

ANTIOXIDANT LOTION FORMULATION WITH A COMBINATION OF ETHANOL EXTRACTS OF BLACK SOYBEAN AND PIGEON PEA

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ABSTRACT

Indonesia's tropical climate with high sun exposure increases the risk of oxidative stress on the skin due to free radicals. Synthetic antioxidants in skincare products are proven effective but may cause long-term side effects. Natural ingredients offer a safer and more promising alternative. This study aimed to formulate an antioxidant lotion using ethanol extracts of black soybean and pigeon pea. Extraction was carried out by maceration using 70% ethanol as solvent. The extracts were formulated into five lotion formulas, consisting of three extract combinations, one positive control, and one negative control. Evaluation included antioxidant activity using the DPPH method and physical stability tests. Formula 3, containing 11,31% black soybean extract and 3,75% pigeon pea extract, shows the highest antioxidant activity with a strong category ($IC_{50} = 73,5 \mu g/mL$). All formulas show good physical stability during 21 days of storage and form oil-in-water emulsions. The combination of black soybean and pigeon pea extracts demonstrates strong antioxidant potential with good formulation stability and is suitable for development in natural cosmetic products.

Keywords : Antioxidant, Black Soybean, Lotion, Physical Stability, Pigeon Pea

ABSTRAK

Iklim tropis di Indonesia dengan paparan sinar matahari yang tinggi meningkatkan risiko stres oksidatif pada kulit akibat radikal bebas. Penggunaan antioksidan sintetis dalam produk perawatan kulit terbukti efektif, tetapi dapat menimbulkan efek samping jangka panjang. Bahan alam menjadi alternatif yang lebih aman dan potensial untuk dikembangkan. Penelitian ini bertujuan merumuskan lotion antioksidan dengan kombinasi ekstrak etanol kedelai hitam dan kacang gude. Ekstraksi dilakukan dengan metode maserasi menggunakan etanol 70%, kemudian ekstrak diformulasikan ke dalam lima formula lotion, terdiri atas tiga formula kombinasi ekstrak, satu control positif, dan satu control negatif. Evaluasi mencakup uji aktivitas antioksidan menggunakan metode DPPH dan uji stabilitas fisik sediaan. Formula 3 (11,31% ekstrak kedelai hitam dan 3,75% ekstrak kacang gude) menunjukkan aktivitas antioksidan tertinggi dengan nilai IC_{50} sebesar $73,5 \mu g/mL$ dan termasuk dalam kategori kuat. Seluruh formula memiliki stabilitas fisik yang baik selama penyimpanan 21 hari dan membentuk emulsi tipe minyak dalam air. Kombinasi ekstrak kedelai hitam dan kacang gude memiliki potensi sebagai antioksidan kuat dengan stabilitas formulasi yang baik, sehingga sesuai untuk pengembangan produk kosmetik alami.

Kata Kunci : Antioksidan, Kacang Gude, Kedelai Hitam, Lotion, Stabilitas Fisik

A. INTRODUCTION

Indonesia's tropical climate, characterised by high solar intensity and humidity, increases the risk of oxidative stress caused by free radicals, particularly Reactive Oxygen Species (ROS), on the skin (1). Continuous exposure to ROS leads to skin cell damage, premature ageing, inflammation, hyperpigmentation, and potential skin cancer (2). Antioxidants serve as protective agents by neutralising free radicals. Although synthetic antioxidants are widely used in skincare products for their efficacy, prolonged use may result in side effects, including irritation, allergies, and hormonal imbalances. Natural antioxidants are increasingly favoured due to their safety profile, environmental friendliness, and suitability for long-term application (3).

Black soybean (*Glycine max* (L.) Merrill) ethanol extract contains flavonoids, isoflavones, and anthocyanins, which are known for their antioxidant activity. Previous studies have reported its IC₅₀ value at 220,42 ppm, indicating moderate antioxidant potential (4). Pigeon pea (*Cajanus cajan* (L.) Huth) extract also demonstrates significant antioxidant properties attributed to its content of flavonoids, saponins, tannins, and phenolic compounds, with an IC₅₀ of 69,67 ppm (5). Both extracts are obtained using 70% ethanol via maceration, a solvent known for efficiently extracting polar and semi-polar compounds while preserving thermolabile constituents (6).

Topical lotions offer favourable physicochemical properties, including ease of application, good spreadability, and enhanced skin absorption. They serve as suitable carriers for antioxidant delivery. For example, a lotion formulated with *Tamarindus indica* L. fruit extract demonstrated in vitro antioxidant activity through the DPPH assay, with IC₅₀ values near 100 µg/mL at a 4% concentration (7). This suggests that increasing the concentration of active extracts enhances antioxidant efficacy in topical formulations.

The present study utilises a novel combination of ethanol extracts from black

soybean and pigeon pea in varying concentrations: 3.91%, 7.61%, and 11.31% for black soybean, and 11.25%, 7.50%, and 3.75% for pigeon pea. These concentrations exceed those typically reported in prior antioxidant lotion studies. Synergistic effects between the two extracts are anticipated to enhance antioxidant activity more effectively than single-extract formulations.

Previous studies have focused primarily on the antioxidant activity of individual plant extracts in topical formulations. There is a lack of data regarding the co-formulation of black soybean and pigeon pea extracts in a lotion base. The current study addresses this research gap by investigating the formulation and evaluation of a lotion containing a combination of these two natural extracts. The potential synergism in antioxidant activity and physical stability of the lotion is also explored, contributing to the development of safe, natural-based cosmetics. This research aims to formulate and evaluate an antioxidant lotion combining ethanol extracts of black soybean (*Glycine max* (L.) Merrill) and pigeon pea (*Cajanus cajan* (L.) Huth) by assessing both antioxidant activity and the physical stability of the formulation.

B. RESEARCH METHOD

Materials

The plant materials used were black soybean (*Glycine max* (L.) Merrill), collected from Kemuning, Ngargoyoso, Karanganyar, Central Java, and pigeon pea (*Cajanus cajan* (L.) Huth), collected from Blora, Central Java. Plant identification was performed at the UPF Yankestrad Laboratory, Tawangmangu, and the specimens were confirmed to match the referenced species. Other ingredients used in the lotion formulation were stearic acid (Brataco), cetyl alcohol (Brataco), liquid paraffin (Brataco), glycerin (Brataco), triethanolamine (Brataco), methylparaben (Brataco), propylparaben (Brataco), fragrance (local supplier, Surakarta), and distilled water (Brataco). Ethanol 70% (Brataco) was used as the solvent for maceration extraction of the plant materials. Reagents for phytochemical screening and antioxidant activity assays included Mayer's reagent, Dragendorff's reagent, FeCl_3 , methylene blue, vitamin C (Brataco), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Brataco).

Methods

1. Preparation and Characterization of Simplisia and Extracts

Dried seeds of *Glycine max* (L.) Merrill and *Cajanus cajan* (L.) Huth were authenticated at the Laboratory of UPF Yankestrad, Tawangmangu, Indonesia. Seeds were cleaned manually, air-dried at room temperature, ground with a grinder, and passed through a 40-mesh sieve (4). The resulting powder was evaluated for organoleptic properties, and loss on drying according to the Indonesian Herbal Pharmacopeia (8). Extraction was carried out by macerating the powder with 70% ethanol for five days with occasional stirring (9). Filtrates were evaporated under reduced pressure using a rotary evaporator (Heidolph Hei-VAP Core, Germany) to obtain viscous ethanolic extracts (4). Organoleptic characteristics and moisture content were assessed (10,11). Phytochemical screening was performed to identify the presence of flavonoids, alkaloids, tannins, saponins, and phenolics. Thin-layer chromatography (TLC) was conducted on silica gel F254 plates using specific spray reagents (12).

2. Lotion Formulation and Preparation

Three test formulations (F1–F3) containing different ratios of black soybean and pigeon pea extracts were prepared. A positive control (F4) containing 1% vitamin C and a negative control (F5) without active ingredients were included. Each formulation used the same lotion base composition. Table 1 shows the composition of each formulation.

Table 1. Composition of Antioxidant Lotion Formulations (% w/w)

Ingredients	Function	F1	F2	F3	F4 (Positive Control)	F5 (Negative Control)
Black soybean extract	Active ingredient	3.91	7.61	11.31	–	–
Pigeon pea extract	Active ingredient	11.25	7.50	3.75	–	–
Vitamin C	Antioxidant	–	–	–	1	–
Stearic acid	Emulsifier	1.25	1.25	1.25	1.25	1.25
Cetyl alcohol	Co-emulsifier	1.00	1.00	1.00	1.00	1.00
Glycerin	Humectant	5.00	5.00	5.00	5.00	5.00
Methyl paraben	Preservative	0.20	0.20	0.20	0.20	0.20
Propyl paraben	Preservative	0.02	0.02	0.02	0.02	0.02
Liquid paraffin	Emollient	7.00	7.00	7.00	7.00	7.00
Triethanolamine	pH adjuster	2.00	2.00	2.00	2.00	2.00
Perfume	Fragrance	3	3 drops	3 drops	3 drops	3 drops

Distilled water	Solvent	drops				
		ad 100	ad 100	ad 100	ad 100	ad 100

The oil phase (stearic acid, cetyl alcohol, liquid paraffin, propyl paraben) was heated to 70°C in a water bath. The aqueous phase (methyl paraben, glycerin, triethanolamine, distilled water) was heated separately to the same temperature. The oil phase was added gradually into the aqueous phase with constant stirring to form an emulsion. The mixture was cooled to 40–45°C before incorporating the extracts or vitamin C, then stirred until homogeneous (13).

3. Antioxidant Activity Assay

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. A 0.1 mM DPPH solution was prepared in methanol. Lotion samples (2.5 g) were dissolved and diluted to concentrations of 2, 4, 6, 8, and 10 ppm. Each sample was mixed with an equal volume of DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). The percentage inhibition and IC₅₀ values were calculated accordingly (11).

4. Physical Evaluation of Lotion

Physical quality tests were conducted on days 0, 7, 14, and 21 for each formulation to assess stability over time. Organoleptic properties, including color, odor, and consistency, were evaluated visually. Homogeneity was examined by spreading the lotion on a glass slide to observe the uniformity of dispersed particles. The pH of the lotion was measured using a digital pH meter (Hanna HI2211, Romania). Viscosity was assessed using a Lamy Rheology viscometer (Model RM 100 CP2000, France) at 30 rpm with spindle L-4. Spreadability was evaluated by placing 0.5 g of lotion between two glass plates and applying a 150 g load for 60 seconds, then measuring the diameter of the spread. Adhesion was tested by applying 0.2 g of lotion between two glass plates, compressing them under a 1 kg weight for 5 minutes, and recording the time until separation. Emulsion type was determined using the methylene blue method, where a uniform blue distribution indicated an oil-in-water emulsion. Organoleptic assessment: Color, odor, and consistency were observed visually (14).

Data Analysis

Observational data from organoleptic evaluation, homogeneity, and emulsion type were analyzed descriptively. pH, viscosity, spreadability, and adhesion data collected on days 0, 7, 14, and 21 were analyzed statistically to evaluate changes over time and differences between formulations. Statistical analysis was performed using SPSS software. One-way analysis of variance (ANOVA) was applied to assess the significance of variations among formulations. A p-value of less than 0.05 was considered statistically significant.

C. RESULTS AND DISCUSSION

RESULTS

1. This Plant Determination

Species identification of black soybean and pigeon pea samples was conducted using organoleptic analysis at the UPF Yankestrad Laboratory, Tawangmangu. The results are presented in Table 1.

Table 1. Determination Results of Black Soybean and Pigeon Pea

Parameter	Result	Test Method / Technique
Black Soybean		
Family	Fabaceae	Organoleptic
Species	<i>Glycine max</i> (L.) Merr.	
Synonym	<i>Phaseolus max</i> L.	
Pigeon Pea		
Family	Fabaceae	Organoleptic
Species	<i>Cajanus cajan</i> (L.) Huth	
Synonym	<i>Cajanus indicus</i> Spreng	

2. Powder Preparation and Yield

Black soybean and pigeon pea samples were dried and ground into powder form. The powder yield is shown in Table 2.

Table 2. Powder Yield of Black Soybean and Pigeon Pea

Sample	Initial Weight (g)	Powder Weight (g)	Yield (%)
Black soybean	400	302	75,5
Pigeon pea	400	300	75

3. Identification of Powder Characteristics

Identification of black soybean and pigeon pea powder characteristics was carried out through organoleptic observation and determination of moisture loss. The results are presented in Table 3 and Table 4.

Table 3. Organoleptic Characteristics of Black Soybean and Pigeon Pea Powder

Organoleptic Parameter	Black Soybean	Pigeon Pea
Shape	Fine powder	Fine powder
Color	Brown	Light brown
Taste	Distinctive, slightly bitter	Distinctive, slightly astringent
Odor	Distinctive	Distinctive

Table 4. Moisture Loss of Black Soybean and Pigeon Pea Powder

Sample	Replication 1	Replication 2	Replication 3	Average (%)
Black Soybean	6,92	7,08	7,15	7,15
Pigeon Pea	6,10	6,05	5,89	6,01

4. Extraction Results

The yield of ethanol extracts from black soybean and pigeon pea is presented in Table 5.

Table 5. Yield of Ethanol Extracts of Black Soybean and Pigeon Pea

Sample	Powder Weight (g)	Extract Weight (g)	Yield (%)
Black soybean	250	34	13,6

ethanol extract			
Pigeon pea ethanol extract	250	33	13,2

5. Extract Characterization

The characteristics of black soybean and pigeon pea ethanol extracts were evaluated through organoleptic observation and yield determination. The results are presented in Table 6, Figure 1 and Table 7.

Table 6. Organoleptic Characteristics of Black Soybean and Pigeon Pea Ethanol Extracts

Sample	Observation Type	Result
Black soybean ethanol extract	Color	Dark brown
	Odor	Distinctive
	Taste	Bitter
	Texture	Viscous
Pigeon pea ethanol extract	Color	Reddish brown
	Odor	Distinctive
	Taste	Bitter
	Texture	Viscous



pigeon pea



black soybean

Figure 1. Ethanol extracts of black soybean and pigeon pea after maceration process

Table 7. Yield of Black Soybean and Pigeon Pea Ethanol Extracts

Sample	Initial Weight (g)	Final Weight (g)	Yield (%)
Black soybean ethanol extract	2	1,56	7,75
Pigeon pea ethanol extract	2	1,68	8,05

6. Phytochemical Screening

Phytochemical screening was conducted to determine the presence of secondary metabolite compounds with antioxidant potential in the ethanol extracts of black soybean (*Glycine max* (L.) Merrill) and (*Cajanus cajan* (L.) Huth). The qualitative phytochemical screening results are presented in Table 8.

Table 8. Phytochemical Screening Results of Ethanol Extracts of Black Soybean and Pigeon pea

No	Compound Class	Black Soybean Extract	Pigeon pea Extract
1	Flavonoids	+	+
2	Saponins	+	+
3	Phenolics	–	+
4	Tannins	–	+
5	Alkaloids	+	–
6	Triterpenoids	+	–

Note: (+) indicates a positive result, (–) indicates a negative result

7. Thin Layer Chromatography (TLC) Results

Thin Layer Chromatography was performed to confirm the presence of specific compound classes by comparing the Retardation factor (Rf) values of the extracts with standard references. The TLC results of the ethanol extracts of black soybean and pigeon pea are shown in Table 9, 10, and Figure 2.

Table 9. Rf Values of Black Soybean Ethanol Extract and Standard References

Compound Class	Rf Value (Extract)	Rf Value (Standard)
Flavonoids	0,73	0,80
Saponins	0,66	0,70

Alkaloids	0,71	0,75
Triterpenoids	0,58	0,61

Table 10. Rf Values of Pigeon Pea Ethanol Extract and Standard References

Compound Class	Rf Value (Extract)	Rf Value (Standard)
Flavonoids	0,68	0,75
Saponins	0,73	0,76
Phenolics	0,44	0,48
Tannins	0,54	0,60

Pigeon Pea



UV 254



UV 360

Black Soybean



UV 254



UV 360

Flavonoids



UV 254



UV 360

Flavonoids



UV 254



UV 360

Saponins



UV 254



UV 360

Saponins



UV 254



UV 360

Phenolics

Alkaloids

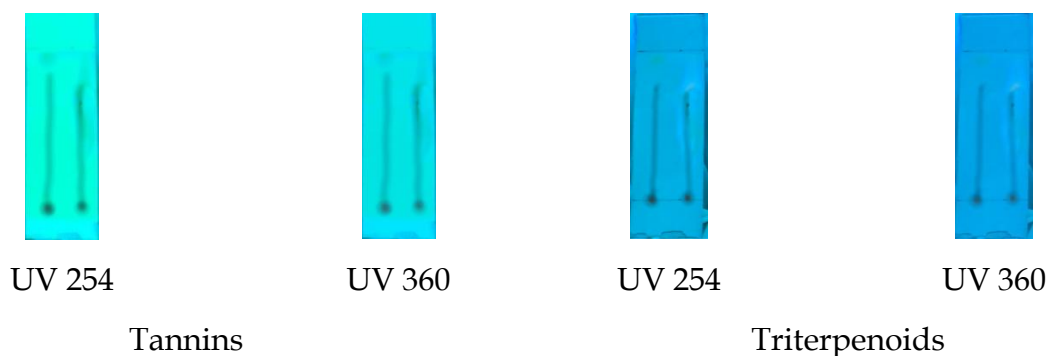


Figure 2. TLC chromatogram of ethanol extracts of black soybean and pigeon pea observed under UV 254 nm and UV 366 nm

8. Preparation of Lotion Formulations

Lotion was formulated using a combination of ethanol extracts of black soybean and pigeon pea in five formulations: Formula 1, Formula 2, Formula 3, Formula 4, and Formula 5. The preparation results are shown in Figure 3.

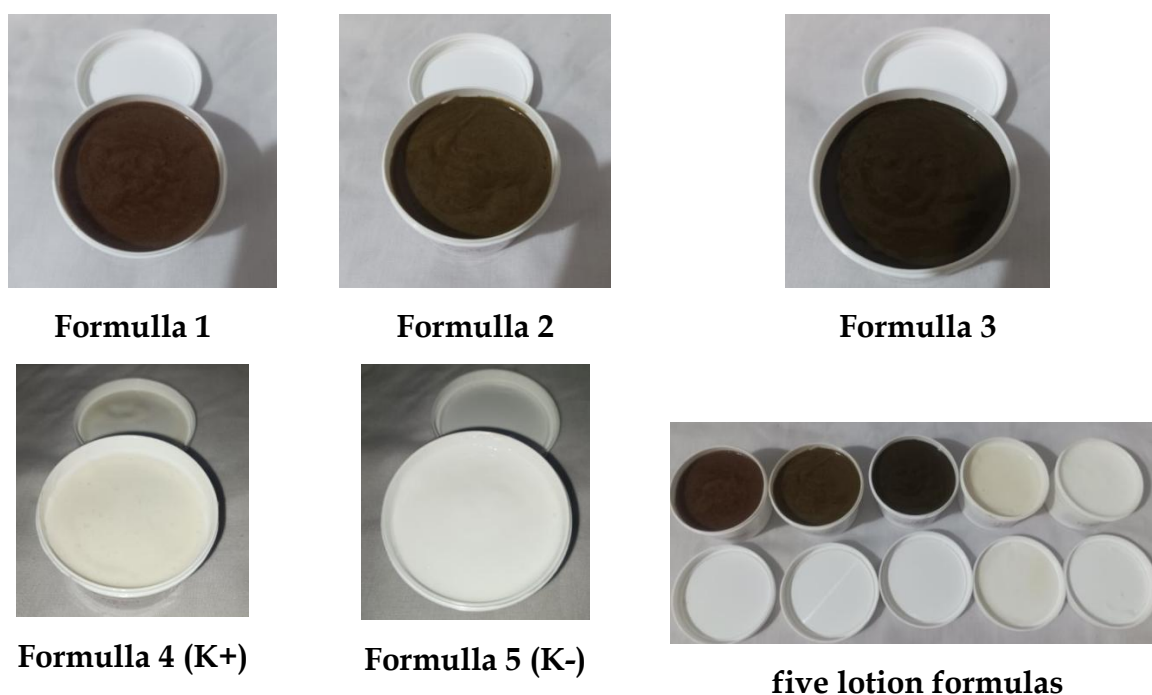


Figure 2. Preparation Results of Lotion Formulations Containing Ethanol Extracts of Black Soybean and Pigeon Pea.

9. Antioxidant Activity

The antioxidant activity of the lotion formulations was evaluated using the DPPH method based on IC_{50} values. The results are presented in Table 11 and Figure 3.

Table 11. Antioxidant Activity of Lotion Formulations Containing Ethanol Extracts of Black Soybean and Pigeon Pea.

Sample	Concentration (ppm)	Absorbance		%Inhibition	Linear regression equation	IC ₅₀ (µg/mL)
		Blanko	Sample			
Formulla 1	2	2,7	1.900	30.48	Y = 0.1153x + 30,228 R ² = 0,9104	171,93
	4	33	1.898	30.55		
	6		1.885	31.03		
	8		1.879	31.25		
	10		1.878	31.28		
Formulla 2	2		1.909	30.15	Y = 0,1645x + 29,755 R ² = 0,9211	123,07
	4		1.907	30.22		
	6		1.891	30.81		
	8		1.879	31.25		
	10		1.878	31.28		
Formulla 3	2		1.923	29.64	Y = 0,2835x + 29,167 R ² = 0,8829	73,5
	4		1.900	30.48		
	6		1.898	30.55		
	8		1.861	31.91		
	10		1.865	31.76		
Formulla 4	2		1.905	30.30	Y = 0,7825x + 28.461 R ² = 0,9869	27,52
	4		1.875	31.39		
	6		1.837	32.78		
	8		1.777	34.98		
	10		1.740	36.33		
Formulla 5	2		2.728	0.18	Y = 0,1615x - 0,151 R ² = 0,9966	310,4
	4		2.719	0.51		
	6		2.711	0.80		
	8		2.703	1.10		
	10		2.692	1.50		

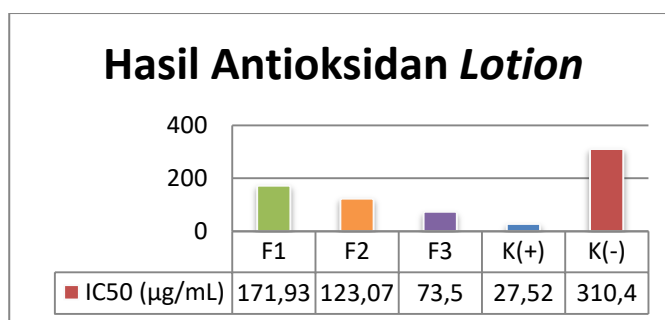


Figure 3. Antioxidant activity (IC₅₀ values) of lotion formulations containing ethanol extracts of black soybean and pigeon pea

10. Physical Quality Evaluation of Lotion Containing Ethanol Extracts of Black Soybean and Pigeon Pea

The physical quality evaluation of lotion containing a combination of ethanol extracts of black soybean and lablab bean was conducted weekly over four weeks, on day 0, 7, 14, and 21. The observed parameters included organoleptic characteristics, homogeneity, pH, viscosity, spreadability, adhesion, and emulsion type. The evaluation results are presented in Tables 12 to 117.

Table 12. Organoleptic Characteristics of Lotion Containing Ethanol Extracts of Black Soybean and Pigeon Pea During 21 Days of Storage

Formulla Organoleptic		Day 0	Day 7	Day 14	Day 21
F1	Form	Smooth, viscous	Smooth, viscous	Smooth, viscous	Smooth, viscous
	Colour	Reddish brown	Reddish brown	Reddish brown	Reddish brown
	Odour	Characteristic	Characteristic	Characteristic	Characteristic
F2	Form	Smooth, viscous	Smooth, viscous	Smooth, viscous	Smooth, viscous
	Colour	Brown	Brown	Brown	Brown
	Odour	Characteristic	Characteristic	Characteristic	Characteristic
F3	Form	Smooth, viscous	Smooth, viscous	Smooth, viscous	Smooth, viscous
	Colour	Dark brown	Dark brown	Dark brown	Dark brown

Formulla	Organoleptic	Day 0	Day 7	Day 14	Day 21
	Odour	Characteristic	Characteristic	Characteristic	Characteristic
F4	Form	Smooth, viscous	Smooth, viscous	Smooth, viscous	Smooth, viscous
	Colour	White	White	White	White
	Odour	Characteristic	Characteristic	Characteristic	Characteristic
F5	Form	Smooth, viscous	Smooth, viscous	Smooth, viscous	Smooth, viscous
	Colour	White	White	White	White
	Odour	Characteristic	Characteristic	Characteristic	Characteristic

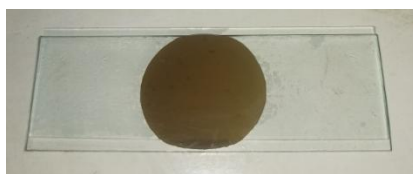
Table 13. Homogeneity Test Results of Lotion Containing Ethanol Extracts of Black Soybean and Pigeon Pea

Fomulla	Day-			
	0	7	14	21
F 1	Homogen	Homogen	Homogen	Homogen
F 2	Homogen	Homogen	Homogen	Homogen
F 3	Homogen	Homogen	Homogen	Homogen
F 4	Homogen	Homogen	Homogen	Homogen
F 5	Homogen	Homogen	Homogen	Homogen

All formulations showed good homogeneity without clumps or phase separation throughout the 21-day storage period.



Formulla 1



Formulla 2



Formulla 3

**Formulla 4****Formulla 5****Figure 4. Homogeneity Appearance of Lotion Formulas 1-5****Table 14. pH Values of Lotion Containing Ethanol Extracts of Black Soybean and Pigeon Pea**

Fomulla	Day-			
	0	7	14	21
F 1	6,30	6,28	6,25	6,22
F 2	6, 33	6,31	6,29	6,27
F 3	6,42	6,41	6,40	6,38
F 4	6,44	6,43	6,41	6,39
F 5	6,39	6,38	6,36	6,34

Table 15. Viscosity of Lotion Containing Ethanol Extracts of Black Soybean and Pigeon Pea

Fomulla	Day-(second)				
	0	7	14	21	Mean
F 1	2373	2347	2319	2294	2333,3
F 2	2291	2264	2239	2215	2252,3
F 3	2188	2163	2139	2116	2151,5
F 4	2062	2041	2020	1999	2030,5
F 5	2033	2011	1989	1968	2000,3

Table 16. Spreadability of Lotion Containing Ethanol Extracts of Black Soybean and Pigeon Pea

Fomulla	Day-				
	0	7	14	21	Mean
F 1	6,0	5,9	5,9	5,8	5,9
F 2	6,1	6,0	6,0	5,9	6,0

F 3	6,2	6,1	6,1	6,0	6,1
F 4	6,5	6,4	6,3	6,2	6,35
F 5	6,7	6,6	6,6	6,5	6,6

Table 17. Adhesion Time of Lotion Containing Ethanol Extracts of Black Soybean and Pigeon Pea

Fomulla	Adhesion Time (second) Day-				
	0	7	14	21	Mean
F 1	7,8	8,0	8,2	8,3	8,08
F 2	7,0	7,2	7,3	7,5	7,25
F 3	6,5	6,6	6,8	7,0	6,73
F 4	6,2	6,3	6,4	6,5	6,35
F 5	5,8	6,0	6,1	6,2	6,03

Emulsion Type of Lotion Containing Ethanol Extracts of Black Soybean and Pigeon Pea. All formulations maintained a stable oil-in-water (O/W) emulsion type throughout the observation period.



Formulla 1



Formulla 2



Formulla 3



Formulla 4



Formulla 5

Figure 5. Emulsion Type Appearance of Lotion Formulas 1-5

11. Data Analysis

ANOVA analysis showed significant differences between formulations for pH, viscosity, spreadability, and adhesion ($p < 0.05$).

DISCUSSION

This study commenced with the determination process of black soybean (*Glycine max* (L.) Merrill) and pigeon pea (*Cajanus cajan* (L.) Huth) plants to ensure the accuracy of sample identity based on morphological characteristics compared with reference literature (15). The determination results confirmed that both materials matched the reported specifications, ensuring the validity of the raw materials. This step is crucial to guarantee that the research is conducted using the correct materials, as also emphasised in other herbal research to minimize misidentification errors that could affect final outcomes.

The subsequent stage involved preparing powdered simplicia from black soybeans and pigeon peas. The yields obtained were 75,5% and 75%, respectively, indicating good quality simplicia as they exceeded the minimum threshold of 10% according to the Indonesian Herbal Pharmacopoeia criteria (Rahadyana et al., 2024)(16). This finding also suggests that the drying and grinding processes were optimal, consistent with the observations of Rahmawan et al. (2021)(17), who reported high yields in dried legume processing.

Organoleptic examination of the simplicia revealed that black soybeans exhibited a dark brown colour, a distinctive bitter taste, and a beany aroma, whereas pigeon peas had a light brown colour, an astringent taste, and a characteristic legume aroma (18)(19)(20)(A. E. Sari et al., 2022; Chaerunnimah et al., 2022; Rahayu and Fidyasari, 2022). These differences are related to variations in pigment composition, phenolic compounds, and alkaloid content in the two materials (Rahmawan et al., 2021; I. A. Y. Lestari et al., 2025).(17) (21)

The moisture content loss results showed values of 7.05% for black soybeans and 6.01% for pigeon peas, both meeting the standard of $\leq 10\%$ (BPOM RI, 2017) (22). Low moisture loss values indicate minimal water content, thereby reducing the risk of microbial growth and active compound degradation (Handoyo and Pranoto, 2020; Nisa et al., 2023).(23)

The extraction process was carried out using maceration with 70% ethanol as the solvent (Khairani et al., 2022)(24) . The yield of black soybean extract was 13.6% and pigeon pea extract was 13.2%, both meeting the minimum requirement of 10%

(25)(Badriyah and Farihah, 2022). Organoleptic analysis of the extracts showed distinctive colours, aromas, and tastes consistent with the literature (Ambari et al., 2021; Mewar, 2023)(11)(26) . The moisture content of the black soybean extract (7.75%) and pigeon pea extract (8.05%) also met the $\leq 10\%$ standard (Mewar, 2023), which is essential for maintaining extract stability (Aini et al., 2023; Rohmanah et al., 2024).(27)(28)

Phytochemical screening using the tube test method revealed that both extracts contained flavonoids as the main compounds, supported by other phenolic compounds such as anthocyanins, polyphenols, saponins, alkaloids, tannins, and triterpenoids ((29)(30)(4)(5)(31)(32)(33)(34) et al., 2021; Fawwaz et al., 2017; Amalia et al., 2021; Ginaris and Faizah, 2023; Yusuf et al., 2019; Putri et al., 2023; Luringunusa et al., 2023; Farikhah et al., 2020). Thin-layer chromatography (TLC) analysis confirmed these findings by showing flavonoid R_f values similar to quercetin (Pratiwi et al., 2023; Ullah et al., 2020).(35)(36)

The lotion was formulated into five variants (F1-F3, K+, K-) with varying extract concentrations. All formulations exhibited good homogeneity, no phase separation, and organoleptic stability during 21 days of storage (Syafitri and Rahma, 2023; Rasydy et al., 2021)(37)(38). Antioxidant activity testing using the DPPH method demonstrated that F3 (black soybean extract 11.31% + pigeon pea extract 3.75%) had the lowest IC₅₀ value of 73.5 ppm (strong category) compared with F1 (171.93 ppm) and F2 (123.07 ppm) (Halisa et al., 2024)(39). The reduced activity in F1 and F2 compared with pure extracts was suspected to result from degradation of active compounds during the formulation process (Nurkhasanah et al., 2023)(40). Vitamin C (K+) served as the best comparator (27.52 ppm), while K- exhibited no significant activity.

Physical quality tests included organoleptic evaluation, homogeneity, pH, viscosity, spreadability, adhesion, and emulsion type. All formulations were identified as oil-in-water (O/W) emulsions, which are easier to apply and remove (S. L. Sari and Zulkarnain, 2024).(41) The pH of all formulations remained within the safe range of 4.5–8 (Karim et al., 2022)(42), with F3 demonstrating the highest stability (6.42–6.38) and F1 showing the greatest decrease. ANOVA results indicated

significant differences ($p < 0.05$) between formulations, with post-hoc analysis revealing that F3 differed significantly from F1 but not from K+.

Viscosity values for all formulations were within the SNI standard range (Husni et al., 2023)(43), with F3 showing the best stability (2151–2036 cP). ANOVA results ($p < 0.05$) indicated that F3 differed significantly from F1, reflecting a more stable emulsion system. Spreadability testing revealed that F3 maintained the most stable values (6.1–5.8 cm), within the ideal range for topical preparations (Aljanah et al., 2022)(44), and statistical analysis confirmed significant differences ($p < 0.05$), with F3 outperforming F1. Adhesion testing showed that all formulations exceeded 4 seconds (Shintyawati et al., 2024)(45), with F3 exhibiting the most balanced adhesion values (6.73–6.55 seconds). ANOVA results again confirmed significant differences ($p < 0.05$) between formulations, with F1 having the highest adhesion values but less favourable spreadability and viscosity profiles.

Overall, the integration of SPSS statistical analysis confirmed that F3 was the most optimal formulation. This superiority was demonstrated by its strong antioxidant activity, reflected in the lowest IC_{50} value, and by its significantly higher stability in pH, viscosity, spreadability, and adhesion compared with the other formulations. The statistical differences were significant ($p < 0.05$) for all parameters, and post-hoc analysis revealed that F3 consistently outperformed F1 while maintaining comparable performance to the positive control in certain aspects, such as pH stability. These findings indicate that the dominant proportion of black soybean extract in F3 contributed to maintaining the chemical stability of active compounds during storage, thereby preserving both functional efficacy and physical performance. Furthermore, the physical characteristics of F3 complied with topical lotion standards, providing an ideal balance between texture, ease of application, and user comfort, thus supporting its potential as a stable and effective antioxidant lotion formulation

D. CONCLUSION

Based on the findings of this study on the antioxidant lotion formulation combining ethanolic extracts of black soybean (*Glycine max* (L.) Merrill) and pigeon

pea (*Cajanus cajan* (L.) Huth), it can be concluded that the combination of these extracts has a positive effect on enhancing antioxidant activity. Formula 3, containing 11.31% ethanolic extract of black soybean and 3.75% ethanolic extract of pigeon pea, demonstrated the highest antioxidant activity with an IC_{50} value of 73.5 ppm, categorised as strong, and was more effective compared with Formula 1 (IC_{50} 171.93 ppm; weak category) and Formula 2 (IC_{50} 123.07 ppm; moderate category). The low IC_{50} value of Formula 3 reflects its optimal ability to neutralise free radicals. In addition, all lotion formulas tested met good physical quality criteria, as evidenced by the parameters of organoleptic properties, pH, spreadability, viscosity, adhesion, and emulsion type, indicating that the extract combination was not only pharmacologically effective but also physically stable in the topical dosage form.

In light of these findings, further research is recommended to investigate the stability of bioactive compounds in pigeon pea extract during the lotion formulation process, particularly concerning the influence of temperature, pH, and interactions with excipients. Further studies should also explore other potential biological effects of the combination of black soybean and pigeon pea extracts, such as anti-inflammatory or anti-ageing activities, to broaden the benefits of this natural-based lotion. Moreover, subsequent research could focus on developing lotion formulations combining ethanolic extracts of black soybean and pigeon pea as anti-ageing products through in vitro evaluation of tyrosinase enzyme inhibition.

E. REFERENCES

- Salsabila S, Rahmiyanti I, Sri Zustaka D. Nilai Sun Protection Factor (SPF) pada Sediaan Lotion Ekstrak Etanol Daun Jambu Air (*Syzygium aqueum*). *Maj Farmasetika*. 2021;6(Suppl 1):123.
- Xing X, Dan Y, Xu Z, Xiang L. Implications of Oxidative Stress in the Pathogenesis and Treatment of Hyperpigmentation Disorders. *Oxid Med Cell Longev*. 2022;12.
- Hafiz Ramadhan, Baidah D, Lestari NP, Yuliana KA. Aktivitas Antioksidan Ekstrak Etanol 96% Daun, Buah dan Kulit Terap (*Artocarpus odoratissimus*) Menggunakan Metode Cuprac. *Farmasains J Ilm Ilmu Kefarmasian*.

2020;7(1):7-12.

- Amalia A, Kusumawinahyu R, Rohenti IR. Studi Potensi Sifat Anti-Aging Ekstrak Kedelai Hitam (*Glycine max* (L.) Merrill) Varietas Detam 1 melalui Uji Antioksidan. *War Akab*. 2021;45(2):43-50.
- Ginaris RP, Faizah NR. The Effectiveness of Flavonoids in Pigeon Pea (*Cajanus cajan*) as Inhibitors of α - Glucosidase Enzyme in Anti-diabetes. *Fitofarmaka J Ilm Farm*. 2023;13(2):139-45.
- Afifah N, Budi Riyanta A, Amananti W. Pengaruh Waktu Maserasi Terhadap Hasil Skrining Fitokimia Pada Ekstrak Daun Mangga Harum Manis (*Mangifera indica* L.). *J Cryst Publ Penelit Kim dan Ter*. 2023;5(1):54-61.
- Isnaini N, Songkro S, Kaewnopparat N, Maneenuan D, Tanmanee N. Formulation And Investigation Of Antioxidant Potential Of O/W Lotions Containing *Tamarindus indica* L. Fruit Pulp Extract. *Matter Int J Sci Technol*. 2019;5(2):100-12.
- Arziyah D, Yusmita L, Wijayanti R. Analisis Mutu Organoleptik Sirup Kayu Manis Dengan Modifikasi Perbandingan Konsentrasi Gula Aren Dan Gula Pasir. *J Hasi Penelit dan Pengkaj Ilm Eksakta*. 2022;1(2):105-9.
- Khairani TN, Fitri K, Novilla L, Shufyani F, Fiska L. Uji Aktivitas Antibakteri Ekstrak Metanol Bunga Tapak Dara (*Catharanthus roseus*) Terhadap Bakteri *Streptococcus pneumoniae* dan Bakteri *Klebsiella pneumoniae*. *J Pharm Sci*. 2022;5(2):438-50.
- Cahyaningrum T, Subhan APB, Rahmawati EN, Zulfa DTNM, Zulfa FA, Erwiyani AR. Paper Soap Daun Belimbing Wuluh Sebagai Skin Moisturizer. *Indones J Pharm Nat Prod*. 2024;07(01):62-71.
- Ambari Y, Saputri AO, Nurrosyidah IHN. Formulasi Dan Uji Aktivitas Antioksidan Body Lotion Ekstrak Etanol Daun Kemangi (*Ocimum cannum* Sims.) dengan Metode DPPH (1,1 - diphenyl-2- picrylhydrazyl). *J As-Syifaa Farm*. 2021;13(2):86-96.
- Oktapiya TR, Pratama NP, Purnamaningsih N. Analisis fitokimia dan kromatografi lapis tipis ekstrak etanol daun rosella (*Hibiscus sabdariffa* L.). *Sasambo J Pharm*. 2022;3(2):105-10.

- Ginaris RP. Lotion Antioksidan Ekstrak Daun Salam (*Syzygium polyanthum* Wight Walp.). *J Kesehat Tujuh Belas*. 2020;2(1):182.
- Setiawan AA, Safitri M, Armayani DT, Herianto G, Marwanta E. Formulation and Antioxidant Effectivity Test of Single Bulb Black Garlic Lotion With DPPH Method (1,1-diphenyl-2-picrylhydrazyl). *Proc 4th Int Conf Sustain Innov 2020–Health Sci Nurs (ICoSIHSN 2020)*. 2020;33(ICoSIHSN 2020):1–7.
- Izza FR, Retnoningsih A, Pukan KKP. Pengembangan Kunci Determinasi Tumbuhan Hasil Eksplorasi Hutan Wisata Guci Kabupaten Tegal Untuk Sekolah Menengah Atas. *Indones J Conserv [Internet]*. 2018;07(01):119–30. Available from: <http://journal.unnes.ac.id/nju/index.php/ijc>
- Rahadyana RZ, Artini KS, Wardani TS. Uji Aktivitas Antioksidan Ekstrak Biji Bunga Matahari (*Helianthus Annuus* L) dengan Menggunakan Metode Dpph (1,1-Diphenyl-2-Picryl Hydrazil). *J Kesehat Tambusai*. 2024;5(3):8049–56.
- Rahmawan A, Mumtaz IJ, Haiefinah R, Hasna LZ, Pareira EM, Riyanto RA, et al. Karakteristik Organoleptik dari tempe modifikasi dengan kacang kedelai hitam (*Glycine max* (L) Merrit). *J Litbang Edusaintech*. 2021;2(2):135–40.
- Sari AE, Oktantia DEN, Kusumowati D, Minati SD. Analisa Organoleptik Produk Es Krim Kedelai Hitam Dengan Penambahan Lidah Buaya. *J Teknol Pangan dan Ind Perkeb*. 2022;2(1):94–9.
- Chaerunnimah, Lestari RS, Asikin H. Fomulasi Minuman Fungsional Berbasis Kacang Gude dan Tempe. *Media Gizi Pangan*. 2022;29(2):53.
- Rahayu LO, Fidyasari A. Organoleptic and Dietary Fiber Quality of Black Pigeon Pea Flour as Bioencapsulation Material. *J Inov Penelit*. 2022;3(4):5911–8.
- Lestari IAY, Ekayani IAPH, Ariani RP. Uji Hedonik Selai Kacang Gude (*Cajanus cajan*). *J Kuliner*. 2025;5(1).
- Badan Pengawas Obat dan Makanan Republik Indonesia. *Farmakope Herbal Indonesia Edisi II*. Jakarta: Badan Pengawas Obat dan Makanan Republik Indonesia; 2017.
- Handoyo DLY, Pranoto ME. Pengaruh Variasi Suhu Pengeringan Terhadap Pembuatan Simplisia Daun Mimba (*Azadirachta Indica*). *J Farm Tinctura*. 2020;1(2):45–54.

- Kusumawulan CK, Rustiwi NS, Sriwidodo S, Bratadiredja MA. Review: Efektivitas Sari Kedelai sebagai Anti-aging dalam Kosmetik. *Maj Farmasetika*. 2022;8(1):1.
- Badriyah L, Fariyah DA. Optimalisasi ekstraksi kulit bawang merah (*Allium cepa* L) menggunakan metode maserasi. *J Sint Penelit Sains, Terap dan Anal*. 2022;3(1):30–7.
- Mewar D. Standarisasi Parameter Spesifik dan Non Spesifik Ekstrak Etanol Daun Gatal (*Laportea decumana*(Roxb.) Wedd) Sebagai Bahan Baku Obat Herbal Terstandar. *J Penelit Kesehat Suara Forikes*. 2023;14(21):266–70.
- Aini DN, Ningsih D, Pramukantoro GE. Uji Efektivitas Patch Ekstrak Daun Sirih Hijau (*Piper betle* L.) pada Penyembuhan Luka Sayat Punggung Kelinci (*Oryctolagus cuniculus*). *J Sains dan Kesehat (J Sains Kes)*. 2023;5(5):837–49.
- Rohmanah A, Sari GK, Hapsari EA. Formulasi dan Uji Stabilitas Fisik Sediaan Serum Ekstrak Etanol 96 % Biji Alpukat (*Persea americana* Mill). *Pratama Med J Kesehat*. 2024;3(2):111–30.
- Tungmunthum D, Drouet S, Lorenzo JM, Hano C. Green extraction of antioxidant flavonoids from pigeon pea (*Cajanus cajan* (L.) Millsp.) Seeds and Its Antioxidant Potentials Using Ultrasound-Assisted Methodology Duangjai. *Molecules* [Internet]. 2021;26(24):1–21. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8703396>
- Fawwaz M, Muliadi DS, Muflihunna A. Kedelai Hitam (*Glycine soja*) Terhidrolisis Sebagai Sumber Flavonoid Total. *J Fitofarmaka Indones*. 2017;4(1):194–8.
- Yusuf M, Agroteknologi PS, Muhammadiyah U, Utara S, Timur M. Pertumbuhan Dan Kualitas Produksi Dua Varietas Kedelai Hitam Akibat Pemupukan Sp-36 Growth and Quality of Production Two Black Soybean Varieties Due To Sp-36 Fertilizer. *J Pertumbuhan*. 2019;22(1):45–51.
- Putri PA, Chatri M, Advinda L, Violita. Characteristics of Saponin Secondary Metabolite Compounds in Plants. *Biol Serambi*. 2023;8(2):251–8.
- Luringunusa E, Sanger G, Sumilat DA, Montolalu RI, Damongilala LJ, Dotulong V. Analisis Fitokimia kuantitatif *Gracilaria verrucosa* Dari Perairan Sulawesi Utara. *J Ilm Platax*. 2023;11(2):451–63.
- Farikhah AN, Mursiti S, Prasetya AT. Uji Aktivitas Antibakteri Senyawa

- Triterpenoid dari Biji Karika (*Carica pubescens*). Indones J Chem Sci [Internet]. 2020;9(2):112–6. Available from: <https://journal.unnes.ac.id/sju/index.php/ijcs/article/view/33173>
- Pratiwi SA, Februyani N, Basith A. Skrining dan Uji Penggolongan Fitokimia dengan Metode KLT pada Ekstrak Etanol Kemangi (*Ocimum basilicum* L) dan Sereh Dapur (*Cymbopogon ciratus*). Pharm Med J. 2023;6(2):140–7.
- Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, et al. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules. 2020;25(22):1–39.
- Syafitri A, Rahma M. Formulasi Dan Uji Efektivitas Sediaan Body Lotion Dari Ekstrak Etanol Daun Asam Jawa (*Tamarindus indica* L .) Sebagai Pelembab Kulit. Best J (Biology Educ Sains Technol. 2023;6(1):501–6.
- Rasydy LOA, Zaky M, Surtiana R. Formulasi dan Evaluasi Fisik Sediaan Hand Body Lotion Ekstrak Etanol Daun Miana (*Pleactranthus scutellarioides* (L.) R. Br.). Pharmauho J Farm Sains, dan Kesehat. 2021;7(1):33–8.
- Halisa N, Frethernety A, Permatasari S, Diana F, Alexandra, Ysrafil. Aktivitas Antioksidan Ekstrak Etanol Bajakah Kalalawit (*Uncaria gambir* (W . Hunter) Roxb) Menggunakan Metode DPPH. J Surya Med. 2024;10(2144–150).
- Nurkhasanah, Bachri MS, Yuliani S. Antioksidan dan Stres Oksidatif. 2023. 1–3 p.
- Sari SL, Zulkarnain AK. Optimasi Formula Lotion O / W Senyawa Oktil Metoksisinamat Dan Uji Aktivitasnya Sebagai Tabir Surya Secara In Vitro. Maj Farm. 2024;20(4):481–9.
- Yulyuswarni. Formulasi dan Evaluasi Lotion Kombinasi Magnesium Oil dan Minyak Biji Kelor (*Moringa Seed Oil*). J Kesehat [Internet]. 2021;12(1):93–100. Available from: <http://ejurnal.poltekkes-tjk.ac.id/index.php/JK>
- Husni P, Ruspriyani Y, Hasanah U. Formulasi dan Uji Stabilitas Fisik Sediaan Lotion Ekstrak Kering Kulit Kayu Manis (*Cinnamomum burmannii*). J Sabdariffarma. 2023;10(1):1–7.
- Aljanah FW, Oktavia S, Noviyanto F. Formulasi dan Evaluasi Sediaan Hand Body Lotion Ekstrak Etanol Daun Semangka (*Citrullus lanatus*) sebagai Antioksidan. Formosa J Appl Sci. 2022;1(5):799–818.
- Shintyawati D, Widiastuti R, Sulistyowati R. Formulasi Dan Uji Stabilitas Fisik

Emulgel Ekstrak Daun Binahong (*Anredera cordifolia*) Sebagai Tabir Surya.
Forte J. 2024;4(1):01-12.