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Research Paper

Plectranthus scutellarioides (L.) R.Br. Leaf Extract as Sunscreen, Skin Lightening, and Antiaging

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Abstract
Plectranthus scutellaroides is a plant with attractive colored leaves. It contains secondary metabolite compounds such as flavonoids and phenolics, which provide skin protection against ultraviolet rays from the sun. The research aims to investigate the activity of *P. scutellarioides* leaf extract from various variants and different solvents on sunscreen, skin lightening, and anti-aging activities. The best extract is formulated in gel dosage form. Each *P. scutellaroides* leaf was extracted using ethanol, ethyl acetate, and hexane as solvents. The obtained extract was assessed for Sun Protecting Factor (SPF), UVA Protection grade (PA), antioxidant activity, and inhibition of tyrosinase, collagenase, elastase, and hyaluronidase. Determination was conducted in vitro using spectrophotometric methods. The highest sunscreen activity was observed in the ethanol extract Va (Va-ET), which exhibited SPF and PA values of 25.618±0.265, and 0.681±0.007 (star 3), respectively at a concentration of 100 μg/mL. The most significant skin-lightening activity was observed in the ethanol extract Vc (Vc-ET), with an IC_{50} value inhibiting tyrosinase of 39.059 μg/mL. Regarding the anti-aging activity of the extracts, as determined by their antioxidant activity and inhibition of collagenase, elastase, and hyaluronidase, the most promising extracts were obtained from ethanol extract Va (Va-ET) and ethyl acetate extract Va (Va-EA), with IC_{50} values of 79.734, 76.838, 143.384, and 122.467 μg/ mL, respectively. The Va-ET was formulated into a gel dosage form, and there was a significant effect of the gelling agent on the SPF value of the extract after formulation ($p < 0.05$). All extracts exhibit activity as sunscreen, skin lightening, and anti-aging, with the Va-ET showing the highest efficacy. Gelling agents significantly influence the SPF value of extracts after formulation into the gel dosage form. Among them, F3, formulated from the Va-ET of *P. scutellaroides* using Carbopol-940 as a gelling agent, exhibits the highest efficacy.

Keywords
Photoprotection, Depigmenting Agent, Antioxidant, Enzyme Inhibition

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1. INTRODUCTION

The skin is the biggest and outermost organ, causing direct exposure to sunlight or ultraviolet (UV) radiation, which can cause sunburn, inflammation, photo-immunosuppression, photoaging, and even skin cancers. For this reason, it is necessary to protect the skin by preventing UV radiation and reducing the amount of UV radiation in the skin. In addition, UV rays are considered beneficial for certain skin-related conditions in medical UV therapy (Tang et al., 2024). UV radiation is a modulator of skin pigmentation that affects melanogenesis. This happens because the UVB produces damage to keratinocyte DNA, causing the expression of p53 which releases the hormone α-melanocytes (α-MSH). α-MSH stimulates melanocortin-1 (MC1R) receptors in melanocytes, which leads to melanogenesis (Yardman-Frank and Fisher, 2021). Exposure to UV light also causes the thickness of the epidermis, dermal elastase, a decrease in the amount of protein (collagen, fibronectin, elastin, and proteoglycan), increased activity of elastase, hyaluronidase, collagenase, and collagen fragmentation, increased inflammatory and telangiectasia. It can be stated that DNA damage in the skin is one of the main events in the photo process. Because of this, a search for potential cosmetic active ingredients that can protect against UV rays or damage caused by UV radiation (Gromkowska-Kepka et al., 2021).

Plectranthus scutellarioides (L.) R. Br. (syn. *Ocimum scutellarioides* (L), *Coleus scutellarioides* (L.) Benth, *Coleus blumei* (Benth), has numerous varieties worldwide, distinguished by their leaf color (Kalita et al., 2020). The *P. scutellarioides* leaf extract contains flavonoids, diterpenoids, alkaloids, tannins, phenolic compounds, and essential oils (Astuti et al., 2019). Secondary metabolite compounds such as phenolics, flavonoids, alkaloids, and carotenoids exhibit activity against UV radiation protection,

as they synthesize special molecules to prevent damage caused by UV radiation (Torres-Contreras et al., 2022). Alkaloids and flavonoids exhibit a protective effect against damage caused by UVB in skin models using human keratinocytes (Chen et al., 2022). Secondary metabolites in plants that function as antioxidants can also serve as sunscreen against both UVA and UVB radiation (Korkina et al., 2018).

Sunscreen prevents the increase in melanin synthesis in melanocytes, a process known as melanogenesis. Dermatological issues arise when excessive melanin production leads to conditions such as freckles, melasma, senile lentigo, pigmented acne scars, and cancer. Inhibiting melanogenesis directly or indirectly targets the tyrosinase enzyme, which is associated with the melanogenesis signaling pathway (Pillaiyar et al., 2018). Phenolic compounds and their derivatives, along with several compounds such as terpenoids, azoles, and benzaldehyde, are characterized as potent tyrosinase inhibitors (Zolghadri et al., 2019). According to in silico and in vitro studies, flavonoids demonstrate strong inhibitory activity against the tyrosinase enzyme (Jakimiuk et al., 2022).

Secondary metabolite compounds derived from plants can protect the skin by preventing UV penetration and acting as antioxidants. These antioxidants can counteract premature aging of the skin caused by the harmful effects of sun exposure (Petruk et al., 2018). UV radiation induces Reactive Oxygen Species (ROS) in the skin, leading to oxidative damage, which contributes to the formation of wrinkles. Concurrently, there is an upregulation in the activity of enzymes such as hyaluronidase, collagenase, and elastase. An increase in hyaluronidase enzyme activity leads to a decrease in hyaluronic acid, while elevated collagenase and elastase activity accelerate the degradation of collagen and elastin. Reduced levels of hyaluronic acid, collagen, and elastin contribute to premature aging of the skin. Consequently, natural ingredients with anti-aging properties are tested based on their ability to act as antioxidants or inhibit the activity of enzymes such as hyaluronidase, collagenase, and elastase (Garg et al., 2017).

The ethanol extract of *P. scutellarioides* leaves contains flavonoids, phenolics, saponins, and triterpenoids (Dwita et al., 2022). Four flavonoid compounds were identified in the ethanol extract of purple variant *P. scutellarioides* leaves, namely quercetin-3-glucoside, quercitrin, quercetin 3-(6"-acetyl glucoside), and quercetin 3-O-acetyl-rhamnoside (Bismelah et al., 2022). The leaf extract of *P. scutellarioides* contains secondary metabolite compounds such as flavonoids, tannins, saponins, alkaloids, and steroids (Yanto et al., 2020). The ethanol and water extracts of *P. scutellarioides* leaves contain flavonoids, saponins, and polyphenols, which exhibit antioxidant activity with IC_{50} values of 227.84 μg/mL and 244.42 μg/mL respectively (Wardojo et al., 2018). The methanol extracts of leaves and stems from *C. blumei* have IC_{50} values of 261 μg/mL and 242 μg/mL, respectively (Zakaria et al., 2008).

Research on the sunscreen, skin lightening, and anti-aging activities of *P. scutellarioides* leaf extract is still limited. Current research on leaf extracts predominantly focuses on antioxidant activity. This study aims to explore the potential of *P. scutellarioides* leaf extract from various variants and different solvents based on their polarity for sunscreen, skin lightening, and anti-aging activities. Investigating these activities is novel for this plant. The extract demonstrating the most promising sunscreen activity will be formulated into a gel dosage form using various gelling agents, and the effect on the SPF value will be evaluated post-gel formulation.

2. EXPERIMENTAL SECTION

2.1 Materials

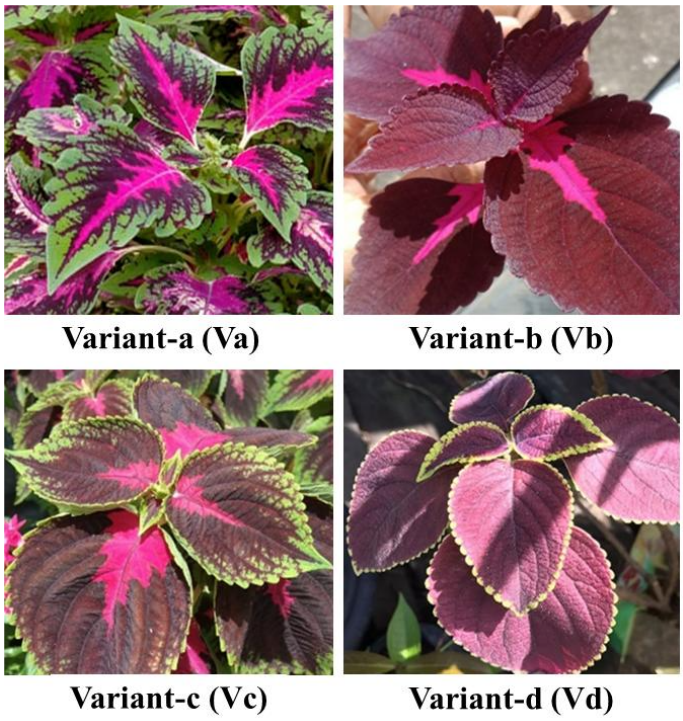

Figure 1. *Plectranthus scutellaroides* (L.) R. Br. Leaf Variants Based on Leaf Color: Variant-a (Va), Variant-b (Vb), Variant-c (VC), and Variant-d (Vd)

Miana leaves were collected from the Tanah Datar District, West Sumatera, Indonesia, with four different varieties distinguished by leaf color (Figure 1). Ethanol, ethyl acetate, hexane, triethanolamine (TEA), and propylene glycol from Bratachem. The following chemicals and reagents were used: Folin–Ciocalteu reagent, gallic acid, quercetin, dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium phosphate monobasic/dibasic, tricine, trizma®, phosphate buffer pH 6.5, bovine serum albumin (BSA), mushroom tyrosinase, L-3,4-dihydroxyphenylalanine (L-DOPA), collagenase from Clostridium histolyticum, N-(3-[2-furyl]acryloyl)-leu-gly-pro-ala (FALGPA), elastase from porcine pancreas, N-SucAla$_3$-p-nitroanilide, hyaluronidase (Sigma H3506), hyaluronic acid, ascorbic acid, retinol, and niacinamide from Sigma-Aldrich. Hydroxyl propyl methyl cellulose (HPMC) from Making Cos-

metics. Polyvinyl alcohol (PVA) from AppliChem. Carbopol-940 from Lubrozol. Dimethylol-5-5-dimethylhydantoin (DM DM-hydantoin) from Nguyen BA.

2.2 Methods

2.2.1 Extraction and Characterization Extract

Identification of *P. scutellarioides* from four different varieties was conducted at the Andalas University Herbarium. Each leaf weighing five hundred grams was macerated with ethanol, ethyl acetate, and hexane. The macerate was concentrated using a rotary evaporator to obtain a thick extract. The resulting extract underwent organoleptic, yield determination, pH measurement, solubility testing, drying loss assessment, determination of ash content, phytochemical screening, and quantification of total phenolic and flavonoid content.

2.2.2 Determination of Sunscreen Activity

For each extract, a concentration series ranging from 25 to 125 μg/mL was prepared, followed by measuring the absorbance using a UV-visible spectrophotometer. The SPF value was determined within the wavelength range of 290 to 320 nm at 5 nm intervals. The PA value was calculated by measuring the UVA/UVB ratio at a wavelength ranging from 290 to 400 nm with 5 nm intervals (Khunkitti et al., 2014).

2.2.3 Tyrosinase Inhibition Assay

Each extract is dissolved in 10% DMSO, then diluted with distilled water to prepare in a concentration series ranging from 12.5 to 200 μg/mL. Using a 96-well plate, the test solution is added to each well, followed by the addition of 200 U/mL mushroom tyrosinase (in phosphate buffer pH 6.5) and 10 mM L-DOPA solution. The plate is then placed in a dark environment for 30 minutes. Absorbance is measured at a wavelength of 475 nm using a microplate reader. Tests were also conducted on blank solutions (without enzymes and extracts). The percentage of tyrosinase inhibition by the extract was calculated. A concentration graph was constructed with the percentage of tyrosinase inhibition, and the IC_{50} (inhibitory concentration, 50%) value was determined (Nurrochmad et al., 2018).

2.2.4 Determination of Antioxidant Activity

Each extract was dissolved in 10% DMSO, added to distilled water, and prepared in a concentration series (12.5 to 400 μg/mL). Add 50 μL of the test solution to each well, followed by the addition of 200 μL of 0.077 mM DPPH solution into each well. Store the plate at room temperature in a dark place for 30 minutes. For the blank, use 250 μL of 0.077 mM DPPH solution. Measure the absorption at a wavelength of 517 nm using a microplate reader. Calculate the percentage of DPPH inhibition by the extract. Generate a concentration graph showing the percentage of DPPH inhibition and determine the IC_{50} value (Wardojo et al., 2018).

2.2.5 Collagenase Inhibition Assay

Each extract was dissolved in 10% DMSO and then diluted with distilled water to prepare a concentration series ranging from 12.5 to 200 μg/mL. The test solution was added to each well, followed by the addition of collagenase at a concentration of 0.1 mg/mL and Tricine buffer 50 mM, pH 7.5, containing 10 mM $CaCl_2$ and 400 mm NaCl). Leave for 20 minutes at 25°C in a dark place. Next, a 1.0 mM FALGPA solution was added and left for another 20 minutes at 25°C in a dark place. Absorbance was measured at a wavelength of 345 nm using a microplate reader. Tests were also conducted on blank solutions (without adding enzymes or extracts). Calculate the percentage of collagenase enzyme inhibition by the extract. Create a concentration graph showing the percentage of collagenase inhibition and determine the IC_{50} value (Jiratchayamaethasakul et al., 2020).

2.2.6 Elastase Inhibition Assay

Each extract was dissolved in 10% DMSO, mixed with distilled water, and prepared in a concentration series ranging from 12.5 to 200 μg/mL. The test solution was added to each well, along with 0.05 U/mL elastase in cold distilled water and Tris buffer solution (100 mM, pH 8.0), and then incubated for 15 minutes at 25°C in a dark place. Add a 0.1 mM N-SucAla3-p-nitroanilide solution in Tris buffer, and let it stand for 15 minutes at 25°C in a dark place. Absorbance was measured at a wavelength of 410 nm using a microplate reader. Tests were also conducted on blank solutions (without adding enzymes or extracts). Calculate the percentage of elastase enzyme inhibition by the extract. Create a concentration graph showing the percentage of elastase inhibition and determine the IC_{50} value (Jiratchayamaethasakul et al., 2020).

2.2.7 Hyaluronidase Inhibition Assay

Each extract was dissolved in 10% DMSO, and then distilled water was added to create a concentration series ranging from 12.5 to 200 μg/mL. Each test solution was added to a well, followed by the addition of hyaluronidase at 6 U/mL and phosphate buffer (pH 5.35). The mixture was allowed to stand for 10 minutes at 37°C. After that, hyaluronic acid was added, and the mixture was incubated for 45 minutes at 37°C in a place dark. An albumin acid solution was subsequently added and allowed to stand at room temperature for 20 minutes. Absorbance was measured at a wavelength of 600 nm using a microplate reader. Tests were also conducted on blank solutions (without adding enzymes or extracts). Calculate the percentage of hyaluronidase enzyme inhibition by the extract. Construct a concentration graph showing the percentage of hyaluronidase inhibition and determine the IC_{50} value (Jiratchayamaethasakul et al., 2020).

2.2.8 Formulation and Determination of Sunscreen Activity of Gel Dosage Form

The extract that exhibited the highest sunscreen activity (the largest SPF value) was formulated into a gel dosage form using three different gelling agents (HPMC, PVA, Carbopol-940).

Table 1. Formulation of Gel Dosage Forms of *P. scutellaroides* Leaf Extracts Va-ET

	Base (%)			Formula (%)		
	$F_0$1	$F_0$2	$F_0$3	F1	F2	F3
Ethanol Extract Variant A	-	-	-	0.125	0.125	0.125
HPMC	3	-	-	3	-	-
PVA	-	2	-	-	2	-
Carbopol-940	-	-	1	-	-	1
TEA	-	-	0.5	-	-	0.5
Propilenglikol	10	10	10	10	10	10
DMDM Hydantoin	0.5	0.5	0.5	0.5	0.5	0.5
Aquadest ad	100	100	100	100	100	100

The formula incorporates an extract concentration of 0.125% (Table 1). The formulated gel dosage form underwent physical evaluation testing, which included organoleptic assessment, assessment of homogeneity, pH uniformity, and viscosity over six weeks. Additionally, stability testing was conducted using the freeze-thaw method for six cycles. Following this, sunscreen activity testing was performed. The gel was weighed and dissolved to achieve a concentration equivalent to 125 μg/mL of extract. The SPF value was determined to assess the effect of the gelling agent on the extract's SPF value after formulating the gel form preparation. The data were statistically analyzed using one-way ANOVA. Tukey's post hoc test was subsequently applied to elucidate the differences in group means, with statistical significance defined as $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 *Plectranthus scutellarioides* Leaf Extracts

P. scutellarioides is an ornamental plant known for its attractive leaf color, boasting hundreds of varieties distinguished by leaf coloration. In this study, four different varieties were utilized. The characterization of *P. scutellarioides* leaf extract from these four varieties is detailed in Table 2. Notably, the ethyl acetate extract of variety d (Vd-EA) exhibited the highest yield, reaching 11.01%. The pH of the extract ranged from 3.02±0.42 to 6.18±0.03. Each extract exhibited varying solubility in water and 96% ethanol. Loss on drying ranged from 5.05±0.89 to 9.36±0.51, while residue on ignition ranged from 0.61±0.11 to 6.78±0.21, indicating compliance with requirements. Ethanol and ethyl acetate extracts from all variants contained flavonoid and phenolic compounds, while hexane extracts from all variants contained alkaloids.

Extraction was carried out on *P. scutellaroides* leaves using the maceration method, a simple method conducted without heating. The solvents used were differentiated based on their polarity. This aimed to assess whether different extracts and variants of *P. scutellaroides* leaves exhibit effects on sunscreen, skin lightening, and anti-aging activities according to the polarity of the extract. The extracts obtained had varying yields depending on the type of solvent used and the variety of *P. scutellaroides* leaves. Among all variants, the highest yield was obtained in ethyl acetate extract, suggesting that *P. scutellaroides* leaves contain numerous semipolar compounds.

The content of secondary metabolite compounds obtained varied in each extract. Generally, the ethanol and ethyl acetate extracts contained flavonoid and phenolic compounds. Terpenoid compounds were not found in all variants. Saponins were exclusively found in Va. The results obtained were almost the same as those carried out by Dwita et al. (2022) where the ethanol extract of *P. scutellaroides* leaves contained flavonoid, phenolic, saponin, and triterpenoid compounds. The secondary metabolite content in plants serves as the primary defense against solar energy, exhibiting activities as photosensitizers and photoprotectors that can be particularly beneficial for human skin. Cosmetics containing sun-protective ingredients are non-toxic to the skin and effectively mitigate the effects of UVA and UVB radiation while providing antioxidant benefits, thereby ameliorating the adverse effects of prolonged sun exposure on the skin (Korkina et al., 2018).

Total phenolic and flavonoid content of *P. scutellaroides* (L.) R. Br. leaf extracts are presented in Table 3. The ethanol extract of Vb exhibits the highest total phenolic content, measuring 715.672±6.506 mg GAE/g extract, while the ethanol extract of Vc demonstrated the highest total flavonoid content, amounting to 462.713±2.218 mg QE/g extract. Phenolic compounds are secondary metabolites found in plants. They possess activity in protecting the skin from exposure to ultraviolet radiation from the sun and are frequently incorporated into cosmetic formulations as sunscreen agents (Torres-Contreras et al., 2022). Flavonoids are antioxidants and photoprotective agents that can prevent damage to the skin (Gebka et al., 2022). Flavonoids are part of the phenolic compounds and exhibit activity as tyrosinase inhibitors. Tests conducted on 44 different flavonoid compounds revealed quercetin, a flavonoid compound, possesses tyrosinase inhibitor activity with an IC_{50} value of 44.38±0.13 μM, surpassing that of kojic acid, used as a control compound (Jakimiuk et al., 2022). Cellularly, flavonoids can prevent premature aging of facial skin (Domaszewska-Szostek et al., 2021). In this study, total phenolic and flavonoid content testing was employed to observe their impact on sunscreen, skin lightening, and antiaging activity. In Citrus unshiu plants, the total phenolic and flavonoid content influence antioxidant activity: as the amount of phenolic and

Table 2. Characteristics of *P. scutellaroides* (L.) R. Br. Leaf Extracts

Test	*P. scutellaroides* (L.) R. Br.					
	Variant-a (Va)			Variant-b (Vb)		
	Ethanol	Ethyl Acetate	Hexane	Ethanol	Ethyl acetate	Hexane
Yield	8.17%	8.25%	1.68%	3.10%	6.45%	0.22%
pH (1% w/v solution)	5.72±1.34	5.31±0.27	6.18±0.03	5.07±0.78	5.16±0.52	3.02±0.42
Solubility in Water	1 in 1750	practically insoluble	practically insoluble	1 in 350	practically insoluble	practically insoluble
Solubility in Ethanol 96%	1 in 700	1 in 1050	practically insoluble	1 in 300	1 in 550	practically insoluble
Loss on Drying	7.71±0.32	8.17±0.43	9.36±0.51	6.14±0.21	6.16±0.35	5.07±0.76
Residue on Ignition	1.60±0.89	2.56±0.56	1.45±0.87	2.44±0.54	2.53±0.87	4.56±0.35
Flavonoids	+	+	-	+	+	-
Phenolics	+	+	-	+	+	-
Saponins	+	+	+	-	-	-
Terpenoids	-	-	-	-	-	-
Steroids	-	-	-	+	+	+
Alkaloids	-	-	+	+	+	+
	Variant-c (Vc)			Variant-d (Vd)		
	Ethanol	Ethyl Acetate	Hexane	Ethanol	Ethyl Acetate	Hexane
Yield	4.04%	7.80%	0.40%	10.12%	11.01%	0.13%
pH (1% w/v solution)	5.51±0.32	4.72±0.16	5.58±0.38	5.35±0.19	5.31±0.18	5.16±0.09
Solubility in Water	1 in 1050	practically insoluble	practically insoluble	1 in 1550	practically insoluble	practically insoluble
Solubility in Ethanol 96%	1 in 750	1 in 850	practically insoluble	1 in 650	1 in 850	practically insoluble
Loss on Drying	7.47±0.65	5.60±0.46	5.67±0.61	5.55±0.24	5.05±0.89	6.32±0.33
Residue on Ignition	3.45±0.67	4.83±0.51	6.78±0.21	1.23±0.42	0.61±0.11	0.92±0.32
Flavonoids	+	+	-	+	+	-
Phenolics	+	+	-	+	+	-
Saponins	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-
Steroids	+	+	-	+	+	-
Alkaloids	+	+	+	+	+	+

flavonoid increases, so does antioxidant activity, albeit not the inhibition of elastase and collagenase enzyme activity. The augmentation in enzyme inhibition is contingent upon the type of bioactive compound present in the plant (Eun et al., 2020).

3.2 Sunscreen Activity of *Plectranthus scutellaroides* (L) R. Br. Leaf Extracts

The sunscreen activity of the extract is presented in Table 4, by measuring the SPF and PA values. Based on the SPF value, the highest value was obtained for the ethanol extract of Va (Va-ET) leaves where at a concentration of 100 μg/mL it reached a value of 25.618±0.265 (minimum SPF for use >15), indicating very good protection. The best PA value was in the ethanol extract of Vd (Vd-ET) leaves which had a value of 1.148 ± 0.0_{50}, classified as the maximum category (four-star).

The sunscreen activity of the extract is determined by examining the SPF and PA values obtained from measurements at a wavelength of 290 – 400 nm. This method is proposed by the Food and Drug Administration (FDA) and measures transmission in vitro using the critical wavelength method. Sunscreen products that protect against UVB rays typically have a critical wavelength of less than 320 nm, whereas those offering protection against UVA rays generally have a critical wavelength ranging between 320 and 400 nm. The critical wavelength represents the point at which 90% of the area under the curve between 290 and 400 nm is obtained, serving as a measure of the extent of sunscreen protection (Donglikar and Deore, 2016).

Based on the total phenolic and flavonoid content of *P. scutellaroides* (L.) R. Br. leaf extracts, along with the SPF and PA values obtained, there was no visible relationship between the high SPF and PA values and the total phenolic and flavonoid content. This is likely due to the varying amounts of secondary metabolites contained in each extract, which affects the SPF and PA values. The ethanol extract (except Vd) at a concentration of 125 μg/mL has shown an SPF value above 15, indicating good protection. Generally, the higher the concentration, the higher the SPF value of the extract will increase. This is not evident in determining the PA value, as increasing the concentration does not increase the PA value. Overall, the hexane extract seems to exhibit a low SPF value, which may also be influenced by the presence of secondary metabolites in the extract, such as alkaloids. However, alkaloids possess activity in protecting the skin from UVB (Chen et al., 2022).

3.3 Skin Lightening Activity of *Plectranthus scutellaroides* (L) R. Br, Leaf Extracts

The *In vitro* skin-lightening activity is demonstrated by the high inhibition value of the extract on the enzyme tyrosinase. Tyrosinase inhibitory activity of *P. scutellaroides* (L.) R. Br. leaf extracts are presented in Figure 2. Based on the IC_{50} value, all extracts exhibit tyrosinase inhibitory activity with the Vc ethanol extract showing the highest inhibitory activity with an IC_{50} value of 39.058 μg/mL. These results are nearly comparable to the IC_{50} value of kojic acid (27.348 μg/mL), used as a reference compound for determining tyrosinase inhibi-

Table 3. Phenolics and Flavonoids Content of *P. scutellaroides* Leaf Extract

Variant	Extracts	Phenolics Content (mg GAE/g extract)	Flavonoids Content (mg QE/g extract)
Va	Ethanol	291.294±6.934	82.445±0.258
	Ethyl Acetate	191.045±10.993	42.064±0.112
Vb	Ethanol	715.672±6.506	22.756±0.433
	Ethyl Acetate	220.398±3.527	417.784±4.216
Vc	Ethanol	316.418±3.949	462.713±2.218
	Ethyl Acetate	202.488± 8.487	162.332±4.507
Vd	Ethanol	450.498±1.878	52.374±0.160
	Ethyl Acetate	293.035±12.694	130.001±0.360

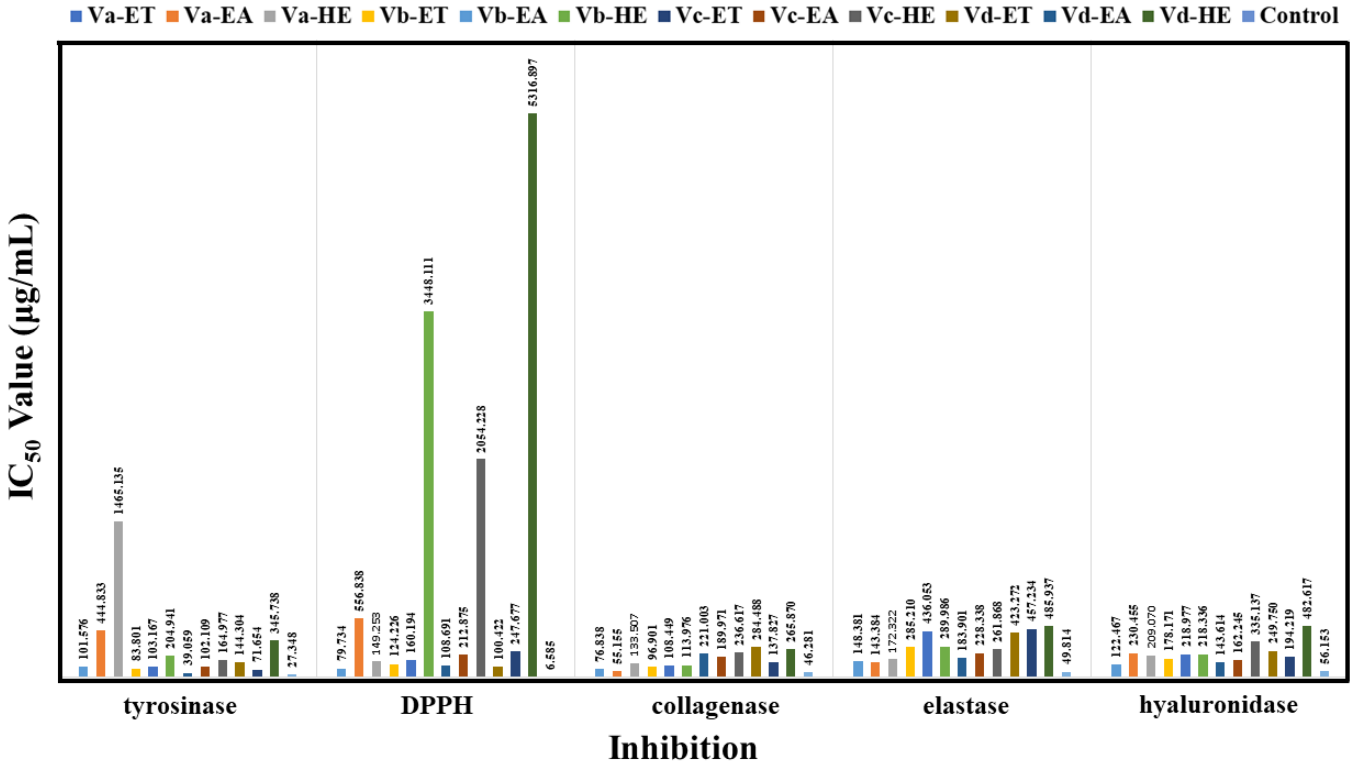

Figure 2. IC_{50} Value of Plectranthus scutellaroides (L.) R. Br. Leaves Extracts. Ethanol Extract Variant-a (Va-ET), Ethyl Acetate Extract Variant-a (Va-EA), Hexane Extract Variant-a (Va-HE), Ethanol Extract Variant-b (Vb-ET), Ethyl Acetate Extract Variant-b (Vb-EA), Hexane Extract Variant-b (Vb-HE), Ethanol Extract Variant-c (Vc-ET), Ethyl Acetate Extract Variant-c (Vc-EA), Hexane Extract Variant-c (Vc-HE), Ethanol Extract Variant-d (Vd-ET), Ethyl Acetate Extract Variant-d (Vd-EA), and Hexane Extract Variant-d (Vd-HE)

tion. Tyrosinase is an enzyme that catalyzes the first two steps in melanogenesis. Melanogenesis is the process of melanin synthesis, which imparts color to skin, hair, and eyes. Continuous exposure to sunlight can stimulate melanogenesis in the skin, leading to hyperpigmentation. There is a concern that hyperpigmentation will diminish the aesthetics of the skin if it spreads unevenly, resulting in black spots or patches appearing on facial skin. This concern has prompted several studies to search for substances or compounds from natural ingredients that can inhibit tyrosinase, thus allowing them to be incorporated into cosmetic formulations as skin lighteners (Neto et al., 2022).

Table 4. Sunscreen Activity of *P. scutellaroides* Leaf Extract

Test	Extracts	Conc. (μg/mL)	*P. scutellaroides* Va	Vb	Vc	Vd
SPF*	Ethanol	25	1.047±0.001	1.708±0.017	1.955±0.002	1.019±0.001
		50	5.939±0.038	2.919±0.091	3.340±0.006	1.321±0.042
		75	11.392±0.054	6.705±0.024	4.974±0.012	2.158±0.003
		100	25.618±0.265	12.768±0.056	6.435±0.009	4.132±0.006
		125	50.111±0.835	22.416±0.037	21.086±0.088	13.875±1.624
	Ethyl Acetate	25	1.445±0.004	1.504±0.013	1.307±0.001	1.072±0.001
		50	1.928±0.004	2.612±0.013	1.416±0.002	1.408±0.002
		75	2.547±0.009	4.237±0.068	1.681±0.001	2.139±0.007
		100	3.365±0.003	6.493±0.034	2.189±0.001	4.372±0.005
		125	4.465±0.008	9.944±0.239	2.897±0.003	9.807±0.054
	Hexane	25	1.201±0.026	1.017±0.001	1.050±0.001	1.012±0.002
		50	1.549±0.005	1.127±0.001	1.068±0.001	1.035±0.001
		75	1.909±0.005	1.162±0.001	1.145±0.001	1.081±0.003
		100	2.413±0.033	1.223±0.001	1.256±0.002	1.158±0.001
		125	3.127±0.098	1.305±0.001	1.401±0.005	1.265±0.002
PA**	Ethanol	25	0.917±0.033	0.916±0.052	0.676±0.001	1.127±0.001
		50	0.820±0.006	0.771±0.056	0.484±0.001	1.148±0.050
		75	0.763±0.007	0.493±0.013	0.363±0.002	0.951±0.002
		100	0.681±0.007	0.368±0.008	0.379±0.001	0.850±0.003
		125	0.615±0.011	0.284±0.003	0.165±0.001	0.475±0.172
	Ethyl Acetate	25	0.952±0.001	0.948±0.007	0.909±0.001	1.084±0.001
		50	0.909±0.003	0.867±0.016	0.888±0.001	1.047±0.001
		75	0.896±0.025	0.809±0.016	0.846±0.001	0.924±0.002
		100	0.877±0.024	0.785±0.028	0.777±0.001	0.867±0.002
		125	0.864±0.007	0.757±0.017	0.724±0.001	0.879±0.009
	Hexane	25	0.985±0.033	0.991±0.001	0.982±0.010	1.003±0.002
		50	0.896±0.022	0.959±0.001	0.969±0.001	1.006±0.002
		75	0.842±0.011	0.950±0.001	0.947±0.001	0.992±0.004
		100	0.786±0.028	0.923±0.001	0.919±0.001	1.001±0.015
		125	0.728±0.032	0.895±0.001	0.887±0.002	0.982±0.003

*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)

Tyrosinase inhibitors are one way to inhibit melanogenesis. Many putative inhibitors were tested in the presence of tyrosine or dopa as enzyme substrates, and their activity was assessed for dopachrome formation in vitro. Another way to inhibit tyrosinase activity is by using alternative enzyme substrates, such as some phenolic compounds, whose reaction products absorb in a different spectral range than dopachrome. When phenolics show good affinity for the enzyme, the formation of dopachrome can be prevented, and this is classified as an inhibition (Chang, 2009). It is suspected that flavonoids provide activity as tyrosinase inhibitors due to the presence of hydroxyl groups at C3 and C7, O^- and C^-glycosylation, methylation, and acetylation of hydroxyl groups (Jakimiuk et al., 2022). Similar to sunscreen activity, in this study, there was no influence of high levels of phenolics and flavonoids on the extract's activity as a tyrosinase inhibitor.

3.4 Antiaging Activity of *Plectranthus scutellaroides* (L) R. Br, Leaf Extracts

Continuous exposure to UV radiation, apart from stimulating melanogenesis, is also considered to be one of the factors causing aging, known as photoaging. Therefore, daily use of sunscreen is highly recommended, and compounds or ingredients with sunscreen activity are often added to cosmetic products

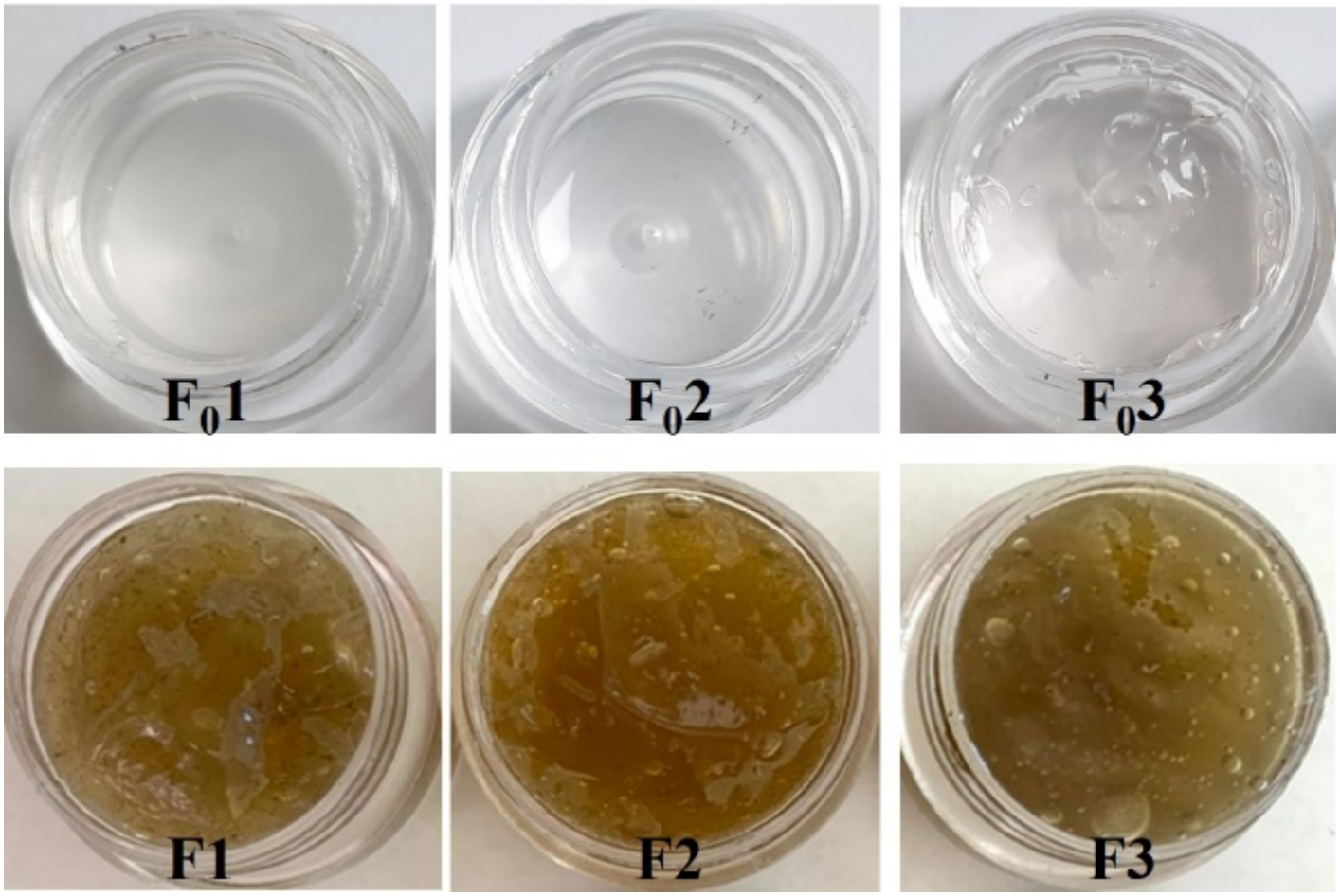

Figure 3. Ethanol Extract Gel Dosage Form of *P. scutellaroides* (L.) R. Br. Leaves Variant-a (Va-ET)

(Gromkowska-Kepka et al., 2021). Exposure to sunlight, especially UVA, triggers the formation of reactive oxygen species (ROS) and photoaging. ROS are free radicals found in the skin. The presence of substances or compounds with antioxidant effects makes overcoming skin pathologies related to premature aging possible. In this case, plants containing phenolic compounds, ascorbic acid, and carotenoids can protect the skin by preventing the penetration of UV rays, reducing inflammation due to ROS, and influencing several signaling pathways involved in the premature aging process, which include various enzymes (Petruk et al., 2018).

The anti-aging activity of the extract was determined by assessing its inhibitory effects against ROS and enzymes involved in the aging process. In this study, we examined the extract's ability to inhibit DPPH as a free radical, as well as enzymes such as collagenase, elastase, and hyaluronidase, which are implicated in the aging process. The inhibitory activity of extracts against DPPH, collagenase, elastase, and hyaluronidase is presented in Figure 2. Based on the IC_{50} value, all extracts exhibited activity as inhibitors of DPPH, collagenase, elastase, and hyaluronidase. The Va-ET extract demonstrated the best antioxidant activity, with an IC_{50} value of 79.734 μg/mL. The results obtained significantly differed from those of ascorbic acid, serving as a control compound with an IC_{50} value of 6.585 μg/mL. The Va-ET extract demonstrated the best collagenase inhibition activity, with an IC_{50} value of 76.838 μg/mL. However, this result was still lower compared to the IC_{50} value of 46.281 μg/mL demonstrated by the comparison compound retinol. The VC-EA extract exhibited the best elastase inhibition activity with an IC_{50} value of 143.384 μg/mL. However, this activity was still lower compared to ascorbic acid, which demonstrated an IC_{50} value of 49.814 μg/mL. Similarly, the VA-ET extract demonstrated an IC_{50} value of 122.467 μg/mL, yet this value was also inferior to that of niacinamide, serving as a comparison compound, which had an IC_{50} value of 56.153 μg/mL.

Assessing antioxidant activity in plants serves as preliminary research to guide subsequent stages of investigation. Testing antioxidant activity, particularly those intended for skin application, aims to address issues stemming from reactive oxygen species, particularly in the context of the aging process. The aging process is a natural phenomenon experienced by the skin as a result of increasing age. However, this process can be accelerated by other factors associated with aging, such

Table 5. SPF Value Gel Dosage Form of *P. scutellaroides* Leaves Extract Va-ET

Sample	Σ AUC (290–320 nm)			SPF			
	1	2	3	1	2	3	Mean
$F_0$1	1.475	1.393	1.433	1.120	1.113	1.116	1.116±0.004
$F_0$2	0.208	0.193	0.208	1.016	1.015	1.016	1.016±0.001
$F_0$3	0.193	0.178	0.178	1.015	1.014	1.014	1.014±0.001
F1	43.038	43.053	43.070	27.201	27.232	27.269	27.234±0.034
F2	31.295	31.345	31.345	11.045	11.087	11.087	11.073±0.025
F3	51.020	51.080	51.130	50.195	50.427	50.621	50.415±0.213
Va-ET (125 μg/mL)	51.223	50.978	50.790	50.982	50.032	49.317	50.111±0.835

as continuous UV exposure, air pollution, stress, and so on. Consequently, some dermatologists take action or administer treatments to address these issues (Ganceviciene et al., 2012). The ethanol extract of the leaves of all variants in this test had better antioxidant activity (below 125 μg/mL) compared to the ethanol extract of the leaves of *P. scutellaroides* tested by Wardojo et al. (2018) which had an IC_{50} value of 227.84 μg/mL. This can occur due to the influence of the leaf variant used, as it possesses a diverse secondary metabolite composition. Aging induced by free radicals can activate collagenase, elastase, and hyaluronidase (Sutjiatmo et al., 2020).

Some plants contain polyphenolic compounds, alkaloids, tannins, saponins, carotenoids, and terpenoids, which exhibit activity in inhibiting the function of enzymes involved in the premature aging process. These enzymes include hyaluronidase, elastase, and matrix metalloproteinase (MMP), commonly referred to as collagenase. Hyaluronidase hydrolyzes hyaluronic acid, reducing its viscosity and increasing permeability. Hyaluro nic acid, a glycosaminoglycan, plays a crucial role in binding and retaining water molecules in the skin, thus ensuring its moisture, smoothness, and softness. A decrease in hyaluronic acid levels in the skin leads to dryness and wrinkles. Elastase hydrolyzes elastin and fibrillin; elastin, a protein, is responsible for maintaining skin elasticity. Elastase has a significant impact on the metabolism of elastic fibers in skin tissue during photoaging. MMP or collagenase is activated only after the skin is exposed to UVB radiation, leading to the degradation of extracellular matrix components in human skin. As a result of this degradation, there is a buildup of fragmented and disorganized collagen fibrils. This collagen degradation product downregulates the synthesis of new collagen, resulting in reduced collagen content in the skin (Garg et al., 2017).

The research conducted showed that the ethanol extract of Va leaves had superior activity in inhibiting collagenase compared to other extracts. However, the results obtained were lower than those of the control compound (retinol), which exhibited an IC_{50} value of 46.281 μg/mL. Elastase inhibition testing of the extract revealed that the ethyl acetate extract of Va leaves exhibited a lower IC_{50} value compared to other extracts. Interestingly, the IC_{50} value obtained was nearly equivalent to that of the ethanol extract of Va leaves. Notably, the results obtained were significantly lower than those of the comparison compound, ascorbic acid, which displayed an IC_{50} value of 49.814 μg/mL. Testing the extract for inhibition of hyaluronidase activity revealed that the ethanol extract of Va leaves exhibited superior activity compared to other extracts. However, this value was lower than that of the comparison compound, niacinamide, which had an IC_{50} value of 56.153 μg/mL. The reference compounds used in testing for the three enzymes were not uniform. Instead, after assessing various reference control compounds, the one demonstrating the highest activity for each enzyme was selected. This differs from testing tyrosinase inhibition, where the reference compound possesses a clear mechanism of action in inhibiting melanogenesis (Pillaiyar et al., 2018). Based on the results of the research conducted, the ethanol extract of Va leaves is utilized in gel dosage form formulations at a concentration of 0.125%

3.5 Gel Dosage Form Formulation and Sunscreen Activity of *Plectranthus scutellaroides* (L.) R. Br. Leaf Extracts

The formulation of the *P. scutellaroides* leaf extract gel dosage form utilizes ethanol extract of VA leaves (Va-ET), which exhibits a high SPF value at the same concentration. The extract used in the dosage form contains 0.125%. Gel dosage form of *P. scutellaroides* (L.) R. Br leaf extracts using HPMC, PVA, and Carbopol 940 as gelling agents are presented in Figure 3. All formulations meet the physical evaluation requirements, demonstrating no alteration in color, shape, or odor. The dosage form remains homogeneous during storage, exhibiting consistent pH and viscosity. Importantly, the dosage form remained stable after the freeze-thaw examination.

The sunscreen activity of the gel dosage form is presented in Table 5, where the influence of the gelling agent is seen on the SPF extract value after being made in the form of the gel dosage form ($p < 0.05$). All bases do not show sunscreen activity (SPF value < 15). Each base with the addition of the extract shows a significant difference in the SPF value, whereas the base added with the extract shows an increase in the SPF value. However, the base used in the extract affects the SPF value, where the a decrease in the SPF value from the extract after it is made in the form of gel dosage using HPMC and PVA as gelling agent. A very large decline in the SPF value when the

extract in the formulation uses PVA, where the SPF value does not reach 15. There is no significant difference ($p = 0.904$) between the extract and the gel dosage form that uses Carbopol-940 as a gelling agent. Between the extract using Carbopol-940 with HPMC, there are also significant differences. So that it can be stated, the extract made using the Carbopol-940 does not affect the SPF value of the extract itself.

4. CONCLUSIONS

Extracting compounds from plants using solvents with varying polarities will influence the secondary metabolite content in the extract. The plant variant used also impacts the total phenolic and flavonoid content of the extract when employing the same solvent. However, it does not affect the extract's efficacy as a sunscreen, skin-lightening, and anti-aging agent. All extracts exhibit sunscreen, skin-lightening, and anti-aging activities. The ethanol extract of Va leaves (Va-ET) demonstrates the most effective sunscreen activity. In contrast, the extract of Vc and Va leaves display superior skin lightening and anti-aging activities, respectively. Hence, the ethanol extract of Va leaves is utilized in the gel dosage form formulation at a concentration of 0.125%. The gelling agent exerts a notable influence ($p < 0.05$) on the SPF value of the extract post-formulation. The gelling agent Carbopol 940 is the optimal choice for formulating the ethanol extract of Va leaves into a gel dosage form. However, this research is currently confined to in vitro testing. It is essential to conduct in vivo testing to supplement data concerning the efficacy of *P. scutellaroides* (L.) R. Br. leaf extracts in terms of sunscreen activity, skin lightening, and anti-aging properties.

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