



FULL REPORT

Development of Cosmeceutical Product from Rice Panicle Extract

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All of the participated volunteers are thankful upon their collaborations during irritation, efficacy and preference evaluations of the developed products.

Lastly, we hope that this research result would delineate the development of prototype cosmeceutical products into the commercial achievement that not only benefits for the cosmetic industrial sessions but also strengthen support the strategy of value added up of economically important crops of Thailand, in particular Khao Dawk Mali 105 rice.

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EXECUTIVE SUMMARY

Thailand is one of the world major producers and exporter of rice with the vast variety of more than 93 high quality cultivars. Rice grain is accounted as the important cereal consumed by more than half of the world population as the main staple with phytonutrients of rice particularly in endosperm and germinating rice including bran are accounted as the main source of natural health promotion actives.

Profiles of biologically active phenolics are differed during grain development of particular determined highest in the flowering state which the rice will be further develop into the milky state and transforming into the grain of which the husk turn to be brown completely. However, the flowering period of each rice cultivar is varying. Research dealing with rice panicle is sparsely to be reported, there was only the research about antioxidant assessment of Jasmine rice (*Oryza sativa* L.) during grain development by means of DPPH, ABTS and FRAP assays. Of which the activity and total phenolic content were remarkably high during the flowering state which are day 0 to day 7 that the panicles are still green. The activities and active principle content were related with *p*-hydroxybenzoic acid and ferulic acid contents. Thereafter the flowering state, activities and actives were reduced on the meantime that the grain is developed. In addition, there was the study of 2 rice cultivars in Taiwan which are red pigmented rice (KFSW) and white rice (TK16), the results were in accord with Jasmine rice that actives content in terms of total phenolics and flavonoids of TK16 and its activity were greatest in the flowering state. While KFSW that is the pigmented rice exhibited the highest antioxidant and active contents at 15 days. However, the comparison about activity and total phenolics content shown that KFSW was about two times higher than TK16 during the time that content and antioxidant activity is at the highest point. This is based on the fact that anthocyanins content will be produced in KFSW at day 18 of the pigmented red rice grain. Furthermore, biologically active phenolics were found in the rice panicle extracts for instance ferulic acid, *p*-coumaric acid and caffeic acid. Moreover, the newspaper and website media informatively mentioned that the content of essential amino acids greatly contained in the mediate stage of rice growth. These phytonutrients are important in health promotion with aesthetical benefits for skin and hair respecting to gamma-oryzanol and oryzamide, the potent antioxidant with anti-inflammatory and UV absorbance properties and additionally retain skin hydration protecting wrinkles of skin accordingly.

Since there is not much research on biological activities in Thailand and foreign countries, our researcher group developed the concise extraction of rice panicle with feasibility in an industrial scale. Antioxidant and enzyme inhibitory activities relevant to skin, which are collagenase, elastase and tyrosinase of the safe rice panicle extracts from different five cultivars, were compared. An active compound of these extracts

was quantitatively analyzed for the quality control and stability assessments. Thereafter, an efficient and safe cosmeceutical prototype as observed in human volunteer will be developed and appraisal for commercialization. The 1st year results revealed that rice panicle of Kaow Dok Mali 105 cultivar is the most potential candidate for further study in the 2nd year due to the highest total phenolics and marker contents, in addition to interesting biological activities and safety. The extract is during to be evaluated on stability prior to product development.

The ML_EtOAc_Direct (Ins) extract was found to be physically stable in similar to chemical stability. Total phenolics contents of was slightly reduced (7.62%) following the accelerated stability test by means of 7 heat-cool cycles. Thereafter, cosmeceutical products in the dosage form of cream containing the extract (0.1-0.3%) were developed. All of the preparations were stable under accelerated test condition. The 0.1 and 0.2% active creams were included for skin irritation and efficacy evaluations in the human volunteers respecting to theirs better appearances. Both of the products showed none of skin irritation sign, thereafter randomized double-blind, placebo-controlled efficacy evaluation was undertaken. The rice panicle creams significantly lighten skin color tone better than cream base ($p < 0.001$ and ≤ 0.004) following the treatment for 4 and 8 weeks. In addition, anti-wrinkle efficiency was exhibited by the improvement of skin firmness and smoothness that significantly superior over the base ($p \leq 0.045$ and ≤ 0.038) after the products were applied for 8 and 12 weeks. Efficacy of the 0.1 and 0.2% rice panicle extract creams was comparable with an insignificant differed ($p \geq 0.622$).

Lastly, we hope that this research result would delineate the development of prototype cosmeceutical products into the commercial achievement that not only benefits for the cosmetic industrial sessions but also strengthen support the strategy of value added up of economically important crops of Thailand, in particular Khao Dawk Mali 105 rice.

บทคัดย่อ

การศึกษาวิจัยครั้งนี้มีวัตถุประสงค์ เพื่อนำสารสกัดรวงข้าวขาวดอกมะลิ 105 คือ ML_EtOAc_Direct (Ins) ที่เตรียมได้จากการศึกษาวิจัยในปีที่ 1 ซึ่งมีปริมาณฟีนอลิกรวมและปริมาณสารสำคัญสูงสุด ตลอดจนแสดงฤทธิ์ทางชีวภาพต่างๆ และมีความปลอดภัยในเซลล์เพาะเลี้ยง มาทดสอบความคงตัวของสารสกัดและการพัฒนาเป็นผลิตภัณฑ์เวชสำอางที่มีความปลอดภัยและมีประสิทธิภาพในอาสาสมัคร

ผลการวิจัยพบว่า สารสกัด ML_EtOAc_Direct (Ins) มีปริมาณฟีนอลิกรวมลดลงร้อยละ 7.62 ภายในสภาวะเร่ง Heating cooling และได้พัฒนาเป็นตำรับเวชสำอางรูปแบบครีมผสมสารสกัดที่ร้อยละ 0.1-0.3 และคัดเลือกครีมผสมสารสกัดที่ร้อยละ 0.1 และ 0.2 ไปทดสอบการระคายเคืองและประสิทธิภาพต่อผิวในอาสาสมัครเพราะมีความคงตัวดี ซึ่งผลการวิจัยพบว่า ครีมผสมสารสกัดทั้งสองตำรับ ไม่ก่อให้เกิดความระคายเคืองผิว สามารถลดรอยหมองคล้ำและลดริ้วรอยผิวได้ดีกว่าครีมพื้นอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$ และ ≤ 0.004) ในสัปดาห์ที่ 4 และ 8 ตามลำดับ นอกจากนี้ยังสามารถกระตุ้นผิวและเพิ่มความเรียบผิวดีกว่าครีมพื้นอย่างมีนัยสำคัญทางสถิติ ($p \leq 0.045$ และ ≤ 0.038) ในสัปดาห์ที่ 12 และ 8 ตามลำดับ และครีมผสมสารสกัดทั้งสองตำรับนั้นมีประสิทธิภาพต่อผิวไม่แตกต่างกันทางสถิติ ($p \geq 0.622$)

คำสำคัญ: ขาวดอกมะลิ 105 / รวงข้าว / เวชสำอาง / รอยหมองคล้ำ / ริ้วรอย / กระชับผิว / ความเรียบผิว

ABSTRACT

This research is aimed to assess rice panicle extract stability and further developed into safe and efficient cosmeceutical products as examined in the human volunteers. The rice panicle extract with the highest phenolics content and actives was obtained in the first year of the research project. The obtaining ML_EtOAc_Direct (Ins) with biological activities and safety due to its non-cytotoxicity in cultured cells was subjected to be studied.

The ML_EtOAc_Direct (Ins) extract was found to be physically stable in similar to chemical stability. Total phenolics contents of was slightly reduced (7.62%) following the accelerated stability test by means of 7 heat-cool cycles. Thereafter, cosmeceutical products in the dosage form of cream containing the extract (0.1-0.3%) were developed. All of the preparations were stable under accelerated test condition. The 0.1 and 0.2% active creams were included for skin irritation and efficacy evaluations in the human volunteers respecting to theirs better appearances. Both of the products showed none of skin irritation sign, thereafter randomized double-blind, placebo-controlled efficacy evaluation was undertaken. The rice panicle creams significantly lighten skin color tone better than cream base ($p < 0.001$ and ≤ 0.004) following the treatment for 4 and 8 weeks. In addition, anti-wrinkle efficiency was exhibited by the improvement of skin firmness and smoothness that significantly superior over the base ($p \leq 0.045$ and ≤ 0.038) after the products were applied for 8 and 12 weeks. Efficacy of the 0.1 and 0.2% rice panicle extract creams was comparable with an insignificant differed ($p \geq 0.622$).

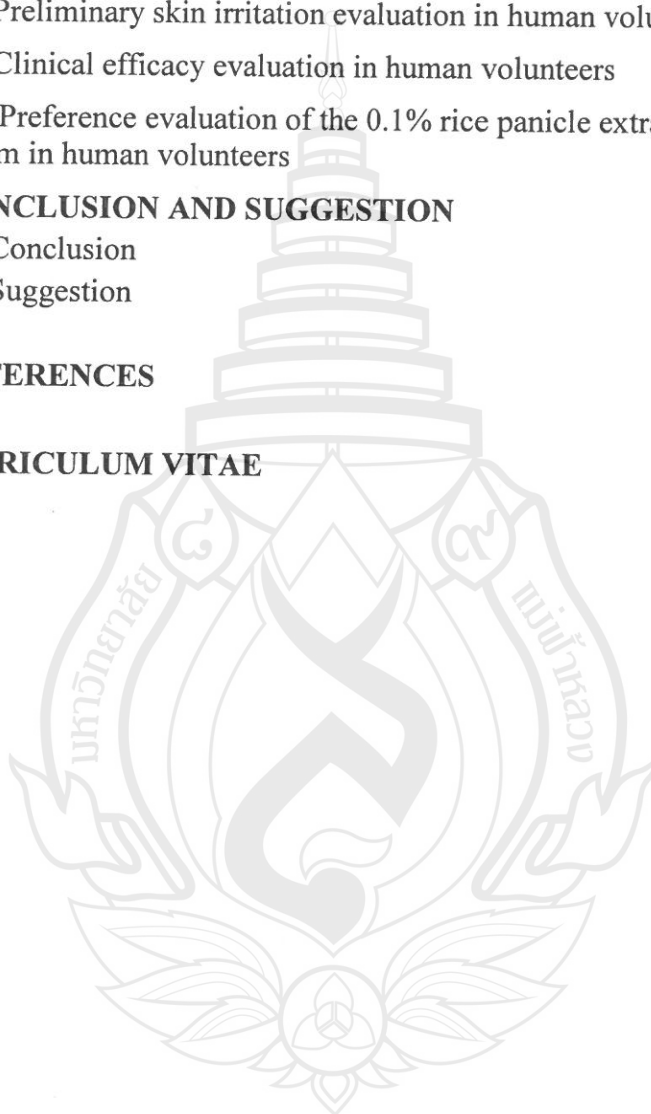
Keywords: Khao Dawk Mali 105 / Rice panicle/ Cosmeceutical/ Dark spot/ Wrinkle/ Skin firmness/ Skin smoothness

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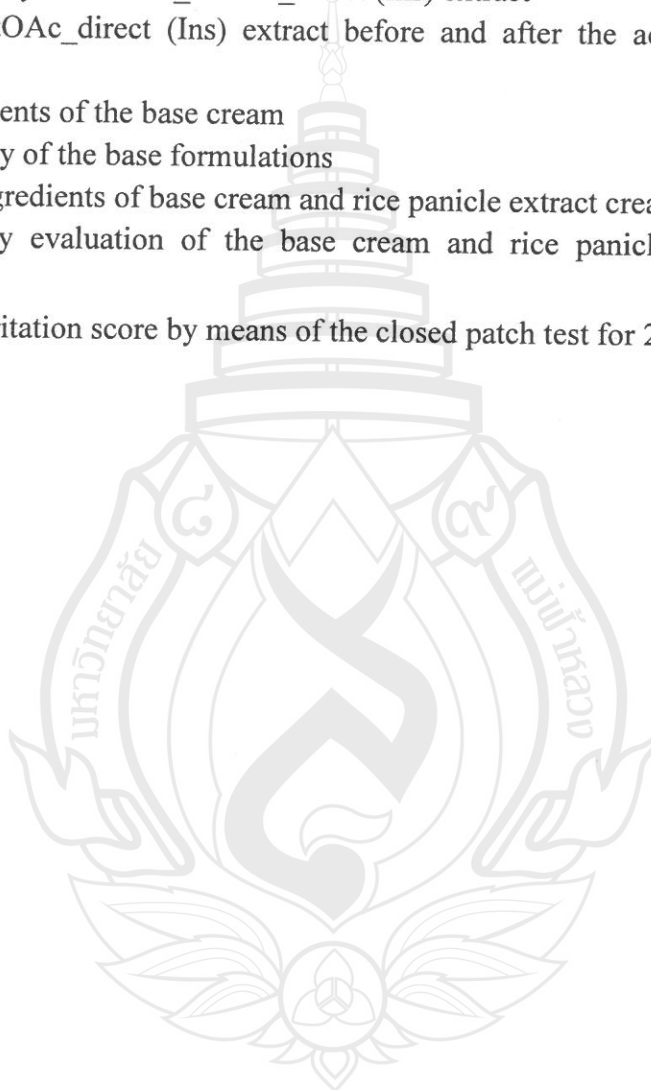
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CHAPTER 1

INTRODUCTION

1.1 Background and rational

Thailand is the country with the vast variety of more than 93 high quality rice cultivars which makes Thailand to be one of the world major producers and exporter of rice (Ministry of agriculture and cooperatives, 2011a). More than half of world population consumes rice grain as the main staple with phytonutrients of rice particularly in endosperm and germinating rice including bran is accounted as the main source of natural health promotion actives. However, research and development in an attempt to apply biologically actives of rice panicle in the forms of health promotion and aesthetic products are sparsely to be presented (Ministry of agriculture and cooperatives, 2011b).

Profiles of biologically active phenolics are differed during grain development of particular determined highest in the flowering state which the rice will be further developed into the milky state and transforming into the grain of which the husk turn to be brown completely. However, the flowering period of each rice cultivar is varying (Lin and Lai, 2011). Research dealing with rice panicle is sparsely to be reported, there was only the research about antioxidant assessment of Jasmine rice (*Oryza sativa* L.) during grain development by means of DPPH, ABTS and FRAP assays. Of which the activity and total phenolic content were remarkably high during the flowering state which are day 0 to day 7 that the panicles are still green. The activities and active principle content were related with *p*-hydroxybenzoic acid and ferulic acid contents. Thereafter the flowering state, activities and actives were reduced on the meantime that the grain is developed (Busat et al., 2009). In addition, there was the study of 2 rice cultivars in Taiwan which are red pigmented rice (KFSW) and white rice (TK16), the results were in accord with Jasmine rice that actives content in terms of total phenolics and flavonoids of TK16 and its activity were greatest in the flowering state. While KFSW that is the pigmented rice exhibited the highest antioxidant and active contents at 15 days. However, the comparison about activity and total phenolics content shown that KFSW was about two times higher than TK16 during the time that content and antioxidant activity is at the highest point. This is based on the fact that anthocyanins content will be produced in KFSW at day 18 of the pigmented red rice grain. Furthermore, biologically active phenolics were found in the rice panicle extracts for instance ferulic acid, *p*-coumaric acid and caffeic acid (Lin and Lai, 2011). Moreover, the newspaper and website media informatively mentioned that the content of essential amino acids greatly contained in the mediate stage of rice growth. These phytonutrients are important in health promotion with

aesthetical benefits for skin and hair respecting to gamma-oryzanol and oryzaceramide, the potent antioxidant with anti-inflammatory and UV absorbance properties and additionally retain skin hydration protecting wrinkles of skin accordingly. However, supportive scientific evidences remained unrevealed (Ministry of agriculture and cooperatives, 2011c).

On the patronage by research support of fiscal year 2013, the research result showed that Khao Dawk Mali 105 (ML) rice panicle in the form of bound phenolics that extracted with 50% ethanol followed by hydrolysis and worked up with ethyl acetate (ML_EtOAc_Direct (Ins)) and the extract obtained by an extraction of the panicle, hydrolysis and partitioned with ethyl acetate afforded statistically insignificant different content of phenolics. The extracts exhibited comparable antioxidant activity as assessed by ABTS and DPPH assays. However, antioxidant on the basis of reducing mechanism (FRAP assay) was significantly ($p < 0.05$) superior in ML_EtOAc_Direct (Ins) ($515.2 \pm 22.06 \mu\text{g FeSO}_4 / 1 \text{ mg extract}$). Both of the rice panicle extracts were found safe towards human dermal skin fibroblast cells. The more potent antioxidant rice panicle extract additionally inhibited tyrosinase, elastase and collagenase (62.40 ± 2.32 , 48.74 ± 4.67 and $79.27 \pm 5.24\%$, respectively). Moreover, sun protection efficacy in UVB range was also exhibited by the ML_EtOAc_Direct (Ins) extract. This extract therefore appointed for cosmeceutical product development in the 2nd year (Kanlayavattanakul and Lourith, 2013) as well as safety testing and efficiency of developed product.

1.2 Research objectives

1. To study upon chemical and physical stability of the rice panicle extract
2. To develop the stable cosmetic preparation containing rice panicle extract
3. To evaluate on the clinical safety and efficiency of the cosmetics containing rice panicle extract

1.3 Research scope

Stability evaluation of the rice panicle extract and development of cosmetic preparation in the dosage form of cream containing rice panicle extract that is stable, safe and efficient and satisfy as assessed in the human volunteers.

1.4 Accomplishment and value of research

Stable, efficient and safe cosmetic product from rice panicle

CHAPTER 2

LITERATURE REVIEW

2.1 Rice and rice panicle

Rice (*Oryza sativa* L.) is the main cereal for consumption in Asia and is the most consumed staple food of Thai people. It is accounted as the important cereal consumed by two-thirds of the world population as the main staple (Wang et al., 2011). In addition to the majorly used as food, rice products in several forms are found their versatile benefits for instance Japanese Sake and rice cosmetics due to phytonutrients of rice that are accounted as the main source of natural health promotion actives (McKevith, 2004). Furthermore, different parts and products of rice with different biologically actives that are efficient, safe and suitable for health promotion product developments for example rice bran oil enriched with gamma-oryzanol, ester derivatives of ferulic and caffeic acids. This antioxidant reduces blood cholesterol with anti-inflammatory activity. Therefore, pharmaceutical and food products containing this rice product are widely developed (Lerma-Garcia et al., 2009). Application of rice product using germinated rice of blood pressure reduction, cancer therapy, stress relief and Alzheimer patients products are also commercialized according to the high content of gamma-aminobutyric acid or GABA as well as ferulic acid (Patil and Khan, 2011).

Although Thailand is represents as the major rice producer for the global market, a lot of innovative rice products in the competitive market are rigorous. Thus, achievement of rice quality in terms of rice productivity per cultivated area especially research and development of innovative rice products are therefore intended to identify adding value up of rice and expand the consumers' benefits such as ready to eat canned rice, health supplements from rice germ, GABA rice, Udon noodles from rice, rice bran oil and milk grain which served as food and health supplement at the same time (Functional food). Healthcare, pharmaceuticals and cosmetics derived from rice products are accounted as a high value addition with a high market potential (Lourith and Kanlayavattanakul, 2013). These innovations will strengthen the potency of Thai rice in the word competitive market on the basis of sustainable utilization of agricultural product that meets the consumers' preference of particular organic agricultural practice (National Innovation Agency of Thailand, 2006).

Rice panicle or rice flower is the rice stem during growth and flowering (0-14 days after following) of more than 80% prior to milk grain (15-21 days) and dough grain (22-28 days) which is the maturity stage and the grain is so much heavy (3/4 of

the panicle) and then fully ripe stage (29-35 days) that the husk becomes all brown. However, the period of each developing stage may be differed in each variety (Lin and Lai, 2011). Rice husk of Khao Dawk Mali 105 cultivated in Mahasarakam were differed in activity during grain development. ABTS, DPPH and FRAP activities including total phenolics content were found to be highest in the beginning of flowering stage (0-7 days) that the panicle still green. The activities were related with the amounts of *p*-hydroxybenzoic acid and ferulic acid. Thereafter, the activities were reduced and minimum at the fully ripe stage (Butsat et al., 2009). This report is in accordance with that of Taiwanese rice, red (KFSW) and white (TK16) rice varieties. Reducing power, total phenolics and flavonoids contents were highest at day 15 of KFSW, whereas those of TK16 were day 6. In similar, the activity and actives contents were later declined. However, total phenolics contents and activity of KFSW were 2 folds higher than TK16 at their best stages. This is roles by anthocyanins content in day 18 of KFSW. Moreover, markers of these two varieties were ferulic, *p*-coumaric and caffeic acids (Lin and Lai, 2011).

Our results studied in a glutinous rice of San Pa Tong 1 (ST), and a longer grain white rice of Phitsanulok 2 (PL) and Suphan Buri 1 (SP) varieties have showed that bound phenolics yielded by the developed extraction method gave high phenolics content extract. In particular, bound phenolics of PL that extracted in 50% ethanol, hydrolysis and partitioned with ethyl acetate (PL_50%EtOH_EtOAc (Ins)). That of PL hydrolyzed and partitioned (PL_EtOAc_Direct (Ins)) and PL extracted with ethyl acetate, hydrolysis and worked up with ethyl acetate (PL_EtOAc (Ins)) were insignificantly differed in term of total phenolics contents in similar to their comparative ABTS and DPPH antioxidant activities. All of the rice panicle extracts were safe as evaluated in human dermal skin fibroblasts. It was noted that PL_EtOAc_Direct (Ins) was significantly better antioxidant assessed by FRAP assay and tyrosinase inhibitory effect. Neuron promotion efficacy was evaluated consequently. The rice panicle extracts were non-cytotoxic in P19 neuron cells. Neuroprotective and neuritogenic activities were significant. In addition, fiber, fat and mineral of PL were significantly greater than the others. The rice panicle extracts contained health benefit phenolics particularly *p*-coumaric, ferulic and caffeic acids. However, essential fatty acids in cosmetics were undetectable (Kanlayavattanakul and Lourith, 2014).

The developed extraction of high rice panicle phenolics was propagated in ML variety. ML_50%EtOH_EtOAc (Ins), ML_EtOAc_Direct (Ins) and ML_EtOAc (Ins) were found insignificantly differed in total phenolics contents with a comparable antioxidant activity by means of ABTS and DPPH assays. Furthermore, the extracts were safe in human dermal skin fibroblasts. Furthermore, it is worthy noted that ML_EtOAc_Direct (Ins) exhibited the significant antioxidant activity assessed by FRAP assay ($515.2 \pm 22.06 \mu\text{g FeSO}_4 / 1 \text{ mg extract}$). In addition to its tyrosinase,

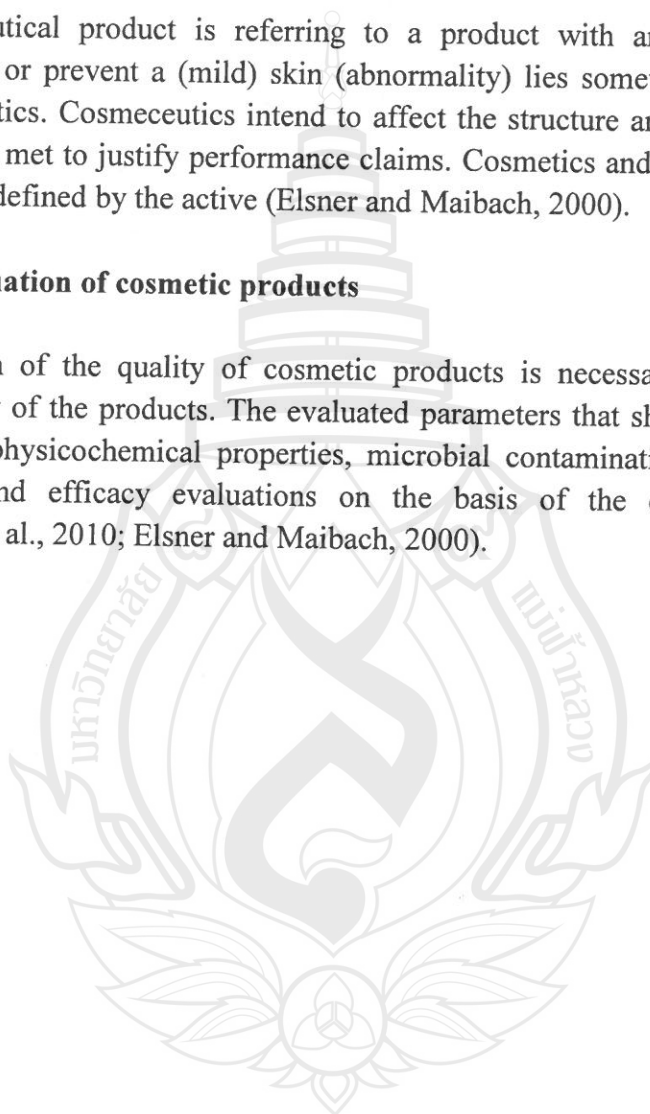
elastase and collagenase inhibitory effects (62.40 ± 2.32 , 48.74 ± 4.67 and $79.27 \pm 5.24\%$, respectively). Moreover, UVB protection efficacy was also exhibited by the ML_EtOAc_Direct (Ins) extract. This extract therefore appointed for cosmeceutical product development (Kanlayavattanakul and Lourith, 2014).

2.2 Cosmeceutical product

Cosmeceutical product is referring to a product with an activity that is intended to treat or prevent a (mild) skin (abnormality) lies somewhere in between drugs and cosmetics. Cosmeceutics intend to affect the structure and function of the skin and must be met to justify performance claims. Cosmetics and cosmeceutics are therefore clearly defined by the active (Elsner and Maibach, 2000).

2.3 Quality evaluation of cosmetic products

Evaluation of the quality of cosmetic products is necessary to ensure the quality and safety of the products. The evaluated parameters that should be assessed are appearance, physicochemical properties, microbial contamination, stability and clinical safety and efficacy evaluations on the basis of the claimed efficacy (Chuarienthong et al., 2010; Elsner and Maibach, 2000).



CHAPTER 3

RESEARCH METHODOLOGY

Base on the research data in the 1st year, the highest total phenolics contents of the ML rice panicle extract, ML_EtOAc_direct (Ins) with the preparation procedure as shown in the flow chart (Figure 3.1) was chosen for the product development.

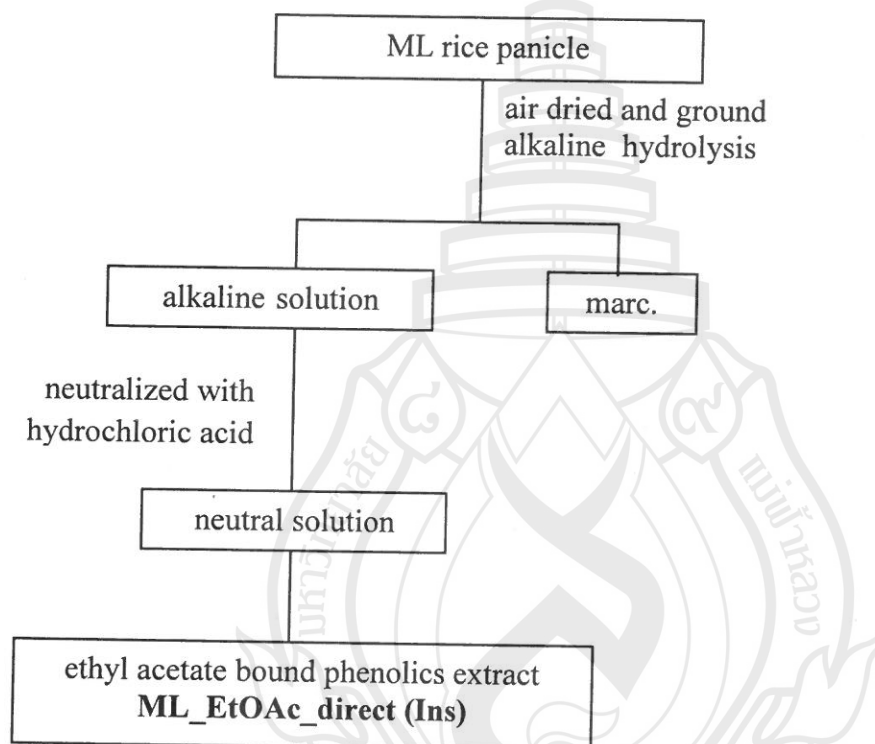


Figure 3.1 Preparation of the ML_EtOAc_direct (Ins) extract

3.1 Evaluation of the ML_EtOAc_direct (Ins) extract properties

Solubility of the extract in the common solvents used in cosmetic preparation that are propylene glycol and glycerine was evaluated as well as pH of the extract in the solvents (Kanayavattanakul and Lourith, 2013).

3.2 Chemical and physical stabilities of the extract under accelerated condition

Testing of the chemical and physical stabilities of the extract under the storage condition of 4 ± 2 °C for 24 h and switched to 45 ± 2 °C for 24 h (counted as 1 cycle)

was undertaken. The heat-cool cycle was repeated for 7 cycles. The physicochemical properties were thereafter observed in color, precipitation and total phenolics contents (Arabshashi et al., 2007; Chang et al., 2006; Juntachote et al., 2007).

3.3 Formulation of base cream and cream containing the extract

Cream base and cream containing the extract were formulated and physicochemically evaluated in terms of color, odor, pH and viscosity and so forth.

3.4 Stability evaluation by means of accelerated test

The cream base and cream containing the extract were preliminary tested by means of centrifugation assay and 7 heat-cool cycles. Consequently, physicochemical properties that are appearance, homogeneity, pH, viscosity and total phenolics contents were determined (Antignac et al., 2011; Kanlayavattanakul et al., 2012).

3.5 Ethical proposal for clinical evaluation in human volunteer approval for the university board of ethic

3.6 Primary irritation test in human volunteers (Futrakul et al., 2010)

3.6.1 The subjects in the study

- 20 healthy volunteers
- Thai nationality
- age between 25-50 years old

3.6.2 Inclusion criteria

- healthy volunteers
- Asian skin type
- none of skin diseases and others
- none of cosmetic allergic report
- voluntary enroll in the study project and signing the informed consent form

3.6.3 Exclusion criteria

- pregnant or lactating volunteers
- skin disease or skin disorder in the tested area
- medication historically used of anti-allergic, anti-inflammatory, antibiotic within 1 week before enrolling in the study, used of steroidal drugs or retinoic

acid derivatives within 4 weeks before enrolling in the study, and used of anti-immune and anti-cancer within 6 months before enrolling in the study

- start/stop/ switch hormonal treatment within 5 weeks before enrolling in the study

- high sensitive skin

- high density of hair, melasma or dark spot or tattoo in the tested area

- severe sickness symptom

3.6.4 Irritation test

Skin irritation of the formulated products is evaluated by means of closed patch test in 20 volunteers using Finn chamber (8 mm) with 20 μ l of the product on the inner arm for 24 h. Thereafter, the chamber is removed, observed and reported. Of which the test products are;

1. Sodium lauryl sulfate 0.5% in water as the positive control
2. Distilled water as the negative control
3. Base cream
4. Cream containing the rice panicle extract at level 1
5. Cream containing the rice panicle extract at level 2

Data interpret

The skin is observed following the 30 min removal of the patch under the same day light condition. None of skin reaction at the tested site refers the end of the irritation test. The volunteers will be later interviewed. In case the skin reaction is observed, the test will be prolonged until the skin will be recovered to the normal condition.

Table 3.1 Human skin irritation test and scoring

Skin response category	Description of skin response	Score
None of irritation	No reaction	0
Doubtful irritation	Barely perceptible erythema	0.5
Weak irritation	Mild erythema or dryness	1
Moderate irritation	Distinct erythema or dryness	2
Strong irritation	Strong and spreading erythema with edema	3
Severe irritation	Severe erythema with edema possibly spreading beyond the treatment site	4

Table 3.2 Skin irritation of the test product on the basis of M.I.I.

M.I.I.	Interpretation
< 0.20	None of irritation
$0.20 \leq \text{M.I.I.} < 0.50$	Slight irritation
$0.50 \leq \text{M.I.I.} < 1.00$	Moderate irritation
≥ 1.00	Irritation

Irritation score is recorded as shown in Table 3.1. The mean irritation index or M.I.I. is thereafter calculated using the followings equation;

$$\text{M.I.I.} = \text{Total irritation score} / \text{Number of the volunteers}$$

3.7 Clinical efficacy evaluation in human volunteers or performance test

- 24 healthy volunteers of 25-50 years old
- instrumental efficacy evaluation by means of non-invasive methods for instances Corneometer® CM 825, Cutometer® MPA 580, Mexameter® MX 18 and Visioscan® VC 98 before the application of the developed cream base and cream containing the extract and after 2, 4, 8 and 12 weeks of the application. The measurement was installed in the acclimated condition (Chuarienthong et al., 2010; Futrakul et al., 2010).

3.8 Preference test towards the preparation in human volunteers

Thirty healthy volunteers of 20-60 years old were included in the preference test of the products by means of questionnaires.

3.9 Data analysis and conclusion

All of the research results are gathered and statistically analyzed for research summarization and conclusion. Data were presented as the means $\pm$ SD in the cream formulation and means $\pm$ SEM in the clinical efficacy evaluation. An ANOVA test was used to evaluate the difference with the level of significance at $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physicochemical properties of the ML_EtOAc_direct (Ins) extract

In addition to the appointed biological activities and health benefit actives of the natural extract that are necessary for cosmetic and personal care application, miscibility or solubility and stability of the extract are required (Antignac et al., 2011). The rice panicle extract of ML_EtOAc_direct (Ins) was therefore observed upon its solubility in propylene glycol and glycerine, separately. The extract (10 mg) was diluted in 10 g of the solvent. A portion of extract (5 mg) was consequently added into the clear solution stepwise until the brownish yellow suspension of the extract is observed on the solvent surface. The solution was left for 24 h, in case the suspension still remained indicates that the solution is saturated. Total amount of the stepwise added of the extract prior to afford the saturated solution was used to calculate the maximum solubility of the extract in the solvent as shown in Figure 4.1. The appearance and pH of the rice panicle extract solutions were recorded (Table 4.1).

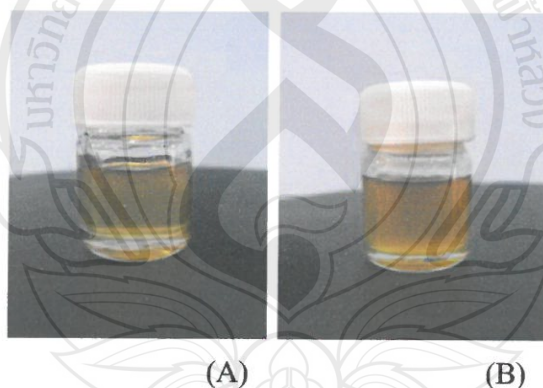


Figure 4.1 ML_EtOAc_direct (Ins) extract dissolved in (A) glycerin and (B) propylene glycol

As shown in Table 4.1 the ML_EtOAc_direct (Ins) extract was highest dissolved in propylene glycol and glycerin at 10 and 8.5% (w/w). Propylene glycol was therefore chosen to be used as a co-solvent in the formulation.

Table 4.1 Solubility of the ML_EtOAc_direct (Ins) extract

Parameters	Solvents	
	Glycerin	Propylene glycol
Extract (g)	850	1,000
pH	4.38 ± 0.51	4.66 ± 0.03
Appearance	Yellowish-brownish clear solution	Yellowish-brownish clear solution
Solubility (%)	8.5	10

4.2 Chemical and physical stabilities of the extract under accelerated test

Application of natural and herbal extracts in cosmetics is necessary to verify the parameters relating with the extract safety in addition to cytotoxicity and skin irritation. The extract is needed to be quantified on its actives as well as biological activities. Therefore, the rice panicle extract was assessed on its stability by means of accelerated test using 7 heat-cool cycles. The methanolic solution (3 mg/ml) of extract was observed upon its physicochemical properties and total phenolics content before and after the accelerated test.

4.2.1 Physical stability following accelerated test

As shown in Figure 4.2, the ML_EtOAc_direct (Ins) extract solution following accelerated stability test was slightly darken in color in similar to a weakly shift of pH (Table 4.2).



Int. HC

Figure 4.2 ML_EtOAc_direct (Ins) solution (3 mg/ml in MeOH) before (Int.) and after accelerated test (HC)

Table 4.2 ML_EtOAc_direct (Ins) extract before and after the accelerated test

Appearance	Accelerated test	
	Before (Int.)	After (HC)
Color	Brownish yellow	Brownish yellow
pH	4.73 ± 0.23	4.68 ± 0.31
Precipitation	Clear solution none of precipitate	Clear solution none of precipitate

4.2.2 Chemical stability following accelerated test

Total phenolics content was assessed in an order to tract the chemical stability of the rice panicle extract due to its concise and economically feasible for the industrial practice (Antignac et al., 2011)

The actives amount by means of total phenolics content of the ML_EtOAc_direct (Ins) extract of the initial stage prior to 7 heat-cool cycles was 22.48 ± 1.24 gGAE/100 g extract. Thereafter, the content was declined to 20.78 ± 0.61 gGAE/100 g extract that could be calculated into percentage of reduction as 7.62% as shown in Figure 4.3.

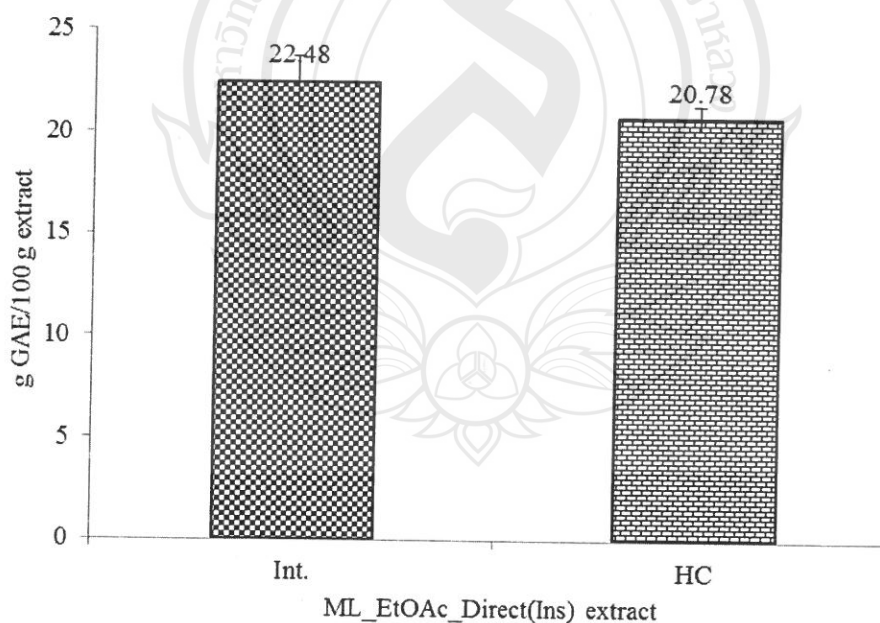


Figure 4.3 Total phenolics content of the ML_EtOAc_direct (Ins) extract before (Int.) and after (HC) accelerated test

4.3 Development of base cream and cream containing the extract

The emulsion in dosage form of oil in water cream was developed. The cream base ingredients in the present of propylene glycol as the co-solvent were listed in Table 4.3. Physicochemical properties of the developed creams (Figure 4.4) were determined and preliminary assessed on the accelerated stability by means of centrifugation assay as shown in Table 4.4. The formulations were subsequently undertaken stability evaluation.

Table 4.3 Ingredients of the base cream

Part	Ingredient	% (w/w)							
		1	2	3	4	5	6	7	8
A	Carbopol Ultrez-21								
	DI water								
	Propylene glycol	86.5	87.5	85.5	85.5	90.5	87.14	87.14	87.14
	4NaEDTA								
B	Cyclomethicone								
	Dub BOIS						9.0	9.0	9.0
	Stearic acid	13.0	14.0	12.0		8.0			
	Jojoba oil				12.0		-	-	-
	Cethyl alcohol					-	-	-	-
	Shea butter	-	-	-		1.00	1.0	1.00	1.00
	Ethylhexyl stearate	-	-	-	-	-	2.0	-	-
	Isodecyl neopentanoate	-	-	-	-	-	-	2.00	-
	Rice bran oil	-	-	-	-	-	-	-	2.00
C	TEA 99%								
D	Vitamin E acetate	0.5	0.5	0.5	0.5	0.5	0.86	0.86	0.86
	Liquid germal plus								

Preparation method

1. Heat the mixture of part A to 70 °C
2. Mix all of the ingredients of part B and heated up to 70 °C
3. Add part B into part A and thoroughly mix using homogenizer for 3-5 min which obtain part AB
4. Cool part AB into 50-55 °C, then add part C that already homogeneously dissolved and further mix with homogenizer
5. Cool down the mixture to 40-45 °C before adding part D using homogenizer

Table 4.4 Stability of the base formulations

Formula								
	1	2	3	4	5	6	7	8
Appearance	Opaque white cream with white plate on skin	Opaque white cream with heavy feeling	Opaque white cream, greasy and and adsorb	Opaque white cream, light texture, easy dried	Opaque white cream, lighter texture, easier dried and adsorb	Opaque white cream, lighter texture, easier dried and adsorb	Opaque white cream, lighter texture, easier dried and adsorb	Opaque white cream, lighter texture, easier dried and adsorb
pH	6.65 ± 0.02	6.52 ± 0.01	6.33 ± 0.01	6.32 ± 0.01	6.30 ± 0.20	6.30 ± 0.20	6.31 ± 0.80	6.39 ± 0.03
Viscosity	++	++	+++	+++	++	++	++	++
Centrifugation	Separate	Separate	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

+ low viscosity; ++ moderate viscosity; +++ high viscosity



Formula 1 2 3 4 5 6 7 8

Figure 4.4 Base creams no. 1 - 8

From Table 4.4 and Figure 4.4, it was found that base cream no. 1 and 2 were separated following centrifugation assay of which base no. 8 was found to be the most suitable cream base for further development of the rice panicle cream. By adding rice panicle ML_EtOAc_direct (Ins) extract into base no. 8 at different concentrations (0.1, 0.2, and 0.3%) to get the rice panicle extract cream (formula 8.1-8.3; Figure 4.5 and Table 4.5) with pH between 5.99 - 5.93 and viscosity about 2,912-2,076 cps.

4.4 Accelerated stability test of the formulation

4.4.1 Physical stability of the formulation

The base cream and rice panicle extract creams that passed the centrifugation assay were subsequently engaged in 7 heat-cool cycles. The products' appearance, pH and viscosity were determined in a comparison with the products at the initial stage.

Table 4.5 The ingredients of base cream and rice panicle extract creams

Part	Ingredients	% (w/w)			
		8	8.1	8.2	8.3
A	Carbopol Ultrez-21				
	DI water				
	Propylene glycol	87.14	87.04	86.92	86.79
	4 Na EDTA				
B	Cyclomethicone				
	Dub BOIS				
	Shea butter	12.0	12.0	12.0	12.0
	Stearic acid				
	Rice bran oil				
C	TEA 99%				
D	Vitamin E acetate	0.86	0.86	0.88	0.91
	Liquid germal plus				
E	ML_EtOAc_direct (Ins) extract	-	0.1	0.2	0.3

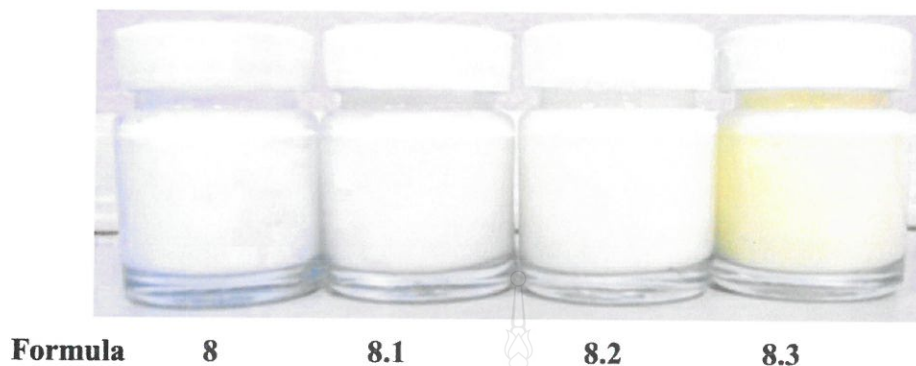


Figure 4.5 Base cream, 0.1, 0.2 and 0.3% of rice panicle extract creams

Figure 4.6 and Table 4.6 show the physicochemical stability of the developed creams (base no. 8) which gives yellowish color of the cream and lower pH but higher viscous. Following accelerated test, the formulations remained homogenous but slightly darken in color. On the means time that, the creams became acidified as a lower pH with a slightly shift of viscosity, of particular the cream containing 0.3% of the rice panicle extract. Therefore, the creams with 0.1 and 0.2% of extract were included for chemical stability evaluation by means of total phenolics content.

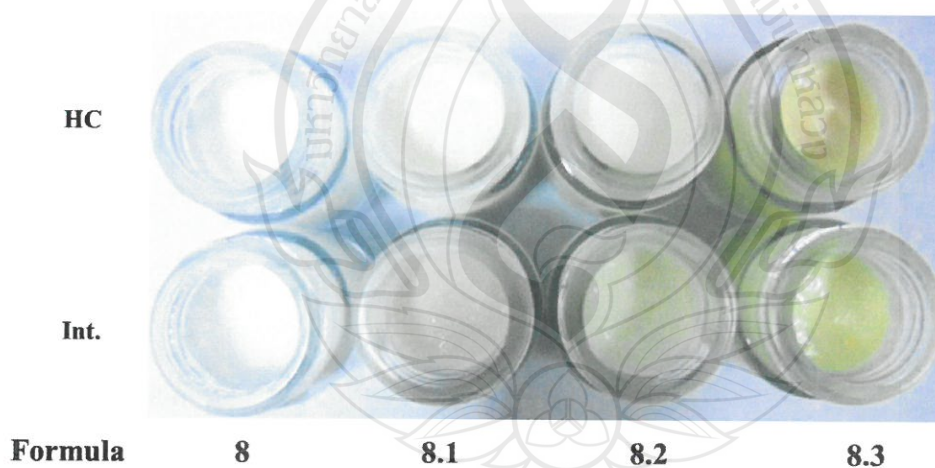


Figure 4.6 Base cream, 0.1, 0.2 and 0.3% rice panicle extract creams before (Int.) and after 7 heat-cool cycles (HC)

Table 4.6 Stability evaluation of the base cream and rice panicle extract creams

Condition	Formula				
		8	8.1	8.2	8.3
Int.	Appearance	Opaque white, light texture, easy dried and adsorb	Opaque light yellowish white, light texture, easy dried	Opaque slight yellowish white, light texture, easy dried	Opaque yellowish white, light texture, easy dried
	pH	6.39 ± 0.03	5.99 ± 0.01	5.93 ± 0.01	5.93 ± 0.01
	viscosity* (cps)	3,051 ± 91.5	2,912.0 ± 87.4	2,094.7 ± 62.8	2,076.0 ± 62.3
HC	Appearance	Opaque white	Opaque light yellowish white	Opaque slight yellowish white	Opaque yellowish white
	pH	6.32 ± 0.01	5.92 ± 0.50	5.91 ± 0.20	5.78 ± 0.03
	viscosity* (cps)	4,159.0 ± 30.7	2,920.3 ± 64.0	2,063.3 ± 61.3	1,553.7 ± 15.9

* spindle no. 5, 120 rpm, % torque > 60

4.4.2 Chemical stability evaluation of the creams under accelerated test

Figure 4.7 shows the total phenolics content of the 0.1 and 0.2% rice panicle extract creams before and after 7 heat-cool cycles. The actives content was found to be 9.6 and 10.1% reduced, respectively. It was noted that the reduction of the total phenolics content in the formulation was worse than the form of the extract. This is in accord with our previous finding that the extract in the liquid medium is easier to be decomposed incorporating with a greater oxidation in the system at a higher extract amount (Kanlayavattanakul and Lourith, 2013). Thus, addition of antioxidant at the same content in each preparation would prevent or delay the decomposition of the extract for instance 0.2% rice panicle extract cream.

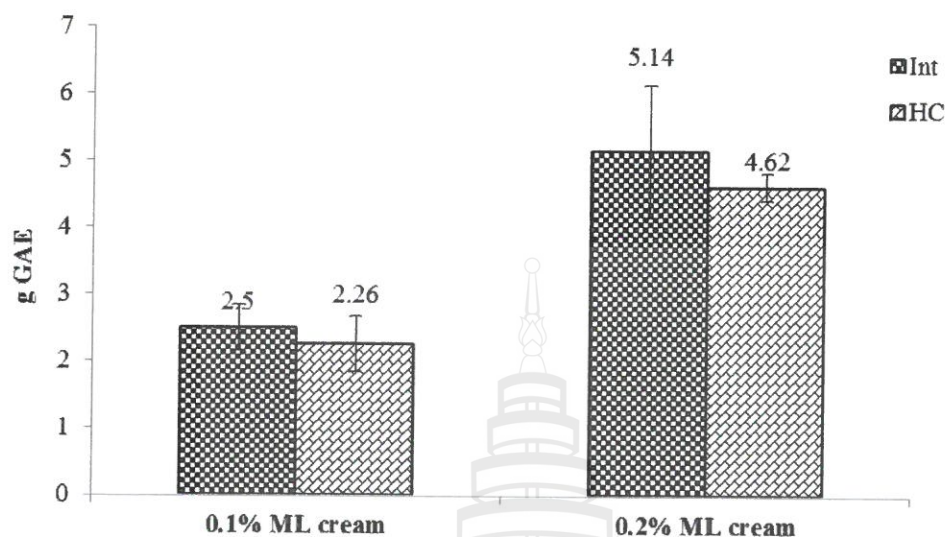


Figure 4.7 Total phenolics content of the 0.1 and 0.2% rice panicle extract creams before (Int) and after 7 heat-cool cycles (HC)

4.5 Preliminary skin irritation evaluation in human volunteers

Closed patch test was adopted for preliminary skin irritation test in 25 healthy volunteers. The 0.5% SLS solution (1), base cream (2) 0.1% rice panicle extract cream (3) 0.2% rice panicle extract cream (4) and water (5) were tested in the volunteers (Figure 4.8). All of the volunteers had none of any sign of irritation with base cream, creams containing rice panicle extract and water respecting to the M.I.I. of 0. On the other hands, that of positive control (0.5% SLS solution) was 0.21 (Table 4.7).

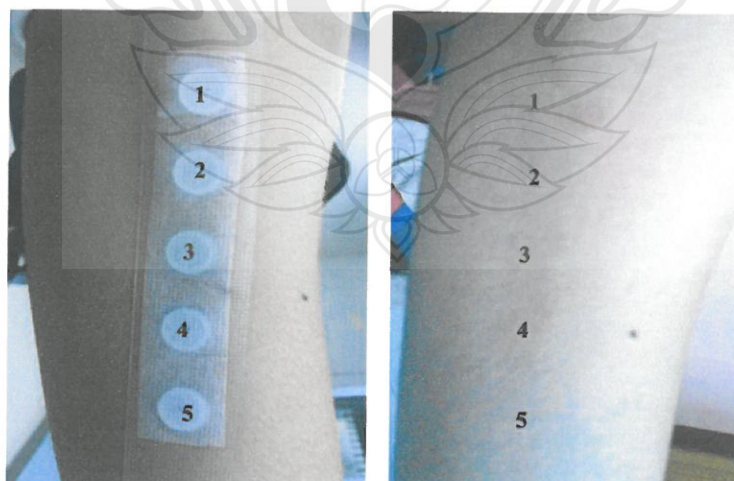


Figure 4.8 Skin irritation test by means of the closed patch test for 24 h

Table 4.8 Skin irritation score by means of the closed patch test for 24 h

Volunteer	No.				
	1	2	3	4	5
	0.5% SLS	Formula 2	Formula 3	Formula 4	Water
1	0.5	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0.5	0	0	0	0
6	0.5	0	0	0	0
7	1	0	0	0	0
8	1	0	0	0	0
9	0	0	0	0	0
10	0.5	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0.5	0	0	0	0
15	0.5	0	0	0	0
16	0	0	0	0	0
17	0.5	0	0	0	0
18	0.5	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
24	0	0	0	0	0
25	0	0	0	0	0
M.I.I.	0.21	0	0	0	0

4.6 Clinical efficacy evaluation in human volunteers

The enrolled 7 male and 17 female volunteers aged 37.46 ± 6.66 years were randomized, double-blind placebo controlled trial using cream base, the 0.1 and 0.2% rice panicle extract creams on randomly left or right forehead (total 3 creams for 3 application spots). Non-invasive instruments were used to track the efficacy of the product at the base line and following the treatments for 2, 4, 8 and 12 weeks, consecutively.

4.6.1 Skin hydrating efficacy

The efficacy of the rice panicle creams to enhance skin hydration in term of moisturizing effect was monitored using Corneometer®. Efficacy of each time interval was exhibited in Figure 4.9.

The 0.1 and 0.2% rice panicle extract creams enhanced skin hydration better than the cream base every the time of measurement. Therefore, the efficacy was compared at each time interval. Application of both rice panicle extract creams for 8 and 12 weeks, significantly ($p \leq 0.032$) improved skin moisture better than 2 weeks of treatment. On the other hands, skin hydrating efficacy of the cream base was insignificantly ($p \geq 0.242$) achieved.

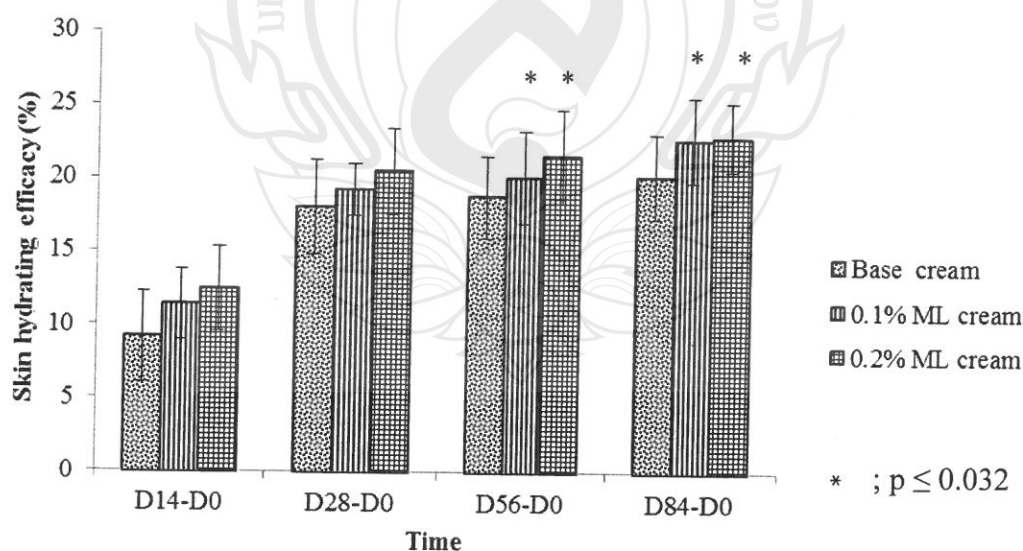


Figure 4.9 Skin hydrating efficacy of the base cream, 0.1 and 0.2% rice panicle extract creams at different time interval

4.6.2 Skin lightening efficacy

The performance of rice panicle cream against dark spot was determined by means of Mexameter[®]. The 0.1 and 0.2% rice panicle extract creams were exhibited to lighten skin color tone as shown in Figure 4.10, of which the result was significant ($p < 0.001$) after 4-week treatment, compared to cream base. In a comparison between 0.1 and 0.2% of rice panicle extract creams at each time interval, their efficacies were insignificantly ($p \geq 0.622$) differed.

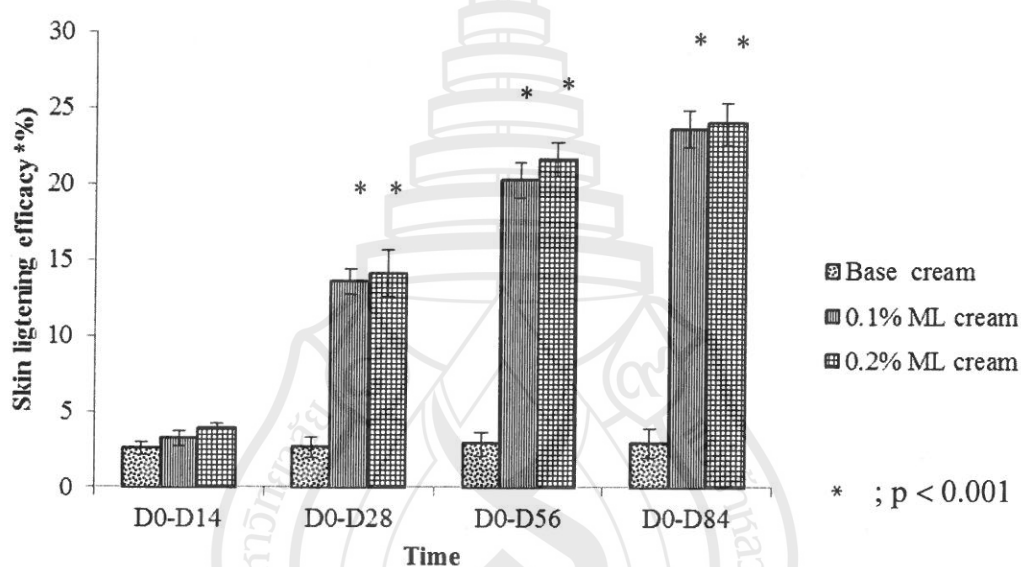


Figure 4.10 Skin lightening efficacy of the base cream, 0.1 and 0.2% rice panicle extract creams at different time interval

4.6.3 Skin firming efficacy

Cutometer[®] was used to evaluate upon skin firming efficacy of the developed creams. After 12 weeks of treatment, both of the rice panicle extract creams significantly ($p \leq 0.045$) increased firmness of skin better than the cream base (Figure 4.11). Additionally, the 0.1 and 0.2% of rice panicle extract creams at each time interval insignificantly improved skin firmness ($p \geq 0.953$).

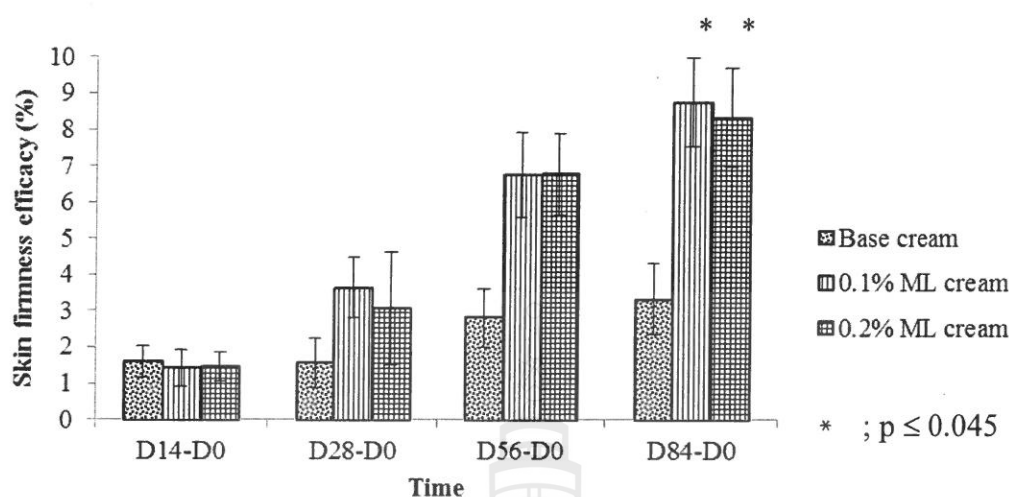


Figure 4.11 Skin firming efficacy of the base cream, 0.1 and 0.2% rice panicle extract creams at different time interval

4.6.4 Skin wrinkle reducing and smoothing efficacies

Efficacy of rice panicle extract creams combating wrinkle and roughness of skin was monitored with Visioscan® as shown in Figure 4.12 and 4.13. The 0.1 and 0.2% rice panicle extract creams were shown to significantly reduce skin wrinkle and induce skin smoothness ($p \leq 0.004$ and ≤ 0.038) after 8-week treatment, compared to cream base. In addition, both of the active creams were similarly suppressed wrinkling of skin and improved smoothness of skin ($p \geq 0.774$ and ≥ 0.979) accordingly at each time interval.

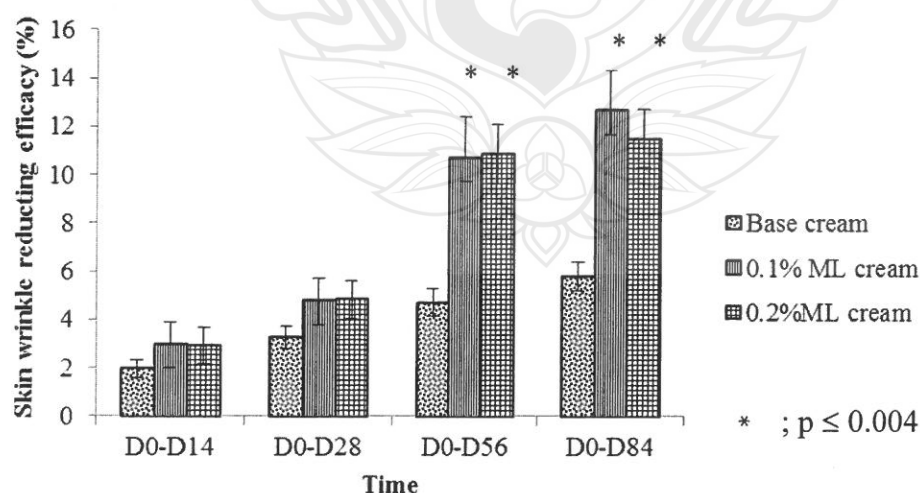


Figure 4.12 Skin wrinkle reducing efficacy of the base cream, 0.1 and 0.2% rice panicle extract creams at different time interval

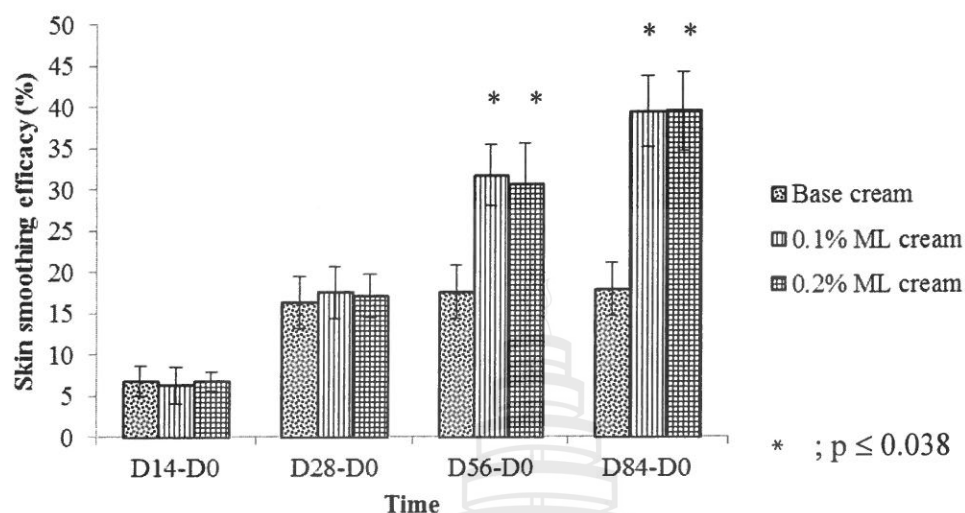


Figure 4.13 Skin smoothing efficacy of the base cream, 0.1 and 0.2% rice panicle extract creams at different time interval

According to efficacy of cosmeceutical product in the form of cream containing rice panicle extract, the preparations composed with 0.1 and 0.2% of the extract were effectively increased skin hydration, firmness and smoothness as well as the efficacies against dark spot and wrinkle in a similar performance but significantly better than cream base. In accordance with physicochemical properties of the 0.1% rice panicle extract cream with light yellowish white, light texture and slightly fragranced and better stable than the 0.2% rice panicle extract cream. The 0.1% rice panicle extract cream is therefore highlighted as the potential prototype cosmeceutical product for further achievement in the commercial feasibility. It was chosen for preference evaluation accordingly.

4.7 Preference evaluation of the 0.1% rice panicle extract cream in human volunteers

Thirty assessors aged between 20-60 years old were included for the sensory test towards the developed cosmeceutical products by means of questionnaires.

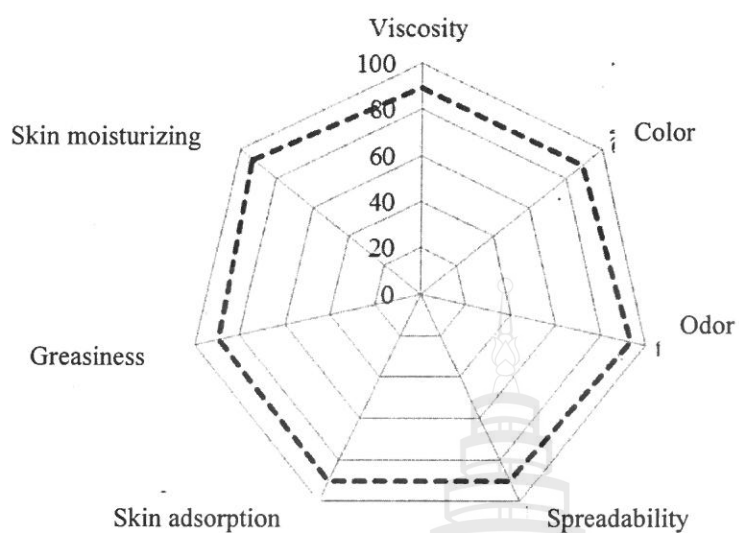


Figure 4.14 Preference of the 0.1% rice panicle extract cream

As shown in Figure 4.14, the assessors preferred the developed cream of more than 80% in each terms of sensory that are viscosity, color, odor, spreadability, skin adsorption, greasiness and skin moisturizing.

CHAPTER 5

CONCLUSION AND SUGGESTION

5.1 Conclusion

5.1.1 Physicochemical properties of the rice panicle extract

Rice panicle extract that was chosen for the continue research in the 2nd year of the project is ML_EtOAc_direct (Ins). The extract was well dissolved in propylene glycol (10% w/w) giving yellowish-brownish clear solution with pH of 4.66 ± 0.03 . This solvent was therefore selected to be used as the co-solvent in the formulation.

5.1.2 Physical and chemical stability of the ML_EtOAc_direct (Ins) extract

Stability of the extract was assessed by means of accelerated test using 7 heat-cool cycles. The extract was found to be slightly darkened in color and slightly change of pH. Furthermore, total phenolics content was found to be reduced only 7.62%

5.1.3 Formulation of rice panicle extract creams and stability evaluation by means of accelerated condition

The ML_EtOAc_direct (Ins) extract was further developed in the dosage form of cream. Cream composing 0.1, 0.2 and 0.3% of the extract as well as base cream were physically stabled and remained homogeneous. However, the 0.3% rice panicle extract cream was more darken in color with a sharp reduction of viscosity following stability test. Thus, 0.1 and 0.2% rice panicle extract creams were chosen for chemical stability evaluation under accelerated test. The 0.1 and 0.2% active creams were 9.6 and 10.1% reduced in the total phenolics content, respectively.

5.1.4 Efficacy evaluation of rice panicle extract creams

The 0.1 and 0.2% rice panicle extract creams were evaluated on their efficacies in terms of skin hydrating, firming, smoothing, lightening and wrinkling. Both of the preparations were found to be similar in these efficacies but significantly better than the cream base. Taken into account with the physicochemical properties that the 0.1% rice panicle extract cream with light yellowish white, light texture and slightly fragranced and better stable than the 0.2% rice panicle extract cream. The 0.1% rice panicle extract cream is therefore highlighted as the potential prototype cosmeceutical product for further achievement in the commercial feasibility.

5.1.5 Preference evaluation of 0.1% rice panicle extract cream

Preferences upon viscosity, color, odor, spreadability, skin adsorption, greasiness and skin moisturizing of the rice panicle cream were more than 80% as assessed in the assessors.

5.2 Suggestion

Preparation of the cosmeceutical product in the form of cream presented in this research offers the potential prototype product. A greater production scale including larger group of the volunteers and assessors are recommended for safety and efficacy assessments in an attempt to engage in the commercial achievements.

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