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Research Article
Antioxidant and UV Protection Activities of Squid (*Loligo sp.*) Ink Powder Lotions

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Abstract
Melanin is a pigment contains in human skin which role as a UV-absorbing agent. One of the exogenous melanins can be obtained from squid (*Loligo sp.*) ink. Squid ink melanin has potent free radical protection activities. This study aimed to determine the physicochemical, stability, antioxidant, and UV protection activities of squid ink powder lotions. Squid ink powders were obtained from the drying process using HCl 0.5M and stored in the climatic chamber. Antioxidant activity was conducted quantitatively using the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method. The best result of the DPPH scavenging activity was 29.2±0.023%, shown from formula III. UV protection activity was conducted by observing erythema scores in animal skin, which exposure to UV. This preparation inhibits the effect of UV exposure. Squid ink powder lotions are potential as a sunscreen product.

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INTRODUCTION
Skin is the largest part of the human body located at the outermost extent, protecting the body from external environments¹. One of the possible environmental factors for skin damaged is ultraviolet radiation (UVR). In continuous exposure, UVR leads to some molecular damage (DNA photodamage) and clinical damage (erythema, tanning, skin cancer, photogingiv) to the skin. UVR activates matrix metalloproteinases (MMPs), which are implicated in photogingiv and collagen breakdown¹. The topical formulation that contains filtering or scattering UVR is called sunscreen, and the efficacy can examine by measuring the sun protection factor (SPF)².

One of the body's photoprotective parts, which role as a broadband UV-absorbing agent, is melanin. Melanin is a human skin pigment that acts as a protection from UVA, UVB, and visible blue light, which has radical scavenging and antioxidant properties³. However, endogenous melanin does not adequately protect the skin, especially in tropical climate areas like Indonesia. Thus, it is necessary to use sunscreens containing exogenous melanin or melanin-related compounds or mimic endogenous melanin⁴.

One of the melanins from natural sources known to have potential as free radical scavengers is squid (*Loligo sp.*) ink melanin⁵. Squid ink plays a significant role in eliminating intracellular excessive reactive oxygen species (ROS) and

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Dyah Rahmasari^{1*}Aulia Juwanti¹Ima Pratiwi¹Novia Zulfa Diana¹Raditya Weka Nugraheni¹Dita Nurlita Rakhma²¹Department of Pharmacy, Universitas Muhammadiyah Malang, Malang, East Java, Indonesia²Department of Pharmacy, Universitas Hang Tuah, Surabaya, East Java, Indonesia*email: dyahrahmasari@umm.ac.id**Keywords:**

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INTRODUCTION

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One of the body's photoprotective parts, which role as a broadband UV-absorbing agent, is melanin. Melanin is a human skin pigment that acts as a protection from UVA, UVB, and visible blue light, which has radical scavenging and antioxidant properties⁶. However, endogenous melanin does not adequately protect the skin, especially in tropical climate areas like Indonesia. Thus, it is necessary to use sunscreens containing exogenous melanin or melanin-related compounds or mimic endogenous melanin⁷.

One of the melanins from natural sources known to have potential as free radical scavengers is squid (*Loligo sp.*) ink melanin⁸. Squid ink plays a significant role in eliminating intracellular excessive reactive oxygen species (ROS) and

improve its antioxidant ability^{9,10}. It also has anti-retroviral, anti-inflammatory, antimicrobial activity, and other traditional uses¹¹. Antioxidants can protect against photo-induced radical reactions, thereby helping sunscreens in inorganic and organic UV filters¹². Also, there was a positive relation and linear correlation between sunscreen and antioxidants¹³.

There is currently no research on using squid ink as an active ingredient in sunscreen products because of its limitation in the low solubility of organic solvent and water⁸. UV-absorbing and antioxidant activity within squid ink may be used as the active compound in sunscreen lotion to increase its benefit and utilization. Lotions are emulsion dosage forms for external application to the skin, which has many characteristics like creams. Lotions consist of an oil-in-water emulsion, water washable, and widely acceptable cosmetically¹⁴. Therefore, this study investigates the physicochemical characteristics, stability, antioxidant activity, and UV protection effectivity of squid ink powder in lotion preparations.

MATERIALS AND METHODS

Materials

Squid from Sendang Biru beach was purchased from the local market in Malang city, East Java then dried to a dry squid ink powder. The squid used was determined in the Department of Biology, Faculty of Science and Technology, Universitas Airlangga. Squid is different from cuttlefish (*Sepia sp.*) morphologically. The color of the ink produced by squid is blue-black, while cuttlefish produces a brownish ink color. Other materials including virgin coconut oil (VCO), cetyl alcohol, triethanolamine (TEA), stearic acid, glycerin, propylene glycol, natrium edetate, butylated hydroxy toluene (BHT), methylparaben, propylparaben, fragrance, and distilled water in technical grade for lotion preparation. The

DPPH (2,2-diphenyl-1-picrylhydrazil), ascorbic acid and methanol pro analysis for the antioxidant test, and *Rattus norvegicus* strain Wistar for SPF determination test's subject.

Methods

Squid ink powder preparations

Freshly obtained squids were dissected, and ink glands were manually removed from the viscera. The 50 g of ink squid was added with 100 mL HCl 0.5 M in a tight light condition. The solution was then stirred using a magnetic stirrer for 30 minutes then stored for 24 hours at 10°C. After 24 hours, the solution was centrifuged for 15 minutes and dried and stored in the climatic chamber at 60°C¹⁵.

Squid ink powder lotion preparation

The oil-in-water lotion was prepared with the composition as shown in Table I. The water-soluble components (part A) and the oil-soluble components (part B) were mixed at 70°C, separately. The water phase was added to the oil phase with continuous stirring. The squid ink powder was mixed to the lotion base and added with fragrance homogeneously¹⁶.

Table I. Squid ink powder lotion formulation

Ingredients	Formula I	Formula II	Formula III
Squid ink powder	1 g	2 g	3 g
VCO	5 g	5 g	5 g
Cetyl alcohol	2 g	2 g	2 g
Stearic acid	10 g	10 g	10 g
TEA	2 g	2 g	2 g
Propylene glycol	5 g	5 g	5 g
Glycerin	8.5 g	8.5 g	8.5 g
Methylparaben	0.1 g	0.1 g	0.1 g
Propylparaben	0.1 g	0.1 g	0.1 g
BHT	0.1 g	0.1 g	0.1 g
Natrium edetate	0.1 g	0.1 g	0.1 g
Citrus fragrance	qs	qs	qs
Distilled water	Until 100%	Until 100%	Until 100%

Physicochemical evaluation of squid ink powder lotion

The physicochemical evaluation involved was organoleptic, determination of pH value, homogeneity, viscosity, and gel spreadability. The organoleptic test acts as a factor in the physicochemical change parameters and

the acceptability of the preparation¹⁷. The organoleptic test was observed as its color, scent, and texture visually. pH value was measured using a digital pH meter, and homogeneity was analyzed by visual inspection for any coarse particle's existence. Viscosity was measured by Brookfield Viscometer, and gel spreadability was determined by applying gel in between two glass slides, then added with some weights¹⁶.

Stability testing of squid ink powder lotion

1. Real-time method

Real-time stability studies of the different formulations were carried out under different temperature conditions (4°±2°C; 30°±2°C; 40°±2°C) and checked the effect on its organoleptic, homogeneity, and pH value. All formulations were stored in vial glass for 30 days¹⁸.

2. Freeze-thaw cycling method

Freeze-thaw cycling stability studies was determined by storing the preparation in a refrigerator at 4°±2°C for 24 hours, then moved into a climatic chamber at 40°±2°C for 24 hours and counted as one cycle. This test was held in six cycles (12 days)¹⁹.

Antioxidant DPPH scavenging activity test

This method was adapted and modified from Fatimah Zaharah and Rabeta²⁰ as well as Saputri *et al.*²¹. The samples with different concentrations of squid ink powder lotions and ascorbic acid as a positive control were reacted with the DPPH radical in methanol solution. About 5 mL of sample (in methanol) was mixed with 1 mL of 0.4 mM DPPH solution. Blank was prepared as 1 mL of 0.4 mM DPPH mixed with methanol until 10 mL. The positive control was prepared with 2 mL of the ascorbic acid solution and mixed with 1 mL of 0.4 mM DPPH and methanol. The mixture was mixed homogeneously using a vortex shaker and incubated for 30 minutes in dark conditions at room temperature. The absorbance was measured at 517 nm using a UV-Vis

spectrophotometer against blank and positive control. The ability of preparations for scavenging DPPH were calculated and expressed in the term of percentage value (%) by using the equation:

$$\%inhibitory = \frac{ABS\ blank - ABS\ sample}{ABS\ blank} \times 100$$

ABS blank : Absorbance of blank
 ABS sample : Absorbance of sample

The half-maximal inhibitory concentration (IC₅₀) values were calculated using their calibration curve until the linear regression equation is obtained.

UV protection effectivity test

This test was determined by observing the erythema on the animal's skin test exposed to UV light. This test was using the male *R. norvegicus* strain Wistar as an experimental animal. The test was carried out by shaving the rats' back hair about 2 cm x 2 cm, then applying the samples and putting them on an Exo Terra lamp for six hours. Rats were divided into five groups (n = 6), a positive control group (using Parasol™), a negative control group (using lotion without active), and three sample test groups (using a lotion with 1%, 2%, and 3% of squid ink powders)¹⁷. Parasol lotion has been clinically tested and contains active ingredients such as ethyl p-methoxycinnamate, benzophenone-3, and titanium dioxide, widely used in sunscreen preparations. This lotion has good protection against sunlight by reflecting and scattering UV radiation. The erythema score used was 0 - 4 which showed no erythema = 0; very little erythema (diameter <25 mm) = 1; erythema is clearly visible (diameter 25 - 30 mm) = 2; moderate erythema (diameter 30 - 35 mm) = 3; and severe erythema (diameter >35 mm) = 4²².

Research ethics approval

This research was approved by the Health Research Ethics Committee of Universitas Muhammadiyah

Malang with Approval Code E.5.a/266/KEPK-UMM/XII/2019.

RESULTS AND DISCUSSION

Physicochemical evaluation of squid ink powder lotion

The squid ink powder lotions had a soft texture, black in color, and citrus scent. The black color of these lotions follows the squid ink powder's color, and formula III has sharper color than formula II and I, as shown in **Figure 1**. Visually, the squid ink powder lotions had no coarse particle, which indicated that the preparations were homogenous. The measurement of pH value, viscosity, and lotion spreadability are shown in **Table II**.

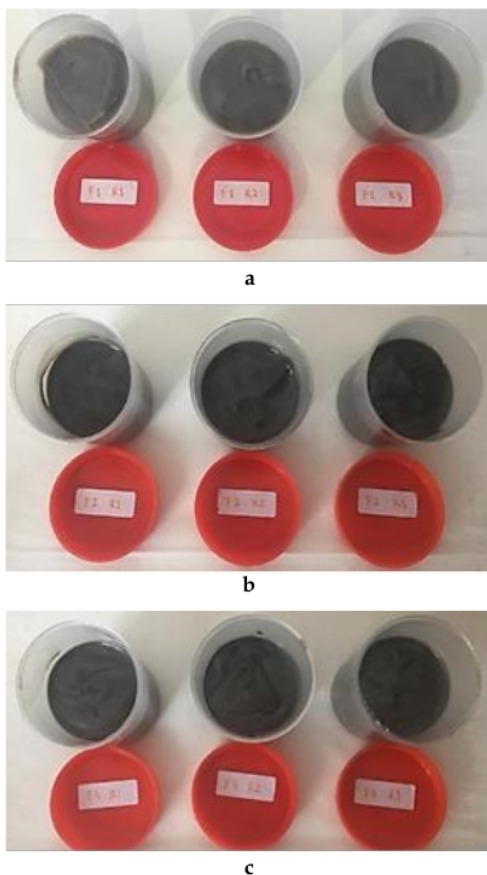


Figure 1. Physical appearance of squid ink powder lotions in three replications of Formula I (a); Formula II(b); and Formula III (c)

Table II. Physical and chemical characteristics of squid ink powder lotions

Formula	pH	Viscosity (cps)	Spreadability (g/cm)
I	7.38 ± 0.04	3,083 ± 629	0.0037 ± 0.0000
II	7.30 ± 0.02	3,583 ± 382	0.0036 ± 0.0000
III	7.04 ± 0.01	4,667 ± 289	0.0033 ± 0.0002

The different squid ink powder concentrations (1%, 2%, and 3%) had different pH, viscosity, and spreadability value. As shown in **Table II**, the higher concentration of squid ink powder resulted in lotion preparation with a lower pH value. This phenomenon occurred when the squid ink powder was made, the solvent (HCl 0.5M) remains; hence the pH value was lower due to high powder content. However, the pH value results still qualify the pH range requirements of the skin tolerance (4–7)²³ and the requirement of pH value for sunscreen preparation (4.5–8)²⁴. The viscosity value was significantly different, and the value was higher with the addition of squid ink powder. The spreadability value of Formula I (1% squid ink powder) was the highest and decreased with the addition of the active ingredient. This phenomenon follows the theory that the higher viscosity, the lower the spreadability value²⁵.

Stability testing of squid ink powder lotion

1. Real-time method

The results of real-time stability testing of squid ink powder lotions showed no changes in color, odor, and phase separation after storage at 4°, 30°, and 40°C. Significantly, the pH values were affected by the addition of squid ink powder and the storage temperature. **Table III** showed that the pH value of squid ink powder lotions was decreased over time, but the preparations were most stable at 30°C storage.

Table III. The pH value in real-time stability of squid ink powder lotion preparations

Formula	1 st day	30 th day		
		4°C	30°C	40°C
I	7.38 ± 0.04	7.22 ± 0.04	7.20 ± 0.07	6.98 ± 0.14
II	7.30 ± 0.02	7.05 ± 0.01	7.15 ± 0.02	6.87 ± 0.03

III	7.04 ± 0.01	6.85 ± 0.02	6.92 ± 0.09	6.58 ± 0.08
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2. Freeze-thaw cycling method

The pH value results of the freeze-thaw cycling test of squid ink powder lotions showed that all formulas did not change organoleptically and showed no phase separation. The pH value measurement results of this evaluation were shown in Table IV. The three formulas' pH values were different, but formula II was most stable than formula I or III.

Table IV. The pH value in freeze-thaw stability of squid ink powder lotion preparations

Formula	1 st day	12 th day
I	7.38 ± 0.04	7.26 ± 0.02
II	7.30 ± 0.02	7.25 ± 0.02
III	7.04 ± 0.01	7.10 ± 0.01

Antioxidant DPPH scavenging activity test

Antioxidant activity was examined by the DPPH scavenging method. The antioxidant will react with DPPH by electron donate mechanism, which stabilizes DPPH by decreasing the intensity of DPPH's violet color and turns into yellow²¹. Ascorbic acid was a positive control, which is well known as a potent antioxidant for DPPH scavenging activity. Squid ink powder showed DPPH scavenging activity, which has an IC₅₀ value of about 46.24 ppm (Table V). These results indicate that squid ink powder has a potent antioxidant activity, which categorized as a very powerful antioxidant (<50 ppm)²⁶.

Table V. IC₅₀ value of ascorbic acid and squid ink powder

Sample	Concentration (ppm)	DPPH scavenging activity (%)	IC50 (ppm)
Ascorbic acid	1	46.92 ± 0.73	1.44
	2	53.35 ± 0.81	
	3	58.94 ± 0.80	
	4	66.76 ± 1.02	
	5	68.99 ± 1.12	
Squid ink powder	5	17.75 ± 0.83	46.24
	10	18.28 ± 1.25	
	20	26.07 ± 1.16	
	50	26.97 ± 1.02	
	100	95.73 ± 0.92	

Table VI showed the scavenging activity of squid ink powder when formulated into lotion preparations. Formula III (squid ink powder 3%) gave out the highest value in antioxidant scavenging DPPH activity compared with Formula I (squid ink powder 1%) and II (squid ink powder 2%). The higher of squid ink powder concentration, the higher scavenging activity obtained. These results indicate that squid ink powder lotions are categorized as a weak antioxidant (250-500ppm)²⁶. The freeze-thaw cycling test implies that the antioxidant DPPH scavenging activity of squid ink powder lotions was decreased, but not significantly.

Table VI. Antioxidant DPPH scavenging activity (%) of squid ink powder lotions

Formula	Before freeze-thaw (%)	After freeze-thaw (%)
I	15.83 ± 0.013	15.18 ± 0.012
II	20.95 ± 0.019	21.13 ± 0.002
III	29.12 ± 0.023	27.08 ± 0.024

UV protection effectivity test

Erythema is induced by UV-B radiation causes cellular immunologic changes that lead to blood vessel dilation²². As shown in Table VII, this study showed that the squid ink powder lotions significantly decrease in the erythema area compare to the negative control group. These lotions were able to inhibit the effects of acute UV exposure. The presence of antioxidant activity can explain this finding. Antioxidant activity has been shown to enhance protection against UV-induced DNA damage by reducing oxidative stress and inhibiting NF-kB. It also neutralizes the UV-induced free radicals. It makes this agent can play a role as potential "non-sunscreen" agents²⁷.

Table VII. UV protection effectivity value

Samples	Erythema area (mm ²)	Erythema Score
Positive control	0 ± 0	0
Negative control	159.27 ± 48.84	4
Formula I	17.79 ± 13.96	1
Formula II	0 ± 0	0
Formula III	0 ± 0	0

CONCLUSION

The 3% squid ink powder lotion showed the best formulation of this study. It has good physicochemical characteristics and stability. It showed antioxidant activity with DPPH scavenging activity of $29.12 \pm 0.023\%$ and was also inhibit the effect of UV exposure. It indicates that this squid ink powder lotions have the potential as a sunscreen product.

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