

Antioxidant Activity and Sun Protection Factor Evaluation for Cream Formulation of Purified Roasted Corn Silk Extracts (*Zea mays* L. *Saccharata*)

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Antioxidant activity and sun protection factor evaluation for cream formulation of purified roasted corn silk (*Zea Mays* L. *Saccharata*) extracts

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ABSTRACT

Free radicals might cause harmful effects on the skin, such as skin irritation, change of skin color, and worst effect. Phenolics play a role as effective sunscreen and antioxidants to prevent UV radiation effects. Corn silk contains flavonoids, which are antioxidants that are potential as sunscreen. Phenolic compounds can be identified by phytochemical screening using the tube method. The study aimed to determine the antioxidant activity of purified extract of roasted corn silks (*Zea Mays* L. *saccharata*) and the SPF level of the cream preparation. The study was conducted by collecting samples of roasted corn silks waste to make dried simplicial, then extracting it with 70% ethanol as solvent, evaporated till condensed extract is gotten. The obtained extract was then purified with n-hexane solvent. The antioxidant activity test was conducted by using the DPPH method. The cream of the purified extract was made in three formulations: 1%, 5%, and 10% concentration. Its SPF levels were then evaluated. The results showed the purified corn silks extract had an IC_{50} value of 256.66 $\mu\text{g/mL}$ better compared to corn silks ethanol extract having an IC_{50} value of 356.17 $\mu\text{g/mL}$ including the weak antioxidant category. Meanwhile, the SPF level test results for concentration of 1%, 5%, and 10% were 1.27 ; 2.41; and 5.94 respectively.

Keywords: corn silks, purified, antioxidants

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INTRODUCTION

The use of plants as cosmetic ingredients is an excellent opportunity for the community to develop natural ingredients potential. Skin health needs to be maintained so that it is not damaged by exposure to solar radiation. Ultraviolet (UV) rays of the sun can accelerate the aging process of the skin. This process is cumulative. Chronic reactions from sun exposure to ultraviolet rays over the years can cause structural skin disorders, especially premature aging of the skin, as well as skin cancer (Walker et al., 2003). The damage caused can be seen clinically, histopathologically, and functionally (Berneburg et al., 2000).

Natural compounds that are reported to act as anti-aging are compounds with aromatic rings such as phenolic groups, especially flavonoids (Maheshwar et al., 2010). One of the plants reported to contain phenolics is corn silk. Corn silk is rich in phenolics, especially flavonoids (Liu et al., 2011). Phenolics are compounds that have one or more hydroxyl groups that are directly attached to aromatic rings. Phenol or carboxylic acid is the structure that underlies all the groups of these compounds in which the aromatic ring is benzene (Scalbert and Williamson, 2000). The test results for the antioxidant activity of the ethanol extract of corn hair had an IC_{50} value of $143.55 \mu\text{g/mL}$ (Nurhanan and Rosli, 2012). The SPF level of fractionation of the ethanol extract of corn silk was 9-25 (Laeliocattleya and Jati, 2014).

Cosmetic preparation preferred by the public, especially women, is cream. Cream preparation is used as a protector for the outer skin layer so that it is protected from sun exposure that can easily damage skin tissues. Other benefits of cream preparations are comfortable to wear, not sticky, and easy to wash by water (Rabima and Marshall, 2017).

So far, the waste of corn silks has not been fully utilized, especially the waste from roasted corn silks (*Zea Mays L. saccharata*) at the Taman Sekar Tajir Roundabout, Kediri. The purified extract of roasted corn silk obtained is formulated into antioxidant creams and sunscreens. The form of purification was chosen to obtain secondary metabolite compounds that are more specific from corn silk waste. The determination of antioxidant activity was carried out using the Diphenylhydrazylpicryl (DPPH) method and the determination of the SPF (Sun Protection Factor) level using the spectrophotometric method.

MATERIALS AND METHOD

Materials

The material used in this study is the waste silk of roasted corn (*Zea Mays L. Sacharata*) in the Taman Sekar Tajir roundabout, Kediri City.

Methods

Simplicia preparation

Samples were sorted, then washed, chopped, dried for three days by aerating them, keeping them away from direct sunlight, then milled (Laeliocattleya and Jati, 2014).

Extraction of corn silks

Corn silk extract was made by maceration: simplicia of corn silk were weighed for 100 g then extracted with 750 mL of 70% ethanol by maceration for five days protected from light, after five days of straining. The filtrate was collected, and the residue was added with 70% ethanol as much as 250 mL, then re-filtered. The filtrate from the maceration results was then evaporated in a rotary evaporator then concentrated in a water bath until a condensed extract was obtained (Nurhanan and Rosli, 2012).

Purified extract

The condensed extract of corn silk was purified by using n-hexane as a solvent. A total of 20 grams of condensed corn silk extract was dissolved using 70% ethanol in a ratio of 1:10, then was put in a separating funnel, shaken until become homogeneous. Then, n-hexane was added with a solvent ratio of 1:2, extracted to form 2 phase layers of ethanol and n-hexane, where this process was repeated until the n-hexane phase looks clear. The separated solution was concentrated in a rotary evaporator with a temperature of 60°C until purified extract was obtained (Zhang et al., 2005).

Phytochemical test of cornsilk purified extract

The purified extract of corn silk was tested for the content of secondary metabolites of flavonoids, alkaloids, tannins, and saponins qualitatively by the tube method. In this method, the identification of flavonoids was conducted using 0.1g of the sample plus 3 mL of 70% ethanol, shaken, filtered then added 2 drops of Mg and 2 drops of concentrated HCl. In this stage, the flavonoid was identified using 0.1g tannin in the sample was added with 5 mL of boiled aquadest, then filtered and added 1% FeCl₃. Identify 0.1g saponin sample plus 5 ml of heated aquadest. After that, it was filtered and shook to observe the formation of foam. The identified alkaloids in the 0.1g sample were added with the reagents of Mayer, dragendorff, and Wagner (Nariya et al., 2013).

Antioxidant activity test by DPPH method

A solution of 0.4mM DPPH was made by dissolving 15.8mg of DPPH powder in 100.0 mL methanol. The extract and the purified extract of corn silk were dissolved in methanol with 1 µg/mL concentration, and the series of dilution was held until 0.05 µg/mL, 0.2 µg/mL, 0.4 µg/mL, 0.6 µg/mL concentrations were obtained. As much as 1.0 mL of 0.4 mM DPPH was added into each volumetric flasks, and methanol was added until 10.0 mL volume was obtained, stirred till it becomes homogenous. The next step was measuring the absorbance of the sample at a wavelength of 500 – 524 nm. The obtained absorbency data are then substituted into the equation (1) and followed by linear regression.

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\% \quad (1)$$

The linear regression equation was calculated by entering the concentration series (ppm) as X and the percent inhibition (%) as Y so that the equation: $Y = bx + a$ was obtained. The X value was changed to 50 to obtain the IC₅₀ value. Thus, a sample concentration was obtained to reduce 50% of DPPH free radicals (Badarinath et al., 2010).

Purified corn silk extract cream production

All required materials were weighed. Materials needed for the formulation were separated into two groups: the oily phase and the watery phase. The oily phase group, which consists of stearate acid 5g, cetyl alcohol 3g, liquid paraffin 7g, olive oil 10g, and nipagin 0,0025g, was melted on the water heater at 70°C - 75°C. Meanwhile, the watery phase group, which consists of TEA 1g, glycerin 7g, and Nipazol (propylparaben) 0,015g, was dissolved in hot *aqua dest* (distilled water). The watery phase group was crushed in a hot mortar; then, the oily phase group was gradually added while stirred until it becomes homogenous, and the cream mass was obtained. The purified extract of corn silk formulation 1 as much 1g, as was added and gradually crushed until it became homogenous. The same way was conducted on formulations 2 and 3 (Ekowati and Ningsih, 2014). The formulation of the anti-aging cream made of purified corn silk extract can be seen in Table 1.

Table 1. Cream formulation of purified corn silk extract

Formula	Composition (Gram)		
	F1	F2	F3
Purified corn silk extract	1	5	10
Stearate acid	3	5	5
Cetyl alcohol	3	3	3
TEA	1	1	1
Liquid paraffin	7	7	7
Olive oil	10	10	10
Glycerin	7	7	7
Nipagin	0.025	0.025	0.025
Nipasol	0.015	0.015	0.015
Aquadest	65,96	61,96	52,96
Total weight	100	100	100

Physical quality test of corn silk purified extract cream

The physical quality test of purified corn silk extract cream included organoleptic, pH, homogeneity, spreadability, adhesion, and cream type. Organoleptic testing was carried out by observing the shape, color, and smell of the cream. The degree of acidity (pH) was determined using a pH meter calibrated in advance with standard buffer solutions of pH 4 and 7. The homogeneity test was conducted by taking enough cream smeared on the glass or object-glass, if there were no lumps then the cream was homogeneous. Testing the spreadability of the cream, weighed 0.5g of cream, placed between 2 layers of glass object that was given a load of 100g, measuring the diameter of the cream was carried out after approximately 1 minute. The adhesion test was weighed with 0.5g of cream placed between the two slides then given a load of 1 kg for 5 minutes. (Bernatoniene et al., 2011). The two objects were separated by pulling the slide on top with a weight of 80g, while the screwdriver below was held up by another load. The length of time was taken to separate the two objects was recorded as sticky time. Test the type of cream using conductivity, one end of the tool was put in the cream then the tool was plugged into the socket. If the lamp in the tool was on, it indicates that the cream was a type of oil in water (O / W) (Damogalad et al., 2013).

Purified corn silk extract cream determination of SPF level

The SPF level was determined by using UV spectrophotometry. Cream of purified corn silk extract was diluted with 70% ethanol in a 10 mL flask to obtain 1000 µg/mL concentration, then diluted to a concentration of 100 µg/mL. First, the UV-Vis spectrophotometer is calibrated using 1 mL of ethanol. The diluted solution was measured for its absorbance at λ 290-320 nm with a measurement interval of 5 nm. The absorbance and the SPF value were then calculated (Dutra et al., 2004). The test was conducted for three times to get an accurate value and calculated using equation 2.

$$Value\ SPF = CF \times \sum_{290}^{320} Abs \times EE \times I \quad (2)$$

Description: EE = Erythral effect spectrum, I = light spectrum intensity, Abs = screen product absorption, CF = correlation factor (Molyneux, 2004). The value of EE x I was a constant, obtained from the wavelength 290 - 320 nm and any 5 nm difference had been determined as shown in Table 2.

Table 2. EE and I constants for the calculation of SPF

Wavelength (nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

SPF values range from 0 - 100, and a sunscreen considered good is above 15 (Nguyen and Rigel, 2005).

Data Analysis

The data analysis of the results of this study included the phytochemical content of the purified corn silk extract, comparing the IC_{50} value of the extract compared with the IC_{50} value of the purified corn silk extract using paired sample t-test analysis, a physical quality test of cream preparations, and the SPF value. The IC_{50} value was calculated by linear regression

RESULT AND DISCUSSION

The results of the phytochemical screening test of purified corn hair extract using the tube method are presented in Table 3. It contained flavonoids, tannins, saponins, and alkaloids. The presence of flavonoids was due to an orange color change after adding concentrated Mg and HCl reagents. The purified extract also contains tannins characterized by a dark blue or blackish green color change after adding 1% $FeCl_3$. Saponin content was indicated by the presence of stable foam. The alkaloid content was indicated by the formation of a brown precipitate for Wegner's reagent, a white precipitate for Mayer's reagent, and a red-orange precipitate for dragendorff's reagent.

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Table 3. The results of the phytochemical screening test of purified corn silk extract

Compound group	Reactor	Result color	Information
Flavonoids	Mg, Concentrated HCl	Orange	Positive
Tannins	$FeCl_3$	Dark blue or blackish green	Positive
Saponins	Aquadeast	There is foam	Positive
Alkaloids	Meyer's	White precipitate	Positive
	Dragendorff's	Orange-red sediment	Positive
	Wagner's	Brownish red sediment	Positive

Antioxidant activity testing was conducted using the DPPH method because the process was fast, sensitive, and using synthetic radical DPPH stable and soluble in polar solvents. Hydrazyl Picrile Diphenyl Molecules might react with hydrogen atoms from one molecule component of the sample to form the Diphenyl Pikril Hydrazine compound which might raise the absorbance value. The results of the antioxidant activity test were the IC_{50} level indicated that the ethanol extract and purified extract of corn hair produced IC_{50} values in the weak category, which can capture 50% free radicals compared to vitamin C via linear regression equation. The results of the IC_{50} level obtained can be seen in Table 4.

Table 4. The results of the antioxidant activity test of ethanol extract and purified corn silk extract

Concentrations (ppm)	Absorbancy		% Inhibition		IC ₅₀ (μg/mL)	
	Extract	Purified Extract	Extract	Purified Extract	Extract	Purified Extract
50	0.46	0.43	28.41	32.08		
100	0.44	0.42	30.08	34.33		
200	0.41	0.35	36.06	44.76	356.18±2.77	256.55±2.68
300	0.38	0.29	41.04	54.61		
400	0.27	0.24	57.49	63.26		

Based on Table 4, it can be observed that there is a significant difference of $p < 0.05$ in antioxidant activity after the T-test is held between ethanol extract and purified extract of corn silk. The IC₅₀ value of corn silk extract was 356.18 μg/mL and the IC₅₀ value of purified extract was 256.55 μg/mL with a comparison of antioxidant compounds, namely vitamin C which had an IC₅₀ value of 37.5 μg/mL (Johnson et al., 2017). A compound is said to be a very strong antioxidant if the IC₅₀ value is less than 50 μg / mL, said to be a weak antioxidant if the IC₅₀ value is more than 200 μg/mL (Molyneux, 2004). The antioxidant activity of corn hair extract and purified extract was smaller than that of vitamin C using the DPPH method (Badarinath et al., 2010).

Table 5. The physical quality test result of anti-aging cream preparation of purified corn silk extract

Extract			Preparation Evaluation				
Formula	Organoleptic		pH	Homogeneity	Dispersion (cm)	Adhesion (second)	Cream Type
F1 (1%)	Form:	Semisolid	5.2±0.23	Homogenous	5.01 ± 0.28	4.45 ± 0.00	Oil in water (O/W)
	Color:	Brownish white					
	Aroma:	Typical of corn					
F2 (5%)	Form:	Semisolid	5.4±0.25	Homogenous	5.03 ± 0.28	4.59 ± 0.00	Oil in water (O/W)
	Color:	Brownish					
	Aroma:	Typical of corn					
F3 (10%)	Form:	Semisolid	5.4±0.36	Homogenous	5.66 ± 0.38	4.87 ± 0.01	Oil in water (O/W)
	Color:	Dark brown					
	Aroma:	Typical of corn					

Here is a picture of corn silk purified extract cream. Based on the physical quality test, the greater the purified extract concentration used, the color was getting brown. The cream color can be seen in Figure 1.

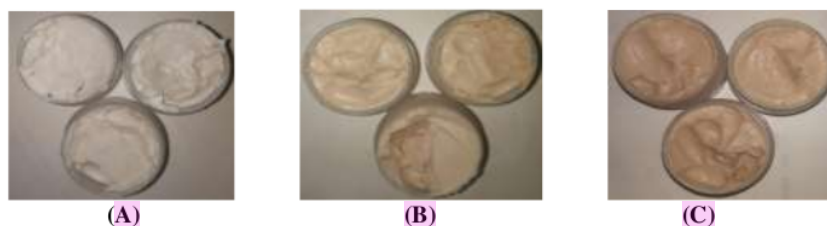


Figure 1. Image of corn silk purified extract cream formulation (A) formulation 1 extract concentration purified 1% (B) formulation 2 concentration of 5% purified extract (C) formulation 3 concentration 10% purified extract

The physical quality test results of anti-aging cream preparations of purified corn silk extract can be seen in Table 5. Judging from the organoleptic form: semisolid, these creams have brownish white to brown and has a distinctive smell of corn. The higher the concentration of the formulation used, the darker the color. The pH cream test was carried out to find out how much the resulting pH value was. The suitability of the pH value affects the skin's acceptance of the preparation. pH preparations that are too acidic will cause skin irritation, while too alkaline can cause skin dryness (Tranggono and Latifah, 2007). The pH evaluation results fulfilled the requirement, which is around 4.5-6.5. The results of the dispersion test, according to the requirements, are around 5-7 cm, the greater the diameter value of the dispersive power, the more surface area the preparation can reach. The adhesion test aims to determine the time it takes for the preparation to adhere to the skin. Test results in adhesion for more than 4 seconds. The longer it takes, the longer the drug's working power. From the results of testing the type of cream with the electric delivery method, the type of cream produced is oil in water (O/W). When tested, the light turns on when the cable is dipped in the cream (Damogalad et al., 2013).

Determination of the SPF level as an anti-aging parameter

The SPF level is one of the parameters which states that the sample has the potential as a UV filter or not. The determination of the SPF level can be determined by UV spectrophotometry. Table 6 below presents the results of the calculation of the SPF level of the sample of purified corn silk extract cream.

Table 6. SPF level of purified corn silk extract cream

I	EE	F1		F2		F3	
		I	EE X I	I	EE X I	I	EE X I
290	0.02	0.21	0.01	0.34	0.01	0.77	0.01
295	0.08	0.16	0.01	0.27	0.02	0.67	0.05
300	0.29	0.14	0.04	0.24	0.07	0.62	0.18
305	0.38	0.12	0.04	0.23	0.08	0.58	0.19
310	0.19	0.11	0.02	0.23	0.05	0.56	0.10
315	0.08	0.11	0.01	0.22	0.02	0.55	0.05
320	0.02	0.11	0.00	0.22	0.00	0.54	0.01
Total			0.12		0.24		0.59
SPF			1.27		2.41		5.94

The SPF level of several extract concentrations is obtained from the total sum of Erythema Efficiency multiplied by the Solar Rays Simulation Spectrum (EE x I) and Correlation Factor (CF), which is 10. The SPF level of each concentration can be seen in Table 6. The SPF level, which describes the product's ability to protect the skin from erythema, can be determined by in vitro. In vitro

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UV absorption activity test can be carried out using a UV spectroscopy technique measured in the UV light wavelength range (290- 320nm) (Dutra et al., 2004). The SPF activity test of anti-aging cream preparations has good results. At a concentration of 1%, it has an average SPF value of 1.27, the minimum protection range. At 5% concentration, it has an average SPF value of 2.41 in the minimum protection range. Meanwhile, the 10% concentration has an SPF value of 5.94 in a moderate protection range. The higher the extract concentration in the cream, the higher the SPF value.

CONCLUSION

The purified extract of corn silk IC₅₀ value of 256.66 µg/mL which was better than the ethanol extract of corn hair which had an IC₅₀ value of 356.17 µg/mL, including the weak antioxidant category. The quality evaluation of creams with concentration 1%, 5%, and 15% showed that they had fulfilled requirements based on pH, dispersion, and adhesion tests. The SPF activity on concentration 1%, has an SPF value of 1.27 within the minimum protection range, concentration 5%, has an SPF value of 2.41 in the minimum protection range, concentration 15% SPF value of 5.94 moderate protection range. The concentration of the purified extract of corn silk in the formulation increased in its SPF level.

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