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Research Paper

Fractionation and Formulation of Face Serum from *Citrus hystrix* DC. Fruit Peel Extract as Sunscreen and Skin-Lightening

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Abstract
While the leaves of *C. hystrix* are commonly traded as cooking spices, their fruit cannot be consumed and is still a waste from the harvest. Scholars strive to take benefit of this fruit, especially after discovering that the *C. hystrix* fruit peel extract has better sunscreen and skin-lightening activity than their leaf and pulp extract. Therefore, this study aims to examine the sunscreen and skin-lightening activity of this fruit peel extract fraction, which was formulated as a face serum. The fractionation process began by separating ethanol extract and ethyl acetate extract of peel using column chromatography. The separation of extract fractions was based on Retardation factor (Rf) values. Extract fractions and peel extracts were then tested to observe the sunscreen and skin-lightening activity by calculating the Sun Protecting Factor (SPF), Protection grade UVA (PA), DPPH, and tyrosinase inhibition using the spectrophotometric method. Following that, face serum was formulated as gel and emulgel using chitosan and Carbopol-940 as gelling agents. The results showed that the best extract fraction was presented by fraction-6 of fruit peel ethanol extract (FEEP-6) with an SPF value of 26.790 ± 0.028 and fraction-1 of fruit peel ethyl acetate extract (FEAEP-1) with a PA value of 0.609 ± 0.021. Good extract fraction was also shown in fraction-5 of fruit peel ethanol extract (FEEP-5) with an IC_{50} value against DPPH and tyrosinase of 159.770 μg/mL and 214.156 μg/mL. FEEP-5 was used in the face serum dosage form with a concentration of 0.05%. All face serum dosages met the physical evaluation requirements. The best SPF and antioxidant values of face serum were presented by F4 of 26.505 ± 0.762 and 61.905 ± 0.571%, while the best tyrosinase inhibitor was formulated by F3 of 64.926 ± 0.090%. This study concludes that FEEP-5 and F4 are the best extract fractions and face serum formulas to be used as sunscreen and skin lighteners. In particular, FEEP-5 can be made into a face serum, such as emulgel, sunscreen, and skin lighteners.

Keywords
Kaffir Lime, Antioxidant, Tyrosinase, Gel, Emulgel

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1. INTRODUCTION

Hyperpigmentation is a widespread skin problem caused by excessive melanogenesis, where dark spots appear on the skin, especially the facial skin. Cosmetic products containing skin-lightening agents are considered an alternative to overcome this problem (Hanif et al., 2020). It is not surprising that such cosmetics are always the most popular products for women because lighter skin becomes a lifestyle and increases social status. As a result, the sales of skin-lightening cosmetic products increase every year. Unfortunately, many active ingredients are added to skin-lightening cosmetics, such as hydroquinone, mercury, and corticosteroids. Generally, these can cause adverse side effects if used continuously (Masub and Khachemoune, 2022).

Myriad studies have investigated the process of melanin formation and reported the negative impacts of UV on skin health. For example, continuous ultraviolet (UV) radiation was claimed to stimulate melanogenesis. In addition, it can cause Reactive Oxygen Species (ROS), a free radical in the skin that causes skin damage such as hyperpigmentation, premature aging, and skin cancer (Gromkowska-Kepka et al., 2021). ROS can stimulate melanogenesis in the formation of melanin. Melanin is an amino acid derivative melanocytes produce through a series of enzymatic reactions using tyrosinase as a substrate. Inhibiting the action of tyrosinase results in the inhibition of melanin production (Wang et al., 2024).

Given these detrimental effects, the human body has a natural defense mechanism called melanin biosynthesis. Melanin biosynthesis is very important to increase the body's defense against the harmful effects of UV radiation, especially on skin

cells. However, excessive production can cause hyperpigmentation of the facial skin in the form of black spots. These black spots affect the aesthetics of the facial skin. To address these issues, scholars strive to find alternatives. They found natural ingredients can be an effective and safe melanogenesis inhibitor compared to synthetic compounds in cosmetic formulations (Neto et al., 2022). Plants contain secondary metabolites, such as phenolic compounds and flavonoids, which are perceived to have photoprotective properties against UVA and UVB rays (Filho et al., 2016). Skin-lightening agents derived from nature are still considered safe for use as cosmetic products, especially when they should be applied for a long period. Bioactive compounds in plants can inhibit melanin production in the skin. These compounds have various mechanisms on the melanin biosynthesis pathway, including inhibiting tyrosinase and melanosome transfer and having antioxidant and anti-inflammatory activities (Hanif et al., 2020). Phenolic compounds, flavonoids, anthocyanidins, curcuminoids, quinones, and phenyl derivatives contained in plants can also inhibit the work of tyrosinase. Tyrosinase is a key enzyme in melanogenesis. Inhibiting tyrosinase activity can inhibit the formation of melanin in the skin (Hassan et al., 2023).

Of several plants, kaffir lime leaves (*C. hystrix* plants) are perceived potential because these plants contain phenolic compounds, flavonoids, alkaloids, and triterpenoids. Dichloromethane fractionation of methanol extract of kaffir lime leaves, containing phenolic and flavonoid compounds, has antioxidant activity with an IC_{50} value of 186.20 ± 4.95 μg/mL and at a concentration of 2 mg/mL has an SPF value of 34.03 ± 0.18 (Fernando and Rajapakse, 2022). Additionally, the fractionation of kaffir lime peel extract using solvents with different polarities produces differences in phenolic and flavonoid compounds and has antioxidant activity (Wijaya et al., 2017). Research also suggests that ethyl acetate fraction of ethanol extract from *C. hystrix* peel demonstrated better antioxidant activity than water fraction and hexane fraction, each having an IC_{50} value of 0.03, 1.09, and 2.37 mg/mL (Irawaty and Ayucitra, 2015).

A facial serum is a skincare product that uses active ingredients that can diffuse deeply into the skin. Facial serums can have a variety of effects, such as hydrating, anti-aging, brightening, exfoliating, soothing, and oil-control properties. Serums are highly concentrated products that are water or oil-based. The small amount of ingredients in a serum is intended to maximize the availability of the active ingredients, which could be a growth factor, vitamin, botanical extract, etc. Serums absorb faster and penetrate the deeper layers of the skin to target different areas and provide the most nourished skin. Serum formulas produce a non-greasy finish, fast absorption, and the capacity to penetrate the deeper layers of the skin (Mankar and Vaidya, 2024).

Although the leaves of *C. hystrix* DC. offer numerous benefits, their fruit becomes waste from the harvest because it cannot be consumed. Testing of sunscreen and skin-lightening activities from *C. hystrix* DC. fruit peel extract is still very limited. From the limited research available, some of them still revolve around testing antioxidant activity. If there is any testing of sunscreen activity, the test is conducted on the plants' leaves. Testing of tyrosinase inhibition from all parts of the *C. hystrix* DC. plant has not been carried out. Therefore, this research introduces the novel determination of SPF, PA, antioxidant activity, and tyrosinase inhibition values from fractionation of *C. hystrix* DC. fruit peel extract, along with the development of face serum formulations derived from subfractions of *C. hystrix* DC. fruit peel extract. The face serum formula was prepared in both gel and emulgel forms, using two different gelling agents, namely chitosan and carbopol-940. This was done to identify the best face serum formula for the *C. hystrix* DC. fruit peel extract fraction.

2. EXPERIMENTAL SECTION

2.1 Materials

This section details the materials and reagents used in the study on *Citrus hystrix* DC. fruit peel extract. The first material was *Citrus hystrix* DC. fruit pee, authenticated in Andalas University Herbarium with authentication number 411/K-ID/ANDA/VII/2023). Silica Gel-60 (107733). Other materials included TLC-Silica Gel 60 F254 (105554), methanol (106009), ethanol (100983), Folin-Ciocalteu's phenol reagent (109001), AlCl3 (801081), Na2CO3 (106392), dimethyl sulfoxide (DMSO) (102952), and gallic acid (GA) (842649) from Merck. The research also involved mushroom tyrosinase (T3824), L-ascorbic acid (AA) (A92902), kojic acid (KA) (K3125), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (D9132), quercetin (Q4951), and L-3,4-dihydroxyphenylalanine (L-DOPA) (D9628) retrieved from Sigma-Aldrich. While chitosan was obtained from Chimultiguna, avocado oil was purchased from Happy Green. Carbopol-940 was obtained from Lubrozol and dimethylol-5-5-dimethylhydantoin (DMDM-hydantoin) was from Nguyen BA.

2.2 Methods

2.2.1 Fractionation of *C. hystrix* DC. Fruit Peel Extract

The preparation and fractionation process of *C. hystrix* fruit peel extracts involved multiple steps to isolate active components. Firstly, *C. hystrix* fruit was cleaned, and the flavedo part of the fruit peel was taken, chopped, and macerated using ethanol, ethyl acetate, and hexane, respectively. This process is illustrated in Figure 1. After that, silica Gel-60 was inserted into the chromatography column, followed by the ethanol extract of *C. hystrix* fruit peel (EEP), which was inserted into the chromatography column. The eluents (hexane and ethyl acetate) were inserted successively with various ratios of hexane to ethyl acetate, while the solution was examined using TLC-Silica Gel 60 F254, and the Rf value of the extract fraction was calculated based on the spots formed. Through these processes, solutions that had the same Rf value were combined. The eluent solution was evaporated so that the extract fraction was obtained. The same method was carried out on the ethyl acetate extract of *C. hystrix* fruit peel (EAEP) (Abubakar and Haque, 2020).

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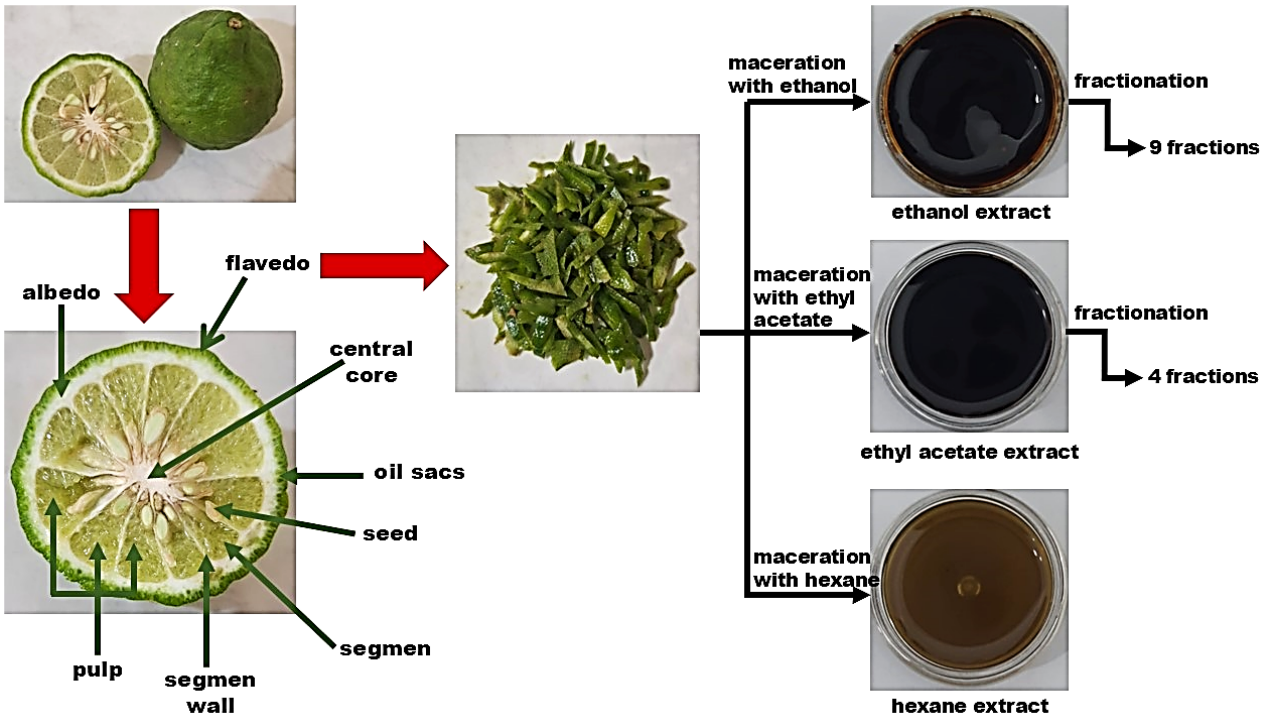

Figure 1. Extraction and Fractionation of *C. hystrix* Fruit Peel

2.2.2 Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The methods used to determine the TPC and TFC in the extracts and fractions involved specific procedures for gallic acid and quercetin. Gallic acid was made in a series of concentrations of 10 to 50 μg/mL. The extract and fraction test solutions were made with a concentration of 5 mg/mL. Each solution was pipetted 0.5 ml, added with 7.5% Folin-Ciocalteu, and left for 8 minutes. Then, 1% Na_2CO_3 was added and incubated for 60 minutes. Following that, this research measured the absorbance at a wavelength of 746 nm. The TPC was determined as mg GAE/g extract. Quercetin was made with a concentration of 10 to 50 μg/mL. Meanwhile, the extract and fraction test solutions were made with a concentration of 5 mg/mL. Each solution was pipetted 0.5 mL, added with ethanol, 10% $AlCl_3$, 1 M Na acetate, and distilled water. The solution was then shaken and let stand for 30 minutes at room temperature. The absorbance was measured at a wavelength of 439 nm. The TFC was determined as mg QE/g extract (Saleem et al., 2023).

2.2.3 Sunscreen Activity Testing

The procedures for measuring the SPF and PA values involved preparing the extracts and fractions at specific concentrations and using a UV-Vis spectrophotometer to measure the absorbance level. During this process, each extract and extract fraction was made at concentrations of 25, 50, and 100 μg/mL in ethanol solvent. Then, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 290-400 nm with an interval of 5 nm. While the measurement of SPF values was conducted at a wavelength of 290-320 nm (UVB), PA values were examined by measuring the UVA/UVB ratio at a wavelength of 290-400 nm (Widyastuti et al., 2024).

2.2.4 DPPH Radical Scavenging Activity

To evaluate the DPPH radical scavenging activity, the extracts and ascorbic acid (AA) were tested at different concentrations. Specifically, the test solution of the extract and extract fraction was prepared at a concentration of 25-400 μg/mL. As a comparison, ascorbic acid (AA) was used with a concentration of 4-20 μg/mL. The test solution was then put into a 96-well plate, and 0.1 mM DPPH solution was added to each well. They were stored at room temperature in a dark place for 30 minutes. As a blank, 0.1 mM DPPH solution was employed. After that, the absorbance was measured at a wavelength of 517 nm. The percentage of DPPH inhibition calculated and made a graph of concentration with the percentage of DPPH inhibition. Finally, the IC_{50} value of each test solution and AA was determined (Guo et al., 2020).

2.2.5 Tyrosinase Enzyme Inhibition Assay

The tyrosinase inhibition activity of the extracts and their fractions was assessed by measuring the inhibition at various concentrations and comparing the results to kojic acid (KA). The extract test solution and extract fraction made a series of 25-400 μg/mL concentrations. As a comparison, kojic acid (KA) 5-100 μg/mL was used. Using a 96-well plate, the solution was put into each well added with 200 UI/mL tyrosinase solution and 10 mM L-DOPA solution in 50 mM phosphate

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buffer pH 6.5. The solution was let at room temperature in a dark place for 60 minutes. The absorbance was then measured at a wavelength of 475 nm. The same work was repeated on the control test solution (without tyrosinase) and the control solution (without extract and fraction of the extract). Then, the percentage of tyrosinase inhibition was calculated and made using a graph of concentration with the percentage of tyrosinase inhibition. Finally, the IC_{50} value of each test solution was determined (Widyastuti et al., 2023).

2.2.6 Face Serum Formulation

The formulation and evaluation of the face serum in gel and emulgel forms were conducted to assess its stability and physical properties. The face serum dosage form was made using chitosan and carbopol-940 gelling agents. The formula used FEEP-5 with a concentration of 0.05%, as shown in Table 1. Then, the gel and emulgel dosage form formulas were subjected to physical evaluation tests, including organoleptic, homogeneity, pH uniformity, viscosity, and emulsion type (emulgel), which were observed for six weeks. Finally, stability testing was conducted using the freeze-thaw method for six cycles (12 days).

2.2.7 Determination of Sunscreen and Skin-Lightening Face Serum

The testing of the face serum preparations for SPF, PA, antioxidant activity, and tyrosinase inhibition was carried out as follows. First, each dosage form of face serum was weighed 1.0 g (equivalent to 0.5 mg of extract). The dosage form was then dissolved with distilled water up to 10 mL. The test solution was put into a cuvette, and the absorbance was measured at a wavelength of 290-400 nm with an interval of 5 nm. After that, the SPF and PA values of the dosage form were determined. The test solution was put into a 96-well plate, added 0.1 mM DPPH solution, and stored at room temperature in a dark place for 30 minutes. The absorbance was measured at a wavelength of 517 nm. The percentage of DPPH inhibition was calculated. Using a 96-well plate, the test solution was added 200 IU/mL tyrosinase and 10 mM L-DOPA solution in 50 mM phosphate buffer pH 6.5. Then, it was let at room temperature in a dark place for 60 minutes. The absorbance was measured at a wavelength of 475 nm.

3. RESULT AND DISCUSSION

3.1 Fractionation of *C. hystrix* DC. Fruit Peel Extract

This study investigated the fractionation of *Citrus hystrix* fruit peel extracts to identify and isolate compounds beneficial for skin protection and lightening applications. In this study, fractionation on the fruit peel's ethanol extract obtained 9 ethanol extract fractions (FEEP) and 4 ethyl acetate extract fractions (FEAEP) of *C. hystrix* fruit peel. The extract fractions were separated based on the Rf values. All extract fractions were subjected to TPC and TFC determination and testing of sunscreen and skin-lightening activities. Building on previous findings that *C. hystrix* fruit peel extract had better sunscreen and skin-lightening activities than *C. hystrix* leaf and pulp extracts, this study focused on the *C. hystrix* fruit peel. Only EEP and EAEP were fractionated because HEP lacked phenolic and flavonoid compounds according to phytochemical screenings. Fractionation using column chromatography aims to separate compounds from a mixture based on their size, shape, and charge, using a mobile phase, which is an extraction solvent, and a dian phase, such as silica gel. The silica gel is used to separate amino acids, sugars, fatty acids, lipids, and alkaloids (Abubakar and Haque, 2020). The obtained extract fractions were subjected to TPC and TFC determination and sunscreen and skin-lightening activity testing.

3.2 TPC and TFC of *C. hystrix* DC. Fruit Peel Extract Fraction

As shown in Table 2, the TPC and TFC of the extract fractions showed that fraction-5 ethanol extract (FEEP-5) had a higher TPC than other extract fractions. There was an increase in TPC content in FEEP-5 compared to EEP. FEEP-5 also had a higher TFC compared to other extract fractions, but the TFC content was lower than EEP. All extract fractions had lower TFC than the extract. TPC and TFC of FEEP-5 were greater than TPC by 13.47 mg GAE/g extract and TFC value of 4.9 mg QE/g extract (Desmiaty et al., 2024). Phenolic compounds in plants are widely used in dermatology and cosmetics. Phenolic compounds overcome ROS in the skin, inhibit melanin synthesis, protect from UV radiation, modulate antioxidant enzyme activity, and prevent skin cancer (Nisa et al., 2024). *C. hystrix* has 78 characterized compound components, where the main compounds include coumarin, flavonoids, phenolic acids, and terpenoids, and have activity on the skin as antioxidants and anti-inflammatories (Zhao et al., 2023; Siti et al., 2022).

In this study, the part of *C. hystrix* used for investigation is the fruit peel. Fruit peel is rich in bioactive compounds, especially polyphenols, which are very useful for overcoming symptoms of damage to the body due to oxidative stress. One example of efficacy is the use of orange peels, which help overcome pollution problems caused by waste disposal (Rafiq et al., 2018). Other research emphasizes that bioactive compounds in plants, such as polyphenols, have effects on the skin, such as increasing elasticity, reducing wrinkles and dryness, and serving as an anti-aging (Michalak et al., 2021). The extract fraction showed better antioxidant activity compared to the extract form. There is a strong correlation between antioxidant activity and TPC and TFC (Lukman et al., 2024). Qualitative analysis of the compounds contained in the *C. hystrix* fruit peel extract was not carried out in this research. This is a limitation of the research conducted.

3.3 Sunscreen Activity of *C. hystrix* DC. Fruit Peel Extract Fraction

The sunscreen activity results provide insight into the protective potential of *C. hystrix* extracts. The sunscreen activity of the extract is presented in Table 3. It measures the SPF and PA values. The test results showed that FEEP-6 had the best SPF

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Table 1. Formula Face Serum Dosage Form

Component	Formula							
	Base (%)				Formula FEEP-5 (%)			
	B1	B2	B3	B4	F1	F2	F3	F4
FEEP-5	-	-	-	-	0.05	0.05	0.05	0.05
Chitosan	3	-	3	-	3	-	3	-
Acetic acid 1%	40	-	40	-	40	-	40	-
NaOH 0.1 N	5	-	5	-	5	-	5	-
Carbopol-940	-	1	-	1	-	1	-	1
TEA	-	0.5	-	0.5	-	0.5	-	0.5
Avocado oil	-	-	5	5	-	-	5	5
Tween-80	-	-	25	25	-	-	25	25
PEG-400	-	-	5	5	-	-	5	5
Propylene glycol	10	10	10	10	10	10	10	10
DMDM Hydantoin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Aquadest ad	100	100	100	100	100	100	100	100

Table 2. TPC and TFC of *C. hystrix* Fruit Peel Extract Fraction

Sample	TPC (mg GAE / g extract)	TFC (mg QE / g extract)
FEEP-1	77.059 ± 0.216	9.951 ± 1.136
FEEP-2	98.866 ± 1.202	0.990 ± 0.223
FEEP-3	103.752 ± 1.568	19.896 ± 0.663
FEEP-4	110.455 ± 2.775	6.945 ± 0.621
FEEP-5	153.009 ± 0.971	11.317 ± 0.739
FEEP-6	142.729 ± 1.978	10.934 ± 0.164
FEEP-7	118.866 ± 2.619	6.563 ± 0.250
FEEP-8	94.255 ± 0.922	1.307 ± 0.177
FEEP-9	83.539 ± 0.601	0.804 ± 0.081
FEAEP-1	31.265 ± 1.095	1.181 ± 0.124
FEAEP-2	61.296 ± 1.522	0.782 ± 0.059
FEAEP-3	98.617 ± 0.748	0.661 ± 0.250
FEAEP-4	91.826 ± 0.863	1.973 ± 0.527
EEP	115.502 ± 1.029	29.623 ± 0.913
EAEP	120.112 ± 1.041	33.612 ± 0.809

TPC = total phenolic content, TFC = total flavonoid content, GAE = gallic acid equivalent, QE = quercetin equivalent, FEE-1= fraction-1 ethanol extract, FEE-2 = fraction-2 ethanol extract, FEE-3 = fraction-3 ethanol extract, FEE-4 = fraction-4 ethanol extract, FEE-5 = fraction-5 extract, FEE-6 = fraction-6 ethanol extract, FEE-7 = fraction-7 ethanol extract, FEE-8 = fraction-8 ethanol extract, FEE-9 = fraction-9 ethanol extract, FEAE-1 = fraction-1 ethyl acetate extract, FEAE-2 = fraction-2 ethyl acetate extract, FEAE-3 = fraction-3 ethyl acetate extract, FEAE-4 = fraction-4 ethyl acetate extract, EEP = ethanol extract of *C. hystrix* peel, EAEP = ethyl acetate extract of *C. hystrix* peel

value. At a concentration of 25 μg/mL, the SPF value was higher than the extract and fruit peel extract fraction. At a concentration of 100 μg/mL, FEEP-6 was classified as very good. However, this was not followed by the PA value, where FEEP-6 had a PA value with a moderate category. The best PA value in EEP at a concentration of 25 μg/mL was 0.861 ± 0.022, which fell into a maximum category (star 4).

Given these findings, it is essential to consider the broader impact of sun exposure on skin health and the role of plant-based compounds in photoprotection. The area of skin exposed to sunlight is susceptible to photoaging. It may cause dry skin, uneven pigmentation, lentigo, hyperpigmentation, wrinkle formation, and decreased skin elasticity. Strikingly, polyphenol compounds in plants have the potential to be photoprotective, where the integration of polyphenol compounds into sunscreen preparations will be able to protect the skin from the adverse effects of UV radiation exposure. In addition, polyphenol compounds can overcome oxidative disorders, inflammatory cascades, and DNA damage to the skin (Sharma et al., 2024).

Sunscreen products protect the skin from UV radiation based on their ability to absorb, reflect, or scatter sunlight. In nature-based products, compounds derived from nature are protective agents that have biological activities such as antioxidant effects and anti-inflammatory. Many studies show that extracts or compounds from natural ingredients have photoprotective activity and are safe and easy-to-use natural sunscreens (Potey et al., 2023). In this study, it was found that the *C. hystrix* fruit peel extract fraction contained phenolic and flavonoid compounds and had better sunscreen activity than fruit peel extract.

3.4 Skin Lightening Activity of C. hystrix DC Fruit Peel Extract Fraction

Skin lightening activity can be identified by measuring the antioxidant activity and tyrosinase inhibition of *C. hystrix* DC. fruit peel extract fraction based on the IC_{50} value obtained. One of the simple ways to determine antioxidant activity is

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Table 3. Sunscreen Activity of *C. hystrix* Fruit Peel Extract Fraction

Sample	SPF value*			PA value**		
	25 μg/mL	50 μg/mL	100 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL
FEEP-1	1.491 ± 0.022	1.528 ± 0.268	4.464 ± 0.139	0.738 ± 0.008	0.553 ± 0.004	0.323 ± 0.007
FEEP-2	2.040 ± 0.046	3.875 ± 0.048	10.832 ± 0.125	0.603 ± 0.010	0.382 ± 0.003	0.191 ± 0.001
FEEP-3	3.285 ± 0.193	8.289 ± 0.215	22.034 ± 0.196	0.422 ± 0.017	0.219 ± 0.004	0.127 ± 0.001
FEEP-4	4.034 ± 0.259	10.956 ± 0.066	25.427 ± 0.236	0.367 ± 0.017	0.187 ± 0.001	0.127 ± 0.001
FEEP-5	3.514 ± 0.107	10.145 ± 0.437	25.983 ± 0.378	0.413 ± 0.008	0.203 ± 0.005	0.128 ± 0.002
FEEP-6	4.040 ± 0.217	11.038 ± 0.204	26.790 ± 0.028	0.378 ± 0.015	0.196 ± 0.002	0.134 ± 0.002
FEEP-7	3.795 ± 0.156	10.226 ± 0.258	24.655 ± 0.296	0.388 ± 0.006	0.196 ± 0.003	0.130 ± 0.001
FEEP-8	3.415 ± 0.066	6.993 ± 0.045	9.161 ± 0.047	0.430 ± 0.008	0.298 ± 0.001	0.363 ± 0.002
FEEP-9	3.358 ± 0.113	6.536 ± 0.059	8.513 ± 0.014	0.433 ± 0.010	0.310 ± 0.001	0.372 ± 0.002
FEAEP-1	1.248 ± 0.066	1.528 ± 0.268	1.969 ± 0.103	0.855 ± 0.029	0.745 ± 0.080	0.609 ± 0.021
FEAEP-2	1.513 ± 0.343	1.775 ± 0.069	2.814 ± 0.038	0.768 ± 0.115	0.677 ± 0.022	0.498 ± 0.006
FEAEP-3	2.312 ± 0.054	4.706 ± 0.059	13.423 ± 0.338	0.544 ± 0.008	0.329 ± 0.003	0.169 ± 0.002
FEAEP-4	2.234 ± 0.347	3.929 ± 0.093	10.487 ± 0.262	0.574 ± 0.059	0.386 ± 0.006	0.208 ± 0.006
EEP	1.364 ± 0.038	1.751 ± 0.094	3.276 ± 0.123	0.861 ± 0.022	0.780 ± 0.011	0.607 ± 0.011
EAEP	1.444 ± 0.025	2.013 ± 0.022	4.189 ± 0.088	0.830 ± 0.014	0.710 ± 0.004	0.508 ± 0.005
EHP	1.662 ± 0.102	2.747 ± 0.119	6.248 ± 0.075	0.669 ± 0.030	0.454 ± 0.016	0.245 ± 0.003

EEP = ethanol extract of *C. hystrix* peel, EAEP = ethyl acetate extract of *C. hystrix* peel, EHP = hexane extract of *C. hystrix* peel *grade of SPF; 15-24 (good protection), 25-39 (very good protection), and > 40 (excellent protection.
**star category description of UVA ratio; < 0.2 (too low for UVA claim), 0.2– < 0.4 (moderate), 0.4– < 0.6 (+ + good), 0.6– < 0.8 (+ + + superior), and ≥ 0.8 (+ + + + maximum) (Donglikar and Deore, 2016).

Table 4. IC_{50} Value of Inhibition of *C. hystrix* Fruit Peel Extract Fraction

Sample	DPPH Inhibition			Tyrosinase Inhibition		
	Linear Equation	R^2	IC_{50} (μg/mL)	Linear Equation	R^2	IC_{50} (μg/mL)
FEEP-1	y = 0.0230x + 2.4180	0.9834	2068.783	y = 0.0631x – 1.0811	0.9503	809.526
FEEP-2	y = 0.0957x + 4.6109	0.9832	467.929	y = 0.0805x + 0.6720	0.9871	612.770
FEEP-3	y = 0.1405x + 8.4647	0.9823	295.625	y = 0.1198x + 1.5686	0.9894	404.269
FEEP-4	y = 0.1620x + 9.0458	0.9491	252.804	y = 0.0952x + 18.612	0.9686	329.706
FEEP-5	y = 0.1611x + 24.2610	0.9596	159.770	y = 0.1256x + 23.102	0.9831	214.156
FEEP-6	y = 0.1970x + 7.1046	0.9642	217.743	y = 0.1567x + 6.2106	0.9642	279.447
FEEP-7	y = 0.1700x + 2.6699	0.9722	278.412	y = 0.1647x + 1.5858	0.9985	293.954
FEEP-8	y = 0.0762x + 1.9180	0.9980	630.997	y = 0.0464x + 2.1304	0.9399	1031.672
FEEP-9	y = 0.1105x + 3.9431	0.9858	416.805	y = 0.0763x + 14.295	0,9621	467.955
FEAEP-1	y = 0.0892x + 23.883	0.9405	292.791	y = 0.0752x + 6.1970	0.9665	582.487
FEAEP-2	y = 0.0657x – 0.2320	0.9972	764.566	y = 0.1152x + 3.4935	0.9910	403.702
FEAEP-3	y = 0.1676x + 10.5540	0.9573	235.358	y = 0.0786x – 1.3239	0.9755	619.289
FEAEP-4	y = 0.1511x + 8.0330	0.9708	277.743	y = 0.0659x + 1.0200	0.9929	743.247
EEP	y = 0.1621x + 8.5992	0.9903	255.403	y = 0.1275x + 4.7157	0.9850	355.171
EAEP	y = 0.1675x + 21.2340	0.9570	171.737	y = 0.1273x + 0.3369	0.9817	390.126
EHP	y = 0.0174x + 9.9223	0.9974	2303.316	y = 0.0593x + 1.9495	0.9438	810.295
AA	y = 5.5258x + 0.5361	0.9990	8.951			
KA				y = 0.4458x + 37.1900	0.9640	28.735

AA = ascorbic acid, KA = kojic acid

using the DPPH analysis. The analysis showed that FEEP-5 had better antioxidant activity than other extract fractions. As shown in Table 4, there is an increase in the antioxidant activity of FEEP-5 compared to EEP, but the antioxidant activity decreased when EAEP was fractionated.

In comparison to other extraction methods and previous studies, the antioxidant profile of *C. hystrix* fruit peel fractions varies. For example, the antioxidant activity was lower than ultrasonicated ethanol extracts with an IC_{50} value of 125.62 μg/mL (Desmiaty et al., 2024). Another study showed sim-

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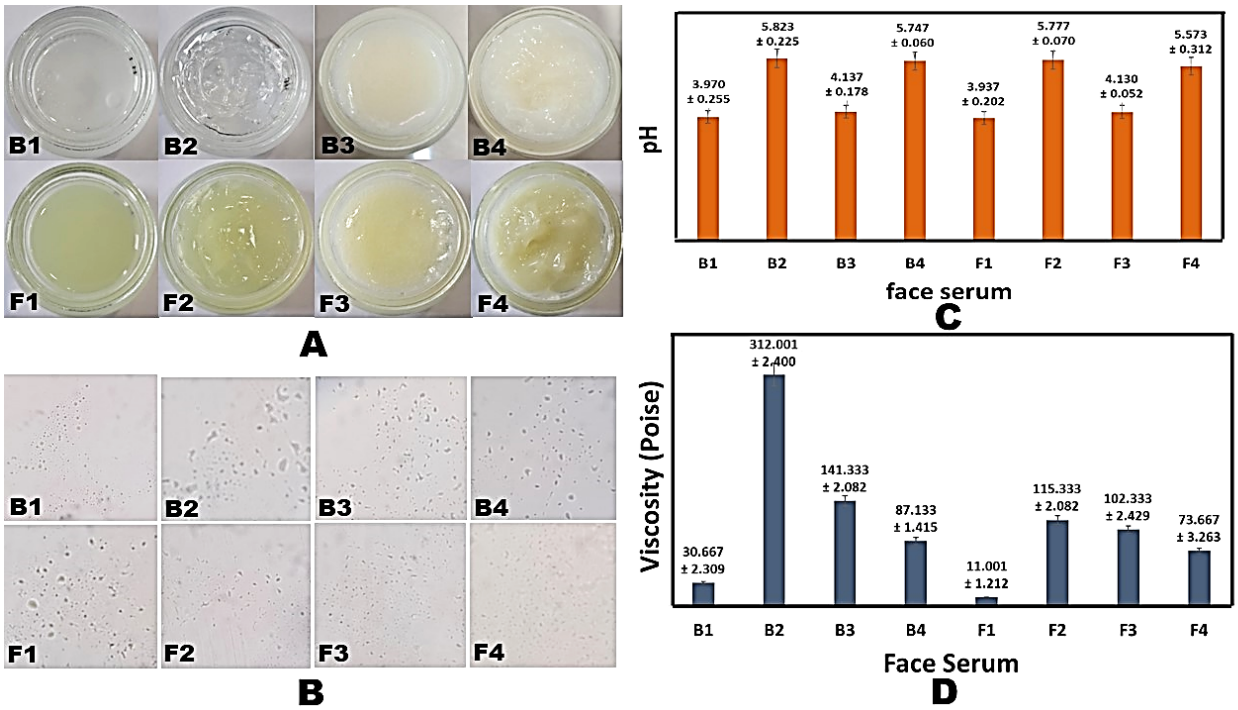

Figure 2. Physical Evaluation of Face Serum Dosage Form. Organoleptic (A), Homogeneity (B), pH (C), and Viscosity (D)

ilar findings as they obtained better antioxidant activity than the ethanol extract of dried *C. hystrix* fruit peel, which had an IC_{50} value of 249.43 μg/mL (Darsono et al., 2024). Additionally, the water extract of *C. hystrix* fruit peel had a high content of phenolic compounds, flavonoids, carotenoids, and anthocyanins, which are associated with its high antioxidant activity (24). The antioxidant activity of the compounds 11-hydroxynoracronycine, (+)-syringaresinol isolated from *C. hystrix* plants and water extract of *C. hystrix* using DPPH assay had IC_{50} values of 190, 32, and 14910 μg/mL. The antioxidant capacity of the fractions decreased in the following order: ethanol extract, chloroform extract, and methanol extract. *C. hystrix* extract with a dose of 50 μg/mL can reduce ROS from the formation of ROS induced by H_2O_2 (Zhao et al., 2023). The butanol fraction of ethanol extract of *C. hystrix* fruit peel has the highest antioxidant activity compared to the hexane and ethyl acetate fractions, which have IC_{50} values of 442.4 μg/mL (Wijaya et al., 2017).

FEEP-5 demonstrated better tyrosinase inhibitory activity than extract and EEP fractions (Table 4). This activity aligns with the higher TPC and TFC in FEEP-5, suggesting that the increase of these compounds may contribute to its enhanced tyrosinase inhibition. Several plant-based compounds, such as phenolics, terpenes, steroids, chalcones, flavonoids, alkaloids, long-chain fatty acids, and coumarins, are known to inhibit melanogenesis by inhibiting the activity of the tyrosinase enzyme reversibly or irreversibly. For instance, aloesin, a compound isolated from aloe vera, inhibits tyrosinase competitively. Hence, they can be used as promising depigmenting agents for cosmetic applications (Kumari et al., 2018).

Moreover, high phenolic compounds and antioxidant activity from plant extracts, like FEEP-5, can support anti-melanogenic effects by inhibiting melanogenesis pathways. These findings emphasize the potential of FEEP-5 and similar extracts in developing natural skin lighteners. Further, in vivo studies can be conducted in the future to assess the safety and effectiveness of these natural ingredients for cosmetic use (Neto et al., 2022).

3.5 Formulation of Face Serum Dosage Form

Although FEEP-6 has a high SPF value, FEEP-5 was used in the formulation of face serum preparations because it had better antioxidant and tyrosinase inhibition activity. The face serum was developed in gel and emulgel forms using chitosan (a natural ingredient) and carbopol-940 (a synthetic polymer) as a gelling agent. As a rapidly growing skin care product, serums are now available in various cosmetic formulations tailored for different skin types, such as oily, dry, or other skin types. The right serum formulation can optimize the use of skin care cosmetics such as moisturizers, cleansers, sunscreens, skin brighteners, and anti-aging. Serums that are added with certain ingredients according to the purpose of use show the desired effects (Shejul and Kudale, 2023). Moreover, serums are favored for their fast absorption and deep penetration into the deepest layer of skin (hypodermis) while maintaining non-greasy results so that they are more comfortable for use. Thus, it is necessary to conduct physical evaluation tests of the preparation, such as physical appearance, pH, viscosity, and stability tests (Rajdev et al., 2022).

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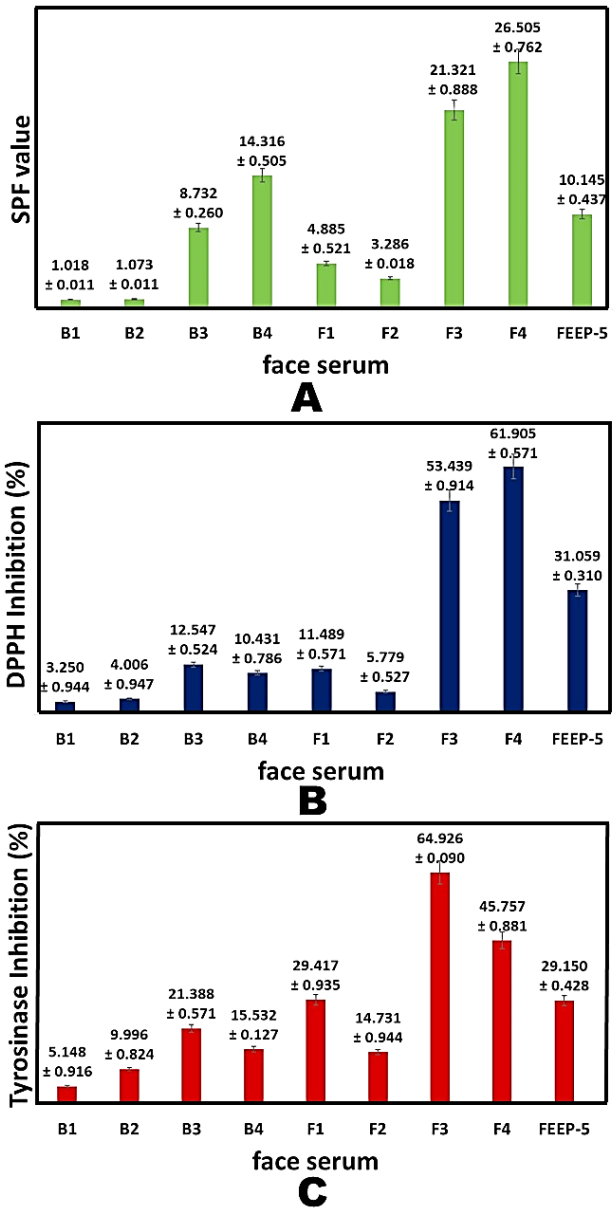

Figure 3. Activity of Face Serum Dosage Form as Sunscreen and Skin-Lighteners. SPF Value (A), DPPH Inhibition (B), and Tyrosinase Inhibition (C)

The face serum formulation containing 0.05% FEEP-5 is designed to function as both a sunscreen and a skin lightener. Physical evaluations indicate that the face serum is a semi-solid dosage form (Figure 2A). Under 400× magnification with a microscope, the face serum dosage form exhibits a homogeneous composition (Figure 2B). The pH of the face serum ranges from 3.937 ± 0.202 to 5.823 ± 0.225, which falls within an acceptable range for skin application (Figure 2C). Although the viscosity of the preparation decreased slightly with the addition of FEEP-5, it remained within the semi-solid category (Figure 2D). Stability tests show that the serum is stable during both storage and freeze-thaw testing. The emulgel version of the face serum has an oil-in-water (o/w) emulsion type, which makes it easily washable and stable during centrifugation. Overall, these evaluations confirm that FEEP-5 can be effectively formulated into face serum gel and emulgel dosage form using chitosan and carbopol-940 as gelling agents.

3.6 Sunscreen and Skin-Lightening Activity of Face Serum

In this study, the best sunscreen activity is presented by F4, with an SPF value of 26.505 ± 0.762, falling into a very good protection category and a PA value of 0.341 ± 0.041 with a moderate category (Figure 3A). The study also observed an increase in the SPF value of FEEP-5 after being formulated as emulgel with carbopol-940 as a gelling agent. Meanwhile, B4 has sunscreen activity, although there is a difference between B4, FEEP-5, and F4.

The present study also argues that the active ingredients added to sunscreen dosage form can be synthetic or natural substances. A number of synthetic agents have serious side effects on facial skin, such as photoallergic dermatitis, environmental pollution, and lack of vitamin D production. In contrast, cosmetic formulations containing the right natural ingredients can increase protection against UV radiation and avoid the side effects of synthetic agents (Mewada and Shah, 2023). The effectiveness of sun protection depends directly on the sunscreen product, how it is used, and the amount used. Factors that influence effectiveness include sun exposure conditions (direct or indirect), level of protection (SPF and PA), the amount of the product, maximum exposure period before reapplication, type of preparation, required layer thickness, coverage, and ability to spread and penetrate the skin (Portilho et al., 2022). Natural ingredients that have been tested for effectiveness and safety on the skin can be developed into a skincare cosmetic product for therapeutic and beauty care purposes (Sharma et al., 2022).

Based on these findings, the best antioxidant activity was given by F4, which had an antioxidant capacity of 61.905 ± 0.571%. There was an increase in the antioxidant capacity of FEEP-5 after it was made into a face serum emulgel form using carbopol-940 as a gelling agent (Figure 3B). Bioactive plant ingredients that have antioxidant effects are often added to cosmetic preparations, especially skincare cosmetics (Arora et al., 2019). Evaluating ingredients added to cosmetic preparations is important to consider the possibility of long-term effects such as contact dermatitis and allergic reactions. Therefore, it is necessary to test the effectiveness and safety of the preparation (Panico et al., 2019). The use of plant-derived ingredients aims to produce products that are safer to use than synthetic ingredients. As a natural ingredient that shows antioxidant activity, it outlines the ability of plants to adapt to survive in a highly oxidative environment. Therefore, the antioxidant activity of plants has the potential to help reduce or protect the skin from the harmful effects of UV radiation (Liu, 2022). Antioxidants can overcome ROS in the skin due to UV radiation exposure, whereas antioxidants can increase the endogenous ROS elimination system in tissue and help overcome skin in-

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fections and aging. In this way, antioxidants can be used as anti-aging for the skin (Neha et al., 2019).

The best tyrosinase inhibition activity of face serum is presented by F3, which has a tyrosinase inhibition capacity of 64.926 ± 0.090%. This study discovered an increase in the tyrosinase inhibition capacity of FEEP-5 after it was made into a face serum emulgel form using chitosan as a gelling agent (Figure 3C). Tyrosinase is an important enzyme in melanogenesis. Therefore, its inhibitors can attract interest from the cosmetic industry as depigmentation agents for skin-lightening products. In general, tyrosinase inhibition is tested in the presence of mono phenolic substrates such as tyrosine or diphenolic substrates such as L-DOPA. Its activity is typically assessed based on the formation of dopachrome. The reactions that occur are oxidation and reduction reactions, so materials that act as antioxidants are likely to have activity as tyrosinase inhibitors (Zolghadri et al., 2019).

Flavonoid compounds are mostly found in orange peel (both in the flavedo and albedo) which have activity as free radical scavengers or as antioxidants (García-Nicolás et al., 2023). Phenolic compounds that have antioxidant activity from natural materials are likely to have anti-melanogenic effects, which suggests that inhibition of melanogenesis can be achieved by substances containing phenolic compounds (Neto et al., 2022). Face serum contains FEEP-5 of 0.05%, equivalent to 0.0765 mg GAE/g face serum. Research conducted by Mapoung et al. (2021) stated that the total phenolic content in cream samples was in the range of 0.0153–1.5973 mg GAE/g cream showing antioxidant activity and tyrosinase inhibitory activity, where the tyrosinase inhibitory capacity was in the range of 2.58–97.94%.

4. CONCLUSIONS

C. hystrix is a plant that is cultivated to harvest its leaves as a cooking spice, causing the fruit to become a waste because it cannot be consumed. Interestingly, previous studies have shown that *C. hystrix* fruit peel extract has better sunscreen and skin-lightening activity than their leaf and pulp extracts. This study followed up on this topic and found that the *C. hystrix* fruit peel extract fraction increased the sunscreen and skin-lightening activity of the fruit's peel extract. The sunscreen activity of FEEP-6 obtained an SPF value of 26.790 ± 0.028 at a concentration of 100 μg/mL with a very good protection category. Meanwhile, the PA value of FEAEP-1 was 0.609 ± 0.021 at a concentration of 100 μg/mL with a superior category (star 3). The best antioxidant and tyrosinase inhibition activity was FEEP-5, with an IC_{50} value against DPPH of 159.770 μg/mL and an IC_{50} value against tyrosinase of 214.156 μg/mL. Then, the best extract fraction overall was FEEP-5. Thus, it can be used in face serum preparations with a concentration of 0.05%. All face serum dosage form have done physical evaluations that meet organoleptic requirements, homogeneity, pH, viscosity, and stability. Face serum in the form of emulgel has an o/w emulsion type. The best SPF and antioxidant values of face serum are presented by F4 of 26.505 ± 0.762 and 61.905 ± 0.571%, while the best skin brightener of face serum is presented by F3 of 64.926 ± 0.090%. FEEP-5 can be made into a face serum in emulgel form as a sunscreen and skin lightener.

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