Biomedical & Pharmacology Journal, December 2024. Vol. **17**(4), p. 2527-2534

Potential of Ethanol Extract *Ulva Lactuca* Cream in Inhibiting Tyrosinase Enzyme Activity as an Anti-Hyperpigmentation Agent in Guinea Pig (Cavia Porcellus) Skin Exposed to Ultraviolet Radiation

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https://dx.doi.org/10.13005/bpj/3045

(Received: 04 September 2024; accepted: 24 October 2024)

The ethanol extract of Ulva lactuca cream was developed to assess its potential as an anti-hyperpigmentation agent by inhibiting tyrosinase enzyme expression and melanin formation. This study aims to evaluate the effectiveness of the cream in preventing hyperpigmentation by inhibiting tyrosinase enzyme activity. This study uses a randomized post-test-only control group design. The subjects were 27 healthy male guinea pigs (Cavia porcellus), aged 3-4 months, weighing 300-350 grams, divided into three groups. The control group received a base cream, while treatment group 1 received a cream with 30% ethanol extract of Ulva lactuca. Treatment group 2 received a cream with 50% ethanol extract. All of the groups were exposed to UVB radiation, three times a week at a dose of 65 mJ/cm² for 65 seconds for two weeks. The cream was applied daily, 20 minutes before and 4 hours after UVB exposure. The levels of tyrosinase enzyme were measured and analyzed using an independent T-test. The average tyrosinase enzyme level in the control group was significantly higher, at 111.92 ng/ml ± 6.56, compared to Group 1 with 91.42 ng/ml ± 0.96, and Group 2 with 58.83 ng/ml ± 1.21. This indicates that tyrosinase enzyme was inhibited in the groups treated with 30% and 50% Ulva lactuca extract cream compared to the control group ($p<0.001$). It can be concluded that the ethanol extract Ulva lactuca creams have significant potential to inhibit the formation of tyrosinase enzyme, which plays a role in hyperpigmentation, in guinea pig skin exposed to UVB radiation.

Keywords: Ethanol extract cream; Guinea Pig; Hyperpigmentation; Tyrosinase Enzym; Ulva Lactuca.

As a tropical country, Indonesia is exposed to sunlight nearly year-round. Sunlight is a source of energy for all living organisms. However, sunlight does not always have beneficial effects. Ultraviolet (UV) rays produced by the sun can have adverse effects on the skin when exposed for prolonged periods. Continuous exposure to ultraviolet rays can cause skin damage, leading to hyperpigmentation. Sunlight produces three types of ultraviolet rays: UVA, UVB, and UVC.[1]

UVA rays are ultraviolet rays with a long wavelength (320-440 nm) and are capable of

penetrating the Earth's ozone layer. Approximately 95% of the UV rays reaching the ground are UVA. Prolonged exposure to UVA rays can damage blood vessels, collagen fibers, and elastic fibers, and contribute to skin aging.[1] UVB rays, with a shorter wavelength (280-325 nm), partially penetrate the ozone layer. The effects of UVB rays include causing skin redness and potentially triggering skin cancer. In contrast, UVC rays, with the shortest wavelength (100-280 nm), do not reach the Earth's surface as they are fully absorbed by the ozone layer.[2]

The most common negative effect of continuous ultraviolet (UV) exposure on the skin is hyperpigmentation, which results in darkening of the skin. UVB exposure has a stronger effect in stimulating the pigmentation process compared to UVA exposure. UVA rays primarily enhance the distribution of pre-existing melanin, leading to intermediate pigmentary darkening, as pigmentation can persist for up to 6-8 hours after exposure. In contrast, UVB rays can increase melanin production, the number of melanocytes, the activity of the tyrosinase enzyme, and melanin distribution. Melanin is a pigment that protects the skin from UV exposure.[3] However, abnormalities in melanin production can lead to hyperpigmentation and result in aesthetic skin issues. One method to prevent or inhibit melanin formation is by actively inhibiting tyrosinase activity.[4] Tyrosinase is an enzyme involved in the formation of skin pigments, a process known as melanogenesis. In melanogenesis, tyrosinase acts as a catalyst in two distinct reactions: the hydroxylation of tyrosine to dihydroxyphenylalanine (L-DOPA), and the oxidation of L-DOPA to DOPA quinone. Tyrosinase in the skin is activated by UV radiation, thereby accelerating melanin production.[5]

An ethanol extract Ulva lactuca cream was developed to assess its potential as an anti-hyperpigmentation agent by inhibiting tyrosinase enzyme expression and melanin formation. Given this potential, this research aims to determine whether the cream also has the capability to treat hyperpigmentation by inhibiting tyrosinase and reducing melanin levels. Traditionally, hydroquinone has been the gold standard for anti-hyperpigmentation treatments; however, it can cause side effects such as ochronosis or rebound phenomena with prolonged use, leading to restrictions on its use.[6] Therefore, an alternative from natural sources with minimal side effects and anti-hyperpigmentation properties is being explored.

Sea lettuce (Ulva lactuca) is a macroalga classified under the division Chlorophyta due to its high content of chlorophyll, phenols, flavonoids, carotenoids, and vitamins A, C, and E.[7] According to research by Putra (2024), ethanol extract of sea lettuce (Ulva lactuca) exhibits the highest antioxidant activity, which can protect the skin from UV exposure, thereby serving as an effective active ingredient in sunscreens.[8,9] The high levels of phenols and beta-carotene not only provide natural sunblocking effects but also have potential as tyrosinase inhibitors in the melanin pigmentation process.[10,11] Sea lettuce is also readily available as it grows abundantly in shallow waters along the coastlines of Indonesia.[7] To date, sea lettuce (Ulva lactuca) has not been extensively utilized in pharmaceuticals or as a cosmetic ingredient, and its use has primarily been limited to food products. Given the components present in Ulva lactuca, the current research aims to investigate whether Ulva lactuca has the potential as a tyrosinase inhibitor for preventing hyperpigmentation caused by ultraviolet exposure. Therefore, it was interesting to explore this potential in the form of an ethanol extract Ulva lactuca cream and testing it *in vivo* using guinea pigs as the experimental model. This study was approved by The Research Ethic Commission of the Faculty of Medicine Udayana University with Ethical Clearance number 1993/UN.14.2.2.VII.14/LT.2024

MATERIALS AND METHODS

The manuscript incorporates all datasets produced or examined throughout this research study. Data sources for this study are ethanol extract of Ulva lactuca and three-month-old brown guinea pigs (Cavia porcellus). The guinea pigs were healthy, with normal eating and drinking behavior. They were obtained from the Laboratory Animal Unit, Pharmacology Department, Faculty of Medicine, Udayana University. The primary material for the research, Ulva lactuca, was extracted at the Faculty of Agricultural Technology, Udayana University. The equipment used includes guinea pig cages, Onyx drinking bottles, Phillips

UVB lamps, Goal brand razors, Tanita digital scales, and surgical tools such as anatomical scissors and B. Braun scalpels.

Preparation of cream base

Formulation of the base cream: Sepigel 305, used as the emulsifier at a concentration of 3%, was mixed with water for 5 minutes. Then, lanolin (2%), dimethicone (2%), and phenoxyethanol (0.5%) were added. Continue mixing until the ingredients form a cream.

Formulation of the Ethanol Extract Ulva lactuca

Fresh sea lettuce is cut into pieces approximately 2 cm x 4 cm in size to facilitate drying and grinding. The sea lettuce pieces are dried in an oven at 50 ± 2°C for 12 hours until the moisture content reaches 7-8%. The dried sea lettuce is then ground using a blender until fine and sieved through a 60-mesh sieve. The extraction of sea lettuce is carried out using the Soxhlet extraction method. First, 20g of sea lettuce powder is weighed and placed into a thimble according to the Soxhlet apparatus size. The thimble is placed into the Soxhlet apparatus, and ethanol solvent with concentrations based on treatment 90% is added, with 200 ml for each concentration, resulting in a powder-to-ethanol ratio of 1:10. The extraction process is carried out for 3, 4, 5, and 6 hours. The solution is then filtered using regular filter paper to remove larger residues, and Whatman No. 1 filter paper is used to filter finer particles, resulting in sea lettuce extract still mixed with the solvent. The result of the filtrate is evaporated to remove the solvent using a rotary evaporator at 50 ± 2°C under a pressure of 100 mBar, yielding a concentrated extract. The evaporation process is halted once the solvent stops dripping.[12]

Examination Antioxidant Level of Ulva Lactuca Extract

Antioxidant activity has a positive linear relationship with the phenolic content in the Ulva lactuca extract. Phenolic compounds, particularly phenolic acids and flavonoids, are natural antioxidants found in fruits, vegetables, and other plants.[13] Antioxidant activity is measured based on the reduction of the purple color, where, when DPPH solution is mixed with an antioxidant substance, a hydrogen atom donation reaction occurs. The hydrogen from the antioxidant is captured by DPPH, which is then reduced to 1,1-diphenyl-2-picrylhydrazine, indicated by a color change from purple to yellow. The parameter used to measure antioxidant activity is the IC50 value (50% Inhibitory Concentration), obtained from the regression equation.[14] This study aims to determine the antioxidant activity of Ulva lactuca extract based on the free radical scavenging method using diphenyl picrylhydrazyl (DPPH) and UV-Vis spectrophotometry, focusing on the IC50 value.

Measurement of Sun Protection Level of Ulva Lactuca Extract

SPF Testing Using UV-Vis Spectrophotometry: A total of 50 mg of the sample was measured three times, then each sample was placed into a 50 ml volumetric flask. The first sample was dissolved in ethanol, the second in ethyl acetate, and the third in chloroform. The three dissolved samples were then filtered using filter paper. From the filtered solutions, 3 ml of each was taken and transferred into a 10 ml volumetric flask. The first solution was topped up with ethanol, the second with ethyl acetate, and the third with chloroform until reaching a final volume of 10 ml. The absorbance was then measured using UV-Vis spectrophotometry at wavelengths ranging from 290-320 nm with 5 nm intervals. The absorbance that appeared on the UV-Vis spectrophotometry was recorded and then the SPF value was calculated.[15]

Treatment of Experimental Animals

A total of 27 guinea pigs were adapted for 1 week. They were randomly divided into three groups: the control group, which was exposed to UVB radiation and treated with the base cream (P0); Group 1, which was exposed to UVB radiation and treated with 30% Ulva lactuca extract cream (P1); and Group 2, which was exposed to UVB radiation and treated with 50% Ulva lactuca extract cream (P2). Each group consisted of 9 guinea pigs.

All guinea pigs in groups P0, P1, and P2 had their dorsal fur shaved. The shaved area was treated with the respective creams, allowed to absorb for 20 minutes, and then exposed to UVB radiation three times a week—on Monday, Wednesday, and Friday—with a dose of 65 mJ/cm² for 65 seconds per guinea pig, for 2 weeks. Topical applications were repeated 4 hours later. On non-irradiation days, topical applications were performed once daily. Skin samples were collected 48 hours after the final UVB exposure.

For sample collection, the fur was shaved from the dorsal area before taking the skin tissue. The area was then cleaned with 70% alcohol on cotton. Anesthesia was induced using a combination of ketamine (50 mg/kg body weight) and xylazine (10 mg/kg body weight) administered intramuscularly. A 4 mm punch biopsy was performed to obtain skin samples, extending to the underlying muscle. The skin tissue was then extracted, and enzyme-linked immunosorbent assay (ELISA) was conducted to measure tyrosinase enzyme levels in each group.

ELISA is a method for detecting and measuring enzymes, proteins, antibodies, or antigens concentrations in biological samples. The biological samples used in this research are guinea pig skin tissues exposed to Ulva lactuca cream and UVB irradiation. The ELISA method operates based on the interaction between antigens and antibodies, where one of the components (either antigen or antibody) is labeled with an enzyme. This research examined the concentration of the tyrosinase enzyme in the treated skin tissue. When this enzyme reacts with its specific substrate, a reaction product is formed that can be detected colorimetrically (through a color change), fluorometrically, or via luminescence.[16] This allows for quantifying tyrosinase levels in the tissue, providing evidence for the hypothesis that Ulva lactuca cream may prevent the increase in tyrosinase, the enzyme responsible for melanogenesis, as the result of UVB exposure.

RESULTS AND DISCUSSION

Antioxidant Levels in Ethanol Extract Ulva lactuca Cream

Testing was conducted to measure the antioxidant levels in ethanol extract Ulva lactuca creams with concentrations of 10%, 30%, and 50%. The results indicated that antioxidant content increased with the higher percentages, as shown in Table 1.

The table above shows that the Ulva lactuca extract cream contains high levels of phenols, vitamin C, beta-carotene, and vitamin E, indicating its potential as an antioxidant. According to various sources, antioxidants such as phenols, vitamin C, beta-carotene, and vitamin E can act as UVB blockers and tyrosinase inhibitors, thereby inhibiting the formation of tyrosinase enzyme responsible for hyperpigmentation.[17]

Sun Protection Factor (SPF) Ability of Ethanol Extract Ulva lactuca Cream

In this study, Ulva lactuca ethanol extract cream was applied to the skin of guinea pigs exposed to UVB radiation at concentrations of 30% and 50%. This was based on preliminary studies, which tested SPF strength at concentrations of 10%, 30%, and 50%. The results indicated that higher concentrations corresponded to increased sun protection capabilities, with SPF levels rising accordingly, as shown in Table 2.

From the information included in table 2, it can be seen that all three concentrations of the cream have SPF values above 15. Sunscreens

Table 1. Antioxidant Levels in Ethanol Extract Ulva lactuca Creams at 10%, 30%, and 50% Concentrations

No.	Percentage of Ulva lactuca Extract Cream	Phenol Content (mg/100g)	Vitamin C Content (mg/100ml)	Beta-Carotene Content (mg/100g)	Vitamin E Content (mg/100g)
1.	10%	0.3965	230.3	308.6	45.1
3.	30%	0.6961	309.3	604.8	168.16
4.	50%	0.7401	429.8	850.7	257

Table 2. Sun Protecting Factor (SPF) Testing Results of Ethanol Extract Ulva lactuca Cream at 10%, 30%, and 50% Concentrations

No.	Concentration of Ulva Lactuca Cream (%)	SPF
1	10	19.909
2	30	21.657
3	50	22.475

that provide extra protection from sunburn and do not cause tanning are categorized as having a Sun Protection Factor (SPF) of 15 or higher.[18] Sunscreen protection is categorized as minimal (2-4), moderate (4-6), high (6-8), maximum (8-15), and ultra (>15).[19] Thus, it can be concluded that the Ulva lactuca cream has a high SPF value, making it a very promising sunscreen.Top of FormBottom of Form

Ethanol extract of Ulva Lactuca cream inhibits the increase in tyrosinase enzyme levels in the skin of guinea pigs (Cavia porcellus) exposed to UVB radiation.

After two weeks of treatment, the dorsal skin of the guinea pigs was shaved before biopsy.. A punch biopsy was performed to obtain skin samples, and then extracted for ELISA examination to measure tyrosinase enzyme level. Tyrosinase enzyme is an enzyme that responsible to pathogenesis of skin hyperpigmentation especially in melanin formation.[8] Tyrosinase is an enzyme that change Tyrosine to Dopa, and Dopa form into Dopaquinon and the last become Melanin. The skin of guinea pigs treated with the placebo cream exhibited darkening or pigmentation, whereas the skin of guinea pigs treated with 30% and 50% Ulva extract creams showed significantly reduced areas of pigmentation.

The results from the treatment of guinea pigs, divided into three groups—Group A with placebo cream, Group B with 30% cream, and Group C with 50% cream—showed that Group C exhibited the strongest inhibition of tyrosinase enzyme activity, followed by Group B, with the lowest inhibition observed in Group A, which received no treatment. These findings are presented in table 3.

This study demonstrated that the average tyrosinase levels in the control (placebo) group were higher than those in the treatment groups.

Table 3. Results of the Mean Difference Test for Tyrosinase Enzyme Levels Between Groups After Treatment (ANOVA Test)

Treatment Group	N	Tyrosinase enzyme levels (ng/ml)	Standard Deviation (SD)	*p* value
A (Placebo)	8	111.92	6.56	
B (30%)	8	91.42	0.96	0.001
C (50%)	9	58.83	1.21	

p significance <0.05

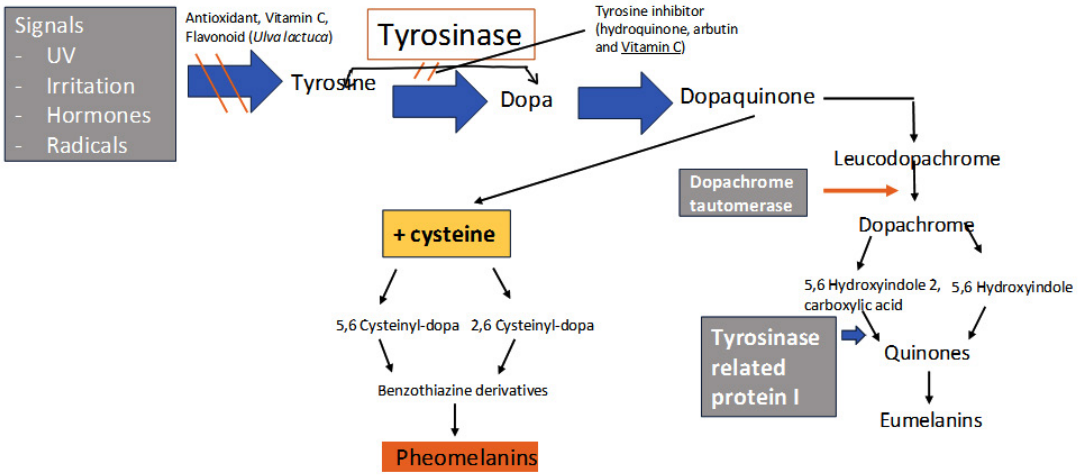

Fig. 1. Mechanism of Action of Antioxidants and Flavonoids in Inhibiting Melanin Formation[23]

This indicates that the formation of tyrosinase was inhibited in the groups treated with 30% and 50% Ulva lactuca extract creams, resulting in lower levels compared to the untreated group ($p<0.001$) (Table 3). The inhibition of tyrosinase formation in the groups treated with 30% and 50% Ulva lactuca extract creams also affected the inhibition of melanin formation. Since melanin synthesis requires tyrosinase, this inhibition may reduce or even prevent hyperpigmentation. The application of Ulva extract creams at concentrations of 30% and 50% has shown to be highly effective in inhibiting the increase in tyrosinase enzyme expression and melanin levels in the skin of guinea pigs (Cavia porcellus) exposed to ultraviolet B (UVB) radiation.

The anti-hyperpigmentation effect of Ulva lactuca extract cream is attributed to its bioactive compounds, including phenols, vitamin C, beta-carotene, and vitamin E, which inhibit the activity of tyrosinase by reducing substances that can cause the oxidation of dopachrome.[20,21] Tyrosinase inhibitors can act either competitively or non-competitively with the tyrosinase substrates, namely L-tyrosine and L-Dopa. Specific tyrosinase inhibitors form covalent bonds with the tyrosinase enzyme, rendering the enzyme inactive during the catalytic reaction.[22]

Previous research has shown that the extract of marigold flowers, with a flavonoid content of 605.48 mg/100g eq, can effectively prevent the increase in melanin levels.[24] Similarly, another study on papaya leaves, which contain alkaloids, tannins, saponins, flavonoids, proteins, fats, vitamin A, vitamin C, vitamin B, and polyphenols, demonstrated similar effects. Papaya leaves are estimated to have tyrosinase inhibitory activity due to their content of vitamin C and flavonoids.[25] This finding supports the results observed with Ulva lactuca extract cream, which also contains bioactive compounds such as flavonoids, vitamin C, beta-carotene, and vitamin E. With an IC50 value of 31.187 ppm, these compounds are classified as strong antioxidants, capable of inhibiting tyrosinase activity by reducing substances that could lead to dopachrome oxidation, thereby potentially inhibiting the hyperpigmentation process.[26]

CONCLUSIONS

Based on the conducted research, it can be concluded that the cream extracted from *Ulva lactuca* contains phenols, vitamin C, beta-carotene, and vitamin E, indicating the presence of antioxidants with potential as inhibitors of the enzyme tyrosinase. The higher the percentage of *Ulva lactuca* extract in the cream, the greater the inhibition of tyrosinase enzyme activity, as evidenced by the lowest tyrosinase enzyme levels in the treatment group with the highest cream percentage (50%), with a significance level of $p<0.001$.

ACKNOWLEDGEMENT

We express high appreciation to Udayana University which provide Research Grant 2024.

Funding Sources

This work was funded by LPPM Udayana University, Bali, Indonesia through the research grant PUPS Udayana No. B/255.262/UN14.4.A/PT.01.03/2024

Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethical approval

Ethical approval was obtained from the Health Ethics Committee Faculty of Medicine, Udayana University with approval number 1993/UN14.2.2.VII.14/LT/2024.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required."

Clinical Trial Registration

This research does not involve any clinical trials.

Authors' contributions

Luh Putu Ratna Sundari: Conceptualization, Methodology, Writing-Original Draft, Funding Acquisition; I Gusti Ayu Widianti: Visualization, Supervision, Project Administration, Writing-Review; Made Alyashanti Radya Bulandari: Data Collection, Writing-

Review & Editing; I Ketut Tunas: Data Collection, Analysis, Resources, Writing-Review & Editing; Each author mentioned has significantly and directly contributed intellectually to the project and has given their approval for its publication.

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