

Carotenoids from female Grapsus albolineatus as potential anti-ageing compounds [version 1; peer review: awaiting peer review]

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RESEARCH ARTICLE

Carotenoids from female *Grapsus albolineatus* as potential anti-ageing compounds [version 1; peer review: awaiting peer review]

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Abstract

Introduction: *Grapsus albolineatus* is thought to have a high concentration of carotenoid pigments. Although studies on male *G. albolineatus* have been conducted, no studies on pigment extraction from female *G. albolineatus* have been carried out. Carotenoids have a high ability to fight free radicals. Previous research has shown that carotenoids can fight free radicals that cause premature skin-aging. The purpose of this research was to find out what kinds of carotenoids are found in *G. albolineatus* and whether they can act as natural inhibitors of proteins that cause premature aging, such as glucogenase, elastase, and hyaluronidase enzymes.

Methods: Carotenoids were extracted from *G. albolineatus* using column chromatography and high-performance liquid chromatography (HPLC); molecular docking and visualization were done with Autodock 4.2 and Discovery Studio/Biovia, respectively.

Results: According to HPLC data, there are carotenoid pigments such as didehydroastaxanthin, tetrahydroastaxanthin, dihydroastaxanthin, diatoxanthin, astaxanthin, and adonixanthin. According to molecular docking experiments, pigment carotenoids from *G. albolineatus* are efficient inhibitors of protein elastase and hyaluronidase.

Conclusions: *G. albolineatus* carotenoids have the potential to be anti-aging since they are more effective as protein elastase and hyaluronidase inhibitors than their native inhibitors.

Keywords

Grapsus albolineatus, glucogenase, elastase, hyaluronidase, carotenoids.

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24 Introduction

Skin is one of the largest organs in the human body and covers almost its entire.^{1,2} Skin has various functions such as protecting muscles, bones, and internal organs.³ Skin can experience aging that is unavoidable due to intrinsic factors, influenced by age, genetics, hormones, and blood sugar levels.⁴ While the majority of extrinsic variables are caused by continuous exposure to ultraviolet (UV) rays, pollution, diet, and smoking.^{5,6}

UV radiation is associated with an increase in oxygen radical species on the skin. Excessive reactive radical species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) can worsen pigmentation and aging of the skin, caused pigmentation disorders, rough skin and skin wrinkling.⁷ In addition to oxidative damage to the skin, ROS are known to activate hyaluronidase, glucogenase, and elastase enzymes.⁸ Increased activity of this enzyme can reduce hyaluronic acid, elastasin, and collagen. These components are key elements of the skin that act on elasticity, flexibility, and moisture provision, allowing the skin to seem young and healthy.^{9,10}

Therefore, to prevent premature aging, the skin needs an antioxidant as well as an enzyme inhibitor which acts as a natural inhibitor of enzymes that are activated due to UV radiation. Carotenoids are a class of compounds that have excellent antioxidant activity.^{11,12} Vegetables and fruits are considered as the most important sources of carotenoids in the human diet; animal products also contain large amounts of carotenoids.^{13,14}

The lightfoot crab from North Sulawesi, *G. albolineatus*, is known to contain high levels of carotenoids. *G. albolineatus* is a large crab with a large population. *Grapsus albolineatus* Latreille has a convex carapace with low tubercles; lateral, rounded margins, with one anterior tooth; the length of the front is equal to the length of the posterior margin of the carapace. The carapace is greenish-blue with paler areas; lateral margins and tubercles white; leg 12 mottled in brown with one orange spot at the tip of meri.¹⁵ This species has markedly arched carapace lateral margins; outer infraorbital angle 12 acute tooth; third maxillipeds with long merus, slightly shorter than the ischium; the male abdomen has five somites about 2.3 times as broad as long; GI is slightly curved. *G. albolineatus* is a large-sized species. Its world distribution includes the Indo-West Pacific: East Africa, Mauritius, Somalia, Socotra, Red Sea, Gulf of Aden, Southern Oman, Persian Gulf, Gulf of Oman, Pakistan, India, Sri Lanka, Bay of Bengal, Nicobar Islands, Andaman Sea, Mergui Archipelago, China, Japan, Indonesia, Singapore, Cocos (Keeling) Islands, Australia, and Hawaii. Its habitat is rocky, intertidal substrate.¹⁶

It is widely distributed in the seas of the South West Pacific. Male *G. albolineatus* have been recognized as having varying quantities of carotenoid pigments, while female *G. albolineatus* have not been studied for the kinds of carotenoid pigments they contain and their separation.¹⁷

Therefore, this study aimed to explore the pigment and separation of carotenoids in female *G. albolineatus* as well as to test carotenoids as anti-aging and natural inhibitors of hyaluronidase, glucogenase, and elastase enzymes using a molecular docking approach.

Methods

Ethical considerations

The researcher submitted a research permit (ethical clearance) to the UB (Universitas Brawijaya) Ethics committee team. Part of this research was conducted at the university.

Ethical clearance was obtained by including a research proposal, in which the method had a 3R stage, namely: 1) Reduction: using 3 animals, 2) Replacement: using 1 gram of the carapace organ, 3) Refinement: To avoid distress in animals, the researcher used 95% alcohol which functions as an analgesic.

5 This research was funded by the Institution of Research and Community Service Sam Ratulangi University (UNSRAT) with assignment letter Number 673/UN12.13/LT/2019, which was signed by the chairman of Institution of Research and community service UNSRAT, after being passing evaluation by the university reviewer.

Total pigment extraction

Sampling and handling

Sampling was carried out by exploring the intertidal area at the lowest low tide at night. The sample was put into a container filled with a little seawater, to ensure the crabs did not die. The dorsal carapace of the 3.7 cm crab in that section was smeared with 95% alcohol, when the crab fainted, surgery was carried out and then extraction was carried out.

Total pigment extraction

Crab samples were first injected to obtain blood from *G. albolineatus* using a five-mil injection and then dissected in order to separate the hepatopancreas and gonads. The next step was to separate the epidermis layer, which is a thin membrane connected to the interior of the carapace, and the black epidermis layer with tweezers and a laboratory knife. The organ carapace was crushed and immersed for five minutes in 2N hydrochloric acid. Then we strained it through filter paper into a separate flask, added petroleum ether and distilled water to make two layers. The absorption peak was then calculated using a UV-Vis spectrophotometer with a wavelength range of 380-550 nm.

Quantitative analysis: Total pigment extract Concentration and quantity

The absorbance of the carapace pigment extract was measured using a UV-Vis spectrophotometer at 380-550 nm wavelength. The spectrogram's shape is a curve with the X axis representing wavelength, and the Y axis representing absorbance, and the highest absorption peak at a certain wavelength can be read. Each spectrogram produced can be used to calculate the amount and concentration of *G. albolineatus* pigments utilized in this investigation.^{18,19}

Qualitative analysis: pigment separation by column chromatography

After that, the entire pigment extract was separated using column chromatography. The n-hexane-acetone was the solvent employed in the mobile phase (70:30). The X fraction was collected, which was then accommodated and examined for absorption with a UV-Vis spectrophotometer at a wavelength of 380-550 nm, yielding the spectrophotometer's highest absorption peak.^{18,19}

High performance liquid chromatography analysis

High performance liquid chromatography (HPLC) type LC.20ADVP with photodiode array detector (PDA; SPD-M20A-Shimadzu). The HPLC column used was C30 (YMC carotenoid, 150 × 4.6 mm I.D) with a column temperature of 30°C and a flow rate of 1 mL/min with a gradient system of H₂O, methanol and methyl tertiary butyl ether. The sample used was 20 µL. Carotenoids were separated in a gradient form for 50 min using a mixed solution of methanol, methyl tertiary butyl ether, and water (80:5:15) at a flow rate of 1 mL/min. After 20 min, the water content was lowered linearly, and the acetone was added to get the acetone proportion up to 20 percent at 50 min after injection. The final composition of the gradient mixture was methanol: methyl tertiary butyl ether (80:20).

Molecular docking analysis of carotenoids with aging receptors

The binding mechanism to the active site of three enzymes was investigated using molecular docking: fibroblast collagenase (1CGL), porcine pancreatic elastase (4YM9), and hyaluronidase (1FCV).²⁰⁻²² These three receptors are available on the Protein DataBank (PDB) website and may be saved as a PDB file.²³

Enzyme preparation was accomplished by eliminating water and cofactors from each enzyme. The protein was first optimized by adding polar hydrogen, followed by the addition of atomic charge with Kolman charge and nonpolar hydrogen. The receptor was then stiffened and stored in PDBQT (Protein DataBank, partial charge (Q), and atom type) format.⁹

The Canonical SmilesY of each ligand utilized in ligand preparation was acquired from Pubchem.²⁴ Subsequently, Canonical Smiles was converted to Chemdraw 2D 16.0 to produce a 2D compound, which was then modified into 3D using Chemdraw 3D and a reduced calculation was done. The file was stored as a PDB file. The ligand's PDB file was then mixed with polar hydrogen and given the gasteiger atomic charge and torque on each ligand, after which the ligand was stored in PDBQT format.

The AutoDock 4.2.6 software was used to simulate docking.²⁵ At each active site, molecular docking was performed. The active site is determined by verifying the docking of each inhibitor and the corresponding receptor. A grid box was created in the middle of the ligand location to determine the coordinates of the receptor's active site. For 1CGL and 4YM9 receptors, dimensions of 50×50×50 points were utilized, while for 1FCV, box dimensions of 60×60×60 points were chosen, with a grid point spacing of 0.375 Å. The Lamarckian Genetic Algorithm was the calculation employed in this docking approach. The optimal conformation was chosen based on the lowest bonding energy among the most conformational populations.

To observe the interactions that occur between the ligand and the receptor, docking visualization was performed using Biovia Discovery Studio 2020.²⁶ Interactions are depicted in three and two dimensions, respectively. Hydrogen bonding and hydrophobicity were utilized to analyze intermolecular interactions.

Results

Quantitative analysis: pigment extraction of female *G. albolineatus*

The highest wavelength of the content and concentration of total pigment extract in *G. albolineatus* crabs, particularly in the carapace, was 474 nm. As a result, the overall pigment extract content and concentration value was 4.33 µg/g, whereas the pigment content in the carapace organ was 4.46 µg/g.

Qualitative analysis: pigment extraction of female *G. albolineatus* using column chromatography

The column chromatography separation was repeated twice with an n-hexane-acetone mobile phase (70:30). A total pigment extract divided into four fractions: F1, F2, F3, and F4. A UV-Vis Spectrophotometer with a wavelength range of 380-550 nm was used to examine the four fractions. The presence of x-carotene pigments was indicated by wavelengths of 426, 448, and 475 nm, whereas the presence of zeaxanthin pigments was indicated by wavelengths of 426, 450, and 475 nm in the second fraction; the pigment types in F3 and F4 were unidentified.²⁷

F1 was not processed through the second round. However, F2, F3, and F4 were separated again using column chromatography: F2.1 was characterized as echinenone and F2.2 as astaxanthin. Furthermore, the pigment was not identified in F3.1 and F4.1. Krococanthin was found in F3.2, alloxanthin was identified in F3.3, and pyrhoanthin was identified in F4.2.

Pigment type analysis with high performance liquid chromatography

To generate 61 maximum absorption peaks, the findings of one column chromatography fraction were separated using HPLC with a propagation time of 50 minutes. The absorption spectra of each peak from the HPLC chromatogram were identified at 463 nm, and those with an area percentage greater than 3% were investigated by examining the spectral pattern using an HPLC UV-Vis absorption spectrophotometer.

The HPLC fraction one findings showed eleven absorption peaks. Table 1 shows the absorption generated at the highest peak. The greatest absorption peak at 473 nm was determined to be a kind of didehydroastaxanthin pigment, while the maximum absorption peak at 476 nm was determined to be a type of tetrahydroastaxanthin. (Tables 1-3). The HPLC separation was carried out at a propagation time of 50 minutes and generated 65 absorption peaks in the findings of the second fraction column chromatography.

At a wavelength of 436 nm, the absorption spectrum region with a larger percentage results in the formation of a maximum absorption peak. The highest absorption in Table 2 helps determine the kind of carotenoid pigment.²⁸

Six spectrum peaks were identified from the separation of nine absorption peaks by HPLC. Only five types of pigments could be identified using the six peak spectral pattern. The HPLC separation resulted in the formation of an absorption peak with a peak area of 3% in Table 3. The greatest absorption peaks were discovered by the absorption of HPLC UV-Vis spectrophotometer, namely the 8th, 14th, 15th, 16th, 17th, and 34th absorption peaks, allowing the kind of pigment to be determined. Figure 1 presents the visualization of the compound structure of the pigment types produced by the HPLC analysis.

Table 1. Types of *G. albolineatus* carotenoid pigments separated by HPLC from the results of the first fraction of the column chromatography.

No	Retention time/Peak no.	Maximum absorption peak	pigment type
1	6.99/11	476	Tetrahydroastaxanthin
2	22.84/27	473	Didehydroastaxanthin
3	24.74/29	476	Tetrahydroastaxanthin
4	33.08/42	473	Didehydroastaxanthin
5	34.74/46	473	Didehydroastaxanthin
6	35.70/48	473	Didehydroastaxanthin
7	36.16/49	473	Didehydroastaxanthin
8	37.17/50	473	Didehydroastaxanthin
9	37.88	473	Didehydroastaxanthin
10	39.47/54	473	Didehydroastaxanthin
11	40.77/56	473	Didehydroastaxanthin

Table 2. Types of *G. albolineatus* carotenoid pigments separated by HPLC from the results of the second fraction of the column chromatography.

No	Retention time/Peak no.	Maximum absorption peak	Pigment type
1	6.224/15	374-468	-
2	6.892/16	477	Dihydroastaxanthin
3	8.296/17	424-446-471	Diatoxanthin
4	9.671/18	425-251-447	-
5	19.901/30	476	Tetrahydroastaxanthin
6	21.132/32	473	Didehydroastaxanthin
7	22.388/34	473	Didehydroastaxanthin
8	22.771/35	473	Didehydroastaxanthin
9	9 24.674/37	478	Astaxanthin

Table 3. Types of *G. albolineatus* carotenoid pigments separated by HPLC from the results of the third fraction of the column chromatography.

No	Retention time/Peak no.	Maximum absorption peak	Pigment type
1	3.376/8	373-467	-
2	6.231/14	373-467	-
3	6.907/15	477	Dihydroastaxanthin
4	8.281/16	425-448-472	-
5	9.643/17	420-451-477	-
6	24.701/34	481	Adonixanthin

Molecular docking analysis of carotenoids from *G. albolineatus* on collagenase protein (1CGL)

The value of the bond energy is generated from the total final intermolecular energy, namely from van der Waals bonds, hydrogen bonds, de-solvation energy, and electrostatic energy, then from the final total internal energy, torsional energy and unbound system's energy. This summation of energy is usually known as the estimated free energy of binding (EFEB). The inhibition constant was also estimated by using Autodock 4.2.6 at 298 K. The low value of the inhibition constant was related to the biological activity.²⁹

The carotenoids discovered by HPLC (Figure 1) were used as test ligands for binding proteins that contribute to skin aging. According to Table 4, after compared to other carotenoid compounds, adonixanthin had the lowest binding energy with -6.61 kcal/mol and the highest inhibition constant with 14.23 mM. The binding energy of these carotenoids, however, is not better than the native ligand (-7.83 kcal/mol with the lowest inhibition constant of 1.81 mM). The visualization of molecular docking results is shown in Figures 2-4.

Molecular docking analysis of carotenoid Compounds from *G. albolineatus* on protein elastase (4YM9)

According to the results of the interaction between carotenoids and the protein elastase, carotenoid pigments have a higher binding energy than natural ligands, which only have a binding energy of -5.55 kcal/mol and an inhibition constant of 85.31 mM (Table 5). Adonixanthin and astaxanthin have the highest binding energies, with binding energies of -9.03 kcal/mol and -9.01 kcal/mol, respectively.

Molecular Docking Analysis of Carotenoid Compounds from *G. albolineatus* on Protein elastase (4YM9)

The carotenoid compounds from *G. albolineatus* had a lower binding energy than the natural hyaluronidase ligand, which had a binding value of -4.17 kcal/mol. Astaxanthin had a binding energy of -2.27 kcal/mol and is only bound to four amino acid residues, whereas didehydroastaxanthin had a binding energy of -7.71 kcal/mol (Table 6).

Discussion

G. albolineatus is a large crab with a large population along the rocky intertidal zone.³⁰ *G. albolineatus* can be found off the coast of Kalasey, Manado Bay, North Sulawesi, in Indonesia. Carotenoid pigments, such as carotene, echinone, and

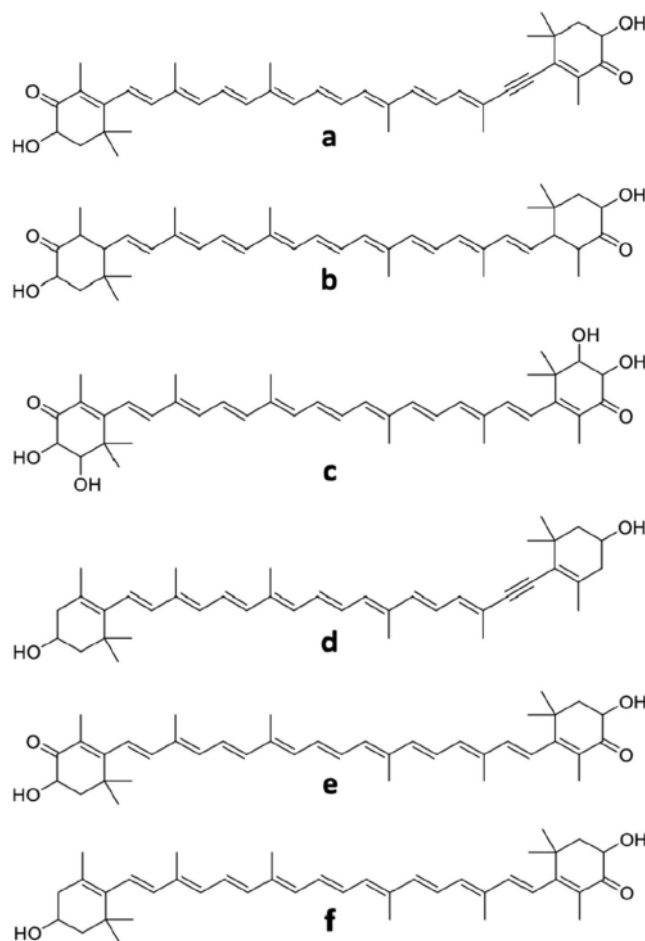


Figure 1. Compounds obtained from *G. albolineatus* and their structures: (a) didehydroastaxanthin; (b) tetrahydroastaxanthin; (c) dihydroxyastaxanthin; (d) diatoxanthin; (e) astaxanthin; (f) adonixanthin.

cantaxanthin, are found in hemocyanin, hepatopancreas, epidermal layer, and outside the carapace in male *G. albolineatus* from Indonesia.¹⁷

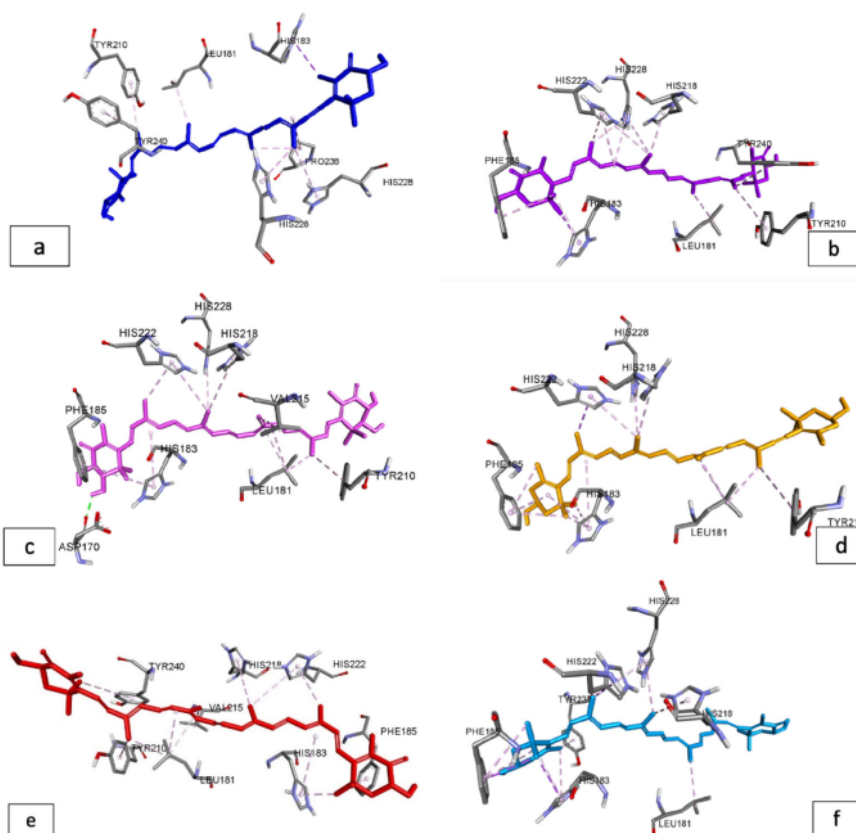
Based on HPLC results from the carapace of the female *G. albolineatus*, several types of carotenoids were found, including didehydrodiastaxanthin, tetrahydroastaxanthin, dihydroastaxanthin, diatoxanthin, astaxanthin, and adonixanthin. Identification of carotenoids from female *G. albolineatus* is a novelty.

Carotenoids provide effective antioxidant protection against peroxy radicals during the photo-oxidation process. Carotenoids have a maximum wavelength of 450 nm due to their compound structure.^{11,31} Several studies have shown that people with high levels of carotenoids in their skin have skin that looks younger than their age, while those with low levels of carotenoids have skin that looks older.³² This is presumably because skin aging is linked to UV radiation, and carotenoids are thought to be effective radical scavengers.³³

Skin exposure to high levels of free radicals can activate collagenase, elastase, and hyaluronidase enzymes which cause a decrease in collagen, elastin, and hyaluronic acid, leading to skin aging of the skin.¹⁴ Molecular docking studies have been used to figure out how carotenoids suppress the enzymes collagenase, elastase, and hyaluronidase, and Table 5 shows the binding energy of each component. Figures 1-3 show an image representation of the bonding that occurs. Native inhibitors had a higher binding energy in collagenase protein (1cg1) than carotenoids derived from *G. albolineatus*. This is because the native inhibitors interact with the amino acid residues His222, His218, and His228 via hydrogen interactions.^{29,34}

Table 4. Estimation of the binding energy, inhibition constant, and interaction type of carotenoids against collagenase enzyme.

Compounds	Binding energy (kcal/mol)	Inhibition constant (mM)	Hydrogen bonding	Hydrophobic bonding
Didehydroastaxanthin	-4.97	225,61	-	Leu181; Pro238; His228; Tyr210; Tyr240; His183
Tetrahydroxanthin	-3.30	3790	-	Tyr210, Tyr240, Leu181, Phe185, His183, His218, His222, His228
Dihydroxyastaxanthin	-3.66	2070	Asp170	Phe185, His183, His222, Tyr210; Leu181; Val215; His218; His228
Diatoxanthin	-4.33	796.35	-	Tyr210, Leu181, His228, His183, Phe185, His[8, His222
Astaxanthin	-4.64	398,40	-	His222; Tyr240; His183; Phe185; His218; Val215; Leu181; Tyr210
Adonixanthin	-6.61	14,23	-	His218, His228, His222, Phe185, His183, Tyr237, Leu181
Native	-7.83	1,81	Pro238; His218; His222; His228	Gly179; Asp175

**Figure 2.** Visualization results of carotenoids as collagenase (1CGL) enzyme inhibitors: (a) didehydroastaxanthin; (b) tetrahydroxanthin; (c) dihydroxyastaxanthin; (d) diatoxanthin; (e) astaxanthin; (f) adonixanthin.

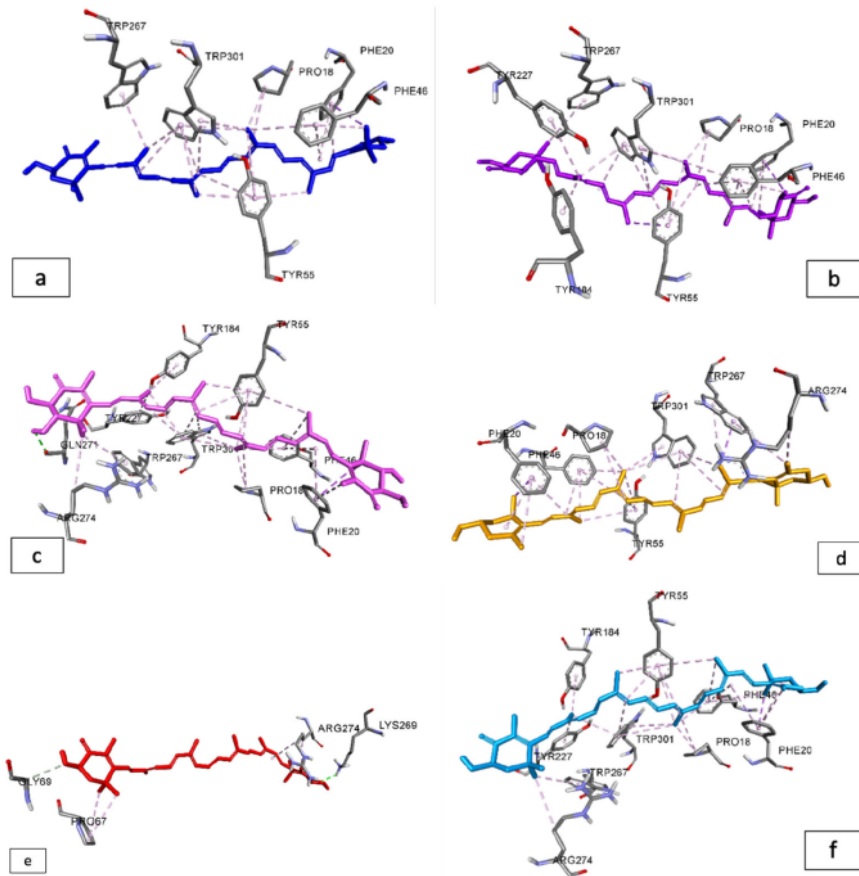


Figure 4. Visualization results of carotenoids as collagenase (1CGL) enzyme inhibitors: (a) didehydroastaxanthin; (b) tetrahydroxanthin; (c) dihydroxyastaxanthin; (d) diatoxanthin; (e) astaxanthin; (f) adonixanthin.

Table 5. Estimation of the binding energy, inhibition constant, and interaction type of carotenoids against elastase enzyme.

Compounds	Binding energy (Kcal/mol)	Inhibition constant (mM)	Hydrogen bonding	Hydrophobic bonding
Didehydroastaxanthin	-8.42	0,673	Tyr35; His40	Leu150; Leu143; Val216; Arg217; Trp171; Ala99; Val99; Thr174
Tetrahydroxanthin	-8.31	0,86	Tyr35	Cys42; Leu143; His40; Leu150; His57; Val216; Arg217; Phe215; Trp171; Ala99; Val99
Dihydroastaxanthin	-7.58	2,78	His40; Tyr35	Ala99; Trp171; Phe215; Val99; His57; Cys42; Leu150
Diatoxanthin	-7.81	1,78	His40	Leu150; Trp171; Arg217; Ala99; Val99; Phe215; His57; Cys42; Leu150
Astaxanthin	-9,01	0,248	Tyr35	His40; Leu143; Trp171; Arg217; Ala99; Val216; Cys58; His57; Cys42
Adonixanthin	-9,03	0,239	His40, Tyr35	Leu143, Ala99, Leu150, Val216, Val99, Arg217, Trp171, Phe215
Native	-5,55	85,31	Cys220; Gln192; Gly193; Ser195	Val216

Table 6. Estimation of the binding energy, inhibition constant, and interaction type of carotenoids against elastase enzyme.

Compounds	Binding energy (kcal/mol)	Inhibition constant (mM)	Hydrogen bonding	Hydrophobic bonding
Didehydroastaxanthin	-7.71	2.25	-	Trp267; Tyr55; Phe46; Phe20; Pro18; Trp301
Tetrahydroxanthin	-7.47	3.35	-	Phe20; Phe46; Pro18; Trp267; Tyr55; Trp302; Tyr184; Tyr227
Dihydroastaxanthin	-6.16	30.56	Gln271	Phe20; Tyr184; Tyr227; Pro18; Trp301; Phe46; Tyr55; Trp267; Arg274
Diatoxanthin	-7.53	3.01	-	Phe20, Tyr55, Phe46, Pro68, Trp301, Trp267, Arg274, Tyr55
Astaxanthin	-2.27	21,79 x 10 ³	Lys269	Arg274; Gly69; Pro67
Adonixanthin	-7.45	3.46	-	Phe20, Pro18, Tyr55, Phe46, Trp305, Tyr227, Tyr184, Trp267, Arg274
Native	-4.17	875.19	Tyr227; Asp111; Ser304; Asp305; Glu113	Tyr184; Trp301; Phe46; Arg47; Ser303

produced a binding energy of -2.27 kcal/mol. As a result of this discovery, it is possible to conclude that astaxanthin has an excessively large inhibition constant, resulting in a low bioavailability value.⁹ Meanwhile, other carotenoids with high binding energy, such as didehydroastaxanthin, tetrahydroastaxanthin, dihydroastaxanthin, diatoxanthin, and adonixanthin, have the potential to be used as natural inhibitors of hyaluronidase protein. Because the test ligands' interactions are anchored in the same location as the active site, these compounds can be used as competitive inhibitors of collagenase, elastase, and hyaluronidase proteins.

Conclusions

The pigment content of the carapace was 4.46 mg based on quantitative measurement of total pigment extraction in female *G. albolineatus*. Qualitatively, the carapace was recognized as containing carotenoid pigments such as didehydroastaxanthin, tetrahydroastaxanthin, dihydroastaxanthin, diatoxanthin, and astaxanthin based on HPLC. Carotenoids are naturally occurring antioxidants that play a vital role in skin regeneration. According to molecular docking results, carotenoid compounds from *G. albolineatus* are stronger inhibitors than native inhibitors, which competitively bind to the same catalytic residues on elastase and hyaluronidase protein. However, *in vivo* and *in vitro* studies are required to confirm this approach so that this data may be utilized as a contender for medication prospects, particularly in the cosmetic area.

Data availability

Underlying data

Figshare: Potential Carotenoids as Anti-aging from Female Grapsus albolineatus, <https://doi.org/10.6084/m9.figshare.19947758>³⁷

This project contains the following underlying data:

- hasil docking-Dr. darus.xlsx
- dock1cgl-native.dlg
- dock4ym9-native.dlg
- dockasta1cgl.dlg
- dockasta4ym9.dlg
- dockbeta1cgl.dlg

- dockbeta4ym9.dlg
- dockdehydro1cgl.dlg
- dockdehydro4ym9.dlg

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Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver \(CC0 1.0 Public domain dedication\)](#)

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References

1. Wang M, Luo Y, Wang T, et al.: **Artificial skin perception**. *Adv. Mater.* May 2021; **33**(19): 2003014. [Publisher Full Text](#)
2. Yadav N, Parveen S, Chakravarty S, et al.: **Skin Anatomy and Morphology**. Singapore: Springer; 2019; 1–10. 9789811325410. [Publisher Full Text](#)
3. Balasubramanian P, Prabhakaran MP, Sireesha M, et al.: **Collagen in Human Tissues: Structure, Function, and Biomedical Implications from a Tissue Engineering Perspective**. Berlin, Heidelberg: Springer; 2013; 173–206. [Publisher Full Text](#)
4. Papakonstantinou E, Roth M, Karak-iulakis G: **Hyaluronic acid: A key molecule in skin aging**. *Dermato-Endocrinology*. Jul 2012; **4**(3): 253–258. [Publisher Full Text](#)
5. Yanpei G, Han J, Jiang C, et al.: **Biomarkers, oxidative stress and autophagy in skin aging**. *Ageing Res. Rev.* May 2020; **59**: 101036. [Publisher Full Text](#)
6. McDaniel D, Farris P, Valacchi G: **Atmospheric skin aging-contributors and inhibitors**. *J. Cosmet. Dermatol.* Apr 2018; **17**(2): 124–137. [Publisher Full Text](#)
7. Chen J, Yang L, Zhao Z, et al.: **Oxidative stress in the skin: Impact and related protection**. *Int. J. Cosmet. Sci.* Oct 2021; **43**(5): 495–509. [Publisher Full Text](#)
8. Jiratchayamaethasakul C, Ding Y, Hwang O, et al.: **In vitro screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals**. *Fisheries and Aquatic Sciences*. Dec 2020; **23**(1): 6. [Publisher Full Text](#)
9. Osorio E, Bravo K, Cardona W, et al.: **Antiaging activity, molecular docking, and prediction of percutaneous absorption parameters of quinoxaline-hydrazone hybrids**. *Med. Chem. Res.* Nov 2019; **28**(11): 1959–1973. [Publisher Full Text](#)
10. Elgamal AM, El Raey MA, Gaara A, et al.: **Phytochemical profiling and anti-aging activities of euphorbia retusa extract: In silico and in vitro studies**. *Arabian J. Chem.* Jun 2021; **14**(6): 103159. [Publisher Full Text](#)
11. Stahl W, Sies H: **Antioxidant activity of carotenoids**. *Mol. Asp. Med.* Dec 2003; **24** (6): 345–351. [Publisher Full Text](#)
12. Fiedor J, Burda K, Vetroslava: **Potential role of carotenoids as antioxidants in human health and disease**. *Nutrients*. Jan 2014; **6**(2): 466–488. [Publisher Full Text](#) | [Reference Source](#)
13. Goodwin TW: **Nature and distribution of carotenoids**. *Food Chem.* Jan 1980; **5**(1): 3–13. [Publisher Full Text](#)
14. Schmitz HH, Poor CL, Gugger ET, et al.: **[11] Analysis of carotenoids in human and animal tissues**. Elsevier; 1993; vol. **214**: 102–116. [Publisher Full Text](#)
15. Poupin J: *A guide to the decapod crustaceans of the South Pacific*. 2010.
16. Naderloo R: *Atlas of Crabs of the Persian Gulf*. Cham: Springer International Publishing; 2017. [Publisher Full Text](#)
17. Paransa DSJ, Mantiri DMH, Lumenta C, et al.: **Morphological and genetic characteristics of lightfoot crab grapsus albolineatus**. *AACL Bioflux*. 2019; **3**: 804–811.
18. Britton G, Young AJ: *Methods for the isolation and analysis of carotenoids*. Dordrecht: Springer Netherlands; 1993; 409–457. [Publisher Full Text](#)
19. *Carotenoids: Handbook*. Basel: Birkhäuser Basel; 2004. [Publisher Full Text](#)
20. Lovejoy B, Cleasby A, Hassell AM, et al.: **Structure of the catalytic domain of fibroblast collagenase complexed with an inhibitor**. *Science*. Jan 1994; **263**(5145): 375–377. [Publisher Full Text](#)
21. Ruivo EFP, Gonçalves LM, Carvalho LAR, et al.: **Clickable 4-oxo-lactam-based selective probing for human neutrophil elastase related proteomes**. *ChemMed-Chem*. Sep 2016; **11**(18): 2037–2042. [Publisher Full Text](#)
22. Markovic-Housley Z, Miglierini G, Soldatova L, et al.: **Crystal structure of hyaluronidase, a major allergen of bee venom**. *Structure*. Oct 2000; **8**(10): 1025–1035. [Publisher Full Text](#)
23. RCSB Protein Data Bank: [Rcsb pdb: Homepage](#). [Reference Source](#)
24. PubChem: [Reference Source](#)
25. Morris GM, Huey R, Lindstrom W, et al.: **Autodock4 and autodocktools4: Automated docking with selective receptor flexibility**. *J. Comput. Chem.* Dec 2009; **30**(16): 2785–2791. [Publisher Full Text](#)
26. [Reference Source](#)
27. Speek AJ, Temallilwa CR, Schrijver J: **Determination of carotene content and vitamin a activity of vegetables by high-performance liquid chromatography and spectrophotometry**. *Food Chem.* Jan 1986; **19**(1): 65–74. [Publisher Full Text](#)
28. Thrane J-E, Kyle M, Striebel M, et al.: **Spectrophotometric analysis of pigments: A critical assessment of a high-throughput method for analysis of algal pigment mixtures by spectral deconvolution**. *PLoS One*. Sep 2015; **10**(9): e0137645. [Publisher Full Text](#)
29. Udrea A-M, Avram S, Nistorescu S, et al.: **Laser irradiated phenothiazines: New potential treatment for covid-19 explored by molecular docking**. *J. Photochem. Photobiol. B Biol.* Oct 2020; **211**: 111997. [Publisher Full Text](#)
30. Paransa DSJ, Mantiri DMH, Paulus JH, et al.: **Exploration study of the pigment types of stone crab (grapsus albolineatus) as pharmaceutical and cosmetic ingredients**. *Ecology, Environment and Conservation*. 2009.
31. Bendich A, Olson JA: **Biological actions of carotenoids 1**. *FASEB J.* Jun 1989; **3**(8): 1927–1932. 0892-6638, 1530-6860. [Publisher Full Text](#)

32. Darvin ME, Sterry W, Lademann J, *et al.*: **The role of carotenoids in human skin.** *Molecules.* Dec 2011; **16**(12): 10491–10506. 1420–3049.
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33. Lademann J, Meinke MC, Sterry W, *et al.*: **Carotenoids in human skin.** *Ex perimental Dermatology.* May 2011; **20**(5): 377–382.
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34. Levison ME, Levison JH: **Pharmacokinetics and pharmacodynamics of antibacterial agents.** *Infect. Dis. Clin. N. Am.* Dec 2009; **23**(4): 791–815.
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35. Halper J, Kjaer M: *Basic Components of Connective Tissues and Extracellular Matrix: Elastin, Fibrillin, Fibulins, Fibrinogen, Fibronectin, Laminin, Tenascins and Thrombospondins.* Dordrecht: Springer Netherlands; 2014; **802**: 31–47. 9789400778924.
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36. Weihermann AC, Lorencini M, Brohem CA, *et al.*: **Elastin structure and its involvement in skin photoageing.** *Int. J. Cosmet. Sci.* Jun 2017; **39**(3): 241–247.
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37. Paransa D: Potential Carotenoids as Anti-aging from Female *Grapsus albolineatus*. figshare. [Dataset]. 2022.
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