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Formulation, Characterization, Antioxidant and Sun Protection Factor Activity of Red Pomegranate (*Punica granatum* L.) Peel Extract in Sunscreen Lotion

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ABSTRACT: Red pomegranate (Punica granatum L.) contains phenolic compounds of ellagic acid as antioxidants and flavonoids and tannins as sunscreens in protecting the skin against ultra violet radiation. The aim of this study was to determine the sunscreen and antioxidant activity of red pomegranate peel extract, also the formation of its stable sunscreen lotion and to determine its SPF and antioxidant activity. Red pomegranate peel was macerated with 70% ethanol and concentrated. Antioxidant activity testing using FRAP method and determination of SPF value, spectrophotometric method was used along with Mansur equation. Lotion formulation was done by adding red pomegranate peel extract as much as 0.06% (F1); 0.12%(F2), and 0.24% (F3). The lotion preparation was evaluated for physical and chemical parameters including organoleptic, homogeneity, viscosity and flow properties, lotion type, pH, spreadability, determination of SPF value and stability test using freeze and thaw method. The results showed the IC_{50} value of red pomegranate peel extract was 12.42 ± 0.01 µg/mL and the value of sunscreen activity at a concentration of 400 µg/mL gave an SPF value of 25.91 ± 0.01. The result of testing the physical properties showed that the lotion type was o/w, viscosity ranges from 10400 to 12000 cPs, pH test from 7.25 to 7.60, spreading power from 6.31 to 6.42 cm. The lotion was stable as there was no phase separation observed after centrifugation, freeze-thaw and thermal stress tests. For the lotion of red pomegranate peel extract showed antioxidant activity with IC_{50} values of 98.73 µg/mL (F1); 68.89 µg/mL (F2); 48.30 µg/mL (F3) and sunscreen values of 13.11 ± 0.02 (F1); 20.78 ± 0.02; 24.78 ± 0.04. It can be concluded that red pomegranate peel extract has very strong antioxidant activity and sunscreen activity with moderate protection, and can be formulated into lotion preparations with good quality parameters.

KEYWORDS: Red Pomegranate; sun protecting factor; antioxidant; FRAP method; lotion.

1. INTRODUCTION

Sunlight exposure can accelerate skin-related damage regardless of the time of year or season, particularly in the tropics [1]. Sunscreen technologies aim to reduce ultraviolet-induced skin cancer by absorbing, scattering, or reflecting radiation [2]. Sunscreens are chemicals that efficiently absorb or reflect ultraviolet light and are applied topically to protect against the adverse effects of sunlight, primarily erythema. The sun protection factor measures how much solar ultraviolet energy protects the skin from sunburn. Currently, natural agents are being developed to address the limitations of conventional sunscreens in protecting against ultraviolet radiation. Furthermore, oral photo protectants do not provide complete skin protection; however, they enhance the body's natural photoprotection mechanisms [3]. Phytochemicals may function in various ways, including absorbing ultraviolet light and acting as filters, stimulating the immune system, triggering gene suppression, halting oxidative DNA damage, detoxifying carcinogens, and initiating specific signalling pathways [4]. Polyphenols are natural compounds widely found in plant-based foods, including fruits, vegetables, nuts, seeds, and flowers. These polyphenols play a significant role in antioxidant as well as anticarcinogenic activity and have been shown to possess substantial protective effects against ultraviolet radiation, including the risk of skin cancers.

Red pomegranate peel is rich in antioxidant flavonoids, phenolic acids, tannins, anthocyanidins, ellagic acid, quercetin, gallic acid, catechins, and vitamin C, which are higher than in the pulp [5]. These antioxidant compounds act as reducers, free radical scavengers, and metal chelators [6,7]. According to

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Saparbekova et al., the highest total phenolics were 20.24% and flavonoids 3.92% in the extract prepared by ethyl acetate extraction. It was also reported that the hydrolyzed tannin content was 21.25 mg per catechin equivalent/g pomegranate peel sample [8]. IC_{50} of ethanol extract of pomegranate peel done by the DPPH method showed a value of 9.58 µg/mL, indicating strong antioxidant activity [9]. The thick red pomegranate peel extracted with 96% ethanol gives an SPF value with a concentration of 0.011% of 16.09, which is included in the ultra-protection category [10].

In this study, red pomegranate peel (*Punica granatum* L.) was utilized due to its protective benefits against skin damage caused by ultraviolet radiation [11]. Compounds that are effective against ultraviolet radiation include flavonoids and tannins, which are polar substances. Polar compounds from red pomegranate peel were extracted using the maceration method with a 70% ethanol solvent. Research conducted by Sintha (2008) indicated that 70% ethanol was chosen because it effectively attracts more tannins than other concentrations, likely yielding a greater quantity of polar compounds from red pomegranate peel, especially flavonoids and tannins [12]. The choice of maceration as an extraction method is based on its ability to protect thermolabile compounds, the simplicity of the required tools, and its overall efficiency without the need for heating.

Some sunscreens still incorporate active substances derived from natural ingredients. Consequently, the researcher plans to develop a sunscreen using active compounds from red pomegranate peel extract. This study aimed to determine the sunscreen and antioxidant activity of red pomegranate peel extract, the formation of its stable sunscreen lotion, and its SPF and antioxidant activity. O/W emulsions, which possess a lighter texture, are non-greasy, simple to prepare, and easy to apply to the skin, providing a moisturizing effect along with a cooling sensation. These dispersed emulsion systems facilitate the incorporation of photoprotective ingredients in one or both phases. The benefit of emulsions is that they quickly absorb into the skin, leaving a thin photoprotective film.

2. MATERIALS AND METHODS

2.1. Materials

The material used was pomegranate peel obtained from the Research Centre for Spice and Medicinal Plants (BALITTRO) in Bogor Regency, West Java. Plants were determined at the Department of Biology Laboratory "Herbarium Depokensis (DEP)" Biota Collection Room at the University of Indonesia, with No.985/UN2.F3.11/PDP.02.00/2022. The mixture contains 70% ethanol, triethanolamine (TEA), stearic acid, cetyl alcohol, glycerine, methylparaben, propylparaben, Whatmann filter paper, and purified water.

The equipment used was analytical balance (Sartorius, Germany), oven, viscometer Brookfield LV, R-205 rotary evaporator (BUCHI, Indonesia), IKA RW-20 homogenizer stirrer, water bath, object glass, UV-Vis spectrophotometer (Shimadzu UV-1900), micropipette (Fraser 100-1000µL), and pH meter (Hanna Instruments).

2.2. Procedure

2.2.1. Preparation of pomegranate peel extraction

Pomegranate peel extract was prepared by maceration method using 70% ethanol solvent. The obtained simplistic powder was put into a vessel and then extracted by maceration using 70% ethanol solvent in a ratio of 1:10 for 3 x 24 hours. The first 6 hours were occasionally stirred, and the next was allowed to stand while occasionally stirring. After that, the material that has been macerated is filtered until the secondary metabolite compounds are completely extracted. The 70% ethanol extract obtained was then concentrated using a rotary evaporator. The extract obtained after the concentration process then evaporated to obtain a thick extract [13].

2.2.2. Antioxidant activity testing of pomegranate peel extract

A total of 20 mg of extract was dissolved in 20 mL of 70% ethanol pa and made in concentrations of 5, 8, 11, 14, and 17 µg/mL, each as much as 10 mL. Each concentration was taken as much as 3 mL and added 1 mL of FRAP reagent. Homogenized and incubated at room temperature during the operating time obtained. The absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength obtained, carried out three times (triple) at each concentration until the IC_{50} value was obtained [14].

2.2.3. Determination of SPF value of 70% ethanol condensed extract of pomegranate peel.

A total of ± 100 mg of a 70% ethanol extract of red pomegranate peel was weighed carefully. It was then put into a 100 mL volumetric flask and diluted with 70% ethanol pa. The extract was then made in concentrations of 50, 100, 200, 400, and 800 µg/mL. To measure the SPF (Sun Protecting Factor) value, the extract was measured by a UV-Vis Spectrophotometer every 5 nm in the wavelength range of 290-320 [15].

$$SPF = CF \times \sum_{320}^{290} \times EE \times I \times Absorbance$$

CF: Correction factor; EE: Receive effect spectrum; I: Solar intensity spectrum

2.2.4. Formulation of sunscreen lotion preparation

The lotion base was prepared by heating the oil phase (stearic acid and cetyl alcohol) and the water phase (purified water, glycerine, and triethanolamine) separately in a water bath at 70-75˚C. Methylparaben was dissolved in a portion of glycerine and then incorporated into the aqueous phase. The oil phase that has been melted is mixed into the water phase slowly, then homogenized with a digital stirrer with rpm 150 for 15 minutes, done until the lotion base is formed. Red pomegranate peel extract was dissolved with enough glycerine and then put into the lotion base. Then, it is stirred until mixed and then homogenized to form lotion [16}.

Table 1. Formulation of pomegranate peel extract lotion

Ingredients	Formula (%)		
	F I	F II	F III
Red pomegranate peel extract	0.06	0.12	0.24
Triethanolamine (TEA)	2	2	2
Stearic acid	5	5	5
Cetyl alcohol	1.5	1.5	1.5
Glycerin	10	10	10
Methylparaben	0.18	0.18	0.18
Propylparaben	0.02	0.02	0.02
Purified water	ad 100	ad 100	ad 100

The lotion preparation was evaluated, including organoleptic, homogeneity, viscosity and flow properties, lotion type, pH, spreadability, sunscreen activity, and antioxidant activity.

2.2.5 Stability testing

The physical stability test was carried out using the freeze and thaw method by storing at a cold temperature of 4˚C for 24 hours, then removing and placing at 40˚C for 24 hours; this process was counted as one cycle. The stability test was carried out up to 6 cycles, and the organoleptic changes, homogeneity, viscosity and flow properties, pH, and spreadability were observed at the sixth cycle [17].

3. RESULTS AND DISCUSSION

3.1 Preparation of pomegranate peel extract

Pomegranate peel extract was prepared by maceration for 3x24 hours with a 70% ethanol solvent. The filtrate was concentrated with a rotary evaporator at 40˚C with 175 mBar pressure until a thick extract was obtained.

The DER-native result is 8.87, and the yield is 11.268, which means that it is known that each gram of thick extract of red pomegranate peel requires 8.87 grams of dry powder. The yield is the ratio between the extract obtained and the initial simple. The higher yield value produced indicates the value of the extract produced. This study's results were less than the requirements in the Herbal Pharmacopoeia Second Edition 2017, which is less than 19.9%. This can be caused by the time of the maceration process, which is not long enough, or the yield value can be increased by maceration.

3.2 Phytochemical screening

Table 2. Phytochemical Screening Result of Red Pomegranate Peel Extract

Screening	Reagen	Results
Alkaloids	Dragendroff	+
Saponins	H_2O + HCl	+
Tannins	$FeCl_3$	+
Flavonoids	Mg + HCl	+
Triterpenoids	CH_3COOH anhidrate + H_2SO_4	+
Steroids	CH_3COOH anhidrate + H_2SO_4	-

Note : + = positive, - = Negative

Phytochemical screening was conducted to determine secondary metabolite compounds. Based on the screening, the pomegranate peel extract tested positive for all of the secondary metabolites except steroids. The screening carried out in this study is in accordance with the results obtained from previous research [18]. Flavonoids contain a free form (aglycone) or are similar to glycosides, such as polyhydroxy aglycones, which are semi-polar, and polymethoxy aglycones, which are non-polar. Flavonoids have the potential to prevent exposure to UV radiation on the skin due to the presence of a chromophore group that can absorb UV A and UV B rays.

3.3 Antioxidant activity of pomegranate peel extracts

FRAP method is used to measure the ability of antioxidants to reduce Fe^{3+} to Fe^{2+}, as seen from the absorbance value. The FRAP reagent consists of a mixture of TPTZ, $FeCl_3$, and acetate diaper, which will form a Fe^{3+}-TPTZ complex compound that is stable at acidic pH, which is likened to free radicals in the body that can damage body cells and will then be reduced by antioxidant compounds. The principle of this method is a reduction reaction in an acidic atmosphere characterized by a color change from yellow to blue, which indicates an increase in reducing power. The amount of reducing power indicates the ability of electron donors so that it is more stable.

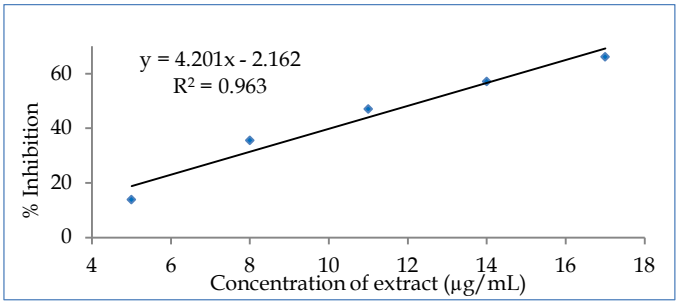

Figure 1. IC_{50} of red pomegranate peel extract

In the antioxidant activity test of vitamin C as a standard comparison, the average IC_{50} result was 4.67 ± 0.01 µg/mL. In the antioxidant activity test of red pomegranate peel, the average IC_{50} result was 12.42 ± 0.01 µg/mL. This result is greater than the previous study, where the IC_{50} result was 9.58 µg/mL using the DPPH method.

3.4 Sunscreen activity of pomegranate peel extract

The determination of SPF value was done using a UV-Vis spectrophotometer at a wavelength of 290-320 nm. SPF measurements were taken at 50, 100, 200, 400, and 800 µg/mL concentrations of pomegranate peel extract. The results of determining the sun protection factor value can be seen in Table 3.

Table 3. SPF value of pomegranate peel extracts

Concentration (µg/mL)	SPF value
50	2.97±0.01
100	5.79±0.01
200	10.67±0.08
400	15.91±0.01
800	17.31±0.04

Based on the data obtained, the greater the concentration of extract used, the greater the SPF value obtained. This is because the compounds in higher concentrations have more compound content, whereas red pomegranate peel contains many tannins and flavonoids that can act as sunscreens [10].

3.5 Stability test of sunscreen lotion preparation

3.5.1 Organoleptic and homogeneity

The organoleptic evaluation of the pomegranate peel extract lotions assessed color, texture, and aroma over 28 days at 40° C. All three formulations maintained stable organoleptic characteristics throughout the study. Formulation F1 exhibited a whitish-brown color, a semi-solid texture, and odourless from day 0 to day 28. Formulation F2 showed a consistent light brown color, semi-solid texture, and odourless throughout the observation period. Similarly, formulation F3 retained a brown color, semi-solid texture, and odourless across all time points. No significant changes in the organoleptic properties were observed during the storage period. In the stability test, the homogeneity of the preparation in each formula did not show any changes.

3.5.2 Viscosity and Rheology

Table 4. Viscosity of pomegranate peel extract lotions

Formula	Viscosity (cPs)	
	Before *Cycling test*	After *Cycling test*
F I	12,800±300.00	12,533±230.94
F II	12,133±230.94	12,000±300.00
F III	10,533±230.94	10,266±311.00

The evaluation of the lotion preparation's viscosity stability during the cycling test showed a decrease in viscosity caused by temperature. An increase in temperature makes the polymer chain easier to break down, causing a decrease in viscosity during measurement. However, this decrease was analyzed using the statistical method of one-way analysis of variance, which showed a Sig>0.05 value of 0.343. This indicates that there was no significant difference in the lotion's viscosity during the cycling test.

Lotion preparations have pseudoplastic thixotropic flow properties; the higher the shear rate, the lower the viscosity.

3.5.3 Type of lotion test

Evaluation of the lotion type is done to see whether or not there is a phase change in the lotion preparation during the stability testing time. Examination of the lotion type was done using the methylene blue and Sudan III dye method, then observed using a microscope. Based on the evaluation of the lotion type in each formula, it was found that the lotion type, both before and after the cycling test, was oil in water (O/W). The O/W type will show a white globule color and a blue outer phase when methylene blue is applied; if Sudan III is applied, the globule will be red, and the outer phase will be white.

3.5.4 pH Lotions

The pH of the lotion preparation was evaluated to determine whether it changed during the stability testing time. The evaluation results are shown in Figure 2.

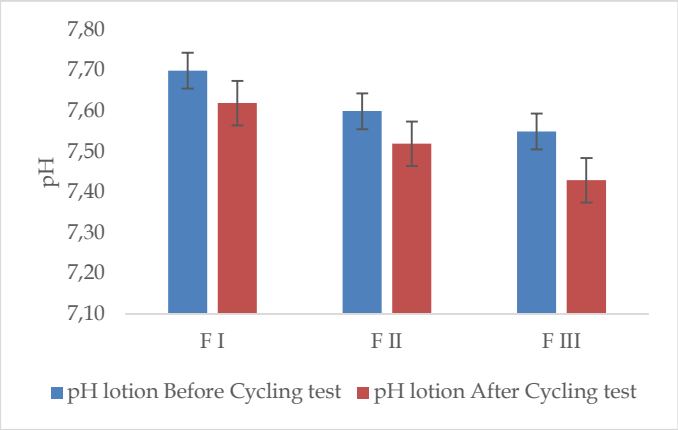

Figure 2. pH Lotions

Based on the statistical analysis of one-way variance, the normality test results were obtained with a Sig. value of 0.189, and the homogeneity test obtained a Sig. value of 0.628, which indicates that the data is regular and homogeneous. In the ANOVA test, the Sig value obtained was 0.088 (>0.05), which indicates there is no significant change in pH value. The pH value obtained after the cycling test is still within the range of pH ranges that are safe for humans, namely 4 - 8.

3.5.5 Spreadability

Table 5. Spreadability of pomegranate peel extract lotions

Formula	Spreadability (cm)	
	Before *Cycling test*	After *Cycling test*
F I	6.31±0.05	6.32±0.02
F II	6.39±0.07	6.42±0.02
F III	6.42±0.09	6.50±0.04

The results show that the spreadability after the cycling test is higher than before the cycling test. This is related to the viscosity value; a decreasing viscosity will give a higher spreadability. This is also due to the effect of temperature, which can reduce viscosity. The results of statistical analysis using one-way ANOVA showed a Sig. value of 0.577 (>0.05), indicating that there were no significant changes in the spreadability of lotion preparations during stability testing.

3.6 Antioxidant and sun protection factor activity of sunscreen lotion preparation

Determination of antioxidant activity was carried out on the three formulas using the FRAP method. This test was conducted triple at each concentration with a wavelength of 594.4 nm and an incubation time of 35 minutes. The determination of SPF value was done on all three formulas. This test determines the SPF category from minimal to ultra. The test was conducted using a UV-Vis spectrophotometer with a wavelength of 280-320 nm. The absorbance obtained was then calculated using the Mansur method, and the SPF obtained from the three formulas ranged from 13.11 to 24.78.

Table 5. Antioxidant and sun protection factor activity of sunscreen lotion preparation

Lotion	Antioxidant Activity (IC_{50}) (µg/mL) of Lotion	Sun Protection Factor (SPF) (µg/mL) of Lotion
F I	98.73 ± 0.11	13.11 ± 0.02
F II	68.89 ± 0.11	20.78 ± 0.02
F III	48.30 ± 0.44	24.78 ± 0.04

The antioxidant activity test with the FRAP method revealed that FI and FII red pomegranate peel extract lotion can be classified as strong antioxidants, and FIII red pomegranate peel extract lotion can be classified as a very strong antioxidant. However, the preparation's antioxidant activity is weaker than that of the extract, which could be due to the reaction between the extract and the ingredients used in the formula (19).

Based on the SPF test results, the highest SPF value was obtained in F III, with a value of 24.78 ± 0.04, which is included in the ultra protection category. In formula I, a lower SPF was obtained, 13.11 ± 0.02, due to the use of a lower concentration of red pomegranate peel extract. This shows that the amount of extract used in the preparation is directly proportional to the SPF value given.

4. CONCLUSION

The higher the concentration used, the higher the antioxidant activity and SPF values in the extract and lotion preparation. The lotion preparation of red pomegranate peel extract meets the requirements of physical and chemical quality parameters.

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