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Protective Factor Evaluation of Purslane (*Portulaca grandiflora*) Magenta Flower Variety Herbs Extract Cream Formula

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Abstract

Background*: Indonesia is an equatorial country that is rich in sunlight all year. UV light is divided into three wavelength groups: UV-A (320-400nm), UV-B (280-320nm), and UV-C (100-290nm). Intracell chromophores in skin cell membranes such as riboflavin, porphyrin, nicotinamide, and enzymes will absorb the UV-A light. The UV-B light penetrates the dermis layer and causes DNA structure changes, which lead to wrinkles and a rising risk of skin cancer. Premature skin aging and skin cancer can be prevented with sunscreen preparation containing compounds that can protect the skin from UV radiation. Flavonoid is one of the purslane (Portulaca grandiflora) active metabolites that have the potency to be developed as sunscreen.* ***Objective****: This research aimed to determine the ability of purslane (Portulaca grandiflora) magenta flower variety herbs extract cream as a sunscreen as indicated by the %Te, %Tp, and Sun Protective Factor value.* ***Methods****: This research was an experimental study with various purslane magenta flower variety herbs extract cream formulas that were tested for their %Te, %Tp, and SPF value with a UV-Vis spectrophotometer.* ***Results****: The sunscreen cream preparation with 2.5% of purslane (Portulaca grandiflora) magenta flower variety herb extract had %Te, %Tp, and SPF values of 25.86 ± 2.41%, 36.05 ± 2.82%, and 3.97 ± 0.35 respectively. At the same time, preparations with 5% concentration of extract had %Te, %Tp, and SPF values of 8.23 ± 0.86%, 16.65 ± 0.92%, and 8.03 ± 0.38, respectively.* ***Conclusion****: The sunscreen activity of all extract concentration creams was significantly different compared to the negative control (cream base) in all parameters. Flavonoids are the compounds responsible for the sunscreen activity of purslane extract.*

Keywords*: sun protective factor, purslane, Portulaca grandiflora, magenta, cream*

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INTRODUCTION

One of the energy sources needed by living creatures is sunlight because of its involvement in every stage of the living process. For example, it is vitamin D generation. Otherwise, sunlight overexposure also has a negative effect.

Sunlight radiation consists of infrared light (wavelength > 760 nm), visible light (400-760 nm), and UV (ultraviolet) light consisting of UV-A (320-400 nm), UV-B (290-320 nm), as well as UV-C (200-290 nm) (Limpiangkanan & Limpiangkanan, 2010). UV-A and UV-B rays are radiation from sunlight that reaches the earth's surfaces and has an impact on the skin (Wang *et al*., 2008). The UV-A light that reaches the skin surface will be absorbed by intracellular chromophores in cell membranes such as riboflavin, porphyrin, nicotinamide, and enzymes. This causes oxidative stress, whereas Reactive Oxygen Species (ROS) production overwhelms the natural skin antioxidant mechanism, leading to a decrease in collagen production and wrinkle appearance (Gragnani *et al*., 2014). The UV-B light that reaches the skin will penetrate the dermis layer and cause DNA structure changes, which lead to wrinkles and rising skin cancer risk (Matsuda *et al*., 2013).

Indonesia is a country in the equatorial area with an abundance of sunlight, which leads to high premature skin aging and skin cancer risk. Premature skin aging and skin cancer can be prevented with sunscreen preparation containing compounds that can protect the skin from UV radiation. The ability to protect skin from sunlight exposure is shown by erythema transmission percentage (%Te), pigmentation transmission percentage (%Tp), and Sun Protective Factor (SPF). Commonly, plants rich in flavonoids have a high SPF value because of their chromophore chemical structure capable of absorbing UV light radiation energy (Saewan & Jimtaisong, 2013). Besides that, flavonoids also have antioxidant activity to prevent oxidative stress so that premature skin aging and cancer risk can be prevented (Chen *et al*., 2012).

Purslane is a plant that has been studied for its health benefits because of its various secondary metabolites. Flavonoid is one of the purslane active metabolites that have the potency to develop as sunscreen. Purslane *Portulaca grandiflora* magenta flower variety has higher levels of flavonoids compared to other varieties, making it suitable to be developed as a sunscreen (Budiawan *et al*., 2023).

Cream is a topical preparation with a semi-solid emulsion system. This preparation is easy to use and gives a comfortable feeling in its application. In addition, with the right cream consistency, the extracts in sunscreen can stick long enough and provide sufficient time for the flavonoid compounds to be absorbed by the skin, providing a maximum sun protection effect.

MATERIALS AND METHODS

Materials

Fresh purslane (*Portulaca grandiflora*) magenta flower variety herb was obtained from Madiun regency, East Java, Indonesia. Ethanol 96% was used the extraction process. Ceto stearyl alcohol, stearic acid, cetyl alcohol, methylparaben, propylparaben, tween 80, and aqua destilata were used to make cream base preparation. Magnesium powder, HCl 2N, C_2H_5OH, NH_3, $CHCl_3$, Dragendorff, Mayer, Bouchardat, $FeCl_3$, H_2SO_4 concentrate, and methanol pro analysis were used as reagents.

Tools

Tools used in this experiment were Spectrophotometer UV-Vis (JaSCO V-730), rotary evaporator, water bath, analytical balance, and glassware (pyrex).

Method

Extract preparation

Two hundred grams of dried purslane (*Portulaca grandiflora*) magenta flower variety herb was extracted with maceration method using 500 ml ethanol 96% for five days. The dregs were re-macerated using the same solvent for another five days. The first and second filtrates were then mixed and thickened with a rotary evaporator at 40°C until one-third of the volume was remaining. The thickened filtrates were then dried in the oven at 50°C for 24 hours until thick extract was obtained.

Phytochemical screening

1. Flavonoids Identification

Half a gram of purslane (*Portulaca grandiflora*) magenta flower variety extract was dissolved into water and transferred into a test tube. Magnesium metal and five drops of HCl 2N were added into the tube, and then the mixture was heated for 5-10 minutes. After filtration, the filtrate waited until it cooled down and was added with amil alcohol and then shaken hard. The reaction was positive if a red color formed in the amil alcohol layer (Harbone, 1987; Hanani, 2017).

2. Alkaloid Identification

Half a gram of purslane (*Portulaca grandiflora*) magenta flower variety extract was basified with 1 mL of ammonia, then chloroform was added and crushed vigorously. The chloroform liquid was filtered, the

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filtrate was placed in a test tube, 2 N HCl was added, the mixture was shaken, and then left to separate. In a separate test tube: Filtrate 1: As much as one drop of Dragendorff reagent solution is dropped into the filtrate, the presence of alkaloids is indicated by the formation of precipitate or turbidity that is coloured brown. Filtrate 2: As much as one drop of Mayer's reagent solution is dripped into the filtrate, the presence of alkaloids is indicated by the formation of a white precipitate or turbidity. Filtrate 3: As a blank or negative control (Harbone, 1987; Hanani, 2017).

3. Saponin Identification

Half a gram of purslane (*Portulaca grandiflora*) magenta flower variety extract was put in a test tube, added hot water and cooled, then shaken for 10 seconds, a stable foam will form in less than 10 minutes, 1-10 cm high, and with the addition of 1 drop of HCl The 2N foam was persistence which indicated the presence of saponins (Harbone, 1987; Hanani, 2017).

4. Tannin Identification

Half a gram of purslane (*Portulaca grandiflora*) magenta flower variety extract was put into a test tube and reacted with FeCl3 1% solution. The extract contains tannins if a green-black or dark-blue color was formed (Harbone, 1987; Hanani, 2017).

5. Terpenoid Identification

Half a gram of purslane (*Portulaca grandiflora*) magenta flower variety extract was put into a test tube and added with chloroform and H2SO4 concentrated. The extract would contain terpenoids if a brown colour was formed (Harbone, 1987; Hanani, 2017).

Cream preparation

The preparation of this research cream was based on the Arisca formula (2018) with minor modifications.

Each material was measured and the oil phase (ceto stearyl alcohol, cetyl alcohol, stearic acid, and propylparaben) was mixed. The water phase (tween 80 and methylparaben) was mixed. Both phases were heated at 80°C with a water bath until dissolved. Cream preparation was done by adding the hot water phase to the hot oil phase and the mixture stirred at 12500 rpm until a cold cream base formed. The purslane (*Portulaca grandiflora*) magenta flower variety herb extract was added to the cream base and stirred at 20 rpm until a homogeneous cream was formed.

Sunscreen activity test

The sunscreen activity test was carried out by determining the SPF value in vitro using the UV-Vis spectrophotometry method. The purslane magenta flower variety herbs extract cream was dissolved into methanol pro analysis to obtain a 1000 ppm concentration of test solution. After that, the test solution transmission was read at 292.5–372.5 nm wavelength (every 5 nm interval). The amount of erythema flux that was transmitted by the sunscreen agent (Ee) is calculated by the formula: Ee = ΣT.Fe while the pigmentation flux is calculated by the formula: Ep = ΣT.Fe. %Te and %Tp value calculated by formula %Te = Ee/ΣEe and %Tp = Ep/Σep, where T= Transmition value, Fe= a constant of flux erythema, Fp= a constant of flux pigmentation (Cumpelick, 1927). SPF value measurement was done by reading the test solution absorbance at 290-320 nm wavelength with 5 nm interval (Mansur *et al*., 1986; Mishra *et al*., 2012) (Table 2). SPF was obtained using the formula:

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

Where: CF: correction factor, EE: erythema effect spectrum, I: light intensity spectrum, Abs: sample absorbance.

Table 2. Value of EE x I

λ (nm)	EE x I
290	0.015
295	0.0817
300	0.2874
305	0.03278
310	0.1864
315	0.0839
320	0.018

Table 1. Purslane magenta flower variety herb extract cream formula

Ingredients	Negative Control Formula (%)	2,5% Extract Formula (%)	5% Extract Formula (%)
Ceto stearyl alcohol	7	7	7
Stearic acid	7	7	7
Cetyl alcohol	6	6	6
Nipagin	0.15	0.15	0.15
Nipasol	0.05	0.05	0.05
Purslane extract	-	2.5	5
Tween 80	0.5	0.5	0.5
Aqua destilata ad	100	100	100

P-ISSN: 2406-9388
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Table 5. %Te value of cream preparation

Replication	Negative Control (Cream Base)	Value of %Te (%) 2.5% Extract	5% Extract
I	97,56	27,36	8,97
II	90,28	23,08	8,44
III	93,06	27,14	7,29
Mean	93,64 ± 3,67	25,86 ± 2,41	8,23 ± 0,86

Data analysis

The %Te, %Tp, and SPF values were analyzed statistically using a one-way ANOVA analytical method with $\alpha = 0.05$ and followed by a post hoc test.

RESULTS AND DISCUSSION

Fresh purslane (*Portulaca grandiflora*) magenta flower variety herb dried and extracted using maceration method until 5.51 gram thick extract was obtained with 10.64% yield (Table 3).

The next step was qualitative phytochemical identification of the obtained extract. The phytochemical screening result is explained in Table 4.

Table 3. Purslane (*P. grandiflora*) magenta flower variety herb extract yield

Simplicia	Powder weight (g)	Extract weight (g)	Yield (%)
Purslane magenta flower variety herb	51.78	5.51	10.64

Table 4. Purslane (*P. grandiflora*) magenta flower variety herb extract phytochemical screening

Test	Reagent	Result
Flavonoid	Mg powder + HCl 2N + C_2H_5OH	+
Alkaloid	NH_3+$CHCl_3$+HCl 2N+ Dragendorff/ Mayer/Bouchardat	+
Saponin	Foam test	+
Tannin	$FeCl_3$	+
Terpenoid	H_2SO_4 concentrate + $CHCl_3$	+

The purslane extract was used as an active ingredient in cream preparation with 2.5% and 5% concentration variations. Cream preparation was chosen because it has benefits such as being easy to use, comfortable, and easy to wash with water. The cream also has various functions as a drug carrier, skin emollient, and protection from different interferences, including sunlight.

Sunscreen preparation could contain active ingredients in an inorganic compound (reflect UV radiation) and an organic compound (absorb UV radiation). The purpose of sunscreen application is not only to protect the skin from UV rays exposure, which can trigger negative effects, but also it's expected to inhibit ROS formation, which triggers gene mutations, premature aging, and carcinogenic effects in the long term. Therefore, active antioxidant compounds that can help increase the physical activity of sunscreen are needed in sunscreen preparations. Purslane (*Portulaca grandiflora*) magenta flower variety herb extract contains various secondary metabolites and has antioxidant activity (Addor *et al*., 2022).

The cream sunscreen activity is determined from the percentage of erythema transmission (% Te), the percentage of penetration transmission (% Tp), and the Sun Protective Factor (SPF) value (Table 5).

The percent erythema transmission value (%Te) describes the amount of UV rays exposure from the sun that hits the skin after using sunscreen, which causes erythema (redness) on the skin (Chen *et al*., 2012). The lower the % Te value, the better the sunscreen protection to prevent erythema. Based on Table 5, the highest %.

Te was in the negative control (preparation basis), which was 93.64 ± 3.67%, followed by preparations containing 2.5% extract, which was 25.86 ± 2.41%, and the lowest was the preparation with 5% extract, which was 8.23 ± 0.86%. Based on the statistical test results, it showed that there was a significant difference in the % Te value both in the negative control preparations with 2.5% and 5% extract. This indicates that the extract concentration determines the erythema transmission value of sunscreen preparations.

The percentage value of pigmentation transmission (% Tp) describes the amount of exposure to UV rays from the sun that hits the skin after using sunscreen, which causes pigmentation of the skin. As with % Te, the lower % Tp value indicates better protection of sunscreen against pigmentation on the skin.

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Table 6. %Tp value of cream preparation

Replication	Negative Control (Cream Base)	Value of %Tp (%) 2.5% Ekstrak	5% Extract
I	98.29	36.69	17.46
II	91.49	32.96	16.83
III	95.66	38.49	15.65
Mean	95.15 ± 3.43	36.05 ± 2.82	16.65 ± 0.92

Table 7. SPF value of cream preparation

Replication	Negative Control (Cream Base)	Value of SPF 2.5% Ekstrak	5% Extract
I	0.18	3.84	7.83
II	0.16	3.71	8.47
III	0.19	4.37	7.79
Mean	0.18 ± 0.02	3.97 ± 0.35	8.03 ± 0.38

Based on Table 6, shows that the highest % Tp was in the negative control (preparation basis), which was 95.15 ± 3.43, followed by preparations containing 2.5% extract, which was 36.05 ± 2.82, and the lowest is a preparation with 5% extract, namely 16.65 ± 0.92. Based on the results of statistical tests, there was a significant difference in % Tp value in the negative control, preparations with 2.5%, and 5% extract with a p>0.05. This shows that the extract concentration in preparation determines the pigmentation transmission value of the sunscreen preparation.

The SPF value states how many times the skin's natural resistance is multiplied so that it is safe in the sun without experiencing sunburn16. Based on the test result, the SPF value also shows an increasing trend with increasing extract concentration in the preparation. Based on Table 7, it can be seen that the lowest SPF value was in the negative control (preparation base), which was 0.18 ± 0.02, followed by preparations containing 2.5% extract, which was 3.97 ± 0.35, and those having the highest was the preparation with 5% extract, namely 8.03 ± 0.38. Based on the results of statistical tests, it showed that there was a significant difference in the SPF value in the negative control, preparations with extracts of 2.5%, and 5% with p>0.05. This shows that the extract concentration determines the SPF value of the sunscreen preparation.

Based on these results, a cream preparation containing 5% purslane herb extract had a higher % Te and % Tp value compared to preparations containing 2.5% purslane herb extract. At the % Tp value, both preparations showed the maximum protection, which was in the sunblock category, while based on % Te, the protection was still in the tanning and suntan categories (Kasitowati *et al.*, 2021). To obtain better protection based on the % Te value, a higher extract content is needed in the preparation.

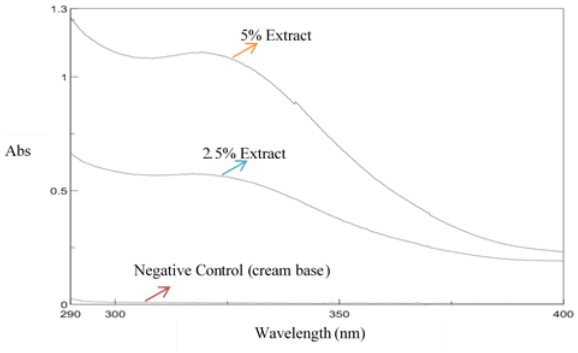

Figure 1. Absorbance spectra at 290-400 nm wavelength of cream preparation

The potential for sunscreen activity can be expressed through the value of the Sun Protective Factor (SPF). The SPF value is determined from the absorption results of the preparation at UV wavelengths between 290 – 400 nm (Figure 1). Based on the research results, it was obtained that the SPF values of cream preparations containing 2.5% and 5% purslane herb extract were 3.97 ± 0.35 and 8.03 ± 0.38, respectively. Both of these SPF values are less than 12, which is in the minimal protection category. This SPF value is not optimal for providing protection because, with an SPF value of less than 15, protection is only given for 1.5 hours (Buso *et al.*, 2017).

Statistical tests were carried out to determine whether there were differences in sunscreen activity in each preparation. Based on statistical tests using one-way ANOVA, a significance value of <0.05 was obtained for both the %Te, %Tp, and SPF values. This means that the purslane herb extract concentration in preparation determines the sunscreen activity. The

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higher the extract concentration in the preparation, the better the sunscreen protection will be.

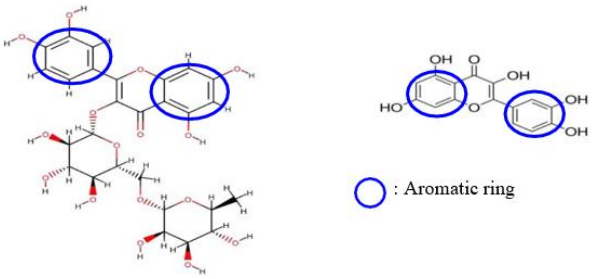

Figure 2. Structure of flavonoid A: rutin, B: quercetin (Ganeshpurkar *et al*., 2017; El-Saber Batiha *et al*., 2020)

Flavonoids are compounds contained in purslane herb extracts that are thought to have sunscreen activity (Cahyani *et al*., 2022). Flavonoids can absorb energy at UV light wavelengths because they have covalent bonds in their structure. Flavonoids can absorb energy at a wavelength of 240-290 nm, and flavonoids that have conjugated covalent bonds can absorb at a wavelength of up to 550 nm (He *et al*., 2021). The Magenta flower variety purslane herb contains the and most significant amount of flavonoids compared to other variants. Therefore, this study used extracts from the purslane herb with the magenta flower variant. Flavonoids contained in purslane (*Portulaca grandiflora*) herb are rutin, quercetin, and isoquercetin compounds (Husein *et al*., 2021). Flavonoids have at least two aromatic rings with a basic carbon skeleton consisting of 15 C atoms forming C6-C3-C6 (Figure 2) (Julianto, 2019).

CONCLUSION

The sunscreen cream preparation with 2.5% of purslane (*Portulaca grandiflora*) magenta flower variety herb extract had %Te, %Tp, and SPF values of 25.86 ± 2.41%, 36.05 ± 2.82%, and 3.97 ± 0.35 respectively. In comparison, preparations with 5% concentration of extract had %Te, %Tp, and SPF values of 8.23 ± 0.86%, 16.65 ± 0.92%, and 8.03 ± 0.38, respectively. The sunscreen activity of all extract concentration creams was significantly different compared to the negative control (cream base) in all parameters. Flavonoids are the compounds responsible for the sunscreen activity of purslane (*Portulaca grandiflora*) magenta flower variety herb extract.

AUTHOR CONTRIBUTIONS

Conceptualization, B. C. K., E. D. C., A. B.; Methodology, B. C. K., E. D. C.; Software, E. D. C., A. B.; Validation, B. C. K., E. D. C.; Formal Analysis, B. C. K., A. B.; Investigation, E. D. C., A. B.; Resources, B. C. K.; Data Curation, B. C. K.; Writing - Original Draft, B. C. K., E. D. C., A. B.; Writing - Review & Editing, A. B.; Visualization, E. D. C.; Supervision, B. C. K.; Project Administration, B. C. K.; Funding Acquisition, B. C. K.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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