Trop J Nat Prod Res, September 2025; 9(9): 4538 - 4545

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Inhibition of Hyperpigmentation by *Padina australis* Hauck on B16F10 Melanoma Cells *In Vitro* and Emulgel Formulation

Wirasti Wirasti[1,2], Retno Murwanti[3], Nanang Fakhrudin[4], Erna P Setyowati[4]*

[1]*Doctoral Program, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[2]*Undergraduate Pharmacy Study Program, Universitas Muhammadiyah Pekajangan Pekalongan, Central Java, Indonesia*
[3]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[4]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia*

ARTICLE INFO

Article history:
Received 23 July 2025
Revised 29 August 2025
Accepted 02 September 2025
Published online 01 October 2025

ABSTRACT

Several types of brown seaweed (Phaeophyceae) Hauck have been studied as active ingredients in skin-brightening cosmetics. However, *Padina australis* has not been specifically investigated as a skin-whitening agent. This study aims to examine the anti-melanogenic effects of *Padina australis Hauck* through ethyl acetate fractionation, purification using Preparative Thin Layer Chromatography (PTLC), and purity analysis with High-Performance Liquid Chromatography (HPLC) using fucoxanthin as a reference. The results showed that the isolate had the same spot pattern as fucoxanthin and a purity level of 96.8%, confirming its identity as fucoxanthin. Melanin content and depigmentation assays on B16F10 murine melanoma cells revealed that the ethyl acetate fraction (EAF) exhibited depigmentation activity of 55.47% without α-MSH (alpha-Melanocyte-Stimulating Hormone) and 38.08% with α-MSH, with no significant difference compared to *kojic acid* (72,09% without and 66.813% with, α-MSH, p>0.05). An emulgel formulation containing EAF as an active ingredient showed a *Sun Protection Factor* (SPF) value of 13.289, categorized as providing maximum protection. Stability testing using a 12-cycle *cycling test* (4ºC and 40ºC) and additional evaluation on day 30 demonstrated that spreadability, adhesiveness, and viscosity remained stable. This study suggests that *Padina australis* has potential as an active skin-brightening agent, with an emulgel formulation offering effective SPF protection and excellent physical stability.

Keywords: Brown seaweed-*Padina australis*, Anti-Melanogenic, Emulgel

Introduction

Hyperpigmentation is characterized by the appearance of dark spots on the skin, which result from increased melanin production in melanocyte cells.[1] Hyperpigmentation is not the same as skin lightening or brightening.Hyperpigmentation refers to a skin condition caused by excessive melanin production. Lightening/Brightening refers to cosmetic approaches aimed at reducing the appearance of pigmentation or making the skin look brighter. Several factors contribute to hyperpigmentation, including exposure to ultraviolet (UV) radiation that stimulates melanin synthesis,[2] as well as the upregulation of tyrosinase enzyme activity through the melanogenesis pathway.[3] Tyrosinase is a rate-limiting enzyme involved in the conversion of the amino acid tyrosine into melanin. Therefore, one of the primary strategies to prevent hyperpigmentation is by inhibiting tyrosinase activity to reduce excessive melanin production. This approach is widely used in the development of cosmetic and dermatological products aimed at skin whitening or evening out skin tone.[4]*Padina australis* Hauck, a type of brown seaweed, has been shown to possess potential in protecting human keratinocyte (HaCaT) cells from damage caused by UVB radiation by enhancing cell viability.[5]

*Corresponding author; Email: erna_prawita@ugm.ac.id
Tel: +6281215312205

Citation: Wirasti , Murwanti R, Fakhrudin N, Setyowati P E. Inhibition of Hyperpigmentation by *Padina australis* Hauck on B16F10 Melanoma Cells *In Vitro* and Emulgel Formulation. Trop J Nat Prod Res. 2025; 9(9): 4538 – 4545 https://doi.org/10.26538/tjnpr/v9i9.56

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

In addition, studies have indicated that *Padina australis* and *Eucheuma cottonii* exhibit sunscreen activity, with Sun Protection Factor (SPF) values ranging from 4 to 5, which falls within the category of moderate protection.[6] Since UVB radiation is one of the key external factors that trigger melanin overproduction leading to hyperpigmentation, the UVB-protective and SPF-related properties of these seaweeds suggest their potential role not only as natural sunscreens but also as preventive agents against UV-induced hyperpigmentation

One of the bioactive compounds found in brown seaweeds such as *Padina australis* is fucoxanthin, a carotenoid pigment known for its strong antioxidant and anti-melanogenic properties.[7] Fucoxanthin has been reported to inhibit melanin production by downregulating tyrosinase expression and interfering with the melanogenesis signaling pathway, making it a promising agent in the treatment and prevention of hyperpigmentation.[8] Its multifunctional role in both UV protection and melanin suppression highlights fucoxanthin's potential as a valuable ingredient in cosmetic formulations targeting skin pigmentation disorders.[9]

Nevertheless, studies on the potential of *Padina australis* as an inhibitor of melanin formation in B16-F10 melanoma cells, as well as its development into an emulgel formulation, are still limited. Therefore, this research aims to explore the anti-hyperpigmentation activity of *Padina australis* and to develop a natural-based emulgel formulation. The findings of this study are expected to contribute to the advancement of knowledge in the field of cosmetics, particularly in the formulation of effective skincare products for addressing hyperpigmentation.

Materials and Method

Material

The main material used in this study was the brown seaweed *Padina australis* Hauck, which was collected along the coastal area of Gunung Kidul, Yogyakarta. The seaweed was harvested between October and November 2022 during low tide. Materials used for the preparation and analysis of the extract included 96% ethanol of pro-analysis grade (E. Merck), ethyl acetate pro-analysis (E. Merck), methanol pro-analysis (E. Merck), acetonitrile, distilled water of pro-analysis grade (PT. Ikapharmindo), fucoxanthin standard (Sigma Aldrich), C18 Zorpax stationary phase column (150 x 2.0 mm), silica powder mesh 80, chloroform pro-analysis (E. Merck), and a 0.45 µm micropore filter. The HPLC instrument used was a Waters brand, model e2695 Separation Module, equipped with a Waters C18 SunChrom column sized 4.6 x 150 mm (Part No. 186002559, Serial No. 02053214413646), and a Waters 2998 photodiode array (PDA) detector. Additional materials and equipment included a tyrosinase inhibitor screening kit (Sigma Aldrich), micropipettes, aluminum foil, 96-well plates, and an ELISA plate reader (Shimadzu). Melanin and α-MSH standards (Sigma Aldrich), as well as B16F10 murine melanoma cells, were also used in the study

Intracellular Tyrosinase Activity In Vitro Inhibition Assay

The intracellular tyrosinase activity inhibition assay was conducted following the method described by Jeon et al.[10] In a 96-well plate, 50 µL of mushroom tyrosinase (EC 1.14.18.1) at a concentration of 700 units/mL in 0.1 M phosphate buffer (pH 6.8) was added to each well. Then, 50 µL of the sample at various concentrations (100, 250, 500 µg/mL) was added to the wells containing *Mushroom tyrosinase*. The mixture was incubated at room temperature for 10 minutes.[11] After incubation, 100 µL of 2.5 mM L-DOPA in 0.1 M phosphate buffer (pH 6.8) was added to each well.[12] The plate was further incubated for 20 minutes at room temperature. The dopachrome formed during the reaction was measured at 475 nm using a microplate reader, and results were compared to a blank (solution without enzyme) and kojic acid at a starting concentration of 100 µg/mL as a standard for tyrosinase inhibition activity. All experiments were performed in triplicate as calculate as follow.

$$\text{Tyrosinase Inhibition} = \frac{A-B}{C-D} \times 100\%$$

A= Absorbance of the sample with mushroom tyrosinase
B= Absorbance of the sample without mushroom tyrosinase
C= Absorbance of mushroom tyrosinase without the sample
D= Absorbance without mushroom tyrosinase and without the sample (only L-DOPA)

Cell Culture

Murine melanoma B16F10 cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) containing 4 mM L-glutamine (Sigma), supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO, USA), 100 units/mL penicillin, and 100 µg/mL streptomycin (Cambrex, East Rutherford, NJ, USA). The cells were maintained in culture flasks and incubated in a CO_2 incubator with 5% CO_2 humidity at 37°C.[13] The culture medium was replaced every 2 days. Cells were harvested by trypsinization when they reached approximately 70% confluency and counted using a hemocytometer. Viable cells were then seeded into culture plates for subsequent treatments.[14]

Melanin content was determined based on a standard curve prepared using synthetic melanin.

The melanin content obtained was then used to calculate the depigmentation index using the following formula:

$$\text{Depigmentation Index (\%)} = \frac{Mc-Mt}{Mc} \times 100\%$$

Mc : control cell melanin content
Mt : sample melanin content

Emulgel Formulation

The preparation of the emulgel consisted of three main stages: preparation of the base gel (Carbopol), preparation of the emulsion, and mixing of the oil and aqueous phases to form a stable emulgel.

Preparation of 3%b/v Carbopol Gel

A total of 9 grams of Carbopol 940 was gradually dispersed into 300 mL of distilled water with gentle stirring to prevent clump formation. The dispersion was then allowed to stand for 24 hours to ensure complete hydration of the Carbopol, forming a stable gel. This process was carried out to prepare the gel base to be used in the emulgel formulation. (This Carbopol dispersion was used for four formulations.)

Emulsion.Preparation

The emulsion preparation process consists of two phases: the oil phase and the aqueous phase.

Oil Phase Preparation

The oil phase was prepared by mixing Span 80 as a surfactant with virgin coconut oil (VCO) at a temperature of 70–80°C. This heating step ensures that all components in the oil phase blend evenly. Once the oil phase was formed, it was gradually added into the Carbopol solution while continuously stirring until optimal homogeneity was achieved.[15]

Aqueous Phase Preparation.

The aqueous phase was prepared by mixing Tween 80 with water at a temperature of 70–80°C. Once a homogeneous solution was obtained, methylparaben and propylparaben—previously dissolved in propylene glycol—were added as preservatives. Triethanolamine (TEA) was then slowly added while stirring to adjust the pH and improve the gel's stability. Butylated hydroxytoluene (BHT) was also added as an antioxidant to prevent oxidation in the formulation.[16]

Additionally, the ethyl acetate-soluble fraction (EAF) was dissolved in 96% ethanol before being incorporated into the aqueous phase. This mixing process was carried out gradually with constant stirring to ensure the homogeneity and stability of the emulsion prior to the final mixing of the oil and aqueous phases in the formation of the emulgel

Mixing of Oil Phase and Aqueous Phase

Once both the oil phase and the aqueous phase were prepared, the oil phase was gradually added to the aqueous phase with continuous stirring using a homogenizer. This mixing process aimed to form a stable emulgel system. The stirring continued until a smooth, homogeneous emulgel with good physical stability was formed, characterized by the absence of phase separation or color change during storage.[15]

In Vitro Sun Protection Factor (SPF) Testing

The purpose of Sun Protection Factor (SPF) testing is to evaluate the effectiveness of a topical formulation in protecting the skin from ultraviolet (UV) radiation exposure. In this study, SPF testing was conducted *in vitro* using the UV-Vis spectrophotometric method. This method is used to determine the formulation's ability to absorb or block UV radiation, particularly within the UVB wavelength range (290–320 nm).

Sample Preparation

The emulgel formulation to be tested was weighed according to the analysis requirements. The sample (extract, fraction, isolate, fucoxanthin,or kojic acid) 250 mg was then diluted with 96% ethanol and homogenized to ensure even distribution of the active ingredients. The resulting solution is stirred until uniform before absorbance measurements are carried out at specific wavelengths relevant for SPF calculation

Absorbance Measurement

The test was performed using a UV-Vis spectrophotometer within the wavelength range of 290–320 nm at 5 nm intervals. The absorbance of the sample was measured at each wavelength to determine how much UV radiation was absorbed by the formulation. These absorbance values form the basis for calculating the Sun Protection Factor (SPF).

SPF Value Calculation

The SPF value was calculated using the equation developed by Dutra,[17] which is widely used for *in vitro* SPF evaluation.[18] The equation is as follows:

The values of EE × I for each wavelength (in nm) are typically obtained from standardized

reference data. The final SPF value reflects the degree of protection provided by the formulation against UVB radiation

Results Interpretation

The *in vitro* SPF values obtained from the emulgel formulation were analyzed to assess the level of protection against ultraviolet radiation, particularly UVB rays.The SPF values were interpreted based on a commonly accepted classification system that categorizes sun protection effectiveness into four levels. SPF values ranging from 2 to 8 are considered toprovide minimal protection against UV radiation. SPF values between 8 and 15 are classifiedas offering moderate protection. High protection is associated with SPF values from 15 to 30,while values exceeding 30 are categorized as providing very high protection.

The higher the SPF value, the greater the formulation's ability to protect the skin from erythema caused by UVB exposure. However, the actual effectiveness of sun protection also depends on factors such as formulation stability, homogeneity of the product, and the thickness and frequency of application on the skin. The authors excluded variables such as formulation stability, product homogeneity, and the thickness and frequency of application by conducting the tests under strictly controlled laboratory conditions. All samples were applied in equal amounts, using a consistent application method, and in a controlled environment.[19] Therefore, the SPF measurements focused on the intrinsic ability of the formulation to absorb or block UVB radiation, without being influenced by those external factors.

Statistical Analysis

Experimental results are presented as mean ± standard deviation. Data were analyzed using ANOVA with a significance level of $p < 0.05$. A *t*-test was employed to compare differences between two samples.

Result and Discussion

The sample collection of *Padina australis* brown seaweed was conducted at Gunung Kidul Beach, Special Region of Yogyakarta, located at coordinates S8º9'2"E110º36'114" to S8º7'5"E110º32'52". The specimen collection took place in October and November 2022, with samples being collected twice daily during low tide, specifically between 13:00-15:00 and 00:00-03:00, to ensure easier access and harvesting. A purposive sampling technique was employed, considering the natural habitat of *Padina australis*, which predominantly grows on rocky substrates in shallow waters.

Environmental factors also play a significant role in the growth of *Padina australis*, affecting both physical and chemical aspects. In this study, the physical parameters analysed, based on data from http://marine.copernicus.eu/, included sea surface temperature. The chemical parameter considered was water salinity. In addition, other environmental factors such as light intensity, ocean currents, and the presence of other organisms also contribute to the development of *Padina australis*.

During the sampling process, the average recorded temperature was 28.4 ± 0.54 ºC, while the salinity was 32.74 ± 0.485 ppt (parts per thousand). According to the Minister of Environment Decree No. 51 of 2004, the water quality standards for marine biota include a temperature range of 28-30 ºC and salinity between 33 and 34 ppt. These measurements indicate that the water conditions where *Padina australis* grows are still close to the standards recommended by the Ministry of Environment of the Republic of Indonesia, Regulation No. 51 of 2004. According to Kadi,[20] macroalgae grow optimally at salinities of 30-33 ppt, while lower salinity levels can hinder growth and decrease population density. Regarding temperature, the thermal energy received from sunlight is used in respiration and nutrient absorption. Several types of macroalgae, including brown and red algae, exhibit a good tolerance to high temperatures during low tide and can even survive up to 32ºC.

The remaceration extraction method was chosen in this study due to its ability to produce a high yield through a process of repeated soaking with solvent replacement. This technique enhances the extraction of secondary metabolites by allowing optimal solubilization of active compounds through prolonged and repeated contact with fresh solvent. Remaceration is also considered a simple method that does not require specialized or sophisticated equipment, making it suitable for laboratory-scale extractions or initial production phases. However, this method has several limitations. These include a relatively long extraction time, lower efficiency compared to techniques involving agitation, reflux, or ultrasonication, and a higher requirement for solvent volume to achieve optimal results. Therefore, the choice of this method reflects a balance between efficiency, equipment availability, and the intended purpose of the extraction process.[21]

In this study, pro analysis ethanol 96%v/v was selected as the extraction solvent due to its high effectiveness in extracting bioactive compounds, such as carotenoids and phenolic compounds, while minimizing the risk of degradation. Pro analysis ethanol offers higher purity compared to technical-grade or 96% ethanol, allowing for more selective and efficient extraction of active constituents.[11] Previous studies have shown that carotenoids are highly sensitive to high temperatures and excessive heating, which can lead to oxidation and a significant reduction in active compound levels. Therefore, choosing the right solvent is essential to maintain the stability of these compounds during the extraction process. Pro analysis ethanol also presents several advantages: it is safe, highly volatile, non-toxic, and environmentally friendly. Furthermore, it is compatible with a wide range of purification techniques for bioactive compounds, such as chromatography and solvent fractionation. Given these benefits, pro analysis ethanol was considered the most appropriate choice in this study to ensure optimal and stable extraction results.

The extract was fractionated using ethyl acetate as the solvent. Ethyl acetate was selected because of its ability to eliminate or separate most polar to semi-polar compounds from the crude extract. This process results in two distinct fractions: the ethyl acetate-soluble fraction (EAF), which contains polar to semi-polar compounds such as flavonoids, phenolics, and tannins; and the ethyl acetate-insoluble fraction (EAIF), which primarily contains non-polar compounds, particularly chlorophyll. This fractionation step is crucial for isolating and concentrating specific groups of bioactive compounds, thereby facilitating further identification, biological activity testing, and potential application in pharmaceutical or cosmetic formulations.

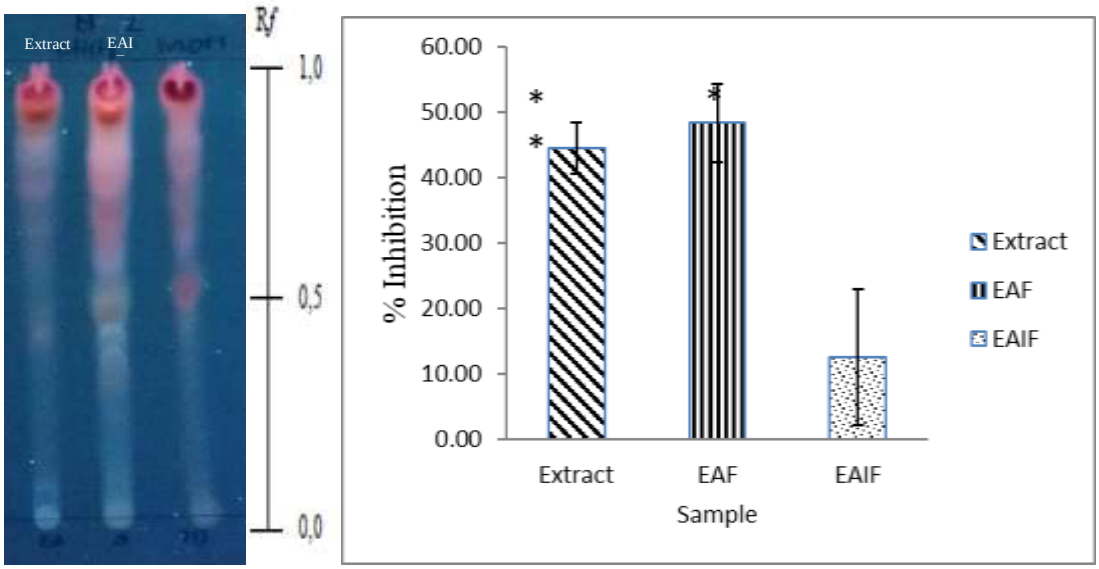
The results of the identification using Thin Layer Chromatography (TLC) with a silica gel GF_{254} stationary phase and a chloroform-methanol (8:2) mobile phase (Figure 1A) showed that both the extract and the ethyl acetate-soluble fraction (EAF) exhibited spots at the same position, indicating the presence of similar components in both samples. Following the tyrosinase activity assay on the crude extract, the ethyl acetate-soluble fraction (EAF), and the ethyl acetate-insoluble fraction, statistical analysis revealed that both the extract and EAF had *p*-values > 0.05. This indicates that there was no significant difference in tyrosinase activity between the extract and EAF. Therefore, it can be concluded that the active compounds responsible for tyrosinase inhibition are more likely concentrated in the ethyl acetate-soluble fraction

The results of the tyrosinase inhibition assay shown in Figure 1B indicate that the extract and the ethyl acetate fraction (EAF) have a p-value > 0.05, suggesting that the difference between them is not significant. Meanwhile, the results of the assay comparing the extract and the fraction eluted from the liquid-liquid extraction analysis (EAIF) show a significant difference. This indicates that the active compounds contributing to tyrosinase inhibitory activity are concentrated in the EAF. The statistical analysis showed that the ethyl acetate fraction (EAF) had a significance value of 0.258 ($p > 0.05$), indicating that the difference was not significant. In contrast, the crude extract had a significance value of 0.02 ($p < 0.05$), indicating a significant difference compared to the positive control. Furthermore, analysis of the active compound content using TLC-densitometry with fucoxanthin as the standard revealed that the fucoxanthin content in the crude extract was 39.157 ± 8.96 µg/mg, while in the ethyl acetate

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

fraction it was 99.105 ± 8.38 µg/mg (Supplementary 1).Therefore, this fraction will be the focus for further purification processes until a pure compound is obtained for testing in the next stage

Purification of the ethyl acetate fraction (EAF) using preparative thin-layer chromatography (PTLC) yielded four subfractions. However, during the solvent evaporation stage, only one subfraction remained stable, while the remaining three were present in quantities too small to



(A) (B)

Figure 1: (A) TLC results using silica gel GF_{254} as the stationary phase and chloroform-methanol (8:2) as the mobile phase. (B) Tyrosinase inhibition test results (% Inhibition) of the Extract, Ethyl Acetate-Soluble Fraction (EAF), and Ethyl Acetate-Insoluble Fraction (EAIF). ** a indicate significant difference p<0,05; * a no significant difference is present when p > 0.05.

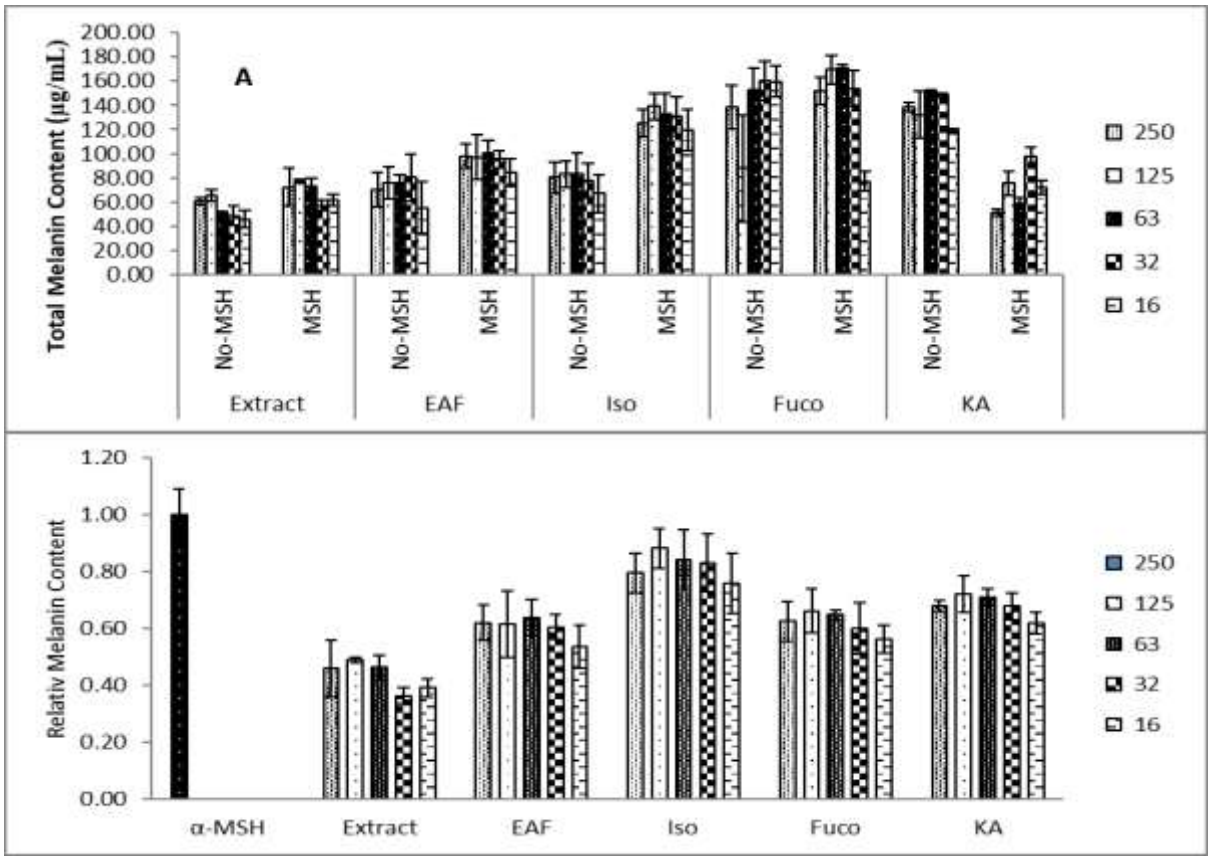


Figure 2: A. Melanin content in B16F10 cells as affected by the inhibition from the extract, ethyl acetate-soluble fraction (EAF), isolate (Iso), fucoxanthin (Fuco),and kojic acid (KA).B. Relative total melanin in B16F10 cells following inhibition by various samples

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

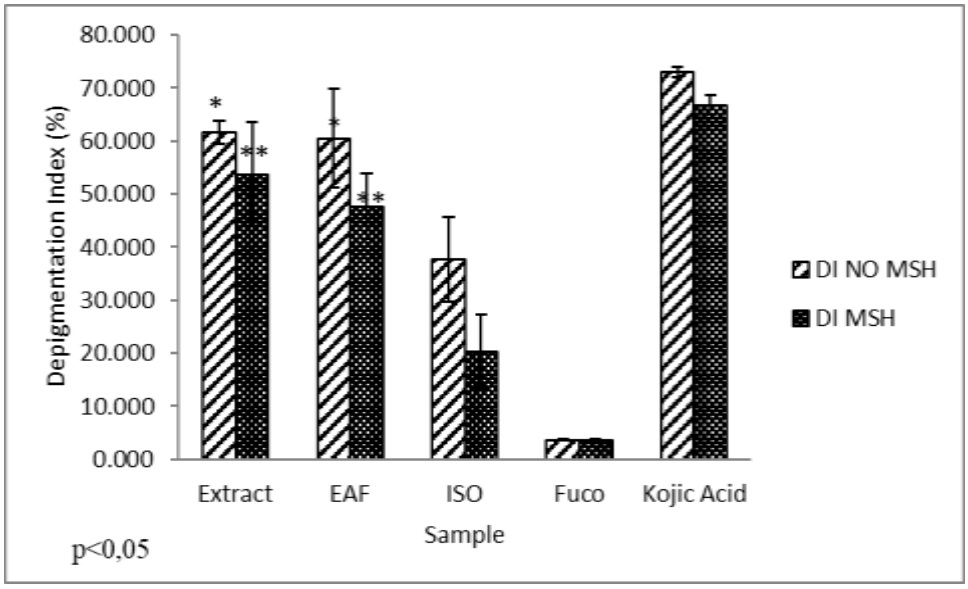


Figure 3: Depigmentation index of the extract, ethyl acetate-soluble fraction (EAF), isolate (Iso), standart compound fucoxanthin (Fuco), and depigmentation standart compound kojic acid (KA). * and ** indicate no significant difference; a significant difference is present when $p < 0.05$

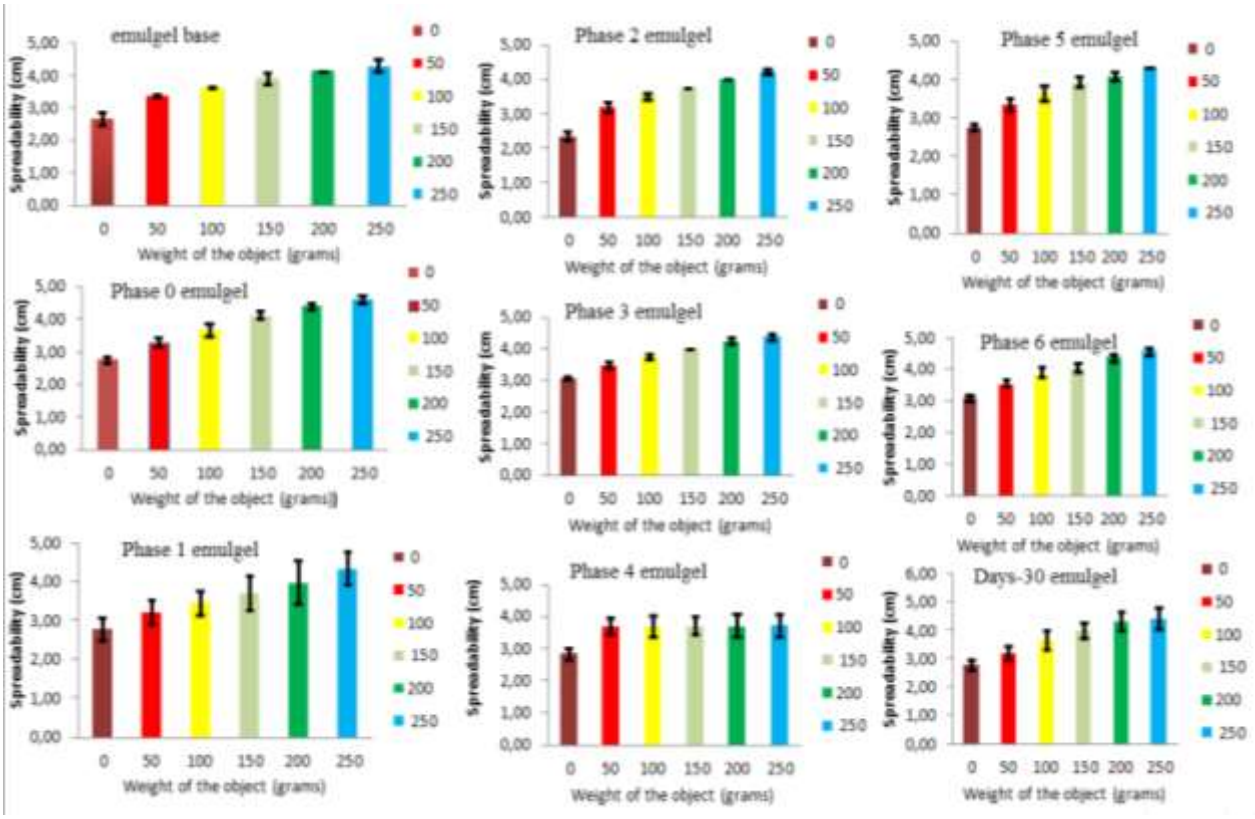


Figure 4: Results of the spreadability test of the emulgel formulation containing the active ingredient FEA under various applied weights (0–250 grams) conducted at each stability cycle. The stability testing was performed using a cycling test, where each cycle involved alternating exposure to cold (4ºC) and hot (40ºC) temperatures for two days, up to the 12th cycle, with an additional evaluation on day 30. The graph illustrates the relationship between increasing weight and the spreadability of the emulgel across each cycle, including the base formulation, cycles 1–12, and day 30, to assess the physical stability of the formulation over time

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

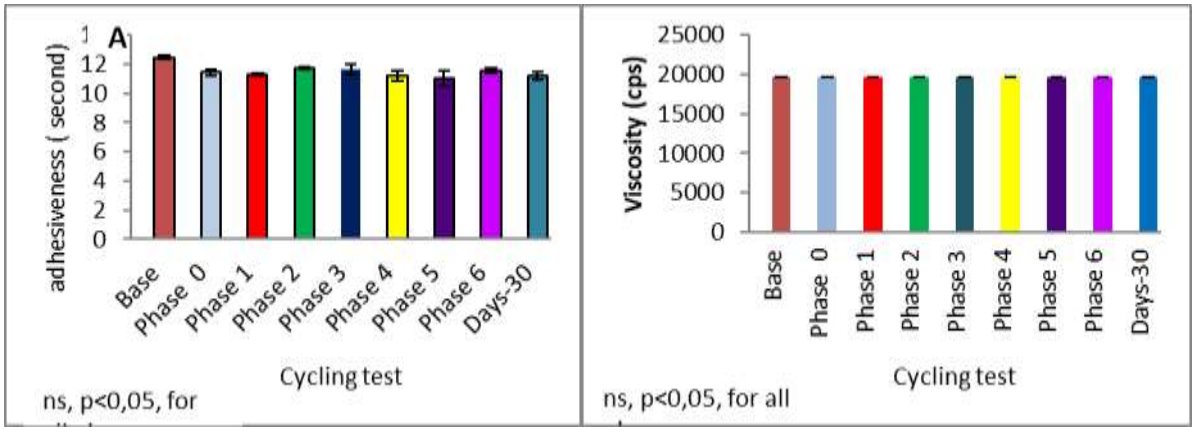


Figure 5: (A) Adhesiveness test results (in seconds) and (B) viscosity of the emulgel formulation containing the active ingredient FEA, evaluated at various testing stages, including the base formulation, stability cycles 0–12 (cycling test), and a follow-up assessment on day 30. This testing aimed to evaluate the stability of the emulgel's adhesiveness and viscosity after alternating exposure to extreme temperatures (cold at 4ºC and heat at 40ºC). Statistical analysis showed no significant differences ($p<0.05$) in either parameter across all testing stages, indicating that both adhesiveness and viscosity remained stable throughout the evaluation period. This stability is essential to ensure user comfort, effective application, and long-term storage reliability of the formulation.

Table 1: Composition of the Emulgel Formulation. [26]

Ingridient	Concentration (w/w%)	Function
Ethyl Acetate Fraction	0.3	Active Ingridient
Carbopol 940 (gel base)	3.0	Gelling agent
Virgin Coconut Oil (VCO)	5.0	Emollient
Span 80	1.5	Surfactant/emulgator
Tween 60	1.0	Surfactant/emulgator
Propylene Glycol	5.0	Humestant
Propyl Paraben	0.01	Antimicrobial preservative
Methyl Paraben	0.03	Antimicrobial preservative
Triethanolamine (TEA)	1.4	Adjuster pH
Butylated Hydroxytoluene (BHT)	0.1	Antioxidant
Distilled Water (ad 100 mL)	q.s. to 100	Hidration

Noted: qs = quantum satis (as needed)

allow further testing. The stable subfraction was then subjected to purity testing using two analytical techniques. The first technique was thin-layer chromatography (TLC) employing a range of mobile phase polarities. The results showed that as the polarity of the mobile phase increased, the migration distance (Rf value) of the subfraction spot decreased compared to that of the fucoxanthin standard. This is consistent with the chemical nature of fucoxanthin, which belongs to the carotenoid class and exhibits semipolar characteristics. The second technique used to assess purity was high-performance liquid chromatography (HPLC). The analysis revealed that the isolated compound had a purity level of 96.8%, which is considered sufficiently high.

The inhibition of melanin formation was evaluated for the crude extract, ethyl acetate fraction (EAF), isolate, and kojic acid, with fucoxanthin used as a reference compound. The inhibition assay was conducted at a range of concentrations: 250, 125, 63, 32, and 16 µg/mL. Melanin levels after α-MSH treatment with the extract, EAF, fucoxanthin isolate, fucoxanthin standard, and kojic acid at a concentration of 250 µg/mL were 97.78 ± 9.85, 72.31 ± 15.7, 125.39 ± 11.2, 151.80 ± 11.31, and 51.63 ± 2.86 µg/mL, respectively. The pattern of increase in these values was consistent across various concentrations. The results demonstrated that the melanin inhibition activity of kojic acid, the extract, and the EAF did not show statistically significant differences (significant, $p < 0.05$). However, a significant difference ($p < 0.05$) was observed when comparing kojic acid with the isolate (Iso) and fucoxanthin (Fuco) across the tested concentrations (**Figure 2A**), under both α-MSH-stimulated and non-stimulated conditions. Based on these findings, the ethyl acetate-soluble fraction (EAF) may be considered a promising active ingredient for emulgel formulation in skin-lightening or anti-hyperpigmentation applications.

Figure 2B illustrates the total melanin content for each sample at various concentrations compared to the control. Statistical analysis revealed that kojic acid (KA), the ethyl acetate-soluble fraction (EAF), and fucoxanthin did not show significant differences in melanin content ($p < 0.05$). In contrast, significant differences ($p < 0.05$) were observed between KA and both the isolate (Iso) and the crude extract. These results indicate that the EAF is a promising candidate as an active ingredient in emulgel formulation, as evidenced by its more effective melanin-reducing activity compared to the extract and the isolate

Figure 3 shows the depigmentation index values, which are calculated based on the difference between the depigmentation levels of the positive control and the sample, divided by the depigmentation level

of the positive control. This index reflects the extent of melanin reduction in murine melanoma cells as a result of treatment with the tested samples. The higher the depigmentation index value, the greater the ability of the sample to inhibit or reduce melanin content in the cells. This indicates that the sample has higher potential as a skin-brightening agent, as it can effectively suppress melanin production or accelerate its degradation within the cells. Therefore, the depigmentation index can serve as an important parameter in evaluating the effectiveness of a skin-lightening agent

The depigmentation index of the ethyl acetate fraction (EAF) did not show a significant difference ($p > 0.05$) compared to the crude extract (Figure 3), but it was significantly different from kojic acid, a well-established and effective skin-brightening agent. Nevertheless, in terms of the depigmentation value achieved, EAF demonstrated greater effectiveness than the extract. This advantage becomes more apparent when compared to the isolate (Iso) and fucoxanthin, as the differences in depigmentation index indicate that EAF has a higher potential for reducing melanin levels in cells.

Based on the results of melanin content and depigmentation tests, the ethyl acetate-soluble fraction (EAF) demonstrated the most effective tyrosinase inhibition activity compared to other fractions. Moreover, the relative melanin levels produced by EAF were not significantly different from those of kojic acid, a widely used skin-whitening agent, showed favourable depigmentation values. Due to its effectiveness in inhibiting melanin formation, EAF holds strong potential as an active ingredient in emulgel formulations. Therefore, to maximize its stability and efficacy in topical applications, the development of an optimized emulgel formulation is essential to ensure optimal delivery and skin penetration of the active compound.

The tyrosinase inhibition assay, along with the measurements of total and relative melanin content, showed that the isolate was consistently less effective than both the ethyl acetate fraction and the crude extract. This may be since tyrosinase inhibition and melanin reduction are not solely attributed to the fucoxanthin-like compound found in the isolate, but also to other synergistic compounds present in the extract or fraction. On the other hand, the isolate still exhibited better tyrosinase inhibition and melanin-reducing activity than the standard fucoxanthin. This difference could be related to the structural isomerism—specifically the cis and trans forms—between the isolate and standard compound. According to Kurinjery et al.[22], the antiproliferative effect of fucoxanthin is significantly influenced by its isomeric structure, where cis-fucoxanthin exhibits higher activity compared to the trans form, likely due to steric hindrance inherent to the cis configuration.

Based on the results of melanin content and depigmentation tests, the ethyl acetate-soluble fraction (EAF) demonstrated the most effective tyrosinase inhibition activity compared to other fractions. Moreover, the relative melanin levels produced by EAF were not significantly different from those of kojic acid, a widely used skin-whitening agent, and also showed favorable depigmentation values. Due to its effectiveness in inhibiting melanin formation, EAF holds strong potential as an active ingredient in emulgel formulations. Therefore, to maximize its stability and efficacy in topical applications, the development of an optimized emulgel formulation is essential to ensure optimal delivery and skin penetration of the active compound.

The selection of the ethyl acetate fraction (EAF) as the active ingredient in the emulgel formulation, rather than the isolated compound, was based on both scientific and practical considerations. The total melanin content, with or without α-MSH induction, was consistently lower in EAF-treated samples compared to the isolate, as shown in Figure 2A. Similarly, the relative melanin content at all tested concentrations demonstrated that EAF exhibited stronger melanogenesis inhibition than the isolate. Moreover, the depigmentation effect of EAF reached 53.77%**,** significantly higher than that of the isolate (20.30%), indicating greater biological activity. From a practical perspective, the isolation process involves more complex, time-consuming, and costly procedures. This presents limitations for large-scale or industrial applications. Taken together, EAF not only shows superior anti-melanogenic efficacy but also offers a more feasible and cost-effective option for formulation development compared to the isolated compound.

Stability testing of the emulgel was conducted using a cycling test, where each cycle involved alternating exposure to cold (4°C) and hot (40°C) temperatures for two days,[23] totaling 12 cycles, along with an additional evaluation on day 30. This test aims to assess the formulation's resistance to extreme temperature variations that could affect its physical characteristics and performance.

The parameters used to evaluate stability included spreadability, adhesiveness, viscosity, and Sun Protection Factor (SPF) value.[24] The spreadability test results, as shown in Figure 4, demonstrate that the greater the applied load, the wider the spread area of the emulgel—a consistent trend observed throughout all cycles, including the base formulation, cycles 1–12, and on day 30. Under the maximum load of 250 grams, the spread area consistently reached 4 cm in all cycles, indicating that the formulation remained stable without significant changes in its spreading characteristics. Furthermore, these findings suggest that the emulgel maintained its stability up to day 30, with no notable alterations in spreadability. This stability is essential to ensure the product's efficacy and user comfort over a specified period.

In addition to spreadability, two other parameters were used to assess the stability of the emulgel: adhesiveness and viscosity. These parameters play a crucial role in determining the quality and stability of the formulation during storage and use.

The results in figure 4A show the adhesiveness test, showing the adhesion time (in seconds) of the emulgel formulation across various testing stages—from the base formula, cycles 0–12, up to day 30. The results indicated no statistically significant differences ($p<0.05$), suggesting that the emulgel maintained consistent adhesiveness despite undergoing exposure to extreme temperatures during the cycling test.

Meanwhile, Figure 5B displays the viscosity measurements of the emulgel across the same stages. The analysis showed that there were no significant changes in viscosity throughout the testing period ($p<0.05$), indicating that the formulation remained stable in terms of thickness, even after repeated heating and cooling cycles.

Collectively, these results confirm that the emulgel maintained both its adhesiveness and viscosity up to day 30. This stability is important, as good adhesiveness ensures the formulation remains on the skin surface for an extended period, while consistent viscosity guarantees user comfort and ease of application. Thus, the EAF-based emulgel demonstrates strong resistance to temperature fluctuations, making it a stable and effective formulation for long-term use.

In addition to stability testing, the Sun Protection Factor (SPF) of the emulgel formulation was also evaluated to assess the product's ability to protect the skin from ultraviolet (UV) radiation. This test aimed to determine how long the emulgel can provide protection against the harmful effects of sun exposure, such as premature aging, hyperpigmentation, and UV-induced skin damage.

The results showed that the emulgel achieved an SPF value of 13.289, which falls within the "maximum protection" category according to the SPF classification standard (8–<15).[25] This indicates that the emulgel provides a reasonably good level of protection against sun exposure for a certain period. The presence of active ingredients in the formulation likely contributes to this protective effect through mechanisms such as UV absorption, reflection, and dispersion.Therefore, in addition to demonstrating good stability under various storage conditions, the emulgel also shows potential as a multifunctional skincare product, offering both brightening effects and UV protection.

Conclusion

The ethyl acetate-soluble fraction (EAF) of the brown seaweed *Padina australis* Hauck demonstrated the most effective tyrosinase inhibitory activity compared to other fractions. Furthermore, this fraction effectively inhibited melanin formation, with results not significantly different from those of kojic acid, and exhibited comparable relative melanin levels. The isolate, as a pure compound, showed lower tyrosinase inhibitory activity than the EAF. In terms of total melanin content, relative melanin levels, and depigmentation values, the isolate produced higher values compared to both the EAF and the crude extract. With these characteristics, the ethyl acetate-soluble fraction has potential as an effective active ingredient in emulgel formulations

and demonstrates good stability, making it a promising candidate for further development in cosmetic or dermatological applications.

Conflict of Interest
The author`s declare no conflict of interest

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements
We would like to express our deepest gratitude to the Faculty of Pharmacy, Universitas Gadjah Mada (UGM), for providing facilities and financial support (1378/UN1/FA/UP/SK/2024). We also extend our sincere appreciation to Universitas Muhammadiyah Pekajangan Pekalongan (UMPP) for their valuable support.

References

1. Chao HC, Najjaa H, Villareal MO, Ksouri R, Han J, Neffati M, et al. Arthrophytum scoparium inhibits melanogenesis through the down-regulation of tyrosinase and melanogenic gene expressions in B16 melanoma cells. Exp Dermatol. 2013;22(2):131–136.
2. Bäsler K, Bergmann S, Heisig M, Naegel A, Zorn-Kruppa M, Brandner JM. The role of tight junctions in skin barrier function and dermal absorption. J Control Release. 2016 Nov;242:105–118.
3. Matsuura R, Ukeda H, Sawamura M. Tyrosinase inhibitory activity of citrus essential oils. J Agric Food Chem. 2006 Mar;54(6):2309–2313.
4. Morais T, Cotas J, Pacheco D, Pereira L. Seaweeds Compounds: An Ecosustainable Source of Cosmetic Ingredients? Cosmetics [Internet]. 2021; Available from: https://www.mdpi.com/962654
5. Yang HL, Lin CP, Gowrisankar YV, Huang PJ, Chang WL, Shrestha S, et al. The anti-melanogenic effects of ellagic acid through induction of autophagy in melanocytes and suppression of UVA-activated α-MSH pathways via Nrf2 activation in keratinocytes. Vol. 185, Biochemical pharmacology. 2021. p. 114454.
6. Nurjanah, Suwandi R, Anwar E, Maharany F, Hidayat T. Characterization and formulation of sunscreen from seaweed Padina australis and Euchema cottonii slurry. IOP Conf Ser Earth Environ Sci. 2019;404(1).
7. Namjooyan F, Farasat M, Alishahi M, ... The anti-melanogenesis activities of some selected brown macroalgae from northern coasts of the Persian Gulf [Internet]. Brazilian Archives of …. SciELO Brasil; 2019. Available from: https://www.scielo.br/j/babt/a/zQXGk77qJbXhKsbtpbk3SLc/?lang=en
8. Hadi AM, Dianursanti. Essence formulation using extract from microalgae Spirulina sp as anti-aging and anti-inflammatory using variance of solvent and sonication time. AIP Conf Proc [Internet]. 2021; Available from: https://aip.scitation.org/doi/abs/10.1063/5.0047569
9. Shimoda H, Tanaka J, Shan SJ, Maoka T. Anti-pigmentary activity of fucoxanthin and its influence on skin mRNA expression of melanogenic molecules. J Pharm Pharmacol. 2010;62(9):1137–1145.
10. Jeon HJ, Kim K, Kim YD, Lee SE. Antimelanogenic activities of piperlongumine derived from Piper longum on murine B16F10 melanoma cells in vitro and zebrafish embryos in vivo: its molecular mode of depigmenting action. Appl Biol Chem [Internet]. 2019;62(1). Available from: https://doi.org/10.1186/s13765-019-0468-7
11. Nurrochmad A, Wirasti W, Dirman A, Lukitaningsih E, Rahmawati A, Fakhrudin N. Effects of Antioxidant, Anti-Collagenase, Anti-Elastase, Anti-Tyrosinase of The Extract and Fraction From Turbinaria decurrens Bory. Indones J Pharm. 2018;29(4):188.
12. Di Petrillo A, González-Paramás AM, Era B, Medda R, Pintus F, Santos-Buelga C, et al. Tyrosinase inhibition and antioxidant properties of Asphodelus microcarpus extracts. BMC Complement Altern Med [Internet]. 2016;16(1):1–9. Available from: http://dx.doi.org/10.1186/s12906-016-1442-0
13. Kim M jin, Kim DS, Yoon H seok, Lee WJ, Lee NH, Hyun C gu. Melanogenesis inhibitory activity of Korean Undaria pinnatifida in mouse B16 melanoma cells. 2014;7(2):89–92.
14. Airin CM, Hana A, Astuti P, Husni A, ... Sargassum crasifolium Extract Could Prevent the Decrese in the Thyroxine Hormone and the Body Weight Loss of Javanese Randu Goats during Transportation. J Food Drugs [Internet]. Available from: https://journal.ugm.ac.id/v3/JFPS/article/view/850
15. Jeengar MK, Rompicharla SVK, Shrivastava S, Chella N, Shastri NR, Naidu VGM, et al. Emu oil based nano-emulgel for topical delivery of curcumin. Int J Pharm [Internet]. 2016;506(1–2):222–36. Available from: http://dx.doi.org/10.1016/j.ijpharm.2016.04.052
16. Chandra D. Uji Fisikokiimia Sediaan Emulsi, Gel, Emulgel Ekstrak Etanol Goji Berry. Jayapangus Press. 2022;11(2):219–228.
17. Dutra EA, Da Costa E Oliveira DAG, Kedor-Hackmann ERM, Miritello Santoro MIR. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Rev Bras Ciencias Farm J Pharm Sci. 2004;40(3):381–385.
18. Sekandi P, Namukobe J, Byamukama R, Nagawa CB, Bacher M, Rosenau T, et al. The antibacterial, antioxidant and sun protection potential of a benzophenone from Dolichopentas decora (S. Moore). Pharmacol Res - Nat Prod [Internet]. 2025;6(December 2024):100135. Available from: https://doi.org/10.1016/j.prenap.2024.100135
19. Okolie NP, Falodun A, Davids O. Evaluation of the Antioxidant Activity of Root Extract of Pepper Fruit (Dennetia Tripetala), and It'S Potential for the Inhibition of Lipid Peroxidation. African J Tradit Complement Altern Med. 2014;11(3):221–7.
20. Kadi A. Interaksi Makroalgae dan Lingkungan Perairan Teluk Carita Pandeglang. Biosfera. 2017;34(1):32.
21. Zhang Y, Xu M, Aðalbjörnsson BV. Sugaring-out: a novel sample preparation method for determination of fucoxanthin in Icelandic edible seaweeds. J Appl Phycol. 2021;33(1):515–521.
22. Kurinjery A, Kulanthaiyesu A. Anti-hyaluronidase and cytotoxic activities of fucoxanthin cis/trans isomers extracted and characterized from 13 brown seaweeds. Process Biochem [Internet]. 2022;122(P2):53–68. Available from: https://doi.org/10.1016/j.procbio.2022.09.024
23. Purwanti RA, Farida Y, Taurhesia S. Formulasi Sediaan Serum Anti Aging dengan Kombinasi dari Ekstrak Buah Tomat (Lycopersicum esculentum L.) dan Ekstrak Kulit Buah Semangka (Citrullus lanatus Thunb.). J Fitofarmaka Indones. 2022;9(2):19–24.
24. Sardinella S, Jusuf H, Hadjarati H, Mustapa MA, Abdulkadir WS, Suryadi AMA, et al. Tropical Journal of Natural Product Research Characterization and Formulation of Anti-Aging Cream Using Collagen Derived from. 2025;9:3013–3020.
25. Yang SI, Liu S, Brooks GJ, Lanctot Y, Gruber J V. Reliable and simple spectrophotometric determination of sun protection factor: A case study using organic UV filter-based sunscreen products. J Cosmet Dermatol. 2018;17(3):518–522.
26. Widyaningtyas Y. Optimasi formula emulgel Sunscreen Ekstrak Etil Asetat Isoflavon Tempe Dengan Carbopol 940 Sebagai Gelling Agent Dan VCO Sebagai Fase Minyak: Aplikasi Desain Faktorial. Sci Rep. 2010;