

ANTIOXIDANT AND ANTI-TYROSINASE ACTIVITIES OF NANOCREAM OF RESAM LEAVES (*Dicranopteris linearis*) AND JERUK KUNCI FRUIT WASTE (*Citrus x microcarpa*) EXTRACT

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Abstract: Premature aging especially among women has become a serious problem that impacts self-confidence. This is caused by exposure to excess ultraviolet radiation which induces the formation of reactive oxygen species and increases the activity of tyrosinase enzymes in the melanogenesis process. This study examined the antioxidant and anti-tyrosinase capabilities of nanocream formulated with *Dicranopteris linearis* (resam) leaves and *Citrus x microcarpa* (jeruk kunci) fruit waste extracts sourced from Bangka Belitung. A stepwise microwave-assisted extraction process was conducted using three solvents n-hexane, ethyl acetate, and ethanol with ethanol proving most effective at extracting bioactive compounds (64.97% yield from resam leaves and 21.12% from jeruk kunci fruit waste). Phytochemical screening showed that ethanol extracts contained the highest levels of active compounds: resam extracts had 179.78 mg GAE/g total phenolics and 90.33 mg QE/g total flavonoids, while jeruk kunci fruit waste extracts contained 135.65 mg GAE/g phenolics and 72.64 mg QE/g flavonoids. Physical characterization confirmed the nanocream met quality standards specified in Indonesian National Standard SNI 16-4954-1998. Biological testing revealed strong antioxidant properties, with the nanocream achieving IC₅₀ values between 7.34 and 16.36 µg/mL markedly better than single extracts alone (IC₅₀ 29.58–72.33 µg/mL). The formulation also showed considerable tyrosinase inhibitory activity at IC₅₀ 100.16 µg/mL. The results indicate the potential of resam leaves and jeruk kunci fruit waste extract nanocream as an innovative natural ingredient-based anti-aging cosmetic preparation.

Keywords: Antioxidant; *Dicranopteris linearis*; *Citrus x microcarpa*; Nanocream; Tyrosinase

Abstrak: Penuaan dini terutama di kalangan wanita telah menjadi masalah serius yang berdampak pada kepercayaan diri. Hal ini disebabkan oleh paparan radiasi ultraviolet berlebih yang menginduksi pembentukan *reactive oxygen species* dan meningkatkan aktivitas enzim tirosinase dalam proses melanogenesis. Penelitian ini bertujuan mengembangkan sediaan nanokrim anti-aging dari ekstrak daun resam (*Dicranopteris linearis*) dan buah jeruk kunci (*Citrus x microcarpa*) asal Bangka Belitung. Ekstraksi bertingkat dengan metode *Microwave Assisted Extraction* menggunakan pelarut n-heksana, etil asetat, dan etanol menunjukkan rendemen tertinggi pada ekstrak etanol (64,97% daun Resam; 21,12% Jeruk Kunci). Ekstrak etanol kedua sampel mengandung senyawa fenolik dan flavonoid tertinggi dengan kadar fenolik total 179,78 mg GAE/g (daun resam) dan 135,65 mg GAE/g (limbah buah jeruk kunci) serta kadar flavonoid

total 90,33 mg EK/g (daun resam) dan 72,64 mg QE/g (limbah buah jeruk kunci). Formulasi nanokrim menunjukkan karakteristik fisik memenuhi standar SNI 164954-1998. Sediaan nanokrim menunjukkan aktivitas antioksidan sangat kuat (IC_{50} 7,34-16,36 $\mu\text{g/mL}$) dengan peningkatan signifikan dibandingkan ekstrak tunggal (IC_{50} 29,58-72,33 $\mu\text{g/mL}$) serta aktivitas anti-tirosinase kategori kuat (IC_{50} 100,16 $\mu\text{g/mL}$) melalui penghambatan enzim tirosinase. Hasil penelitian mengindikasikan potensi nanokrim ekstrak daun resam dan limbah buah jeruk kunci sebagai inovasi sediaan kosmetik anti-aging berbasis bahan alam.

Kata kunci: Antioksidan; *Dicranopteris linearis*; *Citrus x microcarpa*; Nanokrim; Tirosinase

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Introduction

The phenomenon of premature aging of the skin, especially among women, has become a serious problem that needs to be addressed. According to a survey by JakPat (2021), 60% of Indonesian women aged 20 years experience decreased self-confidence due to symptoms of premature aging. The main etiology of this condition is exposure to excess ultraviolet radiation which induces the formation of Reactive Oxygen Species (ROS) and increases the activity of the enzyme tyrosinase in the process of melanogenesis (Sholikha & Wulandari, 2022). This process stimulates an increase in free radicals that have the potential to degrade the dermal matrix (Furi *et al.*, 2022). To overcome this, antioxidant compounds are needed that donate radical hydrogen atoms to radical compounds so that they can prevent the process of premature aging. This study explores the synergistic effects of *Dicranopteris linearis* leaves and *Citrus x microcarpa* fruit waste extracts in nanocream formulation for enhanced skin anti-aging efficacy.

Bangka Belitung is rich in medicinal plants, including *Dicranopteris linearis* (resam) and *Citrus x microcarpa* (jeruk kunci), which have been traditionally used by local communities for skin health and wound healing. These plants are abundantly available as agricultural waste, making them economically viable sources for cosmeceutical development. Ethnobotanically, both are often used in traditional medicine such as resam leaves as a medicine for fever, cough, asthma and an alternative to facial soap and jeruk kunci fruits as a sour flavor in food and drinks (Roanisca *et al.*, 2021). Pratiwi *et al.* (2023) reported that ethanol extract of resam leaves using the MAE method has very strong antioxidant activity with an IC_{50} value of 24.12 $\mu\text{g/mL}$. Meanwhile, according to Gustiriani (2023), the jeruk kunci fruits extract nanocream showed very strong antioxidant activity with an IC_{50} value of 49.021 $\mu\text{g/mL}$. Microwave Assisted Extraction (MAE) method was

chosen to optimize the extraction of compounds due to shorter extraction process, less solvent volume, and higher yield compared to conventional methods.



Figure 1. *Dicranopteris linearis* Burm. **Figure 2.** *Citrus x microcarpa* Bunge.

The effectiveness of antioxidants from natural ingredients as anti-aging can be enhanced through the formulation of topical preparations such as nanocream. Nanocream is a nanoemulsion-based semisolid preparation with material particle sizes ranging from 1-100 nm (Rahman & Herdaningsih, 2021). These nano size characteristics facilitate even dispersion on the skin surface, increase the penetration efficiency of active substances, and minimize side effects. In addition, nanocream formulations also contribute to improved dosage stability and extended shelf life. Based on the literature review, it can be concluded that the antioxidant content in resam leaves extract and jeruk kunci fruits has the potential to prevent premature aging in nanocream preparations.

Method

Materials

Plant materials used in this research, namely resam leaves (*Dicranopteris linearis*) and jeruk kunci fruit waste (*Citrus x microcarpa*), were taxonomically identified and authenticated at the Integrated Basic Laboratory, Universitas Bangka Belitung. Materials used such as resam leaves, jeruk kunci fruits waste, n-hexane (Merck), ethyl acetate (Merck), ethanol (Merck), iron (III) chloride (Merck), magnesium powder (Merck), hydrochloric acid (Merck), methanol (Merck), sodium acetate (Merck), aluminum (III) chloride (Merck), quercetin (Sigma), gallic acid (Sigma), sodium carbonate (Merck), Folin-Ciocalteu reagent (Merck), 2,2-diphenyl-1-picrylhydrazyl (Sigma), Virgin Coconut Oil, tween 80 (Merck), propylene glycol, cetyl alcohol, methyl paraben, propyl paraben, dimethyl sulfoxide (Merck), L-tyrosine (Sigma), tyrosinase (Sigma), phosphate buffer, kojic acid (Sigma), and distilled water.

Instrumentations

The equipment used includes glassware, microwawe digestion (CEM[®]), rotary vacuum evaporator (IKA[®]), UV-Vis spectrophotometer (Shimadzu[®]),

Particle Size Analyze (Biobase[®]), hot plate stirrer (IKA[®]), homogenizer (IKA[®]), pH meter (Hanna[®]), and Rheometer (Benemed[®]).

Extraction of Resam Leaves and Jeruk Kunci Fruit Waste

Resam leaves and jeruk kunci fruits waste obtained from Balunijuk Village, Merawang Subdistrict, Bangka Regency, Bangka Belitung Islands Province were cleaned and dried for 7 days and then mashed to a size of 150 µm. Each simplisia was extracted in stages (n-hexane, ethyl acetate, ethanol) using the Microwave Assisted Extraction method by mixing 2 grams of simplisia in 20 mL of solvent (1:10 b/v) with operating parameters set at 60°C, 1200 W power, and 800 PSI pressure for 10 minutes (Pratiwi *et al.*, 2023). The filtrate obtained was concentrated with a rotary vacuum evaporator at 68.7°C (n-hexane), 77.1°C (ethyl acetate), and 78.4°C (ethanol) at 100 rpm to obtain a thick extract. The extract yield was calculated with the following formula:

$$\text{Yield (\%)} = \frac{\text{Mass of Extract (g)}}{\text{Mass of Simplisia (g)}} \times 100\% \dots \dots \dots (1)$$

Phytochemical Screenings

Qualitative phytochemical screening was carried out to detect the presence of flavonoids using the Wilstater Cyanidin method and phenolics using the Ferri Chloride method. Flavonoid identification was done by reacting 1 mL of sample with 0.1 gram of magnesium powder and 5 drops of concentrated hydrochloric acid. A positive indication of the presence of flavonoids is indicated by a change in the color of the solution to yellow, orange, or red. Meanwhile, phenolic detection is done by reacting 1 mL of sample with 10 drops of 1% iron (III) chloride solution. A positive reaction to the presence of phenolics is indicated by a change in the color of the solution to purple, green, blue, or black (Roanisca *et al.*, 2021).

Total Phenolic and Total Flavonoid Tests

Total phenolic and flavonoid measurements were carried out in duplicate using gallic acid and quercetin standards with concentrations of 20, 40, 60, 80, 100 ppm as well as extracts of resam leaves and jeruk kunci fruit waste with a concentration of 10 mg/10 mL methanol pro analyst. Furthermore, 0.5 mL of standard/extract for total phenolic was reacted with 0.5 mL of Folin-Ciocalteu and 2.5 mL of 7% sodium carbonate and for total flavonoid was reacted with 0.1 mL of 10% aluminum (III) chloride, 1.5 mL of methanol pro analyst, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. Then the whole solution was homogenized and incubated at 37°C for 30 minutes, and the absorbance value of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 760 nm (phenolic) and 435 nm (flavonoid) (Pratiwi *et al.*, 2023). Total phenolics and flavonoids were calculated using the following formula:

$$\text{Total Phenolic/Flavonoid} = \frac{C \times V \times fp}{g} \dots\dots\dots(2)$$

Where C is the phenolic/flavonoid concentration that has been converted to mg/mL, V is the sample volume in mL, Fp is the dilution factor, and g is the sample mass in grams.

Formulation and Characterization of Nanocream Preparations

The nanocream preparation formulation of resam leaves extract and jeruk kunci fruits extract from the modified research method of Sumaiyah & Meyliana (2021) as presented in Table 1 below.

Table 1. Formulation of Nanocream Preparations

	Material	Function	F ₁ (% w/w)	F ₂ (% w/w)	F ₃ (% w/w)
Extract	Resam Leaves	Active Substance	0.25	0.5	0.75
	Jeruk Kunci Fruits		0.75	0.5	0.25
Oil Phase	VCO	Emollient	10	10	10
	Tween 80	Surfactant	36	36	36
	Propylene Glycol	Cosurfactant	6	6	6
	Cetyl Alcohol	Stiffening Agent	0.6	0.6	0.6
	Methyl Paraben	Antimicrobial	0.1	0.1	0.1
Water Phase	Propyl Paraben		0.05	0.05	0.05
	Distilled water	Solvent	Ad 100	Ad 100	Ad 100

Nanocream formulation was carried out using the High Shear Stirring method. The oil phase and water phase were each stirred at 700 rpm using a hotplate stirrer at 55°C for 15 minutes. Then the two phases were mixed with extracts and stirred homogeneously with a homogenizer at 10,000 rpm until a thick emulsion was formed. Evaluation of the nanocream preparation included organoleptic tests of color, shape, aroma, and texture; emulsion type test through 1:100 dispersion in water (M/A) and oil (A/M); pH test through a pH meter with criteria of 4.5-8; spreadability test with a standard of 5-7 cm; viscosity test using a Rheometer with a range of 2,000-40,000 m.Pas; and droplet size test using a Particle Size Analyzer with a target of <100 nm.

Antioxidant Activity Test

Antioxidant testing with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method begins with making a 100 ppm solution of each resam leaves and jeruk kunci fruit waste extract in methanol pro analyst (1:10 b/v) which is diluted into concentrations of 10, 15, 20, 25, and 30 ppm. A total of 1 mL of each test solution was added to 2 mL of methanol pro analyst and 1 mL of DPPH solution into separate test tubes. The blank solution consisted of 1 mL of DPPH and 3 mL of methanol. The mixture was homogenized with a vortex for 30 seconds and incubated at 37°C for 30

minutes. The absorbance value of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 515 nm (Pratiwi *et al.*, 2023).

Anti-tyrosinase Activity Test

Anti-tyrosinase activity was tested by dopachrome method in triplo against tyrosinase enzyme. Samples (5 mg in 50 µL DMSO) were diluted to 10, 15, 20, 25 and 30 ppm. The test solution (100 µL) was mixed with 50 µL of 1 mM L-tyrosine, 50 µL of 50 mM phosphate buffer (pH 6.8), and 20 µL of tyrosinase enzyme, then incubated 5 min at room temperature. Kojic acid was used as a positive control. The blank consisted of 170 µL phosphate buffer and 50 µL L-tyrosine. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer (Amin *et al.*, 2024)

Percent antioxidant and anti-tyrosinase inhibition can be calculated by the following equations (3) and (4).

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance Sample}}{\text{Absorbance of Control}} \times 100\% \dots\dots\dots(3)$$

The IC₅₀ value can be calculated using a linear regression equation, sample concentration (x-axis), and %-inhibition (y-axis). From the equation $y = a(x) + b$, the IC₅₀ value can be calculated using the

$$\text{IC}_{50} = \frac{50 - b}{a} \dots\dots\dots(4)$$

Result and Discussion

Analysis of Extract Yield

Extraction was carried out using the MAE method which utilizes microwave radiation to facilitate the separation of bioactive compounds from the natural material matrix into the solvent, thereby increasing the effectiveness of the interaction between the sample and the solvent (Lidia & Mahardika, 2023). In addition, multistage extraction was implemented to separate compounds in the sample based on their polarity (Riasari *et al.*, 2022). In this study, three types of solvents with different polarities were used, namely n-hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar). This solvent selection aims to extract the target compounds according to their respective polarity levels. The extraction results of both samples are presented in Table 2 below.

Table 2. Extract Yield

Sample	Solvent	Extract (g)	Yield (%)
Resam Leaves	N-Hexane	0.63 ± 0.04	3.97
	Ethyl Acetate	6.26 ± 0.23	39.14
	Ethanol	10.39 ± 0.19	64.97
Jeruk Kunci Fruits	N-Hexane	0.49 ± 0.01	3.07
	Ethyl Acetate	2.59 ± 0.00	16.18
	Ethanol	3.38 ± 0.52	21.12

The quantity of yield produced (Table 2) was influenced by the solubility of the bioactive components, this is related to the principle of “like dissolves like” which states that a compound will dissolve in a solvent with the same polarity (Yani *et al.*, 2023). The highest percentage yield results were obtained in ethanol extracts, indicating that extraction efficiency is more optimal by using highly polar solvents. Ethanol has a hydroxyl group (-OH) with a high oxygen electronegativity value and is able to bind hydrogen, so it can bind to polar molecules and ion molecules (Riasari *et al.*, 2022). In addition, the dielectric properties of the solvent also play an important role in the selectivity and success of microwave-assisted heating, where the absorption of microwave energy will be greater the higher the dielectric constant value of the solvent (Lidia & Mahardika, 2023). Ethanol has the highest dielectric constant compared to ethyl acetate and n-hexane, which are 24.03; 6.02; and 1.89, respectively. With a higher dielectric constant value, microwave energy is able to penetrate the sample surface better. This causes the solvent to enter the sample faster and increase the extraction yield.

Analysis of Qualitative Phytochemical Test

Qualitative phytochemical screening was carried out as a preliminary test to identify the content of compounds in the sample through specific color reactions that appear due to the addition of reagents to certain groups of secondary metabolites. The results of qualitative phytochemical tests on flavonoid and phenolic compounds are shown in Table 3 below.

Table 3. Phytochemical Screening

Compound	Resam Leaves			Jeruk Kunci Fruit Waste		
	N-Hexane	Ethyl Acetate	Ethanol	N-Hexane	Ethyl Acetate	Ethanol
Flavonoid	+	+	+	-	+	+
Phenolic	-	+	+	-	-	+

Note: (+) indicates the presence of the compound; (-) indicates the absence of the compound

Table 3 shows that the secondary metabolite compounds produced are influenced by the type of solvent based on its polarity properties used during the extraction process. Compounds that are polar are phenol group compounds (phenolics, flavonoids, tannins, saponins, and lignans), while alkaloids and steroids are non polar compounds (Yani *et al.*, 2023). The absence of phenolics and flavonoids in non-polar n-hexane extracts is indicated by the low concentration of these compounds in the sample. This phenomenon emphasizes the importance of quantitative analysis to accurately determine the total phenolic and flavonoid levels in both extracts.

Analysis of Total Phenolic and Flavonoid Content

Quantification of total phenolic and flavonoid content was carried out to estimate the antioxidant activity of resam leaves and jeruk kunci fruit waste extracts obtained through extraction using different solvents. Phenolic and flavonoid

compounds have the potential as antioxidants through hydrogen atom donors from hydroxyl groups to free radical compounds thus inhibiting the oxidation process (Muflihah *et al.*, 2021). The results of the total phenolic and flavonoid testing of resam leaves extract and jeruk kunci fruits are presented in Table 4 below.

Table 4. Total Phenolic and Total Flavonoid Content

Sample	Solvent	Total Phenolic (mg GAE/g) ± SD	Total Flavonoid (mg QE/g) ± SD
Resam Leaves	N-Hexane	60.37 ± 0.07	31.00 ± 0.42
	Ethyl Acetate	108.76 ± 0.11	56.60 ± 0.11
	Ethanol	179.78 ± 0.35	90.33 ± 0.53
Jeruk Kunci Fruits	N-Hexane	35.65 ± 0.07	10.85 ± 0.42
	Ethyl Acetate	86.14 ± 0.14	40.03 ± 0.31
	Ethanol	135.65 ± 0.07	72.64 ± 0.21

Based on the results in Table 4, extraction using ethanol solvent resulted in higher total phenolic and flavonoid levels compared to ethyl acetate and n-hexane solvents. This phenomenon can be explained based on the structural characteristics of phenolic and flavonoid compounds, which generally have hydroxyl groups that make them belong to the group of polar compounds. In accordance with the principle of “like dissolves like” in solubility, phenolic and flavonoid compounds show a higher affinity for polar solvents such as ethanol compared to semi-polar ethyl acetate or non-polar n-hexane. The results showed that the highest phenolic and flavonoid levels in ethanol extracts, which indicates that the phenolic and flavonoid components in resam leaves and jeruk kunci fruit waste extracts are dominated by compounds that have polar characteristics.

Analysis of Nanocream Preparations Characterization

Analysis of nanocream preparations characterization is an important stage in the development of nanocream formulations to comprehensively evaluate the quality of nanocream with various physicochemical parameters and preparation stability. The results of organoleptic analysis of nanocream preparations as presented in Table 5 below.

Table 5. Organoleptic Characterization of Nanocream

Formulation	Organoleptic Test Parameters			
	Form	Color	Aroma	Texture
F ₁	Semisolid	Brownish Yellow	Odor of Jeruk Kunci	Not Sticky
F ₂	Semisolid	Light Brown	Odor of Jeruk Kunci	Not Sticky
F ₃	Semisolid	Brownish Green	Slight Odor of Jeruk Kunci	Not Sticky

Organoleptic evaluation (Table 5) showed that the three nanocream preparations were semisolid with yellow to brownish green color (F1-F3) (Figure 3). The aroma of F1 and F2 was dominated by jeruk kunci fruit waste, while F3 had a weaker jeruk kunci aroma. The intensity of aroma and color increased with

increasing extract concentration, reflecting the different ratios of resam leaves and jeruk kunci fruit waste extracts in each formulation. Texture analysis indicated the nanocream preparations were less sticky to the skin, making them more suitable for topical application.



Figure 3. Physical Appearance of Nanocream

Emulsion type test using water solvent (Figure 4) which is hydrophilic and Virgin Coconut Oil (Figure 5) which is hydrophobic.



Figure 4. Emulsion Type Test Results. (A) Water dispersion test: F1, F2, and F3 showing complete solubility in hydrophilic medium

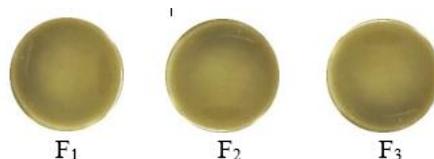


Figure 5. Emulsion Type Test Results. (B) VCO dispersion test: F1, F2, and F3 demonstrating limited dispersion in hydrophobic medium.

The result of the analysis (Figure 4) showed that all three nanocreams were water-soluble, indicating the formation of a reverse micelle type emulsion system or water-in-oil. The configuration (Figure 5) with a hydrophobic external phase has the potential to increase the penetration of active components through skin cell membranes so as to increase the bioavailability and effectiveness of the preparation as an anti-aging agent (Sainakham *et al.*, 2024). All formulations exhibit water-in-oil (W/O) emulsion characteristics with reverse micelle formation, facilitating enhanced skin penetration and bioavailability of active anti-aging compounds.

In addition, the results of other nanocream preparation characterization tests as presented by Table 6 below.

Table 6. Physical Characterization of Nanocream Preparation

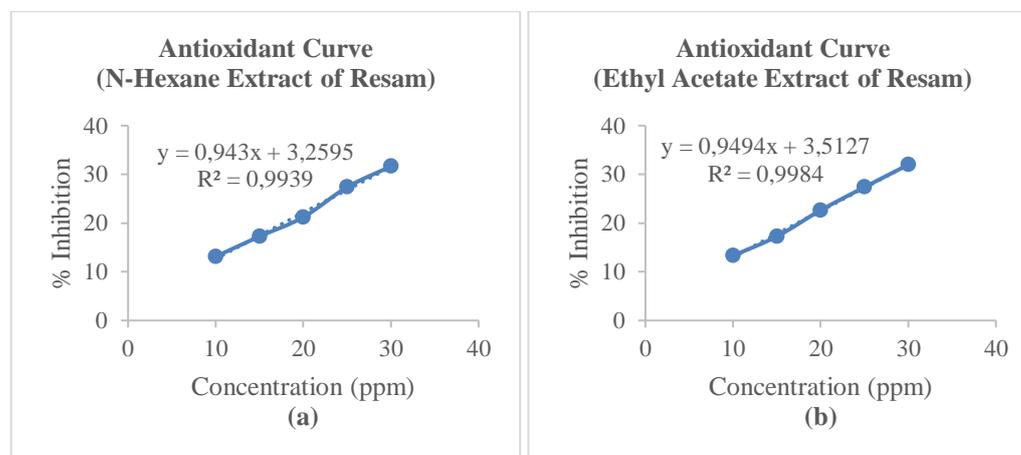
Formulation	Test Parameters				
	pH \pm SD	Spreadability (cm) \pm SD	Viscosity (mPas) \pm SD	Particle Size (nm)	PDI
F ₁	5.46 \pm 0.01	6.18 \pm 0.02	3968.13 \pm 0.07	9.53	0.1436

Formulation	Test Parameters				
	pH ± SD	Spreadability (cm) ± SD	Viscosity (mPas) ± SD	Particle Size (nm)	PDI
F ₂	5.47 ± 0.02	6.37 ± 0.04	3899.32 ± 0.04	10.48	0.1501
F ₃	5.47 ± 0.01	6.63 ± 0.04	3878.17 ± 0.06	9.63	0.1626

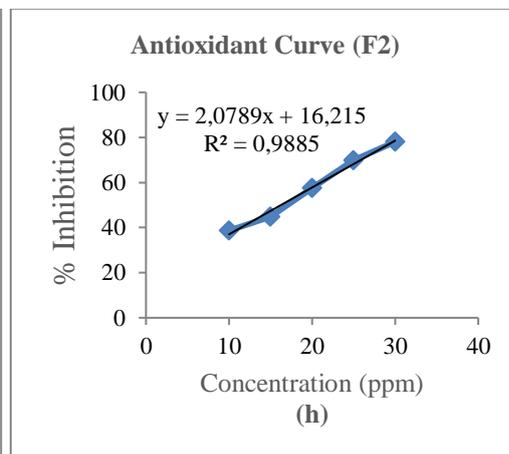
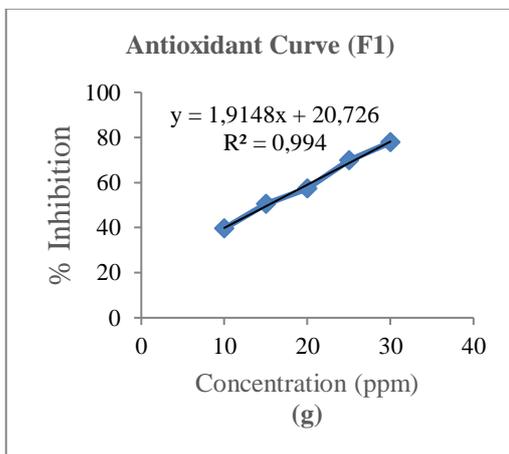
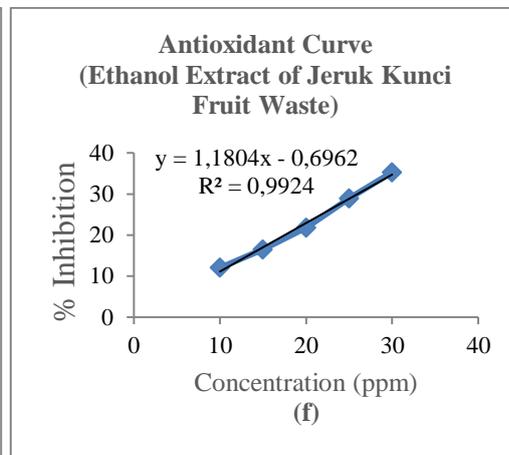
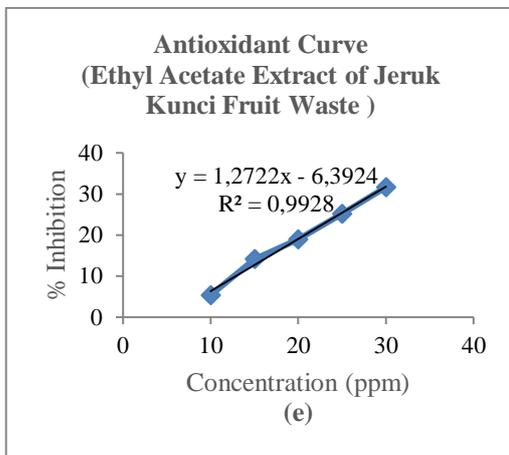
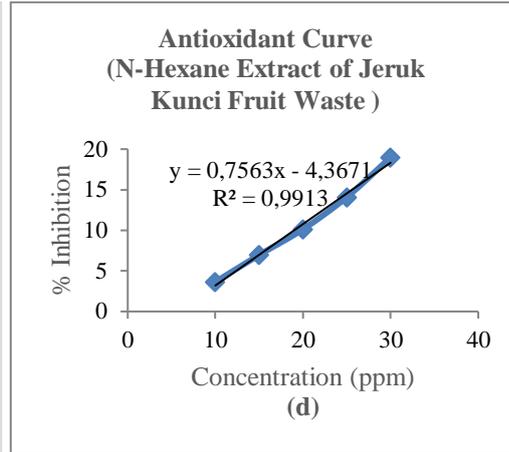
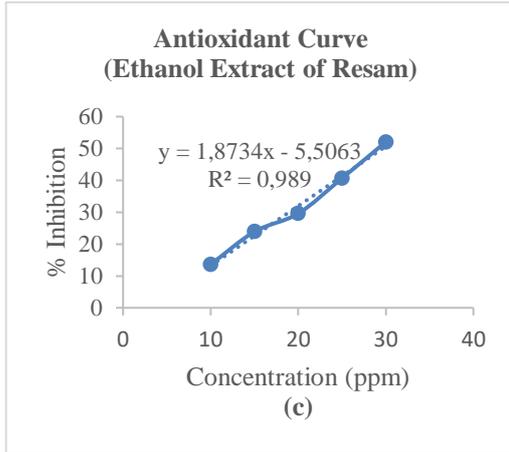
Based on Table 6, the formulations of the three nanocream preparations have met SNI 164954-1998. pH testing using a pH meter shows the results of the three formulations are in the range of 4.5-8 which is the ideal pH for topical preparations. The spreadability of the nanocream preparations also met the requirements of 5-7 cm, while the results of the viscosity test of the three preparations were in the range of 2,000-40,000 mPas in accordance with the requirements of nanocream viscosity. Furthermore, particle size characterization showed results of <100 nm with a Polydispersity Index (PDI) value of less than 0.7 indicating uniform droplet size. These droplet size and homogeneity test results prove that the third formula meets the requirements of nanocream preparations, showing stability and optimal particle size distribution in drug delivery systems (Sainakham *et al.*, 2024).

Analysis of Antioxidant Activity

Evaluation of antioxidant activity was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method and expressed as IC₅₀ (Inhibitory Concentration). Research by Fatmawati *et al.* (2023) classified the level of antioxidant activity based on the IC₅₀ value into four categories: very strong (<50 µg/mL), strong (50-100 µg/mL), moderate (100-250 µg/mL), and weak (250-500 µg/mL). The IC₅₀ value obtained from linear regression analysis is an important parameter that indicates the concentration of the sample required to reduce 50% of DPPH free radicals, so it can be used to compare antioxidant potential between samples or known antioxidant standards. The results of antioxidant activity testing are presented in Table 7 below.



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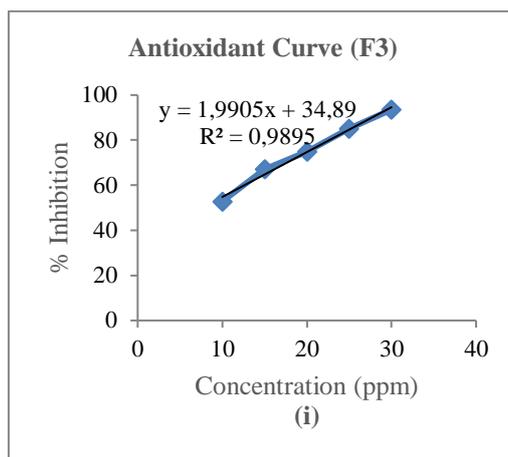


Figure 5. Antioxidant Curve (a). Ethyl Acetate Extract of Resam(b). Ethyl Acetate Extract of Resam (c). Ethanol Extract of Resam (d). N-Hexane Extract of Jeruk Kunci Fruit Waste (e). Ethyl Acetate Extract of Jeruk Kunci Fruit Waste (f). Ethanol Extract of Jeruk Kunci Fruit Waste (g). F1 (h). F2 (i) F3.

Based on the linear regression analysis, the regression equation was established. The antioxidant capacity of the test samples was then calculated using this derived equation.

Table 7. Antioxidant Activity

Sample	Solvent	Antioxidant IC ₅₀ (µg/mL)	Remarks
Resam Leaves	N-Hexane	49.40 ± 0.24	Very Strong
	Ethyl Acetate	48.80 ± 0.24	Very Strong
	Ethanol	29.58 ± 0.07	Very Strong
Jeruk Kunci Fruit Waste	N-Hexane	72.33 ± 0.58	Strong
	Ethyl Acetate	44.37 ± 0.05	Very Strong
	Ethanol	42.96 ± 0.36	Very Strong
F ₁		15.41 ± 0.15	Very Strong
F ₂		16.36 ± 0.14	Very Strong
F ₃		7.34 ± 0.36	Very Strong

Table 7 shows that the extracts of resam leaves and jeruk kunci fruit waste have very strong antioxidant activity, except for the n-hexane extract of jeruk kunci fruit waste. The ethanol extract showed optimum antioxidant activity due to the multistage extraction method using three solvents of different polarities, allowing intensive interaction between the sample and the polar solvent. The MAE method enhances antioxidant activity through the absorption of micro-energy that produces significant localized heating, increasing solvent penetration into the sample matrix, thus facilitating more efficient extraction of bioactive compounds and producing extracts with high antioxidant content (Lidia & Mahardika, 2023). Antioxidant activity is positively correlated with total phenolic and flavonoid levels, in line with the study of Pratiwi *et al.* (2023) which states that antioxidant effectiveness increases as the levels of these two compounds increase. Phenol and flavonoid

compounds with hydroxyl groups in ortho and para positions to -OH and -OR groups function as antioxidants through proton donation and stable radical formation. Radical stability is influenced by electron delocalization through resonance on the aromatic ring, allowing effective interaction with free radicals and neutralizing their negative effects (Muflihah *et al.*, 2021).

The antioxidant activity of ethanol extracts of resam leaves and jeruk kunci fruit waste was significantly improved when formulated in a nanocream preparation. Nanocream formulations provide the advantage of enhancing antioxidant effectiveness through several mechanisms. The nanoparticle size facilitates an increase in contact surface area, thereby enhancing the interaction between the antioxidant active compounds and free radicals. Nanoemulsion-based delivery systems also increase the stability of phenolic compounds and flavonoids that act as antioxidants, protecting them from degradation and maintaining their antioxidative potential. In addition, the nanocream formulation enhances the penetration of active compounds into the skin layers, allowing antioxidant compounds to reach biological targets more efficiently and provide a more optimal protective effect against oxidative stress. The enhanced antioxidant activity in nanocream preparations can also be attributed to the synergy between the carrier components in the formulation and the bioactive compounds, which collectively strengthen the free radical capture capacity and antioxidant mechanism (Alam *et al.*, 2023).

Analysis of Anti-aging Activity

Analysis of the anti-aging activity of tyrosinase enzyme inhibition is an important study in evaluating the potential of cosmetic active ingredients to prevent hyperpigmentation and combat signs of skin aging. This study examines the ability of bioactive compounds to inhibit the action of tyrosinase enzyme, a key enzyme in melanin synthesis that plays a role in the formation of pigmentation spots on the skin due to the aging process. The results of the antioxidant activity test are presented in Table 8 below.

Table 8. Anti-tyrosinase Activity

Sample	Anti-aging IC ₅₀ (µg/mL)	Remarks
Resam Leaves	115.43 ± 0.48	Medium
Jeruk Kunci Fruits	135.25 ± 0.60	Medium
Nanocream	100.16 ± 0.04	Strong
Kojic Acid (Positive Control)	14.76 ± 0.07	Very Strong

The anti-aging activity of resam leaves and jeruk kunci fruit waste extracts formulated in nanocream preparations showed inhibitory ability against the tyrosinase enzyme, although not yet equivalent to the effectiveness of the positive control used. This tyrosinase inhibitory potential indicates that both extracts have prospects as active ingredients in anti-aging products, especially to prevent

hyperpigmentation which is one of the signs of skin aging. Bioactive compounds such as phenolics and flavonoids contained in the extracts play a role in inhibiting tyrosinase activity by forming bonds with the enzyme or neutralizing the oxidation process in the melanogenesis pathway (Hassan *et al.*, 2023). Formulation in nanocream preparations significantly increases the effectiveness of the extract due to the nanoparticle size which provides the advantages of a larger contact surface area, increased stability of active compounds from degradation, and optimal penetration into the epidermis and dermis layers of the skin thereby increasing the bioavailability and efficacy of anti-aging compounds (Sainakham *et al.*, 2024). Nonetheless, the suboptimal inhibitory effectiveness in nanocream preparations suggests the need for formulation optimization efforts.

Conclusion

Based on the research conducted, nanocream formulated from resam leaves extract and jeruk kunci fruit waste showed significant potential as an anti-aging and antioxidant preparation. Multistage extraction using the MAE method produced the highest yield in ethanol solvent, with the highest phenolic and flavonoid contents in ethanol extracts of resam leaves (179.78 mg GAE/g and 90.33 mg QE/g) and jeruk kunci fruit waste (135.65 mg GAE/g and 72.64 mg QE/g). The developed nanocream formulation met the SNI 164954-1998 standard with optimal physical characteristics, particle size <100 nm, and polydispersity index <0.7. The nanocream preparation showed a very strong increase in antioxidant activity (IC₅₀ 7.34-16.36 µg/mL) compared to the single extract (IC₅₀ 29.58-72.33 µg/mL), as well as strong category anti-aging activity (IC₅₀ 100.16 µg/mL) through the mechanism of tyrosinase enzyme inhibition. The results of this study indicate that nanocream from natural ingredients from Bangka Belitung has the potential as an effective anti-aging cosmetic innovation to prevent premature aging of the skin.

Conflict of Interest

The authors declare no conflict of interest related to the publication of this manuscript. Furthermore, all aspects of research ethics including plagiarism, subject consent, ethical violations, data fabrication and/or falsification, duplicate publication and/or submission, as well as redundancy have been fully complied with by the authors.

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