.1.2 After completing skin property measurement, subjects started to use their products. In addition, subjects were evaluated in terms of how to use the products by investigator nurse.

8.1.3 After using the products, subjects waited for 20 minutes then the possible side effects were assessed by subjects themselves and the dermatologist at the first and the last visit.

8.1.4 All subjects received their diaries before they went home every two weeks.

8.1.5 Participants cameback to the setting every week for 8 weeks for follow up the effect of products.

8.1.6 Each skin parameter was measured following time that predefined before start the study (Table4&6).

8.1.7 Only skin color and skin redness were measured on every week while other skin properties were measured on odd or event week as following: Skin moisture, pH, and TEWL of skin were measured on week 2, 4, 6, and 8 while skin elasticity was measured on week 1, 3, 5, 7, and 8 (Table4&6).

8.1.8 At week 8 or the end of study, we evaluated the satisfaction of subjects.

8.2 The diagram of study procedures

This diagram showed all study procedures for this thesis including the study of efficacy and safety of this product (Main study), and the irritation test for this product. For irritation test, it was performed before start the main study (Appendix M)

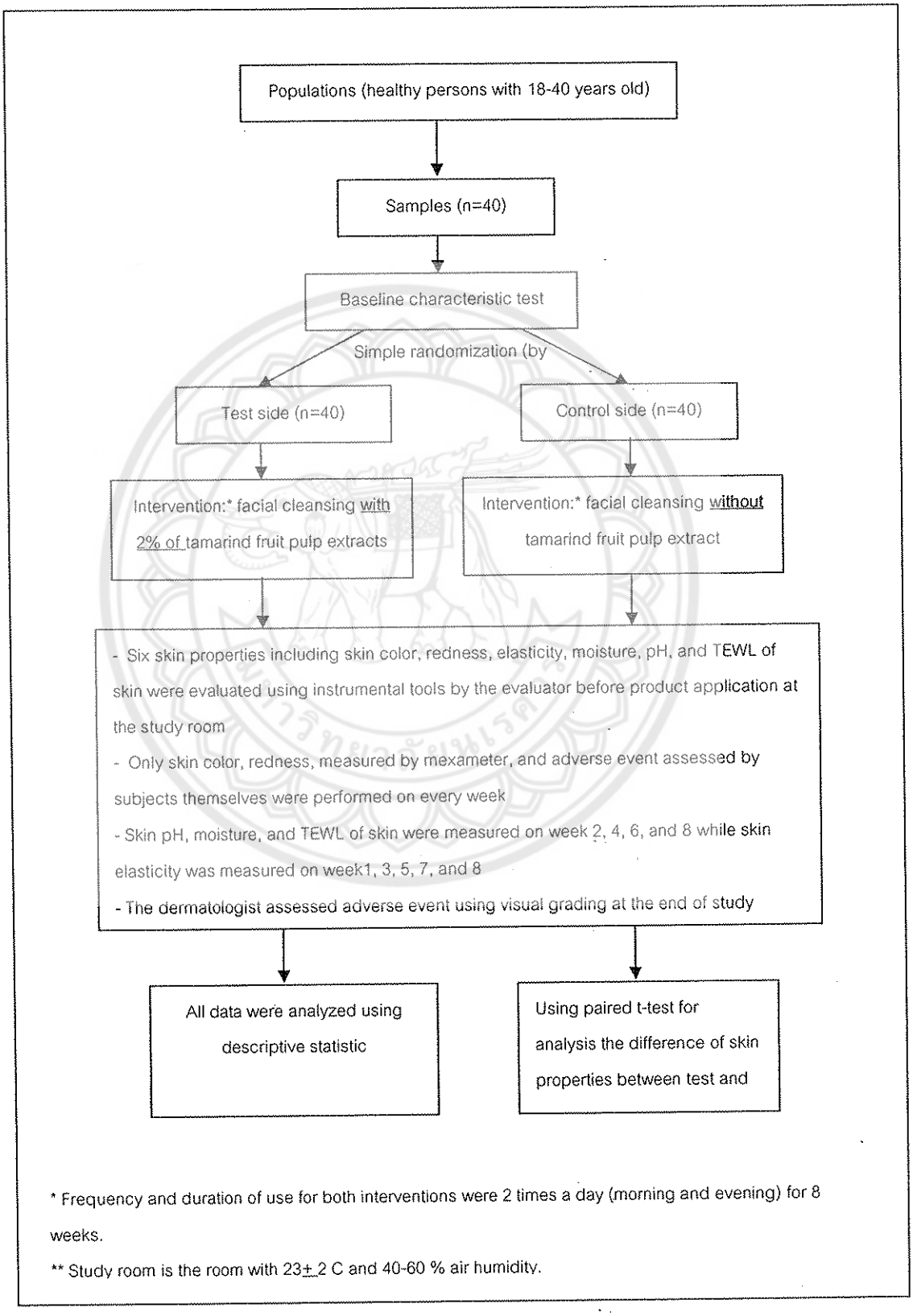
The study procedures at each visit were indicated on following table. Each visit took place at about the same time of day (8.00 to 12.00 a.m.).

Table 4 The diagram of study procedures

Procedure	Visit1	Visit2	Visit3	Visit4	Visit5	Visit5	Visit6	Visit7	Visit8	Visit9	Visit10
	Week -8	Week -1	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
	Screening		Baseline	Follow up							End of study
Recruit subjects	←	→									
Informed consent	←	→									
Screening	←	→									
Irritation test	◆										
Training	↔										
Randomization		↔									
Initial study		↔									
Compliance Check			←								→
Skin color measurement			←								→
Skin redness measurement			←								→
Skin elasticity measurement			↔	↔		↔		↔		↔	↔
Skin pH measurement			↔		↔		↔		↔		↔
Skin moisture measurement			↔		↔		↔		↔		↔
TEWL measurement			↔		↔		↔		↔		↔
Adverse events evaluation			←								→
Satisfaction test											↔

8.3 The framework of the study

The framework of study



* Frequency and duration of use for both interventions were 2 times a day (morning and evening) for 8 weeks.

** Study room is the room with 23± 2 C and 40-60 % air humidity.

9. Outcome assessment

The primary outcome was the skin color measured by the Mexameter. Skin color was chosen as primary outcome because the main action of tamarind extract was stimulating cell renewal, thus resulting in lighter skin appearance. In addition, skin pH, moisture, and elasticity were important parameters to represent a good healthy skin, and there had been demonstrated the effect of AHAs to improve these skin properties. Moreover, skin redness, and TEWL were important parameters to represent the safety of products. Therefore, we assessed these skin parameters as secondary outcomes. Besides six skin parameters, the adverse events and the satisfaction of subjects were assessed as secondary outcome.

9.1 Measurement the skin properties

Six skin properties were measured with five instruments. We predifined 3 fixed area including the center of each cheek and two lateral points with the margin of 2.5 cm from the center to measure skin parameters. Each subject was measured three fixed areas of each face's side (center, left, and right), the average of each value will be calculated.

Table 5 showed the schedule of measurement, and detail of them including skin properties, instruments, area and time of measurements, and the result of measurement in terms of efficacy or safety, respectively. Three skin properties including skin color, redness, and skin moisture were measured 3 times while skin elasticity, pH, and TEWL of skin were measured only one time to reduce the stress of skin due to measurement.

Table 5 The schedule and detail of measurement skin properties

Skin properties	Instruments	Area of measurement on each side of cheek	Time of measurement after use product	Result
1. Skin color	Mexameter	Center, left, right	Week 0, 1-7, & 8	Efficacy
2. Skin redness	Mexameter	Center, left, right	Week 0, 1-7, & 8	Safety
3. Skin moisture	Corneometer	Center, left, right	Week 0, 2, 4, 6, & 8	Efficacy
4. Skin pH	pH meter	Center	Week 0, 2, 4, 6, & 8	Efficacy
5. TEWL of skin	Tewameter	Center	Week 0, 2, 4, 6, & 8	Safety
6. Skin elasticity	Cutometer	Center	Week 0, 1, 3, 5, 7, & 8	Efficacy

9.2 Measurement the adverse events

Dermatologist and subjects themselves using grading scale, and questionnaire, respectively assessed adverse events of product. The grading scale varied in 0 to 3, where 0 was defined as non reaction; 1 was defined as mild; 2 was defined as moderate, and 3 was defined as severe. The side effects were observed as followed: red skin, burning, irritation or itching, rash, papule, swelling or erythema, eczema or blistering, and skin sensitivity.

9.3 Measurement the satisfaction

The satisfaction of subjects to product was evaluated using self-assessment of questionnaire at the end of study. The content validity of this questionnaire was performed (Appendix H).

10. Sample size determination and data analysis

10.1 Sample size determination

The number of expected enrolled participants was calculated to have 80% power to detect the significant difference of color skin measured by Mexameter. Based on within person study designed, the formula for calculating sample size was provided below.

$$N = \frac{[Z_{1-\alpha/2} + Z_{1-\beta}]^2 * Sd^2}{\triangle^2}$$

Where N was the sample size, Sd was standard deviation of the result that was 62.11 unit* for this study, $\triangle$ was the difference of the expect values of skin color between using the test and control products that was 30 unit[2] for this study, $Z_{1-\alpha/2}$ was Z statistic with $\alpha = 0.05$ that was Z value as 1.96, $Z_{1-\beta}$ was Z statistic with the considered power, this study was defined as 80% that was Z value as 0.84. This estimation technique

---

* 62.11 was the standard deviation of the skin color value from 20 participants of the preliminary study which was conducted by Assist.Prof. Jarupa Viyoch, Department of Pharmacy Practice School of Pharmacy, Naresuan University, Phitsanulok, Thailand

[2] 30 was the difference of the expect values of skin color between using the treatment and control product that was expected by Assist.Prof. Jarupa Viyoch who developed and prepared both the test and the control products for this study

resulted in a sample size requirement of 34 participants. However, we decided to recruit 40 participants to allow for lost to follow-up attrition (20%).

10.2 Data analysis

10.2.1 Descriptive statistics

Descriptive statistic was used to report all results of this study in terms of mean (x), standard deviation (Sd), frequency (f), and percentage (%).

10.2.2 Analytic statistics

Each subject was measured three fixed areas of each cheek (center, left, and right), the average of each value was calculated before application at week 0 (baseline value) and week 8 (the end of study).

We analyzed the mean difference of each skin parameter including skin color, skin moisture, skin elasticity, skin pH and transepidermal water loss of skin between the test and the control sides using paired t-test.

If data did not have normal distribution, we would use log transfer to adjust all data or use the non-parametric statistic to analyze them.

11. Quality assurance of study

There were several methods that were performed to ensure the quality of study including the training procedure, regular calibration the instruments for measurement the skin properties, compliance evaluation, validity test of questionnaire or evaluation form before use. In addition, other procedures to minimize the bias or minimize loss to follow up were performed to ensure the quality of study. The details of them were provided below.

11.1 Training the staffs

All staffs were trained about the process of study and assessing procedure for evaluate the outcome. This training included information on: a) using the questionnaire for interviewing and screening process; b) training in the use of products ; c) using adherence assessment techniques; d) using all instruments including Mexameter, Cutometer, Corneometer, Tewameter, and pH merter.

11.2 Training the participants

After recruitment procedure, all participants were instructed and trained in the process of how to use each product by investigator prior to begin the study. The training included information on : a) which product should be apply each area; b) how to use each product; c) amount of product to apply each area; d) time to massage and waiting; e) frequency and duration of use the products.

All participants were divided into two groups for training the process and the use of the products (not more than 20 subjects in each group). If subjects used the products incorrectly, they were advised how to use them properly and reevaluated by the investigator team.

11.3 Compliance of subject

There were several methods to check the compliance of subject including checking diaries of each subject, interviewing subjects when they visit the setting, weighing the product, and evaluating all subjects in terms of how to use the products (Appendix G).

We checked the compliance of subject by checking diaries of them every visit. All subjects were evaluated in terms of how to use the products. If they could not use it correctly, they were advised how to use it properly, and reevaluated by the investigator team. In addition, all products were weighed every 2 weeks. Besides these, we had the protocol to minimize loss to follow up of subject. The detail of this protocol was provided below.

11.3.1 Protocol for follow up the subjects before an appointment time

1) Three days before each appointment date, we contacted each subject to confirm the date by mobile phones or personal home phones.

2) If we cannot contact them at the first time, we will recall until we can contact them or recall them at least 3 times a day for 2 days.

3) If we cannot contact them during 2 days, we will contact them via their friend or the person who can contact them (key personal).

4) If the contact by telephones or the key personal dose not success, we will go to their rooms or their classrooms.

11.3.2 Protocol for follow up the subjects at the visit date

1) First 10 subjects should arrive at the setting before 8.30 a.m.

2) We began to contact subjects who did not arrive the setting within 8.30 a.m. with mobile phone or personal home phone every 10 minutes until we can contact them or recall to them at least 3 times.

3) If we cannot contact them directly, we will contact other persons who can contact them.

4) If the contact by telephones or the key personal dose not success, we will go to their rooms or study rooms.

12. Data processing and management

All data will be managed by the investigator. Data from source documents were collected to the case report form (paper). In addition, hard copy data were transformed from the case report form to the database via screen entry program such as Excel.

12.1 Data validity and reliability

We trained all evaluators, calibrated the instruments before measurement, fixed area for measurement, and assigned the same evaluator to use the same instruments for all visitings during the study period. We performed these procedures to ensure the validity of data. In addition, the data collection form and questionnaire used in this study had already been validated.

12.2 Loss to follow up procedures

To minimize loss to follow up, we had the protocol to follow them as shown in part of subjects' compliance. The address and telephone number of participants were kept with investigator for following and made appointments with them. If the participants miss an appointment, the investigator will try to contact participant in several ways following the protocol within 1-2 days or as soon as possible. If all attempts to contact the subject are failed, the subject will be classified as lost to follow up.

12.3 Handling missing data

We used the imputation-based procedure to handling missing data. The Last-Observation-Carried-Forward (LOCF) was used to estimate the missing data.

13. The facilities

Five biophysical instruments were used in this study including Cutomerter®, Corneometer®, Mexameter®, Skin pH Meter®, and Tawameter® (Figure2).

14. Budget

This study was supported by Research Consortium of Lower Northern Region, Thailand, 2005. An estimated amount of funding was provided below.

Table 6 The management of budget in this study

Item	Cost (baht)
a. Personnel (allowance to be paid)	
Wage of analysis the amount of tartaric acid	15,000
Wage of 40 participants (a total 2500 baht/person)	100,000
Wage of skin physician	15,000
Wage of assessing the efficacy of product in participants	20,000
Total	150,000
b. Equipment and supplies	
Chemical and chemical equipment for:	
- preparing the tamarind extract	10,000
- preparing the product	30,000
Supplies for:	
- preparing the product and packaging	15,000
- analysis the chemical composition of tamarind extract	30,000
Total	85,000
c. Miscellaneous expenditures	
Cost of finding and searching the information	2,000
Cost of reporting the result, publishing, and presentation	8,000
Office equipments	5,000
Total	15,000
d. Management of institution 10%	25,000
Total	250,000