Development of body lotion formula by using pomegranate (*Punica granatum* L.) extract and rice bran oil (*Oryza sativa* L.) as antimicrobial active compounds

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Abstract*—This research focuses on the development of a body lotion by using pomegranate peel extract and Thai jasmine rice bran oil as antioxidant and antimicrobial active compounds. Pomegranate peel was extracted with 95% ethanol. While Thai jasmine rice bran oil was obtained by cold press method. Antioxidant testing was detected by using 2,2-diphenyl-1-picrylhydrzyl (DPPH) scavenging assay. The half maximal inhibitory concentration (IC_{50}) of pomegranate peel and Thai jasmine rice bran were 0.028 and 4.106 mg/mL, respectively. Inhibition of *Staphylococcus aureus* and *Candida albicans* were performed by using Agar diffusion. 2.5% and 5% mixture of pomegranate peel and Thai jasmine rice bran extracted compounds which gave the largest inhibition zone of *Staphylococcus aureus* and *Candida albicans* as 14 mm. The body lotion mixed extracts prepared from a lotion base with 4 levels of mixtures of the extracted compounds at 0%, 1%, 2.5% and 5%, respectively. The highest efficiency to inhibit *Staphylococcus aureus* was 2.5% of the extracted compounds in the lotion formula with a 6 mm inhibition zone. However, none of the lotion formulas could inhibit *Candida albicans*. Freeze Thaw testing was used for the estimation of lotion stability in both chemical and physical properties. Only two body lotion formulas with 2.5% and 5% mixture of the extracted compounds were shown to have the optimal pH range. Finally, it may be concluded that the body lotion containing 2.5% of mixture of extracts showed a suitable level of herbal extract in cosmetic products for the local community.

***Keywords*—pomegranate peel extract, Thai jasmine rice bran oil, antioxidant, antimicrobial, lotion**

I. INTRODUCTION

Nowadays, people need to use cosmetic products to treat and protect gentle skin from pollution in daily life. However, most cosmetic products are made from chemical compounds. Various chemical materials directly affect and damage the users' skin. On the other hand, almost whole phytochemicals in the botanical do not cause any side effects on the human body, but they are able to enrich the body with nutrients and other useful bioactive compounds. Currently, the natural cosmetics composed of phytochemicals from a variety of plant products are becoming popular and are expected to be a new approach in cosmeceutical market. Natural cosmetics contain several bioactive phytochemicals and nutrients necessary can improve the skin conditions and contribute to a healthy skin without any side effects [1, 2]. Two herbs, pomegranate and rice bran oil are referred extensively as effective compounds to heal and treat skin. Pomegranate is ordered as a type of fruit that contains a high level of antioxidant compounds. There is a lot of research to support that pomegranate has a high level of nutrient benefit. [3-5]. Furthermore, rice bran oil was used as an important component for many types of skincare cosmetics [6]. For this work focused on formulation of standard natural anti-aging lotion by use of a mixture of pomegranate extract and rice bran oil as antioxidant active compounds.

II. MATERIALS AND METHODS

A. Plant Material and Extraction

Pomegranate peels were obtained from local markets in Bangkok, Thailand. They were cleaned, and then oven dried at 60 °C for 7 hours. After that, they were ground into powder form. Fifty grams of dried pomegranate peel powder was extracted by soaking with 250 mL of 95% aqueous ethanol (v/v) for 3 days. The filtrate then was separated through Whatman No. 1 filter paper. The dried residue was re-extracted further with the same condition as the first round of maceration. Crude pomegranate peel extract (12.5 g) was obtained from evaporation of filtrate which passed 3 rounds of maceration with a rotary evaporator under reduced pressure. Thai jasmine rice bran was collected from Lopburi province. Six kilograms of rice bran as a by-

product of the milling of rice was cold pressed using a screw oil press machine and then 60.0 g of oil was recovered. The extracts and oil were kept in a refrigerator at 4 °C prior to analysis. They were used for total phenolic, DPPH scavenging, antimicrobial assay and lotion formulation.

B. Total phenolics content

The total phenolic content of pomegranate peel extract and Thai jasmine rice bran oil were determined by using the Folin Ciocalteu assay according to the procedure reported by [7-8]. 62 µL of extract (50 mg/mL) or oil (100 mg/mL) in ethanol were added to 100 µl of distilled water. 62 µL of 10% Follin Cicocalteu reagent, 625 µL of 10% Na_2CO_3 solution and 500 µL of distilled water were added to the mixture. After shaking the mixture with the vortex, reactions were incubated for 90 minutes at room temperature in a dark place. The absorbance was measured at 765 nm with a Microplate Reader against a blank. The concentration of polyphenols in the sample extract or oil was derived from a standard curve of gallic acid ranging from 0.0625 to 2 mg/mL used under the same conditions as shown in **Fig 1**. The total phenolic content was demonstrated as gallic acid equivalents per mg of dry weight (mg / mL GAE g^{-1} DW) Table 2.

C. DPPH scavenging activity

The 2, 2- Diphenyl- 1- picrylhydrazyl (DPPH) radical scavenging activity of sample pomegranate peel extract or Thai jasmine rice bran oil was verified by using the method of Blois 1958 [9]; Anesini *et al.*, 2008 [10]. The sample extract (0.0078 - 0.125 mg/mL) or oil (12.5 - 20 mg/mL) in ethanol was added to 100 µL of 0.2 mM DPPH solution in ethanol. After shaking, the reaction mixture was incubated for 30 minutes at room temperature in a dark place. The decrease in absorbance of DPPH radical (Abs) was measured at 517 nm with a Microplate Reader and compared with butylated hydroxyl toluene (BHT) calibration curve in the range of 0.0313 – 0.5 mg/mL. The same amount of DPPH (100 µL) and ethanol (50 µL) without the sample were used as a control condition. Each sample was performed in triplicate. The percentage of DPPH free radical scavenging activity was calculated by monitoring the decrease in absorbance of the sample using the following equation (1):

$$\% \text{ Scavenging activity} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \quad (1)$$

$Abs_{control}$ is the absorbance of the DPPH solution in ethanol without sample. Abs_{sample} is the absorbance of DPPH solution in ethanol with sample (i.e., extract or oil). Butylated hydroxyl toluene (BHT) solutions of different concentrations were used as positive controls for radical scavenging activity. The IC_{50} value was calculated from the plot of the amount of sample necessary to reduce the absorbance of DPPH radical concentration versus the inhibition percentage at 50%.

D. Formulation of lotion base and lotion mixed extracts

Four lotion bases were formulated using a two-phase heat system under the same ingredients as Table 1. While four lotion formulas were separately added with different amounts of mixture extracts (0, 1, 2.5 and 5.0%), respectively.

Table 1. Formulation of body lotion base and body lotion mixed extracts

Part	Ingredients	Percentage
A	Cremophore A-6	3
	Cremophore A-25	1
	Finsolv tn	5
	White oil	5
	G.M.S.	4
B	Propylene glycol	4
	Water	72
C	Dipotassium	0.2
	Preservative agent	0.5
	Perfume	0.3
	Pomegranate extract	0 - 2.5
	Rice bran oil	0 - 2.5

Briefly, for formulation, the oil soluble ingredients (Part A) and aqueous soluble ingredients (Part B) were continuously heated together two separated containers until 70°C. The melted oil phase was filled into the hot aqueous phase with continuous stirring until the lotion was well formed. The mixture bases were slowly cooled down to 50°C, the residue ingredients (Part C) such as perfume, preservative, anti-inflammatory agent and mixture extracts of pomegranate peel extract and Thai jasmine rice bran oil at a range of 0 to 5.0% w/w were added during this period. The mixture was continuously and slowly stirred until the lotion base became a good emulsion.

E. Evaluation of lotion stability

Two hundred milliliters of each of formulated lotions were kept in 250 mL containers closed with their covers through their physical and chemical stability testing. Freeze Thaw cycle was operated for estimation of lotion stability by using 6 cycles of heating and cooling accelerated condition. One cycle indicated that a rotation of storage condition from heating (45°C for 48 hours) to cooling (4°C for 48 hours). The chemical and physical parameters of lotion such as pH and viscosity, were measured by using a pH meter and a viscometer (Brookfield), respectively.

F. Antimicrobial activity

The antimicrobial activities of pomegranate peel extract, Thai jasmine rice bran oil, lotions base and lotion mixed extract and oil samples were tested against *Staphylococus aureus* (TISTTR 517) and *Candida albicans* (TISTTR 5779) by the agar diffusion method. Both microbial suspensions were prepared to have a turbidity equivalent to 0.5 McFarland turbidity standards. Then 0.1 mL of each microbial suspension was spread in Nutrient Agar (antibacterial tests) and Sabouraud Dextrose agar (antifungal tests). After incubation of microbial at 37ºC for 24

hours. The 60 µL per sample were individually loaded into 5 mm diameter wells. After an incubation period at 37ºC for 48 hours, the diameter of inhibition zone was measured in mm units. All experiments were performed in triplicate. Propylene glycol was used as positive control for both bacteria and fungi, respectively.

III. RESULTS AND DISCUSSION

A. *Total phenolics content*

Two extracted natural products were prepared from pomegranate peel and Thai jasmine rice bran. The pomegranate peel was extracted with 95% ethanol while Thai jasmine rice bran oil was obtained from a cold press method. They were carried out to determine the total phenolic content by using Folin-Ciocalteu assay. Gallic acid was used as a standard and the total phenolic contents were expressed as gallic acid equivalents per gram of dry weight (mg/mL GAE g^{-1} DW) using the standard curve equation $y = 0.6967x + 0.0725$, $R^2 = 0.996$ (Fig. 1).

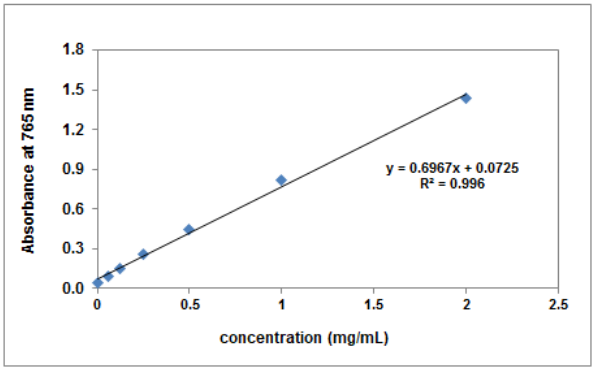

Fig. 1 Standard curve of Gallic acid

The results are presented in Table 2 which show the total phenolic content of pomegranate peel extract and Thai jasmine rice bran oil were 47.20±0.63 and 6.41±0.034 mg GAE/g DW, respectively. Some previous studies reported different result work. Nuamsetti, *et al* 2012 [11] and Tinrat and Singhapol, 2014 [12] who used powdered pomegranate peels extracted by soaking them in 95% ethanol for 18 hours and 3 days, respectively. They reported that the phenolic content of pomegranate peel extract was rather less than this one at 152.65 mg GAE/ 100 g DW and 12.80 mg GAE/g DW, respectively On the other hand, Manasathien *et al.*, 2012 [13] prepared pomegranate peel extract with Soxhlet extraction apparatus for 24 hours. The sample displayed high total phenolic content of 95% ethanol extract at 449.60±4.40 mg GAE/g. The different results of phenolic compound from pomegranate peel extract among those studies may depend on cultivar, preparation of plant extract and timing of extraction. [11-13].

B. *DPPH scavenging activity*

Antioxidant activity of pomegranate peel extract and Thai jasmine rice bran oil were evaluated by using 2,2-diphenyl-1-picrylhydrzyl (DPPH) radical scavenging activity assay. They are presented as IC_{50} value (the concentration of extract was used to inhibit 50% of the initial DPPH free radical) as shown in Table 2.

Table 2. Total phenolic content and IC_{50} were determined in crude extract and oil sample

Sample	**($\overline{X}$ ± SD) mg Gallic acid / g DW**	**IC_{50} (mg/mL)**
BHT	-	0.0220
Pomegranate peel extract	47.20±0.63	0.0275
Thai jasmine rice bran oil	6.41±0.034	4.1063

The pomegranate peel extract exposed high radical scavenging potential with IC_{50} value of 0.028 mg/mL with almost equivalent of standard BHT (butylated hydroxytoluene) as IC_{50} value of 0.022 mg/mL. Whereas the IC_{50} of Thai jasmine rice bran oil was determined as 4.106 mg/mL. The low IC_{50} value of pomegranate peel extract causes the presence of high phenolic compounds in it. Some previous studies found that variation of antioxidant activity of pomegranate peel extracts depended on the amount of the isolated phenolic compounds which was highly correlated with the solvent extraction technique [13-15]. This is similar to previous work [14-15] which indicated that ethanolic extract (rich in phenolics) of pomegranate peels possessed powerful antioxidant activity with large the DPPH scavenging activity was better than that of water extracts. However, methanolic extract showed higher total phenolic compounds [14-15]. The pomegranate peel extract added as an active ingredient for cosmetic products should be prepared from a safe solvent. Ethanol is considered an appropriate solvent for this work.

C. *Evaluation of lotion stability*

The body lotions containing 0, 1, 2.5, and 5% mixture of extracts of pomegranate peel extract and Thai jasmine rice bran oil in base were prepared and kept in containers to undergo the lotion stability process by Freeze Thaw testing. Observation of the physical properties of the lotion base and lotion mixed extracts show that the last one had a more brownish color than the control base. This may be caused by a degradation of extract compounds in the lotion and the higher temperature when passed through Freeze Thaw cycles. Previous studies have also found a change in the color of the cream after a stability test [16]. However, the lotions had a good spread ability on the skin. For the chemical property, the pH value of all formulations showed high correlation with the amount of extracts in the range of 6.7-

7.8 (data not shown) which are accepted levels under natural cosmetic products for Thai Industrial Standards Institute. The low pH values were observed from the lotions contain high amounts of extracts after accelerated condition. This result considered that stress condition and pH values of the extracts cause a decrease of pH range in the lotion. However, there were 2 formulas of lotion containing 2.5% and 5% extracts possessing quite optimal pH value (6.7-7.3) (data not shown) which are nearly the healthy skin pH, of 5-6. Thus, the range of mixture of extracts of pomegranate peel extract and Thai jasmine rice bran oil are 2.5-5.0% in lotion base was considered to be appropriate for lotion formulations for further studies.

D. Antimicrobial activity

These results showed uniformly high inhibition for *Staphylococcus aureus* in high amount of pomegranate peel extract. On the other hand, pure Thai jasmine rice bran oil showed low inhibition against both *Staphylococcus aureus* and *Candida albicans* see Table 3. The study showed that total phenolic content in pomegranate peel extract and Thai jasmine rice bran oil had a concentration- dependent activity for *Staphylococcus aureus* more than *Candida albicans*. The results were in accordance with previous work, which have reported that at a high level of pomegranate peel extract limited antibacterial activity [17]. Interestingly, a mixture of pomegranate peel extract and Thai jasmine rice bran oil demonstrated a greater antimicrobial effect on both of them than pure extract. This result suggested that the combining extracts may be more useful for the inhibition of *Staphylococcus aureus* and *Candida albicans* than the pure extract see Table 3.

Table3. Antimicrobial activity of crude extracts and lotions

Sample	Diameter of inhibition zone (mm.)	
	Staphylococcus aureus	***Candida albicans***
Propylene glycol	NA	2
PPE 2.5%	13	NA
PPE 5%	14	NA
TJRO 100%	2	1
Mixture of PPE and TJRO 1.0%	12	4
Mixture of PPE and TJRO 2.5%	14	14
Mixture of PPE and TJRO 5.0%	14	14
Lotion base without extracts	2	NA
Lotion with 1% mixture of extracts	3	NA
Lotion with 2.5% mixture of extracts	6	NA
Lotion with 5% mixture of extracts	4	NA

PPE = pomegranate peel extract, TJRO = Thai jasmine rice bran oil, NA = not available

This antimicrobial activity was considered the optimal mixture of extracts in the ranges of 0-5% as an active ingredient in lotion formulation. There were four lotion formulas consisting of four different levels of mixture extracts (0, 1, 2.5 and 5%). Antimicrobial activity of lotions performed differently for each mixture extract. All of them showed only antimicrobial activity on *Staphylococcus aureus* at lower inhibition than the pure mixture extract without lotion. Furthermore, 2.5% of mixture extracts in lotion demonstrated the highest effect, while both 1% and 5% of mixture extract in lotions tended to lower the effect than that of 2.5% mixture extracts see Table 3. This result may be explained that at 2.5% of mixture extracts is limiting level for inhibition of *Staphylococcus aureus*. On contrary, inhibition of *Candida albicans* was not found for all lotion formulas. Mixture extracts reduced antimicrobial activity with ingredients effective in lotion when compared with pure oil showed strong inhibition. However, observation of antimicrobial activity found that the lotion formula containing 2.5% mixture extracts was proposed to be a suitable lotion formula. Additionally, this formula was also not found to irritant the skin of testers.

IV. CONCLUSIONS

The total phenolic content of the pomegranate peel extract and Thai jasmine rice bran oil demonstrated antioxidant activity by DPPH assay. The IC_{50} value of pomegranate peel extract was closely similar to BHT which is one of the approved antioxidant agents in cosmetic products. In addition, antimicrobial activity of the mixture extracts had a greater impact than pure crude extract. All of the lotion added mixture extracts (1-5%) displayed inversely much lower antibacterial activity than that of pure mixture extracts. In summary, the lotion containing a mixture of extracts at 2.5% was considered as the most appropriate amount that showed the highest efficiency level of herbal extract as well as having good chemical and physical properties after stability testing. Those are accepted levels under natural cosmetic products for the local community.

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