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การพัฒนาตำรับผลิตภัณฑ์เครื่องสำอางบำรุงผิวที่มีส่วนผสมของสารสกัดเตยทะเล

Development of Cosmetic Emulsion Containing *Pandanus odoratissimus* Extract

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EXECUTIVE SUMMARY

Development of Skincare Cosmetic Emulsion Containing *Pandanus odoratissimus* Extract

Pandanus is a genus of monocots with about 600 known species. Plants vary in size from small shrubs less than 1 m tall, up to medium-sized trees 20 m tall, typically with a broad canopy and moderate growth rate. The plant grows prolifically in tropical areas, including the pacific islands, Africa, South Asia and South East Asia. *Pandanus amaryllifolius* and *Pandanus odoratissimus* are of example species found in Thailand. Pandan is said to be a restorative, deodorant, indolent and phylactic, promoting a feeling of wellbeing and acting as a breath sweetener or used as a preservative on foods (Bhattacharjee et al., 2005). It is also said to have healthful properties, including antiviral, anti-allergy, anti-inflammatory, and antitumor and antioxidant (Ooi, Sun, and, Ooi V., 2004; Cheeptham and Towers, 2002; Jong and Chau, 1998). Additionally, the results obtained from our previous study show that *P. odoratissimus* root collected from Trung Province, Thailand possesses antioxidative activity (Jimtaisong and Mookriang, 2010) and therefore we aim to further study the possibility of incorporating the extract into topical emulsion. In this work, oil-in-water emulsions containing the extract were developed and the physiochemical properties of the product were evaluated. The stability of the product containing the extract has been investigated. Primary irritation of the product containing the extract was studied. Finally the sensory evaluation of the product was collected. The results of this work will be useful for development of commercial cosmetic product containing *P. odoratissimus* root extract.

The dried aerial root of *P. odoratissimus* was extracted in ethanol and propylene glycol solvent. Antioxidant activities of the extract investigated by the DPPH free radical-scavenging activity showed that propylene glycol extract possessed about 20% higher in activity than the ethanol extract. The heat stability testing of the extract was evaluated by DPPH assay at 70 ^{o}C for 5h and it was found that at 3h %inhibition decreased from the beginning about 15%. The stability of the extract was also tested

at different storage conditions, i.e., ambient temperature, 4 °C and 45 °C for 4 weeks. DPPH assay of all conditions decreased about 13-22%. The oil-in-water cosmetic emulsion containing the extract at 5, 10, 15 and 20% were developed and the physiochemical properties of products were studied. All products showed no phase separation under centrifugation (3000 rpm, 30 min). The pH of products decreased while viscosity and yellow color increased when product has more extract concentration (ambient temperature, 45 °C, 4 °C and heating-cooling cycle). The product containing up to 15% extract had the cosmetically acceptable properties and it was subjected to accelerated stability test for 4 weeks at different conditions. The results showed that the viscosity of the products possessed small changes from the initial. The color was recorded in L*a*b* values. The L* values of all storage conditions showed slightly change about 4-8%. The product showed the higher a* values and lower b* values than the initial product but the changes are not considerable different from the initial when visually observed. Moreover, the microorganism contamination test carried out by using Mikrocount® combi test kits showed that the products had no microorganism contamination. In addition, the sensory evaluation of product performed by 20 volunteers showed that 89% like the overall features of the product. Finally, the safety of product containing 5 and 15% of *P. odoratissimus* root extract was tested by single patch test and the product with *P. odoratissimus* root extract 5% or lower is non-irritation and safe for use as antioxidant active in the topical cosmetics.

Development of Skincare Cosmetic Emulsion Containing *Pandanus odoratissimus* Extract

ABSTRACT

Pandanus odoratissimus belongs to pandanaceae family and distributes in the south of Thailand. The aerial root was previously reported to contain steroids, lignan, benzofuran derivatives and phenolic compounds. In this work, dried aerial root of *P. odoratissimus* was extracted in ethanol and propylene glycol solvent. Antioxidant capacities of extracts investigated by the DPPH free radical-scavenging activity showed that propylene glycol extract had about 20% higher in activity than the ethanol extract. The heat stability testing of *P. odoratissimus* root extract evaluated by DPPH assay (70 °C, 5 h) showed that at 3 h %inhibition decreased about 15%. The stability of the extract was also tested at ambient temperature, 4 and 45 °C for 4 W and it was found that the DPPH activity of all conditions decreased about 13-22%. The oil-in-water topical emulsion containing *P. odoratissimus* root extract at 5, 10, 15 and 20% were developed and the physiochemical properties of products were studied. All products showed no phase separation under centrifugation (3000 rpm, 30 min). The pH of products decreased while viscosity and yellowish color increased when product has more extract concentration. The product containing 15% extract possessed suitable texture, pH, color and viscosity and it was subjected to accelerated stability test for 4 W at different conditions (ambient temperature, 45 °C, 4 °C and heating-cooling cycle) and there is only slightly change in viscosity and color compared with initial. Moreover, the products had no microorganism contamination after tested by using Mikrocount® combi test kits. In addition, the sensory evaluation of product performed by 20 volunteers showed that 89% like the overall features of the product. Finally, the safety of product containing 5 and 15% of *P. odoratissimus* root extract was tested by single patch test and the product with *P. odoratissimus* root extract 5% or lower is non-irritation and safe for use as antioxidant active in the topical cosmetics.

การพัฒนาตำรับผลิตภัณฑ์เครื่องสำอางบำรุงผิวที่มีส่วนผสมของสารสกัดเตยทะเล

บทคัดย่อ

เตยทะเล (*Pandanus odoratissimus* Linn) อยู่ในวงศ์เตย (pandanaceae family) พบได้ทั่วไปในพื้นที่ภาคใต้ของประเทศไทย รากประกอบไปด้วยสารเคมีหลายชนิด เช่น steroidal compounds, lignin, benzofuran derivatives และ phenolic compounds ในการวิจัยนี้ได้สกัดรากเตยทะเลแห้งด้วย ethanol และpropylene glycol และศึกษาฤทธิ์ต้านอนุมูลอิสระของสารสกัดด้วยวิธี 1, 1-diphenyl-2-picrylhydrazine (DPPH) assay, reducing power และ thiocyanate methods พบว่า เมื่อใช้ propylene glycol เป็นตัวทำละลายจะให้ค่าที่สูงกว่าethanol ถึง 20% และได้ทำการทดสอบความคงตัวของสารสกัดต่อสภาวะความร้อน 70 $^{\circ}$C พบว่าสารสกัดมีค่า DPPH activity ลดลง 15% หลังจากให้ความร้อน 3 ชั่วโมง นอกจากนี้ ค่า DPPH activity ของสารสกัดลดลง 13-22% ภายใต้สภาวะการเก็บที่ อุณหภูมิห้อง, 45 °C, 4 °C นาน 1 เดือน และได้พัฒนาตำรับอิมัลชันชนิดน้ำในน้ำมัน โดยมีปริมาณสารสกัด *P. odoratissimus* 5, 15, 15 และ 20% ซึ่งผลิตภัณฑ์ที่ได้มีลักษณะเป็นครีมเจล สีขาวอมเหลือง เมื่อปริมาณสารสกัดเพิ่มขึ้นผลิตภัณฑ์มีค่า pH ลดลง ค่าความหนืดเพิ่มขึ้นและมีสีเหลืองขึ้น และทุกตำรับไม่มีการแยกชั้นหลังจากการปั่นเหวี่ยง 3000 รอบต่อนาที นาน 30 นาที จากนั้นนำไปทดสอบความคงตัวที่สภาวะต่างๆ (อุณหภูมิห้อง, 45 °C, 4 °C และ heating-cooling cycle) เป็นเวลา 4 สัปดาห์ พบว่าความหนืดและสีของผลิตภัณฑ์เปลี่ยนแปลงเล็กน้อย ผลทดสอบการปนเปื้อนเชื้อจุลินทรีย์โดย Mikrocount® combi และไม่พบการปนเปื้อน นอกจากนี้ได้ทดสอบด้านประสาทสัมผัส (sensory test) ของผลิตภัณฑ์พบว่าอาสาสมัครร้อยละ 89 พึงพอใจกับลักษณะโดยรวมของผลิตภัณฑ์ และทดสอบความปลอดภัยของผลิตภัณฑ์ที่มีสารสกัด 5 และ 15% ด้วยวิธี single patch test พบว่าผลิตภัณฑ์ที่มีสารสกัด 5% ไม่ก่อให้เกิดการแพ้ต่อผิว ดังนั้น จึงสามารถนำมาเป็นสารออกฤทธิ์ต้านอนุมูลอิสระในเครื่องสำอางได้

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ABBREVIATIONS

%	Percent
°C	Degree Celsius
cP	Centripoint
i.e.	id est
O/W	Oil-in-water
cm	Centimeter
nm	Nanometer
m	Meter
rpm	Round per minute
mg	Milligram
g	Gram
kg	Kilogram
min.	Minute
h.	Hour
ml	Milliliter
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
UV-Vis spectrophotometer	Ultraviolet-Visible spectrophotometer

Chapter 1
Introduction

1.1 Background

Pandanus is a genus of monocots with about 600 known species. Plants vary in size from small shrubs less than 1 m tall, up to medium-sized trees 20 m tall, typically with a broad canopy and moderate growth rate. The plant grows prolifically in tropical areas, including the pacific islands, Africa, South Asia and South East Asia. *Pandanus amaryllifolius* and *Pandanus odoratissimus* are of example species found in Thailand. Pandan is said to be a restorative, deodorant, indolent and phylactic, promoting a feeling of wellbeing and acting as a breath sweetener or used as a preservative on foods (Bhattacharjee et al., 2005). It is also said to have healthful properties, including antiviral, anti-allergy, anti-inflammatory, and antitumor and antioxidant (Ooi, Sun, and, Ooi V., 2004; Cheeptham and Towers, 2002; Jong and Chau, 1998). Additionally, the results obtained from our research show that *P. odoratissimus* root collected from Trung Province, Thailand possesses antioxidative activity (Jimtaisong and Mookriang, 2010) and therefore we aim to further study the possibility of incorporating the extract into topical emulsion. In this work, an oil-in-water emulsion containing the extract will be developed and the physiochemical properties of the product will be evaluated. The stability of the product containing the extract will be investigated. Primary irritation of the product containing the extract will be studied. Finally the sensory evaluation of the product will be collected. The results of this work will be useful for development of commercial cosmetic product containing *P. odoratissimus* root extract.

1.2 Objectives

1. To prepare standard extract of *P. odoratissimus* as natural antioxidant ingredient for cosmetic application.
2. To study the stability of the extract at various temperatures.
3. To develop cosmetic emulsion product containing *P. odoratissimus* extract.
4. To study the stability of the cosmetic product containing *P. odoratissimus* extract.

5. To study the primary irritation of the cosmetic product containing the extract.

1.3 Conceptual Framework

Cosmetics are commercially available products that are used to improve the appearance of the skin. The skin is the largest organ; as our primary external barrier, it is on the forefront of the battle with external causes of damaging free radicals. Free radicals are highly reactive molecules with an unpaired electron that result in damage to surrounding molecules and tissues. It is thought that additional, topical use of antioxidants in cosmetics can better protect and possibly correct the damage by neutralizing these free radicals. Antioxidants may be synthetics, such as BHA and BHT, or of natural origin, such as phenolics as well as polyphenolics. Phenolic compounds are found in all foods of plant origin. Recently, there has been an increase in the use of polyphenolic compounds in cosmetics (Padilla et al., 2005; Juliano et al., 2005; Wang et al., 2006; Peschel et al., 2006). Such data suggest a need for further studies and possible incorporation of similar compounds into cosmetic formulation. Natural antioxidative substances from the polyphenols of edible herbs are believed to be safer and may provide additional health benefits, compared to synthetic antioxidants. It is an area worth investigating due to current consumer concerns about health.

Pandan plants have long been used in food industry (Bhattacharjee et al., 2005). Traditional folk medicine has incorporated pandan as one of the component in the formula and scientists have been trying to isolate active compounds and prove for their activities (Cheeptham and Towers, 2002). In view of cosmetic application, there has been less scientific study. This project thus will focus on the preparation of *P. odoratissimus* root extract as natural antioxidant ingredient for cosmetics. Stability of the extract will be evaluated. Cosmetic emulsion containing the extract at various concentrations will be prepared. The physio-chemical properties will be studied. The product will be subjected to accelerated stability test. Primary irritation test of the product containing the extract will be investigated using patch test method. Finally, preference on the product containing *P. odoratissimus* root extract will be collected using the sensory test.

1.4 Scope of research

1. Collect the sample and perform the extraction.
2. Prepare standard extract for cosmetic formulation.
3. Study the stability of the extract at accelerated temperatures.
4. Develop cosmetic emulsion containing the extract.
5. Perform stability test of the developed product.
6. Determine the antioxidant activity of the extract in emulsion system.
7. Perform primary irritation of the emulsion product containing the extract.
8. Perform a sensory evaluation of cosmetic emulsion containing the extract.

Chapter 2
Literature Review

2.1 *Pandanus odoratissimus*

Pandanus odoratissimus (Synonym: *P. fascicularis*) belongs to pandanaceae family. It is erect branched small tree, growing 3-5 meters, the trunk bearing many support roots. Leaves are spirally crowded toward the ends of the branches, linear lanceolate, slenderly long-acuminate, up to 1.5 meters long, 3-5 cm wide, the margrins and midrib armed with sharp spiny teeth pointing toward the apex of the leaf. Fruit is solitary, pendulous, ellipsoid to globose-ellipsoid, about 20 cm long, composed of 50-75 obovoid, angular, fibrous and fleshy drupes, 4-6 cm long, narrow below and truncate at the apex, Figure 2.1.

Figure 2.1 *Pandanus odoratissimus*

Pandanus is said to be a restorative, deodorant, indolent and phylactic, promoting a feeling of wellbeing and acting as a breath sweetener or used as a preservative on foods (Bhattacharjee et al., 2005). It is also said to have healthful properties, including antiviral, anti-allergy, anti-inflammatory, anti-tumor and antioxidant (Londonkar, Kambel and Reddy, 2010). *P. odoratissimus* is one of the plants listed in ayurvedic anticancer treatment. It is said that a paste of *P. odoratissimus* with sugar was applied externally for the cancer treatment (Balachandran and Govindarajan, 2005). The ripe fruits of *P. odoratissimus* owe their scent to an essential oil dominated by esters: besides geranyl acetate, a couple of hemiterpenoid esters were found: isopentenyl (3-methylbut-3-enyl) and, to a lesser degree, dimethyl allyl (3-methylbut-2-enyl) acetates and cinnamates (Vahirua-lechat et al., 1996). It has been reported that methanolic extract of *P. odoratissimus* flourishes in southern Taiwan possessed great antioxidative activities. The chemical components were isolated and identified by comparing their data with authentic materials on the basis of their mass, UV, IR and ^{1}H and ^{13}C NMR spectra. Chemical component analysis of the root parts of *P. odoratissimus* led to the isolation of a total of 15 compounds and some compounds are listed in Table 1. The structures of some compounds are shown in Figure 2.2 (Jong and Chau, 1998).

Table 2.1 Chemical components isolated from *P. odoratissimus.*

Phytochemical Type	Chemical name
Steroids	α-spinasterol and stigmast-7-en-3β-ol mixture , α-spinasterol caproate, stigmast-4-en-6β-ol-3-one
Phenolic compound	Vanillin (**1**), 2(E)-3-(3'-methoxy-4-hydroxyphenyl)-prop-2-enal (**2**), 4-hydroxy-3-(2',3'-dihydroxy-3'-methyl-butyl)-benzoic acid methyl ester (**3**)
Benzofuran derivative	3-hydroxy-2-isopropenyl-dihydrobenzofuran-5-carboxylic acid methyl ester **(4)**
Lignan	Eudesmin (**5**), kobusin (**6**), pinoresinol (**7**), epipinoresinol (**8**), de-4'-O-methyleudesmin (**9**),3,4-bis(4-hydroxy-3-methoxy-benzyl)-tetrahydrofuran (**10**)

Antioxidant activity of constituents isolated from *P. odoratissimus* root has been studied. Among them, 3, 4-*bis*(4-hydroxy-3-methoxybenzyl) tetrahydrofuran (**10**) showed strong antioxidative activities when BHA was used as a standard in the thiocyanate method. Antioxidant activity of *P. odoratissimus* leaves was also studied and the leaf extract demonstrated moderate activity of antioxidant, reducing power and scavenging activity (Londonkar and Kamble, 2009). Moreover, an anti-inflammatory activity of leaves extracted in methanol was estimated by carrageenan-induced acute and formalin-induced chronic paw edema models in rats. The results explained that *P. odoratissimus* leaves showed a significant anti-inflammatory activity at the dose of 100 mg/kg (Londonkar, Kambel and Reddy, 2010).

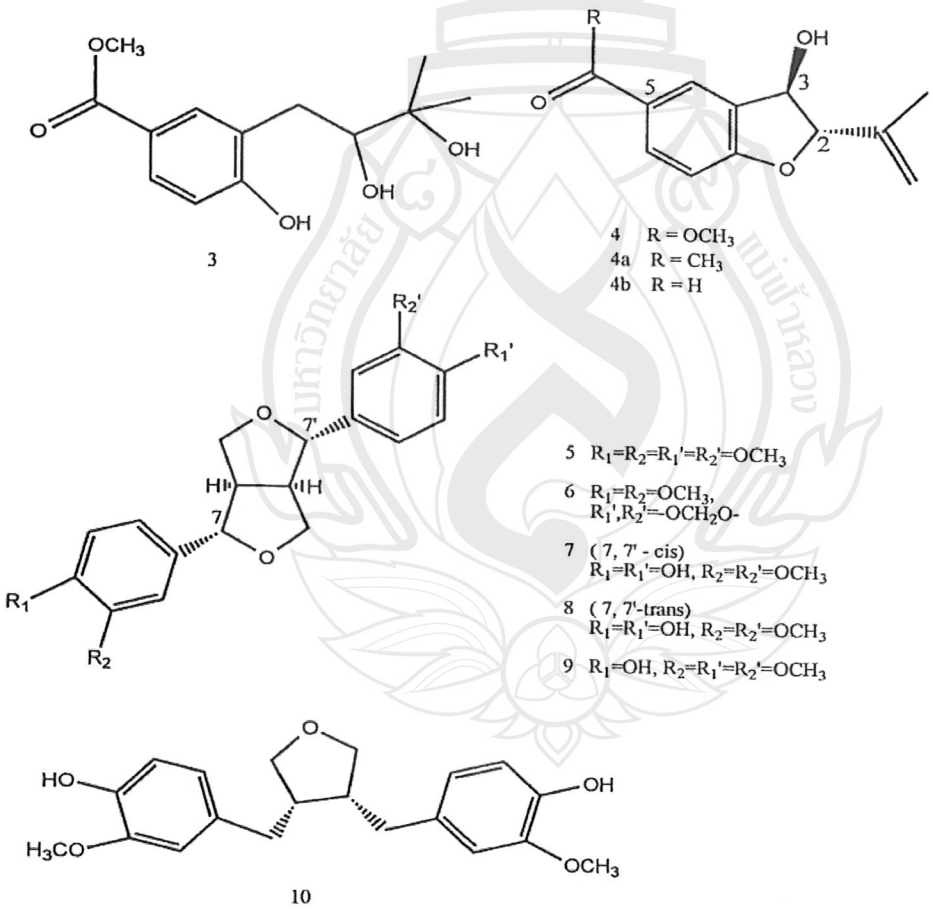


Figure 2.2 Chemical structure of compounds isolated from *P. odoratissimus.*

2.2 Topical oil-in-water emulsion

Emulsions are by far the most popular delivery form for skincare cosmetics. Most emulsions consist of droplets, which form the internal or dispersed phase, that are uniformly distributed into a continuous phase. Generally, the dispersed phase is composed of oil or oil soluble ingredients and the continuous phase is composed of water or water soluble ingredients. This is considered an oil-in-water (O/W) emulsion. Since water soluble and oil soluble ingredients do not mix, an emulsifier is incorporated to reduce the interfacial tension between the oil and water phase by adsorbing to the oil/water boundary and thus acting as a barrier to coalescence. The properties that are most important in a cosmetic emulsion are appearance, feel and odor on application, and effectiveness and having these properties the same each time the product is purchased and used. For any given formula, these properties will depend on the components or ingredients and their properties, the type of emulsion, and the ratio of the major phases. The appearance and feel of a cosmetic depends on a variety of more specific properties such as viscosity or consistency and stability, especially under application conditions.

Stability study of cosmetic emulsion

Stability test was performed to ensure that the products meet the intended physical, chemical and performance characteristics when they stored under various conditions. Stability prediction is usually performed by accelerated storage conditions (Tadros et al., 2004). Before initiating the stability studies, the product should be submitted to a centrifugation test at 3,000 rpm for 30 minutes (Anchisi et al., 2001). Signs of instability of product would be observed and if the product does not change, it may be stable. The stability study is divided into 1) preliminary stability test 2) accelerated stability test, and 3) shelf test. Preliminary stability test is known as the screening test, or short term test. It aims to assist the choosing of formula with a reduced duration. It uses extreme temperature conditions to accelerating reactions on the components and appearance for observed the characteristics of product. The formulations under test are submitted to stress conditions: heating in ovens and cooling in refrigerators. The duration of the study is generally fifteen days. Accelerated stability test is known as normal or exploratory stability test. It provides the data to foresee the stability of the product and used to

estimate the expired date of the product. It generally uses less extreme conditions than the preliminary test. The samples can be submitted to heating in ovens, cooling in refrigerators, exposure to light radiation and to the environment. The duration is generally of ninety days, some cases can be extended for six months or even one year. Shelf test is known as the long-term test which validates the stability limits of the product. This study is carried out over a period equivalent to the time of expiry estimated to evaluate the behavior the product under normal storage conditions. There are many factors that influence the stability of cosmetic products as summarized in Table 2.2.

Table 2.2 Factor that influence to stability testing

Extrinsic factors	**Intrinsic factors**
Time	Physical incompatibility
Temperature	Chemical incompatibility
Light and oxygen	-pH
Humidity	- Reactions of oxidation-reduction
Containing material	- Hydrolysis reaction
Microorganism	- Interaction among formulation ingredients
Vibration	- Interaction between ingredients and containing materials

Safety Test

Clinical trial is the research study conducted to evaluate a new treatment in human. Each study is designed to learn about a potential treatment and its effect on human. Clinical trial is separated into safety test, efficacy test and consumer test. Safety test is the test that performed to ensure the safety of a cosmetic product. The example of safety tests include single patch tests, determination of irritating effect "non-irritation", human repeat insult patch test (HRIPT), determination of allergic effect "hypoallergenic". Single patch testing is the determination of irritating effect on the skin, by applying allergens under occlusion on intact skin of patients. This test may be used for new or novel formulations with known raw materials, and for novel formulations that have been shown to be safe to the skin in an open patch test (Walker

et al., 1996). The test products are applied diluted or undiluted to the skin of, for example, the upper arm or back for periods up to 48 hours under occlusive or semi-occlusive patches and evaluations are performed, for example, l, 24 and 48 hours after removal of the patch. The evaluation is performed visually, assessing, for example, redness, scaling, following the exposure period (Colipa Guidelines, 1997).

Sensory evaluation

Sensory evaluation is defined as a scientific discipline used to measure, analyze and interpret reactions to those characteristics of products as they are perceived by the senses of sight, smell, taste, touch and hearing. Sensory evaluation may be subjective or objective evaluation. Subjective test is based on consumer expectation and results will express in form acceptance or preference. Objective sensory methods are those controlled test variables such as environment, sample handling and respondent (panelist) selection and training. Affective test is of one type of sensory evaluation used to measure preference for products or magnitude of like/dislike for a product. It can be used for consumers or trained panelists, for example, Hedonic test. The word 'hedonic' is of Greek origin and relates to degree or magnitudes of like or dislike. So, it is important to conduct the right type of sensory evaluation in order to find out opinions of consumers and panelist in regards to the quality attributes of products.

Chapter 3
Methodology

3.1 Plant sample and reagents

P. odoratissimus aerial root was collected from Trung province, Thailand. The fresh root was cut and dried by hot air oven at 40°C for 48 hours to obtain dried samples. Solvents for plant extraction (95% ethanol and propylene glycol) and vitamin E acetate are of cosmetic grade. All chemicals and reagents for activity study were of A.R. grade. All ingredients for emulsion preparation are cosmetic grade which comprised of deionized water, xanthan gum, propylene glycol, tween-80, methylparaben, dimethicone, sesame oil, rice bran oil, cetyl alcohol, propylparaben, beeswax, span-80, silicone crosspolymer and cyclopentasiloxane.

3.2 Preparation of solution extracts

The solvent systems were ethanol and propylene glycol. A portion of 20 grams dried *P. odoratissimus* aerial root sample was thoroughly immersed into 200 ml solvent and macerated at 50 °C for 4 hours under sonication (Ultrasonic washer 690D, Crest). The extraction was performed 5 times for each portion. The solution extract was filtrated though Whatman NO.1 and kept in refrigerator at 4°C.

3.3 DPPH radicals scavenging assay

The scavenging activities of the extracts were measured on DPPH radicals according to PhoenCy et al. (2009) with some modifications. Ascorbic acid (0.0073, 0.0156, 0.03125, 0.0625 and 0.125mM) was used as a standard and the range of concentrations were used to create a standard curve. 3 ml of DPPH radicals in absolute ethanol (0.1 mM) was added to the sample (1 ml) or standards (1 ml). The reaction mixture was mixed at ambient temperature, following immediately, incubated at 37 °C for 30 minutes and then the absorbance (Abs) was determined at 517 nm using a UV-Vis spectrophotometer (Libra S22, Biochroms). The scavenging

activity (%SA) on DPPH radicals was calculated from $\%SA = \{Abs_{control} - (Abs_{sample} - Abs_{blank})/Abs_{control}\} \times 100$.

3.4 Development of emulsion product containing the extract

The oil in water emulsion was formulated at the different amount of ingredients to provide smoother and softener emulsion product. Physical properties of the developed products were evaluated by both visual observation and equipments.

Stability study

The products was centrifuged (Mikro 22 R, Hettich) at 3000 rpm for 30 min. Then, accelerating stability test was done under ambient temperature, 4 °C in refrigerator, 45 °C in hot oven and heating-cooling cycle (4 °C 24 h and 45 °C 24 h) for 1 month. The properties or changes of the products were monitored every week.

Microbiological evaluation

Mikrocount® combi test kits were used for determination of microbial contamination of cosmetic emulsion. It is a plastic slide coated on one side with TTC agar (bacterial growth) and on the other side with Rose-bengal-agar (yeast and mould growth). Emulsion (0.2 grams) was applied on both sides of slide and incubated at ambient temperature. After incubation for 24 – 48 h, the TTC agar result (bacteria) is available. For slow-growing microorganisms, the result should be checked again after 48 h. Yeasts and moulds grow after an incubation period of 72 h.

3.5 Single patch test

The tolerance of skin to irritation of the developed product was determined by a single patch test using Finn Chamber®. The product (0.02 grams) was applied on forearm of each volunteer (20 persons) for 24 h. The grading result (0, 0.5, 1, 2 and 3) of irritation was evaluated by comparing with negative control as shown in Table 3.1.

Table 3.1 The grading for irritation zone

Score	Quotation	Criteria	
		Erythema (E)	Odema (O)
0	Absent	No erythema	No edema
0.5	Doubtful	Very slightly erythema (quiet pinked coloration of tested area)	Very slightly edema (palpable, barely visible)
1	Slight	Slightly erythema (rather visible on tested area)	Slightly edema (palpable, visible)
2	Obvious	Obvious erythema (clear erythema covering all of the tested area)	Obvious edema with or without papules
3	Important	Important erythema (severe erythema covering all of tested area)	Important edema (extended area outside the tested area with or without vesicles or blisters)

The calculation of the mean irritation index (M.I.I) was used to classify the potential irritation of the product. The interpretation of the calculated M.I.I scale is shown in Table 3.2.

$$\text{M.I.I} = \frac{\Sigma \text{ of the grade (erythema + odema)}}{\text{Number of subjects}}$$

Table 3.2 The potential irritation scales of the product

M.I.I	Class
M.I.I<0.20	Non irritating (NI)
0.20≤M.I.I<0.50	Slightly irritating (SI)
0.50≤M.I.I<1	Moderately irritating (MI)
M.I.I≥1	Irritating (I)

3.6 Sensory evaluation

The sensory evaluation was performed by using 20 volunteers, males or females, aged between 20-40 years old. They were asked to use the samples and answered on the affective test questionnaire.

Chapter 4
Results

4.1 Sample collection and preparation

P. odoratissimus aerial root was collected from Trung province, Thailand. The fresh root was cut and dried by hot air oven at 40°C for 48 h to obtain dried sample. Ethanol and propylene glycol were selected as solvent for this study. The sample used was 20 grams per 200 ml of solvent and the extract was collected every 4 hours. Tthe extraction was performed five times in order to obtain the highest active components. *P. odoratissimus* root extracts are slightly different in color in different solvents. The pH values of the extracts were slightly different, Table 4.1, and all solution extracts have sweet pandan odor. The pH of extracts increased constantly from the first extraction and the color of extracts slightly decreased from yellow to light yellow. The results indicate the first extraction of *P. odoratissimus* extract may contain higher concentration of active component than the laters.

Table 4.1 Physical properties of *P. odoratissimus* root extracts.

Solvent	Extraction condition	Color	pH
Ethanol	First extraction	Yellow	5.74
	Second extraction	Light yellow	6.06
	Third extraction	Light yellow	6.21
	Fourth extraction	Light yellow	6.42
	Fifth extraction	Light yellow	6.57
Propylene glycol	First extraction	Yellow	5.80
	Second extraction	Yellow	5.84
	Third extraction	Yellow	6.01
	Fourth extraction	Light yellow	6.07
	Fifth extraction	Light yellow	6.27

4.2 DPPH radical-scavenging activity of the extract

The DPPH radical-scavenging activities of *P. odoratissimus* root extract in different systems are shown in Table 4.2. It was found that the first extraction has higher antioxidant activity than that of second, third, fourth and fifth one. It can be seen that the use of propylene glycol as solvent resulting in higher antioxidant activity than that of ethanol about 20%.

Table 4.2 DPPH radical-scavenging activities of *P. odoratissimus* root extracts.

Extraction condition	DPPH (% inhibition)	
	Ethanol	Propylene glycol
First extraction	91.66	93.11
Second extraction	68.53	87.23
Third extraction	53.76	71.08
Fourth extraction	35.89	41.72
Fifth extraction	26.81	33.25

4.3 Preparation of standard extracts *of P. odoratissimus*

According to the antioxidant activity studies, it is sensible to propose the conditions for preparation of the standard extracts as shown follows:

Conditions	**Select suitable condition**
Plant part	Aerial root
Temperature	50 °C
Sonication	Sonication-assisted condition
Extraction time	4 h (x3)
Solvent systems	Ethanol/Propylene glycol (1:1)

After extraction, the extract was collect and ethanol was removed by evaporator. The extract possessed a yellow-viscous liquid with pH 6.30±0.50 and freely soluble in polyols such as ethanol, propylene glycol and glycerin and water.

4.4 Stability of extract

The heat stability testing of *P. odoratissimus* extract was evaluated by DPPH assay at 70 °C, 5 h and DPPH assay was evaluated at initial, 1, 3, and 5 h. This test evaluated the stability of extract when used under heating condition for emulsion formulation process. At 3 h, % inhibition decreased from the beginning about 15% and the value was constant to 5 h as shown in Figure 4.1. The stability was also tested at ambient temperature, 4 °C and 45 °C for 4 weeks in order to check the stability at different storage conditions. The DPPH assay was evaluated every week. % inhibition in all conditions decreased about 13-22% as shown in Figure 4.2.

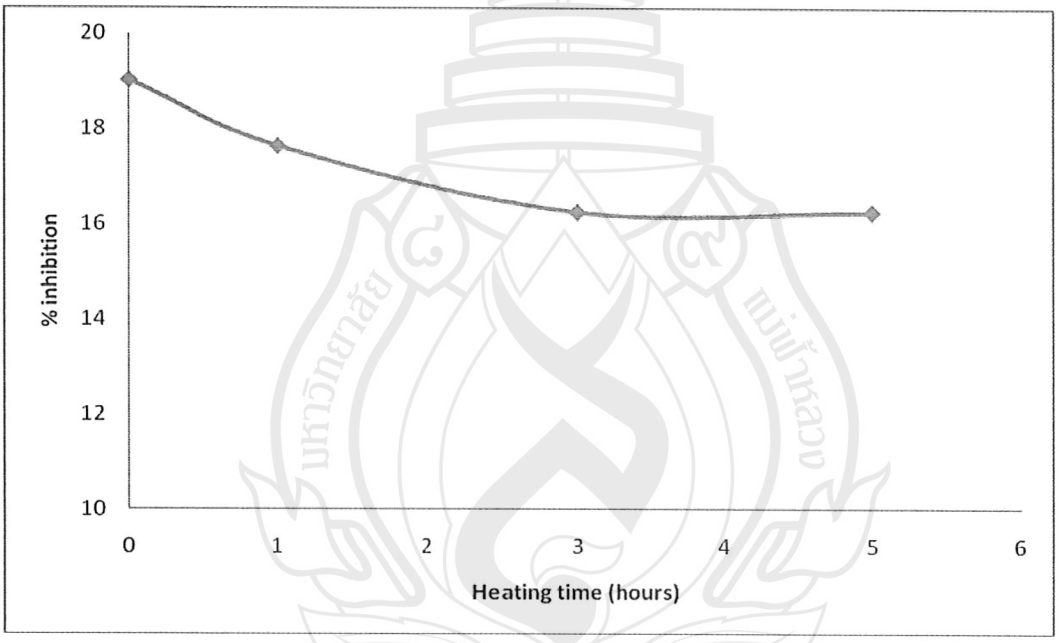


Figure 4.1 The heat stability of *P. odoratissimus* evaluated by DPPH assay at 70 °C.

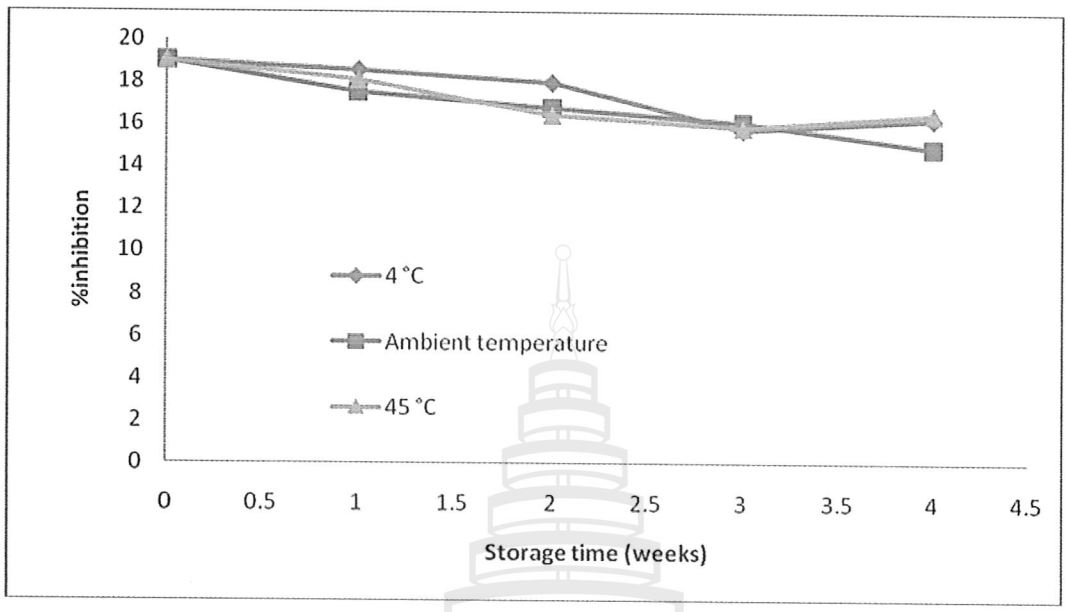


Figure 4.2 The stability testing of *P. odoratissimus* evaluated by DPPH assay at 4 °C, ambient temperature and 45 °C.

4.5 Determination of antioxidant activity of the extract in emulsion system

Antioxidant activity of emulsion containing the extract was investigated by modified lipid hydroperoxide method. A series of emulsions was prepared as shown in Table 4.3. The products were stored at 45 °C and antioxidant activity was determined every day interval and the results are shown in Figure 4.3.

Table 4.3 Emulsion composition for antioxidant activity measurement

Ingredient	Control	BHT	Vit E acetate	Extract	Extract+ Vit E acetate
Linoleic acid	10.0	10.0	10.0	10.0	10.0
Propylparaben	0.2	0.2	0.2	0.2	0.2
Water	86.6	86.3	86.3	81.6	81.3
Methylparaben	0.2	0.2	0.2	0.2	0.2
Extract	0	0	0	5.0	5.0
Vit E Acetate	0	0	0.3	0	0.3
BHT	0	0.3	0	0	0
Sepigel 305	3.0	3.0	3.0	3.0	3.0
Total	100.0	100.0	100.0	100.0	100.0

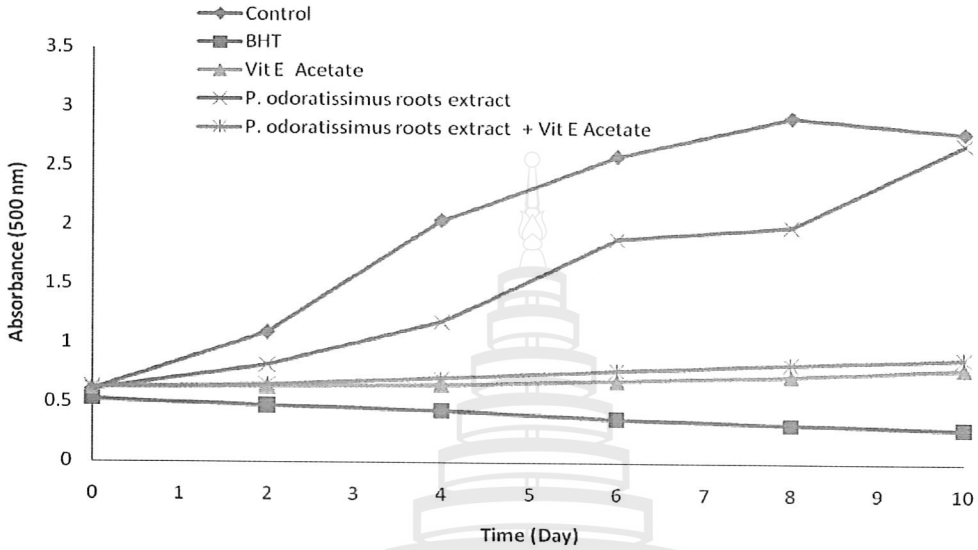


Figure 4.3 Antioxidant activity of emulsion by lipid hydroperoxide method at 45 °C

As it can be seen in Figure 4.3, an emulsion containing only the extract (5%) showed lower absorbance than that of control (at 8 days) and it pointed out that the extract added into the emulsion can help slow down the oxidation of the lipid or polyunsaturated oils in the system at high temperature. It was also found that an emulsion that contains the extract together with the commercial vitamin E acetate has higher efficacy in slowing down the oxidation of the system.

4.6 Oil-in-water emulsion formulation

First, base formulation was developed under the concept of smooth, light, non-greasy, and fragrance free emulsion. The product was prepared as oil-in-water (O/W) emulsion. The percentage of extract added into each formulation is 5%, 10%, 15% and 20%. The texture, color, odor, pH, viscosity and phase separation of the product were observed, Figure 4.4. All products showed no phase separation under centrifugation at 3000 rpm for 30 minutes. The pH of products decreased while viscosity increased when product has more extract concentrations. Moreover, the increase in b* value of product gave appearing in yellow color, Table 4.4. The results showed that the product containing 15% extract had the suitable texture, pH, color

and viscosity. The product was then subjected to stability test and stable product was prepared for safety and sensory evaluation.

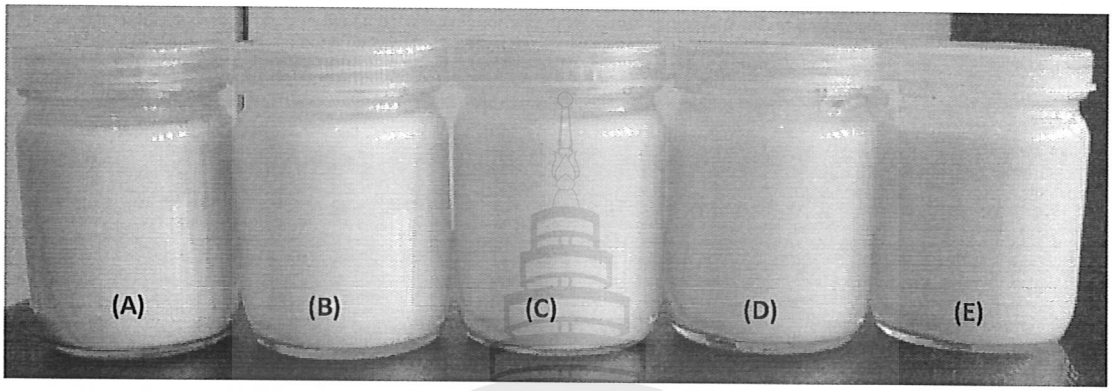


Figure 4.4 Cosmetic emulsion containing *P. odoratissimus* root extract: (A) No extract, (B) 5% extract, (C) 10% extract, (D) 15% extract, (E) 20% extract.

Table 4.4 The physical properties of cosmetic emulsion containing *P. odoratissimus* root extract.

Extract (%w/w)	Physical properties	Color	pH	Viscosity (cP,RV03; 10 rpm)
0	White, creamy-gel and characteristic soybean-milk odor	L* 66.93 a* -2.04 b* 3.72	5.97	5190
5	White, creamy-gel and characteristic soybean-milk odor	L* 64.79 a* -2.17 b* 3.21	5.74	6410
10	White, creamy-gel and characteristic soybean-milk odor	L* 62.49 a* -2.14 b* 3.94	5.67	7330
15	Yellowish, creamy-gel and characteristic slightly sweet pandan odor	L* 63.92 a* -1.87 b* 3.77	5.57	8450
20	Yellowish, creamy-gel and characteristic slightly sweet pandan odor	L* 62.63 a* -1.77 b* 4.07	5.56	9510

Microbial contamination test

Mikrocount® combi test kit was used to determine the microbial contamination of the product. The result showed that the product had no colonies on both sides.

Stability of the emulsion

The preliminary stability test was studied by centrifugation at 3000 rpm, 30 min and the products showed no phase separation. Therefore, the products were chosen for accelerated test at different storage conditions. They were kept in different conditions for 1 month, i.e. at 4 °C in refrigerator, ambient temperature, 45°C in hot air oven and heating-cooling cycle (4°C 24 h, 45°C 24 h). These samples were monitored every week with respect to changes in appearance, color, pH and viscosity.

The viscosity of products was measured using a viscometer (RVDV-II+P, Brookfield) and the results were graphically shown in Figure 4.5. Emulsion products stored at 45 °C and heating-cooling cycle condition showed viscosity reduction compared with that of the beginning. At room temperature and 4 °C, the viscosity of the products increased from the initial value, but the textures showed only small changes.

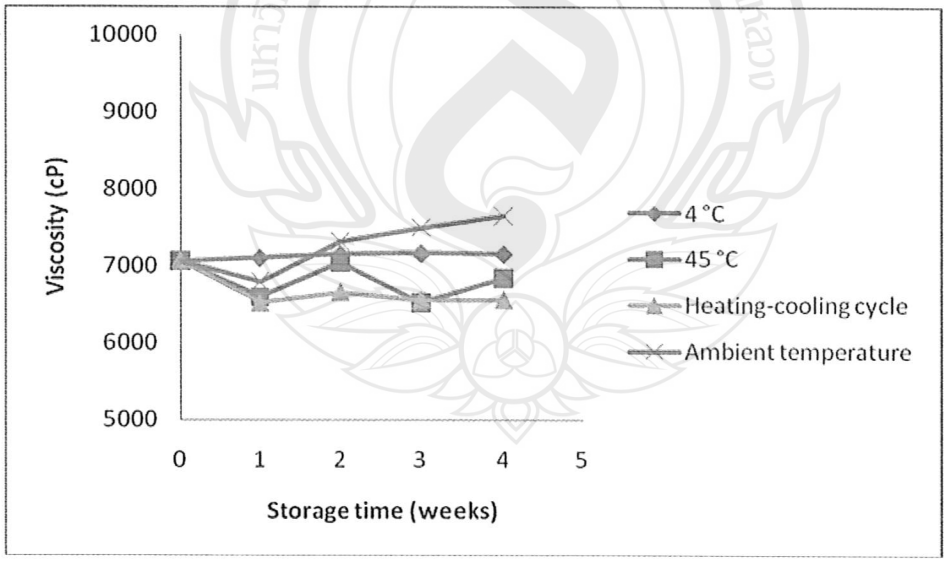
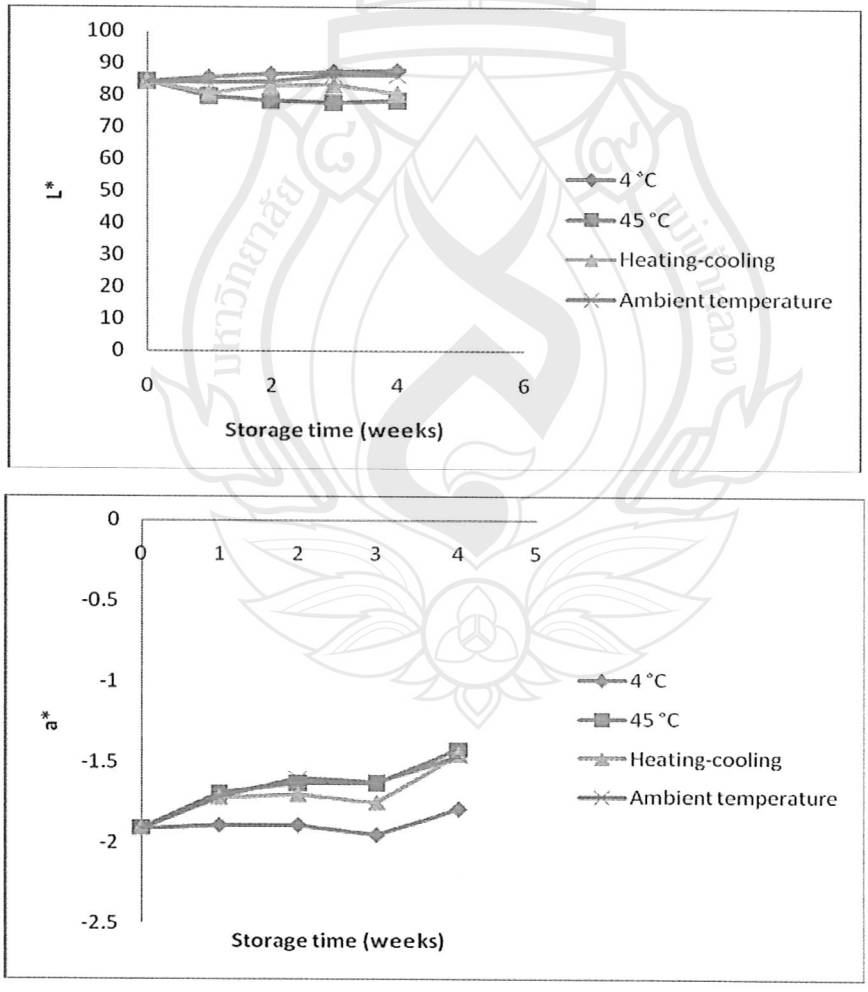


Figure 4.5 The viscosity of the product at different storage condition.

The color of the product was visually observed and also measured by a chromameter (Minolta, CR-400) every week and the results were recorded in L*a*b* values, as shown in Figure 4.6. The L* describes the lightness of the product, the high values show more lightness. It was found that after 4 weeks, the L* values of the product stored at 45 °C and heating-cooling cycle condition were slightly lower (darker) than that of the initial. At 4 °C and ambient temperature condition, the products were slightly lighter than that of the initial. The a* is the color correlation between green (-) and red (+). After 3 weeks, the products stored in all conditions have less negative values of a* than initial. The change of the product's color can be visually observed as a lighter green compared with that of the initial. The b*values refer to blue (-) and yellow (+) color. The b* values of all conditions showed only slightly decreased over 4 weeks.



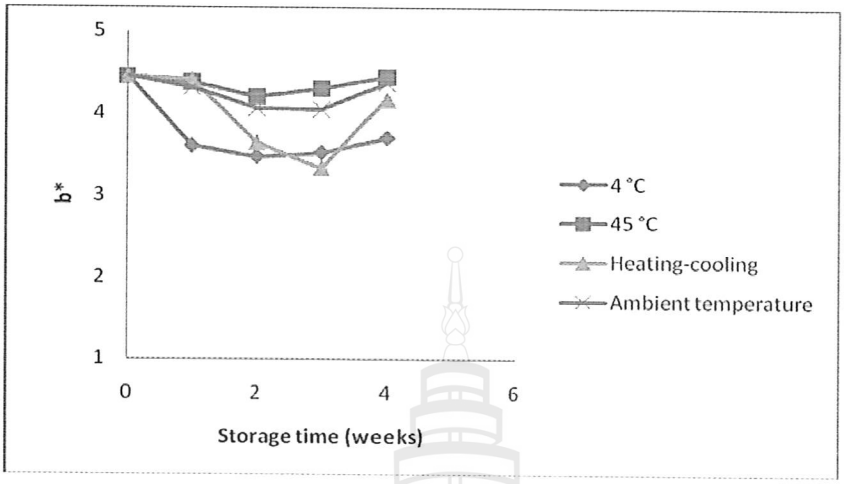


Figure 4.6 L*a*b* values of product at various temperature.

4.7 Primary skin irritation

Single patch test was performed on 20 volunteers using occlusive patch test (Finn chamber) to determine the potential irritation of the product with 15% extract. The mean irritation index (M.I.I) was calculated according to an equation below and the results are shown in Table 4.5. The M.I.I values of all volunteers are 0.5, 0.025,0 and 0 after removed patch at 0, 24, 48 and 72h, respectively. Therefore, the product with 15% extract may be classified as moderate irritation on the skin.

However, single patch test was performed again using the product with only 5% extract and it showed no signs of irritation. Thus, the optimum quantity of the extract in cosmetic emulsion that will not cause any irritation effects is ≤5%.

$$\text{M.I.I} = \frac{\Sigma \text{ of the grade (erythema + odema)}}{\text{Number of subjects}}$$

Table 4.5 The M.I.I of the product containing 15% *P. odoratissimus* root extract.

Subject No.	M.I.I. values at 30 min	M.I.I. values at 24 h.	M.I.I. values at 48 h.	M.I.I. values at 72 h.
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	1	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	2.5	0	0	0
13	2.5	0.5	0	0
14	2	0	0	0
15	2	0	0	0
16	0	0	0	0
17	0	0	0	0
18	0	0	0	0
19	0	0	0	0
20	0	0	0	0
overall	0.50	0.025	0	0

4.8 Sensory evaluation

The sensory test of the product containing 15% extract is performed by 20 volunteers aged 20-40 years old and the results are shown in Table 4.6. It was found that about 89% were moderately to extremely like the overall feature of product. But, volunteers were not satisfied with the odor of the product as it is a fragrance free emulsion. It suggested that the fragrance may be added to improve its odor.

Table 4.6 The sensory evaluation of the product by 20 volunteers.

Properties	Like extremely	Like moderately	Like slightly	Dislike slightly	Dislike extremely
Appearance	5	14	0	0	0
Color	7	10	2	0	0
Odor	1	5	12	1	0
Spreadability	10	7	1	1	0
Lightness	7	8	4	0	0
Penetration (dryness)	5	10	4	0	0
Overall properties	3	14	2	0	0

Chapter 5
Conclusions

Pandanus odoratissimus belongs to pandanaceae family and distributes in the Southern part of Thailand. The root contains steroids, lignan, benzofuran derivatives and phenolic compounds. In this study, dried aerial root of *P. odoratissimus* was extracted in ethanol and propylene glycol solvent. Antioxidant activities of the extract investigated by the DPPH free radical-scavenging activity showed that propylene glycol extract possessed about 20% higher in activity than the ethanol extract. The heat stability testing of the extract was evaluated by DPPH assay at 70 ^{o}C for 5h and it was found that at 3h %inhibition decreased from the beginning about 15%. The stability of the extract was also tested at different storage conditions, i.e., ambient temperature, 4 °C and 45 ^{o}C for 4 weeks. DPPH assay of all conditions decreased about 13-22%. The oil-in-water cosmetic emulsion containing the extract at 5, 10, 15 and 20% were developed and the physiochemical properties of products were studied. All products showed no phase separation under centrifugation (3000 rpm, 30 min). The pH of products decreased while viscosity and yellow color increased when product has more extract concentration. The product containing up to 15% extract had the cosmetically acceptable properties and it was subjected to accelerated stability test for 4 weeks at different conditions. The results showed that the viscosity of the products possessed small changes from the initial. The color was recorded in L*a*b* values. The L* values of all storage conditions showed slightly change about 4-8%. The product showed the higher a* values and lower b* values than the initial product but the changes are not considerable different from the initial when visually observed. Moreover, the microorganism contamination test carried out by using Mikrocount® combi test kits showed that the products had no microorganism contamination. In addition, the sensory evaluation of product performed by 20 volunteers showed that 89% like the overall features of the product. Finally, the safety of product containing 5 and 15% of *P. odoratissimus* extract was tested by single patch test and the product with *P. odoratissimus* extract 5% or lower is non-irritation and safe for use as antioxidant active in the topical cosmetics.

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5. Publications

1. **Ampa Jimtaisong**, Arisa Surinpao and Auchara Yachaimun "Development of Sunscreen Product in Water-in- Silicone Emulsion Form" **Proceeding:** The 1st National Conference on Industrial and Research Projects for Undergraduate Students –IRPUSCON-01 ,Paragon Hall Bangkok, March 27-29, 2009.

2. **Ampa Jimtaisong** , Linsheng Feng , Soumya Sreehari , Craig A. Bayse and Rudy L. Luck Rational Synthesis of Molybdenum(V) TetramersConsisting of $[Mo_2O_4]_2$+ Dimers Held Together by Bridging Phosphinate Ligands and the Tungsten(VI) Dimer $[(CH_3O)_2(O)W(l\text{-}O)(l\text{-}O_2PPh_2)_2W(O)(CH_3O)_2]$:Structural and Theoretical Considerations *Journal of Cluster Structure* **2008**, 19, 181-195. (Impact factor 1.014)

3. **Ampa Jimtaisong** and Rudy L. Luck Synthesis and Catalytic Epoxidation Activity with TBHP and H_2O_2 of Dioxo-, Oxoperoxo- and Oxodiperoxo Molybdenum(VI) and Tungsten(VI) Compounds containing Monodentate or Bidentate Phosphine Oxide Ligands: Crystal Structures of $W(O)_2(Cl)_2(OPMePh_2)_2$, $W(O)(O)_2(Cl)_2(OPMePh_2)_2$,

$Mo(O)_2(Cl)_2dppmO_2 \cdot C_4H_{10}O$, $W(O)_2(Cl)_2dppmO_2$, $Mo(O)(O_2)_2dppmO_2$ and $W(O)(O_2)_2dppmO_2$ *Inorganic Chemistry*, **2006**, 45, 10391-10402.(Impact factor 3.911)

4. Craig A. Bayse, **Ampa Jimtaisong**, Anil K. Kandalam, Rudy L. Luck, Ravindra Pandey, Mark J. Stevens Reexamination of the Structure of $MoO(O_2)_2(H_2O)$(hmpa), hmpa = hexamethylphosphoramide by Crystallographic and Theoretical Means *Journal of Molecular Structure* **2005**, 754, 96-99.(Impact factor 1.44)

5. **Ampa Jimtaisong** and Rudy L. Luck Synthesis, Crystal Structure and Reactivity of $Mo_4O_6(O_2)_2(\mu_3\text{-}O)_2\{(\mu_2\text{-}O,\mu_3\text{-}OC_8H_{14}\}_2(OPCH_3(C_6H_5)_2)_2$: A Self-Assembled Tetranuclear Cluster containing Two Peroxide and Two Trans Cyclooctanediol Ligands *Journal of Cluster Science*, **2005**, 16, 167-184.(Impact factor 1.315)

6. Ge Wang, **Ampa Jimtaisong**, and Rudy L. Luck Crystal structures of $MoCl_2(O)(O_2)(OPMePh_2)_2$ and mixtures of $MoCl_2(O_2)(OPPh_3)_2$ and $MoCl_2(O)(O_2)(OPPh_3)_2$: allylic alcohol isomerization studies using $MoCl_2(O)(O_2)(OPMePh_2)_2$ *Inorganica Chimica Acta*, **2005**, 385, 933–940. (Impact factor 1.606)

7. Ge Wang, **Ampa Jimtaisong**, and Rudy L. Luck "Allylic Alcohol Isomerization and Mechanistic Considerations with CH_3ReO_3" *Organometallics*, **2004**, 23, 4522-4525. (Impact factor 3.196)

Presentation:

1. **Ampa Jimtaisong** and Rudy L. Luck Epoxidation of Cis-Cyclooctene using Hydrogen Peroxide in Ethanol with Catalysts $MCl_2O_2(OPR_3)_2$, (M = Mo ,W and OPR_3 = $(OPMePh_2)$, ½ $dppmO_2$) and $WO(O_2)_2(dppmO_2)$: Catalytic Reactivities and Crystal Structures of $WCl_2O_2(dppmO_2)$ and $WO(O_2)_2(dppmO_2)$: **Poster presentation** The 231st ACS National Meeting, Atlanta, GA March 28, **2006**.

2. Ge Wang, **Ampa Jimtaisong** and Rudy L. Luck ^{18}O Labeled Allylic Alcohol Isomerization Studies Using $MoCl_2(O)(O_2)(OPR_3)_2$, OPR_3 = $OPCH_3Ph_2$, $OPPh_3$, and CH_3ReO_3 :**Poster presentation** The **2003** Student

Research Symposium in Chemistry and Related Fields, Michigan Technological University.

3. **Ampa Jimtaisong**, Arisa Surinpao and Auchara Yachaimun "Development of Sunscreen Product in Water-in- Silicone Emulsion Form" **Poster presentation:** The 7th Industrial and Research Projects for Undergraduate Student IRPUS52 Paragon Hall Bangkok, March 26-29, 2009.

4. **Ampa Jimtaisong**, Arisa Surinpao and Auchara Yachaimun "Development of Sunscreen Product in Water-in- Silicone Emulsion Form" **Oral presentation:** The 1st National Conference on Industrial and Research Projects for Undergraduate Students –IRPUSCON-01 ,Paragon Hall Bangkok, March 27-29, 2009.

5. **Ampa Jimtaisong** Suphinya Yodsrikham "Development of *Oroxylum indicum* as Natural Antioxidant for Skincare Cosmetics: **Poster presentation** The 6th Naresuan Research Conference July 29-31, 2010.

6. **Ampa Jimtaisong,** Siritat Mookriang "Study of Antioxidant Activity of *Pandanus odoratissimus* Linn. Extract" **Poster presentation** The 3rd Annual PSU Phuket Research Conference, November 17-19, 2010.

7. Atcharapan Chantaboon and **Ampa Jimtaisong** "Development of *Moringa oleifera* Leaves Extract as Natural Antioxidant for Topical Application" **Poster presentation** The International Conference on Agriculture and Agro-Industry (ICAAI2010) *Food, Health and Trade* 19-20 November 2010

8. **Ampa Jimtaisong*,** Rungkamol Kraisitthipanich, Waranya Suklim, Panvipa Krisdaphong

"Thai Pandan (*Pandanus amaryllifolius* Roxb.) Extract as Natural Antioxidant in Topical Oil-In-Water Emulsion" **Poster presentation** นักวิจัยรุ่นใหม่พบเมธีวิจัยอาวุโส ครั้งที่ 10, October 14-16, 2010.

9. **Ampa Jimtaisong**, Nisakorn Saewan, Sirirat Mookriang, Panvipa Krisdaphong "Antioxidant capacities of Thai *Mimosa pudica* L. rich bee pollen extract for topical application" **Poster presentation** The 10th AAA Conference and Api Expo, BEXCO Conference Center, Busan, Korea, November 4-7, 2010.

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1998	ตรี	วท.บ. วิทยาศาสตรบัณฑิต	เคมี	มหาวิทยาลัย สงขลานครินทร์	ไทย

5. ประวัติการทำงาน

ปี 2548 อาจารย์สำนักวิชาวิทยาศาสตร์เครื่องสำอาง มหาวิทยาลัยแม่ฟ้าหลวง

6. สาขาวิชาการที่มีความชำนาญพิเศษ (แตกต่างจากวุฒิการศึกษา) ระบุสาขาวิชาการ

- เคมีของสารผลิตภัณฑ์ธรรมชาติ (Natural Products Chemistry)
- เคมีอินทรีย์สังเคราะห์ (Organic Synthesis)

7. ประวัติการนำเสนอผลงานวิจัย

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2. Nisakorn Saewan, Kan Chantrapromma. "Biological activities of *Alpinia mutica* extract" BRT 2009 (October 2009)
3. Kanokwan Iamtan, Rataporn Chaninsat, and Nisakorn Saewan "Development of Sunscreen from *Naringi crenulata* Extract" IRPUSCON-01 (March 2009)

4. Nisakorn saewan; Kan Chantrapromma and John D. Sutherland "New Antimalarial Tetranortriterpenoids from the Seeds of *Lansium domesticum* Corr." RGJ-Ph.D. Congress VI.
5. Nisakorn saewan and Kan Chantrapromma. "Isolation and Biological Activity of the Constituents from *Lansium domesticum* and Their-Structure-Activity Relationships". RGJ-Ph.D. Congress III

8. ประวัติการเผยแพร่ผลงานวิจัย

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2. Nisakorn Saewan; Michael A. Crowe; Madeleine Helliwell; Jim Raftery; Kan Chantrapromma and John D. Sutherland. 2005. "Exploratory Studies to Investigate a Linked Prebiotic Origin of RNA and Coded Peptides Part IV Further Observations Concerning Pyrimidine Nucleoside Synthesis by Stepwise Nucleobase Assembly", *Chem.Biodiv* 2, 66-83.
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