Tyrosinase Inhibitory Activity of Methanolic Extract and Fractions from Green Seaweed *Ulva lactuca*

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Abstract

Ulva lactuca, a widely available green seaweed, has demonstrated promising bioactivity as a tyrosinase inhibitor. This study aimed to evaluate the tyrosinase inhibitory activity of *U. lactuca* extracts and identify the active compounds responsible. The seaweed was extracted using methanol via maceration, followed by partitioning with hexane, chloroform, and water. Total phenolic and flavonoid contents were determined using UV-VIS spectrophotometry, and tyrosinase inhibition was assessed in vitro. Among the tested fractions, the hexane fraction exhibited the highest tyrosinase inhibitory activity with an IC_{50} value of 584.34 ± 35.07 µg/mL, classifying it as an active inhibitor. Although this activity was weaker than kojic acid (IC_{50} = 11.07 ± 0.86 µg/mL), the hexane fraction showed relatively high phenolic (11.66 ± 0.53 mg GAE/g) and flavonoid (9.75 ± 0.30 mg QE/g) contents. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified several bioactive compounds, notably 2,4-dimethoxycinnamic acid, chalcone, and derivatives such as 2,6-dihydroxybenzoic acid and phloretin. These findings highlight the potential of *U. lactuca*, particularly its hexane fraction, as a source of natural tyrosinase inhibitors. This opens avenues for its application in the pharmaceutical and cosmetic industries, especially in formulations targeting hyperpigmentation and skin brightening.

Keywords: pharmaceutical, total flavonoid, total phenolic, whitening agent

Introduction

Indonesia's equatorial location means it gets a lot of strong sunlight. This prolonged UV exposure can harm skin by causing oxidative stress and hyperpigmentation (Mumtazah *et al*., 2020). Ahmad & Damayanti (2018) note that UV-induced free radicals speed up skin aging (photoaging) and boost melanin production by increasing tyrosinase activity, possibly leading to hyperpigmentation. Melanin, which gives skin its color, protects against UV rays. Its production starts with tyrosinase enzymes converting L-tyrosine to DOPA, and then DOPA to dopaquinone (Paudel *et al*., 2019). This process creates reactive oxygen species (ROS) that can increase melanin production (Hindun *et al*., 2017). Because tyrosinase is crucial for pigmentation, it's a main focus for treatments that aim to reduce skin discoloration.

Cosmetics often use synthetic tyrosinase inhibitors like hydroquinone, kojic acid, and mercury to treat hyperpigmentation. Kojic acid is a strong inhibitor, but its use is now limited due to side effects such as skin irritation, thyroid issues, and possible cancer risk at high levels (Purnamasari & Sagala, 2020). This has led to a search for safer, natural options that can effectively inhibit tyrosinase.

Seaweeds are packed with bioactive compounds like polyphenols and flavonoids, known for their antioxidant and enzyme-inhibiting effects. *Ulva lactuca*, a green seaweed common in Aceh, Indonesia, is a promising option (Kurniawan *et al*., 2019). *U. lactuca* contains considerable amounts of phenolics (4.59%) and flavonoids (0.59%), which are effective natural tyrosinase inhibitors because they can bind to metals and mimic substrates (Furi *et al*., 2022). Although other seaweeds have been studied, *U. lactuca*'s potential for

tyrosinase inhibition is less explored. Specifically, there's a lack of thorough research on the tyrosinase-inhibiting properties of methanol extracts and their fractions from *Ulva lactuca*. This gap underscores the novel and important nature of investigating *Ulva lactuca* as a natural source for skin-lightening agents.

Methanol is a good solvent for extracting various compounds from seaweeds (Dolorosa *et al*., 2019). Lee *et al*. (2022) have reported that methanol was the best solvent for extracting tyrosinase inhibitors from *Undaria pinnatifida* compared to ethanol and hot water. This justifies the use of methanol in this study, which aims to test the tyrosinase-inhibiting ability of methanol extract from *Ulva lactuca* in lab conditions.

Materials and Method

Chemical and Instrument

The material used in this study was the green seaweed *Ulva lactuca* obtained from the Coast of Ulee Lheue, Banda Aceh, Indonesia, methanol (Merck KgaA, Germany), chloroform (Merck KgaA, Germany), n-hexane, kojic acid (Tokyo Chemical Industry, Japan), mushroom tyrosinase (Sigma Aldrich, USA), L-DOPA (Tokyo Chemical Industry, Japan), dimetil sulfoksida (DMSO) (Merck KgaA, Germany), potassium phosphate buffer, quercetin (Sigma Aldrich, USA), $AlCl_3$ (Sigma Aldrich, USA), kalium acetate (Merck KgaA, Germany), Na_2CO_3 (Sigma Aldrich, USA), gallic acid (Sigma Aldrich, USA), Folin Ciocalteau reagent (Merck KgaA, Germany). The instruments were used includes spectrophotometer (GENESYS 10S UV-Vis, Germany), GC-MS (SHIMADZU-QP2010S, Japan), fluorescence microplate reader (GlOMAX DISCOVER, Germany), plate 96 well microplate sterile (BIOLOGIX), and incubator (Memmert IN 110, Germany).

Determination and Sample Preparation

Ulva lactuca was collected from Ulee Lheue Beach, Banda Aceh, Indonesia (5.559671°S, 95.284885°E) on March 23, 2022, using a modified method from Dolorosa *et al*. (2019). Fresh samples were cleaned of debris and washed with seawater, then air-dried in the shade. For identification, seaweed samples were sent in cool boxes to the Department of Fisheries, Universitas Gadjah Mada. Intact thalli were chosen and preserved in 70% ethanol at the Department of Pharmaceutical Biology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Identified samples were oven-dried at 40-45°C for 3-4 days, then ground, weighed, and prepared for extraction and fractionation.

Sample Extraction

Secondary metabolites were extracted from *Ulva lactuca* using a slightly modified maceration method from Dolorosa *et al*. (2019). This involved soaking the sample in methanol at a 1:5 (g/mL) ratio at room temperature to obtain bioactive compounds. Methanol was chosen as the solvent because it's a polar protic solvent capable of dissolving a wide variety of polar and moderately non-polar compounds, effectively extracting phenolics, flavonoids, alkaloids, saponins, and glycosides (Thruong *et al*., 2019).

A total of 300 grams of *U. lactuca* powder were soaked in 1500 mL of methanol in a covered Erlenmeyer flask and agitated to ensure full contact. Maceration was performed for 24 hours, and the extract was filtered using Whatman no. 42 paper. The remaining powder was re-soaked twice more in 1500 mL of methanol for 24 hours each time, with filtration after each soak. The three filtrates were combined and evaporated at 40°C and 60 rpm until viscous. The resulting extract was then freeze-dried for 24 hours and stored at -20°C to maintain compound stability.

Liquid-liquid Partition

The methanol extract underwent fractionation using liquid-liquid partitioning, based on Rawa *et al*. (2019), with solvents of increasing polarity: n-hexane (non-polar), chloroform (semi-polar), and water (polar). The n-hexane and chloroform fractions were concentrated in a 45°C water bath, while the water fraction was concentrated at 60°C.

Total Phenolic Content Assay

The Folin-Ciocalteu method was used to determine the total phenol content, following a slightly modified Alagan *et al*. (2017) procedure.

Total Flavonoid Content Assay

The total flavonoid content in this study was assessed following a slightly modified Alagan *et al*. (2017) procedure, which included testing a standard quercetin solution and the flavonoid content of the extract.

In Vitro Tyrosinase Inhibitory Activity Assay

The tyrosinase inhibitory activity of methanol extracts, hexane, chloroform, and aqueous fractions of *Ulva lactuca* was evaluated by measuring their ability to inhibit melanin formation using mushroom tyrosinase and L-DOPA as a substrate. Kojic acid served as a positive control. The assay followed a modified Arguelles & Sapin (2020) method.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted using a GC-MS QP2010S (SHIMADZU), with slight modifications to the Hasan *et al*. (2019) method, to identify compounds in methanol extracts and hexane fractions of *Ulva lactuca*. Samples were dissolved in 20 µL of DMSO, and a 10 ìL portion was injected into a 300°C injection port. The interface and ion source temperatures were set at 300°C and 250°C, respectively, with a split ratio of 1:53. Helium was used as the carrier gas at a flow rate of 0.49 mL/min through a GC SH-I-5Sil MS Capillary column (30 m × 0.25 mm × 0.25 µm). The column temperature started at 70°C, increased by 10°C/min to 320°C, and was held for 5 minutes. Ionized and separated components were detected, producing fragmentation patterns that were then compared to the WILEY and NIST databases for identification.

Statistical Analysis

This study employed a Complete Random Planning (RAL) with methanol extracts, hexane, chloroform, and aqueous fractions as independent variables, and tyrosinase inhibitory activity, total phenol content, and total flavonoid content as dependent variables. Data were analyzed using Microsoft Excel 2021 and IBM SPSS Statistics 26. One-way ANOVA was used to analyze the data from the total phenolic content assay, total flavonoid test, tyrosinase inhibitory activity test, and partition yield. Significant ANOVA results ($p<0.05$) were further analyzed using the HSD-Tukey test to identify differences between treatments. Significant results are presented as mean ± standard deviation (SD).

Results and Discussion

The Yield of Liquid-Liquid Partition

Liquid-liquid partitioning of *Ulva lactuca* methanol extract with hexane, chloroform, and water resulted in yields of 11.02±1.18% for n-hexane, 12.36±1.75% for chloroform, and 68.08±3.49% for the water fraction. The different yields reflect the varying solubilities of compounds in the different solvents. The highest yield in the water fraction indicates that *U. lactuca* extract mainly contains highly polar compounds, than in semi-polar or nonpolar ones. The yields of the fractions are detailed in Table 1.

The water fraction showed the highest yield, followed by the n-hexane and then the chloroform fraction (Table 1). This aligns with findings from Gazali *et al*. (2024) and Ghareeb *et al*. (2019), who also reported the highest yields in the water fraction of *U. lactuca* (56.00% and 55.36%, respectively), followed by n-hexane (43%) and chloroform (18%). Variations in yield across studies can arise from differences in the initial solvent type and concentration, extraction conditions, environmental factors, biomass sources, and fractionation techniques (Ghareeb *et al*., 2019).

Total Phenolic Content

An acid calibration curve served as the standard for measuring the total phenol content in the *Ulva lactuca* methanol extract and its fractions. Acids react well with the Folin-Ciocalteu reagent, producing a sensitive blue complex (Gazali *et al*., 2024). Table 2 shows the total phenol content for each fraction.

Table 2 indicates that the solvent type significantly affects the total phenolic content of *U. lactuca* extract.

Table 1. Yield between fractions from partition results of *Ulva lactuca*

Fractions	Yield (%)
n-hexane	$11.02\pm1.18_a$
Chloroform	$12.36\pm1.75_a$
Water	$68.08\pm3.49_b$

Note: Each values stated as mean±SD in triple treatment. Values with different alphabets (a-c) indicate a tangible difference of each treatment at $p<0.05$ analyzed with HSD-Tukey.

Table 2. Total phenolic content of *U. lactuca* methanol extract and its fractions

Samples	Total Phenolic Content (mgGAE/g)
Methanol extract	$2.10\pm0.15_a$
n-hexane fraction	$11.66\pm0.53_c$
Chloroform fraction	$4.86\pm0.61_b$
Water fraction	$1.09\pm0.15_a$

Note: Each values stated as mean±SD in triple treatment. Values with different alphabets (a-c) indicate a tangible difference of each treatment at $p<0.05$ analyzed with HSD-Tukey.

The hexane fraction exhibited the highest phenolic content, followed by the chloroform fraction. Conversely, polar solvents (methanol extract and water fraction, although values are not shown) yielded the lowest phenolic content. This suggests that the phenolic compounds in *U. lactuca* are more soluble in the non-polar solvent hexane, contrary to the general solubility of polyphenols in polar solvents (Arguelles & Sapin, 2020). This observation aligns with Gazali *et al.* (2024) finding that n-hexane extract of *Ulva* sp. had a higher total phenol content than its methanol extract.

Total Flavonoid Content

The total flavonoid content of the *Ulva lactuca* methanol extract and its fractions was measured because flavonoids, as secondary metabolites, can inhibit excessive melanin production. Flavonoids can directly inhibit tyrosinase activity in melanogenesis due to their á-keto group, which is structurally similar to the dihydroxyphenyl group in DOPA (Sari *et al*., 2019). Table 3 presents the total flavonoid content in the methanol extract, hexane, chloroform, and water fractions.

Table 3 shows solvent type significantly impacts *Ulva lactuca* flavonoid content. The hexane fraction had the most flavonoids (9.75±0.30 mgQE/g), followed by chloroform (6.55±0.51 mgQE/g). This suggests *U. lactuca* is rich in non-polar flavonoids, as flavonoid aglycones are less polar and more soluble in non-polar solvents, even though glycosides are typically polar (Prayoga *et al*., 2019). Some flavonoids, particularly those with detached benzene rings, are exclusively soluble in non-polar solvents (Wulandari *et al*., 2022). Thus, non-polar flavonoids like isoflavones, chalcones, and anthocyanidins likely dominate in *U. lactuca*. The substantial flavonoid content in the chloroform fraction also indicates considerable semi-polar flavonoids, such as free flavones, flavanones, and flavonols (Wulandari *et al*., 2022). Conversely, the lowest flavonoid content in the water fraction and methanol extract suggests fewer slightly polar flavonoids are present in *U. lactuca*.

Tyrosinase Inhibitory Activity

Tyrosinase inhibition was tested for methanol extract and *Ulva lactuca* fractions at 625-10000 ìg/mL. Kojic acid, a known potent inhibitor and pure compound, was tested at lower concentrations (50-250 µg/mL). Figure 1 shows the tyrosinase inhibition results for the extracts, fractions, and kojic acid.

Table 3. Value of total flavonoid extracts and fractions of *U. lactuca*

Samples	Total Flavonoid Content (mgQE/g)
Methanol extract	$5.51\pm0.41_b$
N-hexane fraction	$9.75\pm0.30_d$
Chloroform fraction	$6.55\pm0.51_c$
Water fraction	$3.81\pm0.12_a$

Note: Each values stated as mean±SD in triple treatment. Values with different alphabets (a-c) indicate a tangible difference of each treatment at $p<0.05$ analyzed with HSD-Tukey.

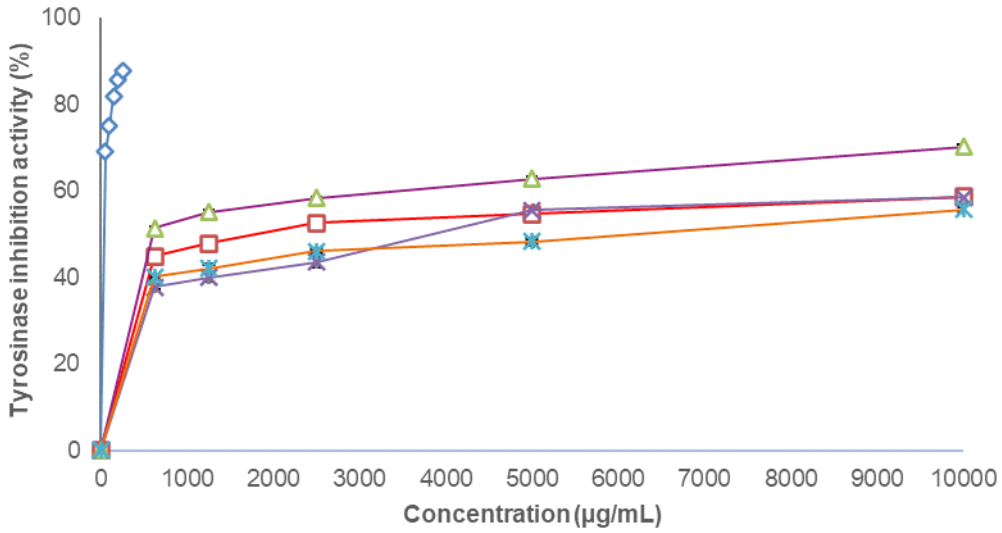

Figure 1. Effects of concentrations of methanol extract (□), hexane fraction (▲), chloroform fraction (x) and water fraction (*) as well as kojic acid (◊) on tyrosinase inhibitory activity.

Figure 1 shows varying percentages of tyrosinase inhibition across treatments, with inhibition increasing as concentration rises. Tyrosinase inhibitors block the interaction between tyrosinase and L-DOPA, reducing dopachrome formation and the brown color intensity. The n-hexane fraction showed the strongest inhibition (51.32±0.49% to 70.13±0.46%). Methanol extracts also inhibited tyrosinase (44.83±0.56% to 58.65±0.14%), as did chloroform fractions (37.82±0.52% to 58.49±1.10%) and the aqueous fraction (40.06±0.33% to 55.70±0.60%). Compared to kojic acid (positive control), the inhibition by the *U. lactuca* extract and fractions was lower. Kojic acid, a pure commercial inhibitor, strongly inhibited tyrosinase (69.01±0.74% to 87.66±0.12%) at much lower concentrations (50-250 µg/mL) (Sholikha *et al*., 2023).

IC_{50} values, representing the concentration needed for 50% tyrosinase inhibition (Sagala *et al*., 2019), were calculated for methanol extract, n-hexane, chloroform, and water fractions using linear regression of their inhibition percentages. Table 4 displays the IC_{50} values for the *U. lactuca* samples and the kojic acid positive control.

Ulva lactuca fractions showed varied tyrosinase inhibition (Table 4). The n-hexane fraction was the most active (IC_{50} = 584.34 ± 35.07 µg/mL), qualifying as an active inhibitor (<1000 µg/mL) per Prasetyo (2021). While its IC_{50} was lower than other *U. lactuca* extracts and fractions (methanol: 1783.06 ± 63.9 µg/mL; chloroform: 3578.23 ± 51 µg/mL; water: 4860.46 ± 27.05 µg/mL), it was significantly higher than kojic acid (11.07 ± 0.86 µg/mL), a highly active commercial inhibitor. This indicates *U. lactuca* is less effective than kojic acid, consistent with prior research on *Sargassum plagyophyllum*, *Eucheuma cottonii* (Dolorosa *et al*., 2019), and *Sargassum* sp. (Gazali, 2018).The *U. lactuca* n-hexane fraction shows moderate tyrosinase inhibition compared to arbutin and aloesin (Jung *et al*., 2001). While arbutin competitively inhibits L-DOPA, aloesin acts differently on tyrosinase pathways (Jung *et al*., 2001; Draelos, 2007). Despite a lower yield, the n-hexane fraction's higher inhibition is likely due to its rich phenolic (11.66 ± 0.53 mg GAE/g) and non-polar flavonoid (9.75 ± 0.30 mg QE/g) content. Non-polar flavonoid aglycones have a strong affinity for tyrosinase's hydrophobic active site (Furi *et al*., 2022). Flavonoids competitively inhibit tyrosinase by their hydroxyl groups interacting with copper ions at the active site (Chang, 2009). However, more polar, glycosylated flavonoids are less inhibitory as their polarity hinders active site access (Furi *et al*., 2022). Thus, the abundance of non-polar flavonoids in the *U. lactuca* n-hexane fraction likely drives its potential as a natural tyrosinase inhibitor.

GC-MS Analysis

GC-MS was used to identify bioactive compounds in *Ulva lactuca*. This technique separates and quantifies compounds (GC) while analyzing their molecular structures (MS) (Melati, 2021). Results are shown as chromatograms, with peaks representing different compounds. We initially screened the methanol extract to identify its general constituents. Then, the n-hexane fraction, which showed high tyrosinase inhibition, was analyzed to pinpoint its active compounds. The *U. lactuca* methanol extract's GC-MS analysis revealed 31 peaks (Figure 2), and its phenolic profile is in Table

Table 4. IC_{50} of tyrosinase inhibitory activity

Samples	IC_{50} (µg/mL)
Methanol extract	$1783.06±63.90_c$
n-Hexane fraction	$584.34±35.07_b$
Chloroform fraction	$3578.23±51.00_d$
Water fraction	$4860.46±27.05_e$
Kojic Acid	$11.07±0.86_a$

Note: Each values stated as mean±SD in triple treatment. Values with different alphabets (a-c) indicate a tangible difference of each treatment at $p<0.05$.

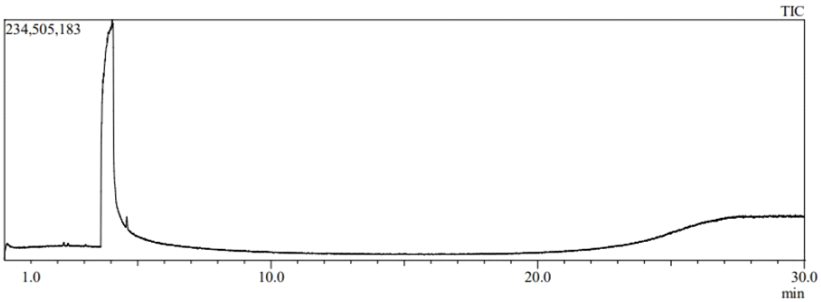

Figure 2. Chromatogram of *Ulva lactuca* methanol extract.

5. The n-hexane fraction analysis showed 33 peaks (Figure 2), with its phenolic chemical profile also in Table 5.

GC-MS analysis identified phenolic compounds in *U. lactuca* extracts based on retention time, molecular formula, and weight, confirmed by peak area (relative concentration) and RT (Melati, 2021). These phenolics exhibit significant biological activity and potential tyrosinase inhibition. Differences in identified compounds between methanol and hexane extracts are likely due to solvent polarity affecting solubility (Suradji *et al*., 2016). Methoxy-cinnamic acids, especially 2,4-dimethoxycinnamic acid (with its crucial hydroxyl group for antioxidant and anti-tyrosinase activity), have previously demonstrated tyrosinase inhibitory effects (Salah *et al*., 2017).

Compounds like 2,4-dihydroxybenzoic acid, 3TMS derivative and 2,5-dihydroxybenzoic acid, 3 TMS derivative, found in both methanol and hexane fractions, likely contribute to anti-tyrosinase activity. Kim et al. (2013) showed that dihydroxybenzoic acid from *Origanum vulgare* inhibited tyrosinase by 32.9% (at 1000 µg/mL) by suppressing the expression of MC1R, MITF, TYR, TRP-2, and TRP-1. Since MITF controls tyrosinase transcription, its inhibition reduces tyrosinase activity (Park *et al*., 2021). Additionally, as MC1R stimulates melanin production, its inhibitors also decrease melanin. Cunhakant and Chaicharoenpong (2019) further reported that dihydroxybenzoic acid inhibits tyrosinase in both the monophenolase (IC_{50} 64.54±0.65 µM) and diphenolase pathways (IC_{50} 84.66±0.90 µM).

Benzenepropanoic acid, a TMS derivative, found only in the methanol extract, is also suspected to inhibit tyrosinase (El-Hady *et al*., 2016). Compounds identified solely in the hexane fraction and believed to inhibit tyrosinase include adenosine, 2'-O-methyl-; phloretin, tetra (trimethylsilyl) ether; and 3-phenyllactic acid, 2TMS derivative. Adenosine at 400 µM was shown to inhibit tyrosinase and melanin production (Kim *et al*., 2014). Phloretin at 0.05 mM inhibited tyrosinase activity by 4.2% (monophenolase) and 28.32% (diphenolase) (Ortiz-Ruiz *et al*., 2015). Phenylacetic acid compounds at 9 mM inhibited tyrosinase activity by 55.44% (monophenolase) and 58.08% (diphenolase).

Table 5. Results of identification of phenolic compounds on methanol extract *Ulva lactuca*

Peak	RT	Component	Molecules formula	MW	% Area
2	21.425	2,4-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	208	2.12
4	24.334	Thymol, TMS derivative	$C_{13}H_{22}OSi$	222	2.11
5	25.442	Chalcone	$C_{15}H_{12}O$	208	1.71
		3-Nitrophthalhydrazide	$C_8H_5N_3O_4$	207	
6	25.550	Acetamide, N-(4-bromo-2-chlorophenyl)-	$C_8H_7BrClNO$	247	1.63
7	25.825	Ethyl gallate, 3TMS derivative	$C_{18}H_{34}O_5Si_3$	414	4.51
		5-Methylsalicylic acid, 2TMS derivative	$C_{14}H_{24}O_3Si_2$	296	
9	26.008	Benzeneacetic acid, .alpha.,3,4-tris[(trimethylsilyl)oxy]-, methyl ester	$C_{18}H_{34}O_5Si3$	414	7.05
		2,4-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	370	
11	26.333	Benzeneacetonitrile, 3,4,5-trimethoxy-	$C_{11}H_{13}NO_3$	207	7.58
		Benzenepropanoic acid, TMS derivative	$C_{12}H_{18}O_2Si$	222	
13	26.625	3-Methylsalicylic acid, 2TMS derivative	$C_{14}H_{24}O_3Si_2$	296	3.29
14	26.692	Hydroquinone, 2,5-di-tert-butyl- (DtBHQ)	$C_{14}H_{22}O_2$	222	2.69
15	26.825	2-Propenoic acid, 3-[4-(acetyloxy)-3-methoxyphenyl]-, methyl ester	$C_{13}H_{14}O_5$	250	2.15
		Chalcone	$C_{15}H_{12}O$	208	
16	26.908	Ethyl gallate, 3TMS derivative	$C_{18}H_{34}O_5Si_3$	414	4.09
18	27.067	3,4-Dihydroxymandelic acid, 4TMS derivative	$C_{20}H_{40}O_5Si_4$	472	1.78
19	27.109	Xanthine, 1,3,7,8-tetramethyl-	$C_9H_{12}N_4O_2$	208	1.73
21	27.272	2,6-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	370	4.43
23	27.700	2-Propenoic acid, 2-[(trimethylsilyl)oxy]-3-[4-[(trimethylsilyl)oxy]phenyl]-, trimethylsilyl ester	$C_{18}H_{32}O_4Si_3$	396	1.64
24	28.350	Homovanillyl alcohol, 2TMS derivative	$C_{15}H_{28}O_3Si_2$	312	1.50
		3-(4-Hydroxy-3-methoxyphenyl)propionic acid, 2TMS derivative	$C_{16}H_{28}O_4Si_2$	340	
27	28.950	Acetic acid, (2,4-dichlorophenoxy)-, ethyl ester	$C_{10}H_{10}Cl_2O_3$	248	1.55
29	29.325	Propylparaben, TMS derivative	$C_{13}H_{20}O_3Si$	252	2.00
		Mephenesin, 2TMS derivative	$C_{16}H_{30}O_3Si_2$	326	
		o-Toluic acid, TMS derivative	$C_{11}H_{16}O_2Si$	208	
30	29.458	Ethanone, 1-(2,3,4-trichlorophenyl)-	$C_8H_5Cl_3O$	222	
31	29.599	Benzenepropanoic acid, 3-methoxy-.alpha.,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{19}H_{36}O_5Si_3$	428	2.47

Note: RT: retention Time, MW: Molecular weight

Chalcones and dihydroxybenzoic acid derivatives inhibit tyrosinase due to structural features that interact with the active site. Hydroxyl groups, especially in the 2,4-dihydroxy arrangement, enhance copper chelation in chalcones, with some even outperforming kojic acid (Gryn-Rynko *et al*., 2022). Dihydroxybenzoic acid derivatives also chelate copper ions via hydroxyl groups, while their aromatic structure allows ð-ð interactions, forming stable tyrosinase complexes (Vontzalidou *et al*., 2022). Phenolic compounds are generally polar due to hydroxyl groups. The presence of phenolics in the non-polar hexane fraction can be explained by: 1) Structural modifications like methylation or lipophilic substituents increasing non-polar solubility (Pan *et al*., 2011); 2) Complexation with lipids or terpenoids enhancing non-polar solubility; and 3) Extraction conditions potentially distributing semi-polar or modified phenolics into the hexane fraction (Nazir *et al*., 2020).

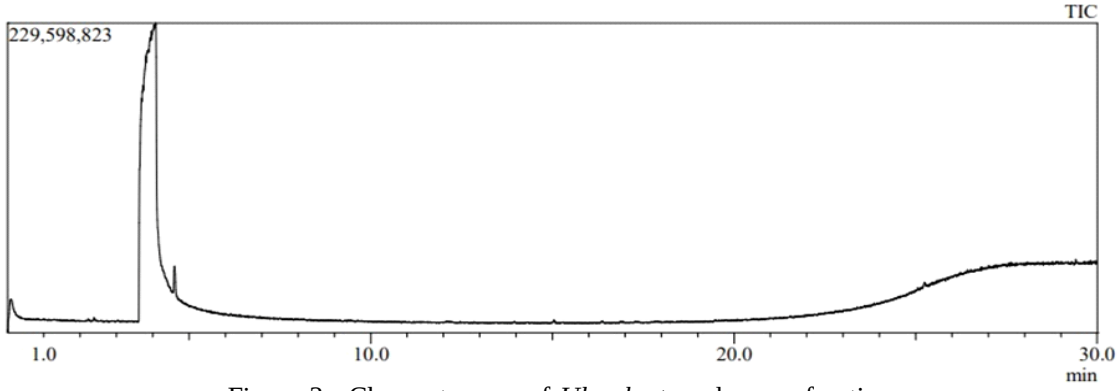

Figure 3. Chromatogram of *Ulva lactuca* hexane fraction.

Table 6. Results of phenolic compound identification on *Ulva lactuca* hexane fraction

Peak	RT	Component	Molecules formula	MW	% Area
2	24.575	Phenylglyoxylic acid, TMS derivative	$C_{11}H_{14}O_3Si$	222	1.29
		delta-Tocopherol, TMS derivative	$C_{30}H_{54}O_2Si$	474	
		3,4-Dihydroxymandelic acid, 4TMS derivative	$C_{20}H_{40}O_5Si4$	472	
7	25.263	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	2.65
8	25.333	2,3,6-Trichlorobenzoic acid, TMS ester	$C_{10}H_{11}Cl_3O_2Si$	296	1.30
11	25.642	Hydroquinone, 2,5-di-tert-butyl- (DtBHQ)	$C_{14}H_{22}O_2$	222	1.40
12	25.675	2,5-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	370	2.18
13	25.808	1,3,5-Benzetriol, 3TMS derivative	$C_{15}H_{30}O_3Si_3$	342	2.65
15	25.967	2-Propenoic acid, 3-(2,3-dimethoxyphenyl)-,	$C_{11}H_{12}O_4$	208	4.67
		2,4-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	208	
16	26.075	Phloretin, tetra(trimethylsilyl) ether	$C_{27}H_{46}O_5Si_4$	562	2.45
		3-Methylsalicylic acid, 2TMS derivative	$C_{14}H_{24}O_3Si_2$	296	
18	26.342	Chalcone	$C_{15}H_{12}O$	208	10.41
19	26.575	10,11-Dihydro-10-hydroxycarbamazepine, N-trimethylsilyl-, trimethylsilyl ether	$C_{21}H_{30}N_2O_2Si_2$	398	2.60
		4-Hydroxybenzoic acid, 2TMS derivative	$C_{13}H_{22}O_3Si_2$	282	
20	26.650	2,3-Dihydroxybenzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	370	1.73
21	26.750	Ketoprofen methyl ester	$C_{17}H_{16}O_3$	268	2.85
22	26.825	2,5-Dimethylbenzophenone	$C_{15}H_{14}O$	210	7.55
		3-Hydroxybenzoic acid, 2TMS derivative	$C_{13}H_{22}O_3Si_2$	282	
24	27.167	Benzeneacetic acid, .alpha.,3,4-tris[(trimethylsilyl)oxy]-, methyl ester	$C_{18}H_{34}O_5Si_3$	414	5.02
		1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	$C_{11}H_{12}O_3$	192	
25	27.342	Thymol, TMS derivative	$C_{13}H_{22}Osi$	222	3.82
26	27.475	3,4-Dihydroxymandelic acid, 4TMS derivative	$C_{20}H_{40}O_5Si_4$	472	4.81
29	27.890	Adenosine, 2'-O-methyl-	$C_{11}H_{15}N_5O_4$	281	4.38
		3-Phenyllactic acid, 2TMS derivative	$C_{15}H_{26}O_3Si_2$	310	
31	28.232	2-Butanone, 4-[3-methoxy-4-[(trimethylsilyl)oxy]phenyl]-	$C_{14}H_{22}O_3Si$	266	1.76
		Benzenepropanoic acid, 3-methoxy-.alpha.,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{19}H_{36}O_5Si_3$	428	
		3-(4-Hydroxy-3-methoxyphenyl)propionic acid, 2TMS derivative	$C_{16}H_{28}O_4Si_2$	340	
32	28.386	Terephthalic acid, diisopropyl ester	$C_{14}H_{18}O_4$	250	2.10
		6-Nitrocoumarin	$C_9H_5NO_4$	191	
33	28.583	β-asarone	$C_{12}H_{16}O_3$	208	1.17

Note: RT: retention time, MW: molecular weight

Conclusion

Methanol extracts and fractions of *Ulva lactuca* show inhibitory activity against the tyrosinase. Hexane fraction exhibited the highest tyrosinase inhibitory activity with an IC_{50} value of 584.34 ± 35.07 µg/mL, classifying it as an active inhibitor. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified several bioactive compounds, notably 2,4-dimethoxycinnamic acid, chalcone, and derivatives such as 2,6-dihydroxybenzoic acid. These findings highlight the potential of *Ulva lactuca*, particularly its hexane fraction, as a source of natural tyrosinase inhibitors. This opens avenues for its application in the pharmaceutical and cosmetic industries, especially in formulations targeting hyperpigmentation and skin brightening.

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