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3 pesan

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25 April 2023 pukul 17.5(

Dear Darus

Carotenoids from female *Grapsus albolineatus* as potential anti-ageing compounds
Paransa DSJ, Kemer K, Mantiri DMH, Kepel RC, Suleman DP, Soemantri AD and Kumia D

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Thank you for your email.

Your article has been reviewer by one reviewer. They gave the first version of your article an Approved with reservations status, then updated the status to Approved for the version 2.

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Sent: 26 April 2023 02:30
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Darus Saadah J. Paransa <darusparansa@unsrat.ac.id>

Reviewers suggested for your article 122649

1 pesan

editorial@f1000research.com <editorial@f1000research.com>
Kepada: darusparansa@unsrat.ac.id

9 Juni 2023 pukul 17.4

Dear Darus

Carotenoids from female *Grapsus albolineatus* as potential anti-ageing compounds
Paransa DSJ, Kemer K, Mantiri DMH, Kepel RC, Suleman DP, Soemantri AD and Kumia D

I hope you're well. We would like to apologise for the delays that your article has experienced in peer review. We are aware that our current author-led peer review process has not been working as we had hoped for some of our authors, and we are in the process of revising and refreshing our model in order to address these problems. In the meantime, to help prevent further delays we have referred your article to our specialist team, who have identified some suitable reviewers and will continue to do so for you. You are also welcome to continue to suggest reviewers in addition to this, if you wish. We hope that this will help secure additional peer review feedback for your article as soon as possible.

We have checked these reviewers against our peer review criteria, however we would be grateful if you could please view the Suggestions Awaiting Approval section of your Suggest Reviewers page to confirm that this is the case. You will be able to approve or reject our suggestions - please provide as much detail as possible if we have suggested someone who is not suitable to review. In order to avoid delays, we'll invite these people to review your article in two days if we have not yet back from you, please let us know if that will be an issue.

Best regards,

On behalf of

Daniel
The Editorial Team, F1000Research

RESEARCH ARTICLE

REVISED Carotenoids from female *Grapsus albolineatus* as potential anti-ageing compounds [version 2; peer review: 1 approved]

Darus Saadah Johanis Paransa[1], Kurniati Kemer[1], Desy Maria Helena Mantiri[1], Rene Charles Kepel[1], Dininurilmi Putri Suleman[2], Ara Deani Soemantri[3], Dikdik Kurnia[3]

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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.
Aim: 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 with binding energy range -7.58 kcal/mol - -9.03 kcal/mol and -6.16 kcal/mol - -7.71 kcal/mol, respectively.
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.

Open Peer Review

Approval Status ✓

	1
version 2 (revision) 14 Apr 2023	✓ view
version 1 08 Dec 2022	? view

1. **Mosad A. Ghareeb**, Theodor Bilharz Research Institute, Giza, Egypt
Sami Nasr, Theodor Bilharz Research Institute, Giza, Egypt
Hend Okasha, Theodor Bilharz Research Institute, Giza, Egypt
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Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the Plant Science gateway.

Corresponding author: Darus Saadah Johanis Paransa (darusparansa@unsrat.ac.id)

Author roles: Paransa DSJ: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Kemer K**: Data Curation, Formal Analysis, Investigation, Methodology, Validation; **Mantiri DMH**: Data Curation, Formal Analysis, Investigation, Validation; **Kepel RC**: Investigation, Supervision; **Suleman DP**: Investigation, Supervision; **Soemantri AD**: Data Curation, Methodology, Software, Validation, Visualization, Writing – Review & Editing; **Kurnia D**: Methodology, Resources, Software, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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First published: 08 Dec 2022, **11**:1457 https://doi.org/10.12688/f1000research.122649.1

REVISED **Amendments from Version 1**

New versions of this article have been improved according to the reviewers' comments and suggestions. In the abstract section we have added the sub-heading Aim before the purpose of this study. We have also added the binding energy range to the docking results. Improvements to the method have been reinforced with relevant citations. In Results Table 1 we have changed the "Pigment type". Conclusion: We have added binding ranges and catalytic residues which are influential in increasing the binding energy in the interaction between *G. albolineatus* compounds and elastase and hyaluronidase. In Abbreviations we have added the abbreviations in our article 1CGL, fibroblast collagenase; 1FCV, hyaluronidase; 4YM9, porcine pancreatic elastase; HPLC, High performance liquid chromatography; GDP, Protein Data Bank; PDBQT, Protein Data Bank, partial charge (Q), and atom type; RNS, reactive nitrogen species; ROS, reactive oxygen species; UV, ultraviolet ; UV-Vis, ultraviolet visible. References section we've edited the mention reference Ref. 10: *Euphorbia retusa*, Ref. 17: *Grapsus albolineatus*, Ref. 20: *Grapsus albolineatus*.

Any further responses from the reviewers can be found at the end of the article

Abbreviations

1CGL: fibroblast collagenase
1FCV: hyaluronidase
4YM9: porcine pancreatic elastase
HPLC: High performance liquid chromatography
PDB: Protein Data Bank
PDBQT: Protein Data Bank, partial charge (Q), and atom type
RNS: reactive nitrogen species
ROS: reactive oxygen species
UV: ultraviolet
UV-Vis: ultraviolet visible

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 in 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; legs mottled in brown with one orange spot at the tip of meri.[15] This species has markedly arched carapace lateral margins; outer infraorbital angle with 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; G1 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.

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.

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.

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-mile 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 hydrodrochloric acid. Then we strained it through filter paper into a separator 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.[17]

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 H_2O, 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 10 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).[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).[21–23] These three receptors are available on the Protein DataBank (PDB) website and may be saved as a PDB file.[24]

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 Data Bank, partial charge (Q), and atom type) format.[9]

The Canonical Smiles of each ligand utilized in ligand preparation was acquired from Pubchem.[25] 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.[26] 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 side. Then, for 1CGL and 4YM9 receptors, dimensions of $50\times50\times50$ points were utilized, while for 1FCV, box dimensions of $60\times60\times60$ 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.[27] Interactions are depicted in three and two dimensions, respectively. Hydrogen bonding and hydrophobicity were utilized to analyze intermolecular interactions.

Results

Quantiative 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.[28]

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 astase. Furthermore, the pigment was not identified in F3.1 and F4.1. Krocoxanthin was found in F3.2, alloxanthin was identified in F3.3, and pyrhoxanthin 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 per-centage 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.[29]

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

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

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.

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

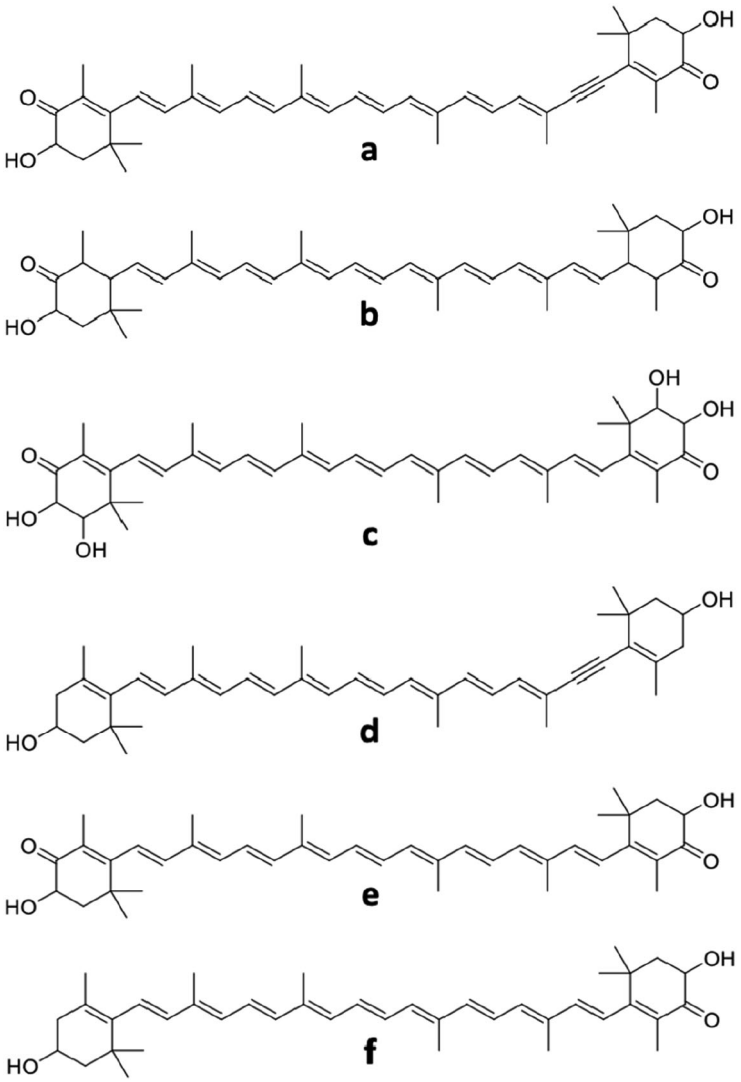

Figure 1. Compounds obtained from *G. albolineatus* and their structures: (a) didehydroastaxanthin; (b) tetrahydroxanthine; (c) dihydroxyastaxanthin; (d) diatoxanthin; (e) astaxanthin; (f) adonixanthin.

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.[30]

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.

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.

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).

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; His218; 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

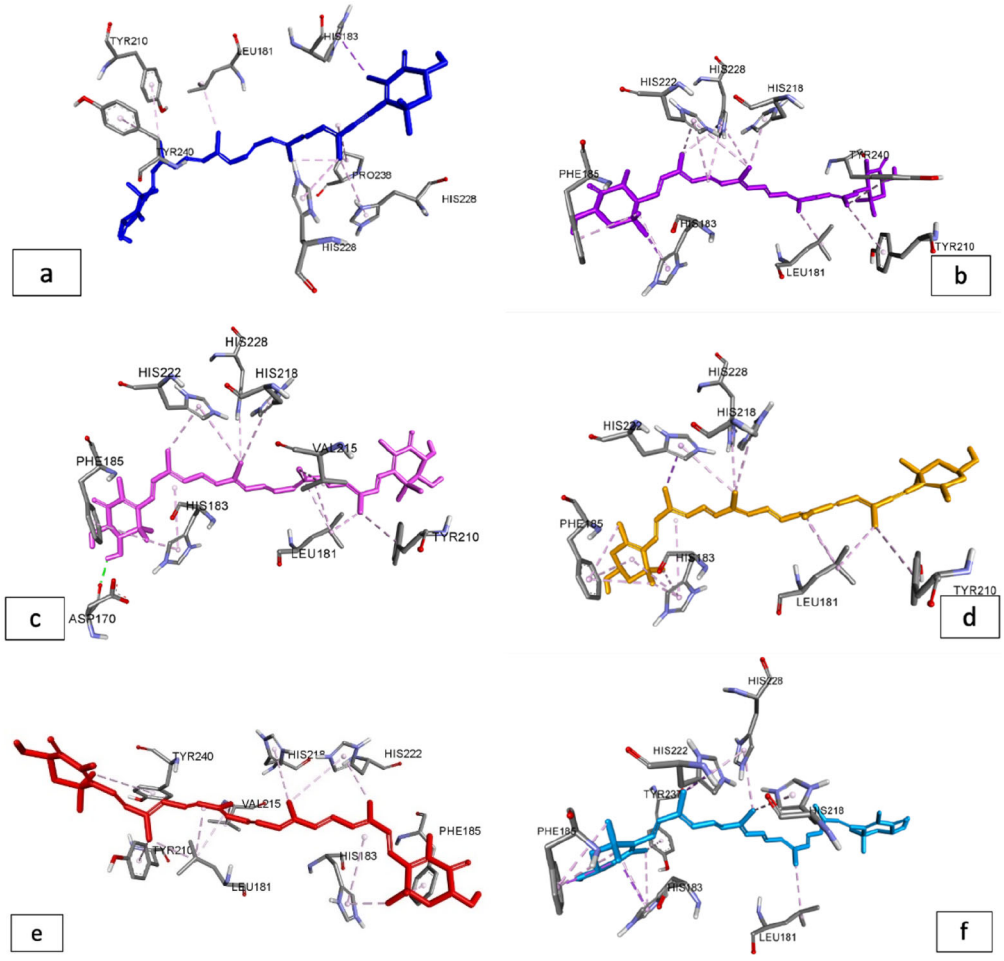

Figure 2. Visualization results of carotenoids as collagenase (1CGL) enzyme inhibitors: (a) didehydroastaxanthin; (b) tetrahydroxanthine; (c) dihydroxyastaxanthin; (d) diatoxanthin; (e) astaxanthin; (f) adonixanthin.

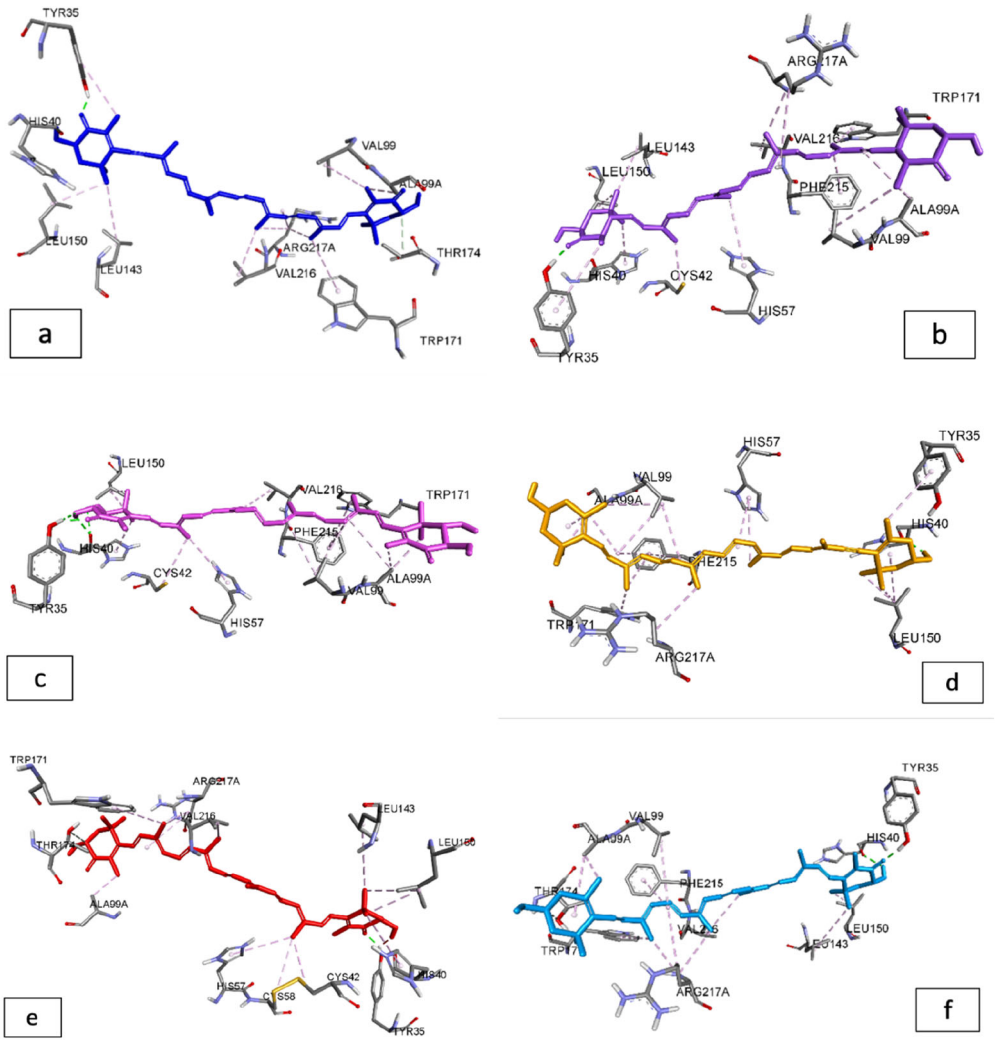

Figure 3. Visualization results of carotenoids as collagenase (1CGL) enzyme inhibitors: (a) didehydroastaxanthin; (b) tetrahydroxanthine; (c) dihydroxyastaxanthin; (d) diatoxanthin; (e) astaxanthin; (f) adonixanthin.

Discussion

G. albolineatus is a large crab with a large population along the rocky intertidal zone.[20] *G. albolineatus* can be found off the coast of Kalasey, Manado Bay, North Su-lawesi, in Indonesia. Carotenoid pigments, such as carotene, echinonen, and 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 peroxyl radicals during the photo-oxidation process. Carotenoids have a maximum wavelength of 450 nm due to their compound structure.[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 exposition to high levels of free radicals can activate collagenase, elastase, and hyaluronidase enzymes which cause a decrease in collagen, elastin, and hyaluronic acid, causing aging of the skin. Molecular docking studies have been used to figure out how carotenoids suppress the enzymes collagenase, elastase, and hyaluronidase, and Table 5 show the

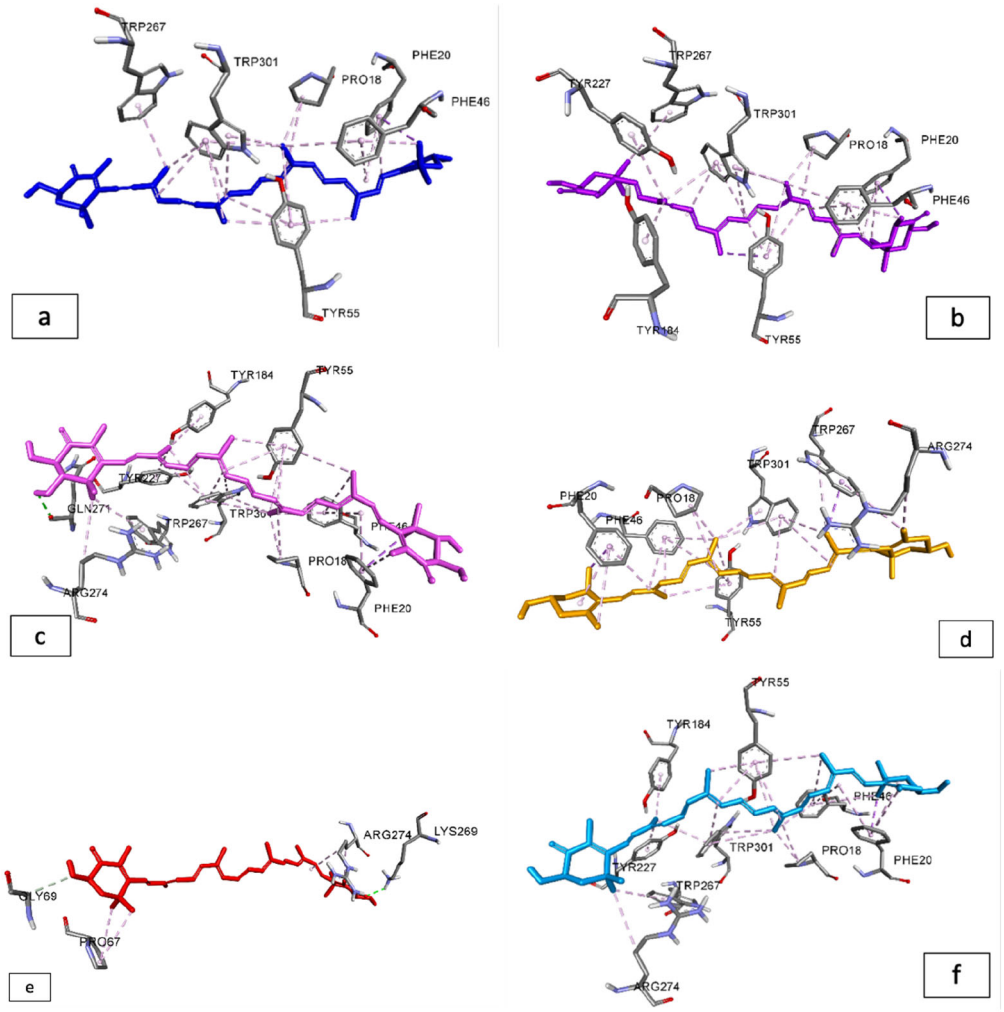

Figure 4. Visualization results of carotenoids as collagenase (1CGL) enzyme inhibitors: (a) didehydroastaxanthin; (b) tetrahydroxanthine; (c) dihydroxyastaxanthin; (d) diatoxanthin; (e) astaxanthin; (f) adonixanthin.

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 Hyaluronidase 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	21.79×10^3	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

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 (1cgl) than carotenoids derived from *G. albolineatus.* This is because the native nhibitors interact with the amino acid residues His222, His218, and His228 via hydrogen interactions.[34]

Meanwhile, the interaction between these residues in the interaction between carotenoid compounds and the collagenase receptor is only found in hydrophobic interactions. Torsion energy is another factor that influences binding energy. A positive value for the torsional energy between carotenoids' interactions that is too large is thought to have an effect on the small binding energy produced. However, adonixanthin (Table 4) has a relatively high binding energy and an inhibitory value of 14.23 mM, which determines the bioavailability of this component for use as a drug[29] (Figures 2 and 3).

According to the literature, elastases are serine proteases whose main function is the cleavage of peptide bonds of many proteins, including elastin, which is responsible for the elasticity of connective tissues and is primarily found in the lungs, arteries, and ligaments.[35,36] It has been identified that binding to Tyr35, His40, and Val216 has a significant influence on the strength of the binding energy between the ligand and the target receptor in the molecular docking interaction between carotenoids and elastase. The docking results revealed that astaxanthin and adonixanthin had the highest binding energies, *i.e.* -9.01 and -9.03 kcal/mol, respectively. Based on binding energy results, the carotenoid compounds from *G. albolineatus* in Table 6 can be used as elastase inhibitors when compared to native inhibitors (Figure 3).

In the interaction that occurs between the complex ligand and protein hyaluronidase, from the results when compared with native inhibitors, residues Tyr227, Phe46, and Pro18 are thought to have an effect on increasing the binding energy of the complex formed, and hydrophobic bonds are preferred. The absence of interaction between native and Pro18 reinforces that the interaction that occurs has a major influence on the resulting bond energy. In addition, astaxanthin only 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, 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 such as Tyr35, His40, and Val216 on elastase and Tyr227, Phe46, and Pro18 on hyaluronidase protein with binding energy range -7.58 kcal/mol – -9.03 kcal/mol and -6.16 kcal/mol – -7.71 kcal/mol, respectively. However, *In vivo*, *In vitro,* toxicological investigations 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

Acknowledgements

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Open Peer Review

Current Peer Review Status: ✔

Version 2

Reviewer Report 25 April 2023

https://doi.org/10.5256/f1000research.144248.r169713

✔ **Mosad A. Ghareeb**
Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt

The authors have improved the manuscript as suggested and addressed all of the requested comments raised by the reviewers. Accordingly, I recommend the indexing of this article in its present form.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 02 February 2023

https://doi.org/10.5256/f1000research.134670.r159375

? **Mosad A. Ghareeb**
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Amal Saad
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The current study includes the chemical characterization of female *Grapsus albolineatus* for the presence of bioactive carotenoids including didehydroastaxanthin, tetrahydroastaxanthin, dihydroastaxanthin, diatoxanthin, astaxanthin, and adonixanthin. The study also deals with evaluation of these carotenoids as natural inhibitors of proteins that cause premature aging, such as glucogenase, elastase, and hyaluronidase enzymes via molecular modeling like docking.

The article is well organized, the introduction is sufficient, the methods and tools used are sufficient, and the references are recent and appropriate to the subject of the study.

Moreover, the study is considered a new addition to the field of drug discovery from natural sources as a safe alternative to synthetic drugs.

We recommend in the future that these compounds should be re-evaluated *in vivo* using experimental animal models and conducting further toxicological investigations.

Abstract:

1. The section ''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'' should be located under the sub-title ''Aim''.

2. Binding energy range should be mentioned in the docking results.

Methods:

Total pigment extraction

- This section should be reinforced by a relevant citation.

High performance liquid chromatography analysis

- This section should be reinforced by a relevant citation.

Results:

- Table 1: The column title ''pigment type'' should be written as ''Pigment type''.

Conclusion:

- The conclusion should be supported by the results.

Abbreviations:

- List of abbreviations should be inserted by the end of the manuscript before references.

References:

1. All scientific names species should be written in italic fonts:-
    - Ref. 10: euphorbia retusa.
    - Ref. 17: grapsus albolineatus.

- Ref. 30: grapsus albolineatus.

2. The first letter of genus name should be written in uppercase letter:-
   - Ref. 10: euphorbia retusa.
   - Ref. 17: grapsus albolineatus.
   - Ref. 30: grapsus albolineatus.

3. The word ''in vitro'' should be written in italic font.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Chemistry of natural products and their biological applications

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 06 Feb 2023

Darus Paransa

Thank you very much reviewing the manuscript. We really appreciate your time and assessment, which will help us to maintain quality of our contents.

Competing Interests: No competing interests were disclosed.

ARTIKEL PENELITIAN

Karotenoid dari betina *Grapsus albolineatus* sebagai potensi senyawa anti penuaan [versi 1; peer review: menunggu rekan tinjauan]

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v1 **Pertama kali diterbitkan:**08 Des 2022,**11**:1457
https://doi.org/10.12688/f1000research.122649.1
Terbaru diterbitkan:08 Des 2022,**11**:1457 https://doi.org/10.12688/f1000research.122649.1

Abstrak

Pengantar: *Grapsus albolineatus* diperkirakan memiliki konsentrasi pigmen karotenoid yang tinggi. Meskipun studi pada laki-laki *G. albolineatus* telah dilakukan, tidak ada penelitian tentang ekstraksi pigmen dari wanita *G. albolineatus* telah dilakukan. Karotenoid memiliki kemampuan tinggi untuk melawan radikal bebas. Penelitian sebelumnya telah menunjukkan bahwa karotenoid dapat melawan radikal bebas penyebab penuaan kulit dini. Tujuan dari penelitian ini adalah untuk mengetahui jenis karotenoid apa saja yang terdapat di dalamnya *G. albolineatus* dan apakah mereka dapat bertindak sebagai penghambat alami protein yang menyebabkan penuaan dini, seperti enzim glukogenase, elastase, dan hyaluronidase.
Metode: Karotenoid diekstraksi dari *G. albolineatus* menggunakan kromatografi kolom dan kromatografi cair kinerja tinggi (HPLC); docking molekuler dan visualisasi masing-masing dilakukan dengan Autodock 4.2 dan Discovery Studio/Biovia. **Hasil:** Menurut data HPLC, terdapat pigmen karotenoid seperti didehydroastaxanthin, tetrahydroastaxanthin, dihydroastaxanthin, diatoxanthin, astaxanthin, dan adonixanthin. Menurut percobaan penambatan molekuler, pigmen karotenoid dari *G. albolineatus* adalah penghambat protein elastase dan hyaluronidase yang efisien. **Kesimpulan:** *G. albolineatus* karotenoid berpotensi sebagai antipenuaan karena lebih efektif sebagai inhibitor protein elastase dan hyaluronidase daripada inhibitor aslinya.

Kata kunci

Grapsus albolineatus, glukogenase, elastase, hyaluronidase, karotenoid.

Buka Peer Review

status persetujuan *MENUNGGU PEER REVIEW*

Setiap laporan dan tanggapan atau komentar pada artikel dapat ditemukan di bagian akhir artikel.

Penulis yang sesuai: Darus Saadah Johanis Paransa (darusparansa@unsrat.ac.id)

Peran penulis: Paransa DSJ: Konseptualisasi, Kurasi Data, Analisis Formal, Akuisisi Pendanaan, Investigasi, Metodologi, Administrasi Proyek, Sumber Daya, Pengawasan, Validasi, Visualisasi, Penulisan – Penyusunan Draf Asli, Penulisan – Review & Editing;**Kemer K**: Kurasi Data, Analisis Formal, Investigasi, Metodologi, Validasi;**Mantiri D**: Kurasi Data, Analisis Formal, Investigasi, Validasi;**Kepel RC**: Penyidikan, Pengawasan;**Suleman DP**: Penyidikan, Pengawasan;**Soemantri AD**: Kurasi Data, Metodologi, Software, Validasi, Visualisasi, Penulisan – Review & Editing;**Kurnia D**: Metodologi, Sumber Daya, Perangkat Lunak, Pengawasan, Penulisan – Review & Editing

Minat yang bersaing: Tidak ada kepentingan bersaing yang diungkapkan.

Informasi hibah: Penelitian ini didukung oleh dukungan dana dari Universitas Sam Ratulangi, Manado, Indonesia, melalui Skema Penelitian Dasar Universitas Unggul, 2019 (RDUU: dengan No. 673/UN12.13/LT/2019).

Para penyandang dana tidak memiliki peran dalam desain studi, pengumpulan dan analisis data, keputusan untuk menerbitkan, atau persiapan naskah.

Cara mengutip artikel ini: Paransa DSJ, Kemer K, Mantiri D*et al.***Karotenoid dari betina *Grap sus albolineatus* sebagai senyawa antipenuaan potensial [versi 1; tinjauan sejawat: menunggu tinjauan sejawat]**F1000Penelitian 2022,**11**:1457 https://doi.org/10.12688/f1000research.122649.1

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pengantar

Kulit adalah salah satu organ terbesar dalam tubuh manusia dan menutupi hampir keseluruhannya.[1,2]Kulit memiliki berbagai fungsi seperti melindungi otot, tulang, dan organ dalam.[3]Kulit dapat mengalami penuaan yang tidak dapat dihindari karena faktor intrinsik, dipengaruhi oleh usia, genetik, hormon, dan kadar gula darah.[4]Sedangkan variabel ekstrinsik mayoritas disebabkan oleh paparan sinar ultraviolet (UV) secara terus menerus, polusi, pola makan, dan merokok.[5,6]

Radiasi UV dikaitkan dengan peningkatan spesies radikal oksigen pada kulit. Spesies radikal reaktif yang berlebihan seperti spesies oksigen reaktif (ROS) dan spesies nitrogen reaktif (RNS) dapat memperburuk pigmentasi dan penuaan kulit, menyebabkan gangguan pigmentasi, kulit kasar dan kerutan kulit.[7]Selain kerusakan oksidatif pada kulit, ROS diketahui mengaktifkan enzim hyaluronidase, glukogenase, dan elastase.[8]Peningkatan aktivitas enzim ini dapat menurunkan asam hialuronat, elastasin, dan kolagen. Komponen-komponen ini adalah elemen kunci dari kulit yang bertindak berdasarkan elastisitas, kelenturan, dan penyediaan kelembapan, membuat kulit tampak muda dan sehat.[9,10]

Oleh karena itu, untuk mencegah penuaan dini, kulit membutuhkan antioksidan sekaligus inhibitor enzim yang berfungsi sebagai penghambat alami enzim yang teraktivasi akibat radiasi sinar UV. Karotenoid merupakan golongan senyawa yang memiliki aktivitas antioksidan yang sangat baik.[11,12]Sayuran dan buah-buahan dianggap sebagai sumber karotenoid terpenting dalam makanan manusia; produk hewani juga mengandung karotenoid dalam jumlah besar.[13,14]

Kepiting lightfoot dari Sulawesi Utara,G. albolineatus,diketahui mengandung karotenoid tingkat tinggi.G. albolineatus merupakan kepiting besar dengan populasi yang besar.Grapsus albolineatusLatreille memiliki karapas cembung dengan tuberkel rendah; lateral, tepi membulat, dengan satu gigi depan; panjang bagian depan sama dengan panjang tepi belakang karapas. Karapas berwarna biru kehijauan dengan area lebih pucat; margin lateral dan tuberkel putih; kaki berbintik-bintik coklat dengan satu bintik oranye di ujung meri.[15]Spesies ini memiliki margin lateral karapas yang sangat melengkung; sudut infraorbital luar dengan gigi akut; rahang atas ketiga dengan merus panjang, sedikit lebih pendek dari iskium; perut laki-laki memiliki lima somit sekitar 2,3 kali lebih lebar; G1 sedikit melengkung.G. albolineatusmerupakan spesies berukuran besar. Distribusi dunianya meliputi Indo-Pasifik Barat: Afrika Timur, Mauritius, Somalia, Socotra, Laut Merah, Teluk Aden, Oman Selatan, Teluk Persia, Teluk Oman, Pakistan, India, Sri Lanka, Teluk Benggala, Kepulauan Nicobar, Laut Andaman, Kepulauan Mergui, Cina, Jepang, Indonesia, Singapura, Kepulauan Cocos (Keeling), Australia, dan Hawaii. Habitatnya berbatu, substrat intertidal.[16]

Itu didistribusikan secara luas di lautan Pasifik Barat Daya. PriaG. albolineatustelah diakui memiliki jumlah pigmen karotenoid yang bervariasi, sedangkan betinaG. albolineatusbelum dipelajari untuk jenis pigmen karotenoid yang dikandungnya dan pemisahannya.[17]

Oleh karena itu, penelitian ini bertujuan untuk mengeksplorasi pigmen dan pemisahan karotenoid pada betinaG. albolineatusserta untuk menguji karotenoid sebagai anti aging dan inhibitor alami enzim hyaluronidase, glucogenase, dan elastase menggunakan pendekatan molecular docking.

Metode

Pertimbangan etis

Peneliti menyerahkan izin penelitian (ethical clearance) kepada tim komite Etik UB (Universitas Brawijaya). Bagian dari penelitian ini dilakukan di universitas.

Ethical clearance diperoleh dengan memasukkan proposal penelitian, dimana metode tersebut memiliki tahapan 3R, yaitu: 1) Reduksi: menggunakan 3 ekor hewan, 2) Penggantian: menggunakan 1 gram organ karapas, 3) Penyempurnaan: Untuk menghindari distres pada hewan , peneliti menggunakan alkohol 95% yang berfungsi sebagai analgesik.

Penelitian ini dibiayai oleh Lembaga Penelitian dan Pengabdian Masyarakat Universitas Sam Ratulangi (UNSRAT) dengan surat tugas Nomor 673/UN12.13/LT/2019. yang ditandatangani oleh ketua LPPM UNSRAT, setelah lulus penilaian oleh reviewer universitas.

Ekstraksi pigmen total Pengambilan
sampel dan penanganan

Pengambilan sampel dilakukan dengan menjelajahi daerah intertidal pada saat surut terendah pada malam hari. Sampel dimasukkan ke dalam wadah berisi sedikit air laut, untuk memastikan kepiting tidak mati. Karapas punggung kepiting berukuran 3,7 cm pada bagian tersebut diolesi alkohol 95%, pada saat kepiting pingsan dilakukan pembedahan kemudian dilakukan ekstraksi.

Ekstraksi pigmen total

Sampel kepiting pertama kali disuntikkan untuk diambil darahnyaG. albolineatusmenggunakan suntikan sepanjang lima mil kemudian dibedah untuk memisahkan hepatopankreas dan gonad. Langkah selanjutnya adalah memisahkan lapisan kulit ari, yaitu selaput tipis yang terhubung dengan bagian dalam karapas, dan lapisan kulit ari hitam dengan pinset dan pisau laboratorium. Karapas organ dihancurkan dan direndam selama lima menit dalam asam klorida 2N. Kemudian disaring melalui kertas saring ke dalam labu pemisah, ditambahkan petroleum eter dan air suling untuk membuat dua lapisan. Puncak serapan kemudian dihitung menggunakan spektrofotometer UV-Vis dengan rentang panjang gelombang 380-550 nm.

Analisis kuantitatif: Ekstrak pigmen total (Konsentrasi dan kuantitas)

Absorbansi ekstrak pigmen karapas diukur menggunakan spektrofotometer UV-Vis pada panjang gelombang 380-550 nm. Bentuk spektogram adalah kurva dengan sumbu X yang mewakili panjang gelombang, dan sumbu Y yang mewakili absorbansi, dan puncak serapan tertinggi pada panjang gelombang tertentu dapat dibaca. Setiap spektogram yang dihasilkan dapat digunakan untuk menghitung jumlah dan konsentrasiG. albolineatuspigmen yang digunakan dalam penelitian ini.[18,19]

Analisis kualitatif: pemisahan pigmen dengan kromatografi kolom

Setelah itu, seluruh ekstrak pigmen dipisahkan menggunakan kromatografi kolom. N-heksana-aseton adalah pelarut yang digunakan dalam fase gerak (70:30). Fraksi X dikumpulkan, kemudian ditampung dan diperiksa serapannya dengan spektrofotometer UV-Vis pada panjang gelombang 380-550 nm, menghasilkan puncak serapan tertinggi pada spektrofotometer tersebut.[18,19]

Analisis kromatografi cair kinerja tinggi

Kromatografi cair kinerja tinggi (HPLC) tipe LC.20ADVPdengan photodiode array detector (PDA; SPD-M20A-Shimadzu). Kolom HPLC yang digunakan adalah C30 (YMC carotenoid, 150 - 4.6 mm ID) dengan suhu kolom 30°C dan laju alir 1 mL/menit dengan sistem gradien H2O, metanol dan metil tersier butil eter. Sampel yang digunakan sebanyak 20 µL. Karotenoid dipisahkan dalam bentuk gradien selama 50 menit menggunakan larutan campuran metanol, metil tersier butil eter, dan air (80:5:15) dengan laju alir 1 mL/menit. Setelah 10 menit, kadar air diturunkan secara linier, dan aseton ditambahkan untuk mendapatkan proporsi aseton hingga 20 persen pada 50 menit setelah injeksi. Komposisi akhir campuran gradien adalah metanol: metil tersier butil eter (80:20).

Analisis docking molekul karotenoid dengan reseptor penuaan

Mekanisme pengikatan ke situs aktif tiga enzim diselidiki menggunakan penambatan molekuler: fibroblast collagenase (1CGL), elastase pankreas babi (4YM9), dan hialuronidase (1FCV).[20–22]Ketiga reseptor ini tersedia di Protein DataBank (PDB)situs webdan dapat disimpan sebagai file PDB.[23]

Persiapan enzim dilakukan dengan menghilangkan air dan kofaktor dari masing-masing enzim. Protein pertama kali dioptimalkan dengan menambahkan hidrogen polar, diikuti dengan penambahan muatan atom dengan muatan Kolman dan hidrogen nonpolar. Reseptor kemudian dikakukan dan disimpan dalam format PDBQT (Protein DataBank, partial charge (Q), dan atom type).[9]

The Canonical SmilesY dari setiap ligan yang digunakan dalam persiapan ligan diperoleh dari Pubchem.[24]Selanjutnya, Canonical Smiles diubah menjadi Chemdraw 2D 16.0 untuk menghasilkan senyawa 2D, yang kemudian dimodifikasi menjadi 3D menggunakan Chemdraw 3D dan dilakukan pengurangan perhitungan. File disimpan sebagai file PDB. File PDB ligan kemudian dicampur dengan hidrogen polar dan diberi muatan dan torsi atom gasteiger pada masing-masing ligan, setelah itu ligan disimpan dalam format PDBQT.

Perangkat lunak AutoDock 4.2.6 digunakan untuk mensimulasikan docking.[25]Di setiap situs aktif, dilakukan docking molekuler. Situs aktif ditentukan dengan memverifikasi docking masing-masing inhibitor dan reseptor yang sesuai. Kotak kisi dibuat di tengah lokasi ligan untuk menentukan koordinat sisi aktif reseptor. Kemudian, untuk reseptor 1CGL dan 4YM9 digunakan dimensi 50-50-50 titik, sedangkan untuk 1FCV dipilih dimensi kotak 60-60-60 titik, dengan jarak titik grid 0,375 Å. Algoritma Genetika Lamarckian adalah perhitungan yang digunakan dalam pendekatan docking ini. Konformasi optimal dipilih berdasarkan energi ikatan terendah di antara populasi yang paling konformasi.

Untuk mengamati interaksi yang terjadi antara ligan dan reseptor, dilakukan visualisasi docking menggunakan Biovia Discovery Studio 2020.[26]Interaksi masing-masing digambarkan dalam tiga dan dua dimensi. Ikatan hidrogen dan hidrofobisitas digunakan untuk menganalisis interaksi antarmolekul.

Hasil

Analisis kuantitatif: ekstraksi pigmen betinaG. albolineatus

Panjang gelombang tertinggi kandungan dan konsentrasi ekstrak pigmen total padaG. albolineatuskepiting, khususnya di karapas, adalah 474 nm. Hasilnya, kandungan ekstrak pigmen dan nilai konsentrasi secara keseluruhan adalah 4,33 µg/g, sedangkan kandungan pigmen pada organ karapas adalah 4,46 µg/g.

Analisis kualitatif: ekstraksi pigmen betinaG. albolineatusmenggunakan kromatografi kolom Pemisahan kromatografi kolom diulang dua kali dengan fase gerak n-heksana-aseton (70:30). Ekstrak pigmen total dibagi menjadi empat fraksi: F1, F2, F3, dan F4. Spektrofotometer UV-Vis dengan rentang panjang gelombang 380-550 nm digunakan untuk memeriksa keempat fraksi tersebut. Adanya pigmen x-karoten ditunjukkan dengan panjang gelombang 426, 448, dan 475 nm, sedangkan keberadaan pigmen zeaxanthin ditunjukkan dengan panjang gelombang 426, 450, dan 475 nm pada fraksi kedua; jenis pigmen pada F3 dan F4 tidak teridentifikasi.[27]

F1 tidak diproses melalui putaran kedua. Namun, F2, F3, dan F4 dipisahkan lagi menggunakan kromatografi kolom: F2.1 dikarakterisasi sebagai echinenone dan F2.2 sebagai astase. Selanjutnya, pigmen tidak teridentifikasi pada F3.1 dan F4.1. Krocoxanthin ditemukan pada F3.2, alloxanthin diidentifikasi pada F3.3, dan pyrhoxanthin diidentifikasi pada F4.2.

Analisis tipe pigmen dengan kromatografi cair kinerja tinggi

Untuk menghasilkan 61 puncak serapan maksimum, temuan fraksi kromatografi satu kolom dipisahkan menggunakan HPLC dengan waktu propagasi 50 menit. Spektra serapan setiap puncak dari kromatogram HPLC diidentifikasi pada 463 nm, dan yang memiliki persentase luas lebih besar dari 3% diselidiki dengan memeriksa pola spektral menggunakan spektrofotometer serapan HPLC UV-Vis

Fraksi HPLC satu temuan menunjukkan sebelas puncak serapan.Tabel 1menunjukkan serapan yang dihasilkan pada puncak tertinggi. Puncak serapan terbesar pada 473 nm ditentukan sebagai jenis pigmen didehydroastaxanthin, sedangkan puncak serapan maksimum pada 476 nm ditentukan sebagai jenis tetrahydroastaxanthin. (Tabel 1-3). Pemisahan HPLC dilakukan pada waktu propagasi 50 menit dan menghasilkan 65 puncak serapan pada temuan kromatografi kolom fraksi kedua.

Pada panjang gelombang 436 nm, daerah spektrum serapan dengan persentase yang lebih besar menghasilkan pembentukan puncak serapan maksimum. Penyerapan tertinggi diMeja 2membantu menentukan jenis pigmen karotenoid.[28]

Enam puncak spektrum diidentifikasi dari pemisahan sembilan puncak serapan oleh HPLC. Hanya lima jenis pigmen yang dapat diidentifikasi menggunakan pola spektral enam puncak. Pemisahan HPLC menghasilkan pembentukan puncak serapan dengan luas puncak 3% inTabel 3. Puncak serapan terbesar ditemukan pada serapan spektrofotometer HPLCUV-Vis, yaitu puncak serapan ke-8, 14, 15, 16, 17, dan 34 sehingga dapat ditentukan jenis pigmennya.Gambar 1 menyajikan visualisasi struktur senyawa dari jenis pigmen yang dihasilkan oleh analisis HPLC.

Tabel 1.Jenis dariG. albolineatuspigmen karotenoid dipisahkan dengan HPLC dari hasil fraksi pertama kromatografi kolom.

Tidak	Waktu retensi/Puncak no.	Puncak penyerapan maksimum	jenis pigmen
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

Meja 2.Jenis dariG. albolineatuspigmen karotenoid dipisahkan dengan HPLC dari hasil fraksi kedua kromatografi kolom.

Tidak	Waktu retensi/Puncak no.	Puncak penyerapan maksimum	Jenis pigmen
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

Tabel 3.Jenis dariG. albolineatuspigmen karotenoid dipisahkan dengan HPLC dari hasil fraksi ketiga kromatografi kolom.

Tidak	Waktu retensi/Puncak no.	Puncak penyerapan maksimum	Jenis pigmen
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

Analisis docking molekul karotenoid dariG. albolineatuspada protein kolagenase (1CGL)

Nilai energi ikatan dihasilkan dari total energi akhir antarmolekul, yaitu dari ikatan van der Waals, ikatan hidrogen, energi desolvasi, dan energi elektrostatik, kemudian dari energi dalam total akhir, energi torsi, dan energi sistem tak terikat. Penjumlahan energi ini biasanya dikenal sebagai estimasi energi bebas pengikat (EFEB). Konstanta inhibisi juga diperkirakan dengan menggunakan Autodock 4.2.6 pada 298 K. Nilai konstanta inhibisi yang rendah terkait dengan aktivitas biologis.[29]

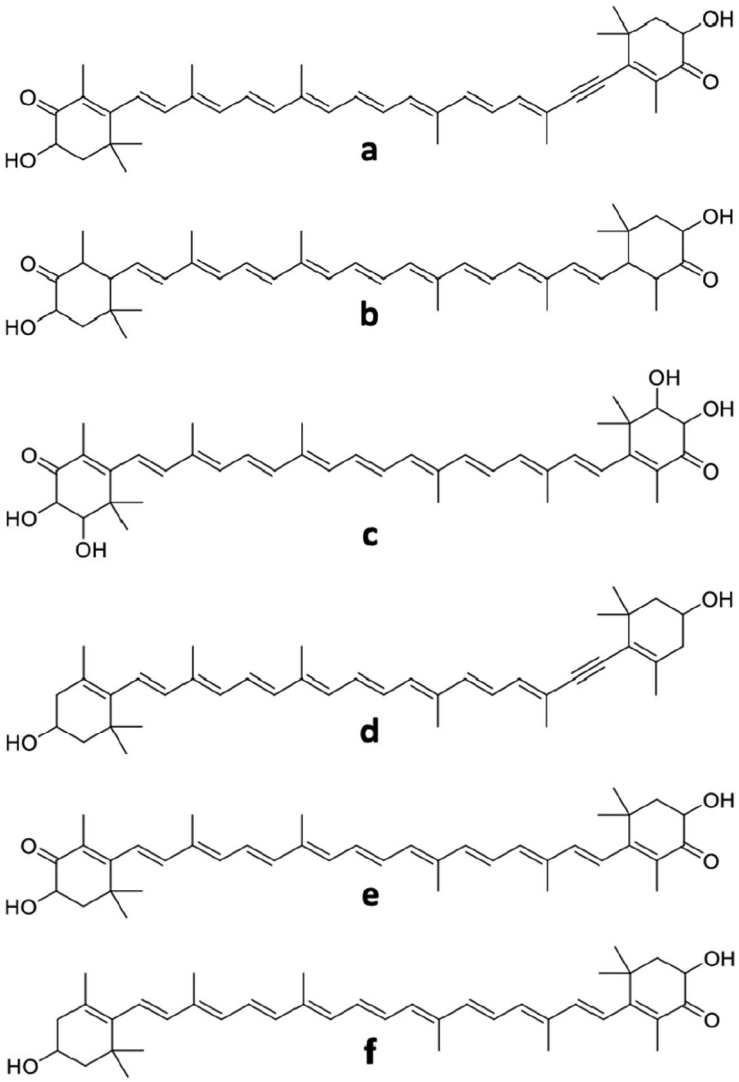
Karotenoid yang ditemukan oleh HPLC (Gambar 1) digunakan sebagai ligan uji untuk mengikat protein yang berkontribusi terhadap penuaan kulit. BerdasarkanTabel 4, setelah dibandingkan dengan senyawa karotenoid lainnya, adonixanthin memiliki energi ikat terendah dengan -6,61 kkal/mol dan konstanta penghambatan tertinggi dengan 14,23 mM. Namun, energi ikat karotenoid ini tidak lebih baik dari ligan asalnya (-7,83 kkal/mol dengan tetapan inhibisi terendah 1,81 mM). Visualisasi hasil docking molekul ditunjukkan padaAngka 2-4.

Analisis docking molekul Senyawa karotenoid dariG. albolineatuspada protein elastase (4YM9) Berdasarkan hasil interaksi antara karotenoid dengan protein elastase, pigmen karotenoid memiliki energi ikat yang lebih tinggi dibandingkan ligan alam yang hanya memiliki energi ikat sebesar -5,55 kkal/mol dan konstanta inhibisi sebesar 85,31 mM (Tabel 5). Adonixanthin dan astaxanthin memiliki energi ikat tertinggi, dengan energi ikat masing-masing sebesar -9,03 kkal/mol dan -9,01 kkal/mol.

Analisis Docking Molekuler Senyawa Karotenoid dariG. albolineatuspada Protein elastase (4YM9) Senyawa karotenoid dariG. albolineatusmemiliki energi ikat yang lebih rendah dibandingkan ligan hyaluronidase alam yang memiliki nilai ikat -4,17 kkal/mol. Astaxanthin memiliki energi ikat -2,27 kkal/mol dan hanya terikat pada empat residu asam amino, sedangkan didehydroastaxanthin memiliki energi ikat -7,71 kkal/mol (Tabel 6).

Diskusi

G. albolineatusadalah kepiting besar dengan populasi besar di sepanjang zona intertidal berbatu.[30]G. albolineatusdapat ditemukan di lepas pantai Kalasey, Teluk Manado, Su-lawesi Utara, di Indonesia. Pigmen karotenoid, seperti karoten, echinonen, dan



Gambar 1.Senyawa diperoleh dariG. albolineatusdan strukturnya: (a) didehydroastaxanthin; (b) tetrahidroksantin; (c) dihidroksiastaxantin; (d) diatoksantin; (e)astaxanthin; (f) adonixanthin.

cantaxanthin, ditemukan di hemocyanin, hepatopancreas, lapisan epidermis, dan di luar karapas pada priaG. albolineatus dari Indonesia.[17]

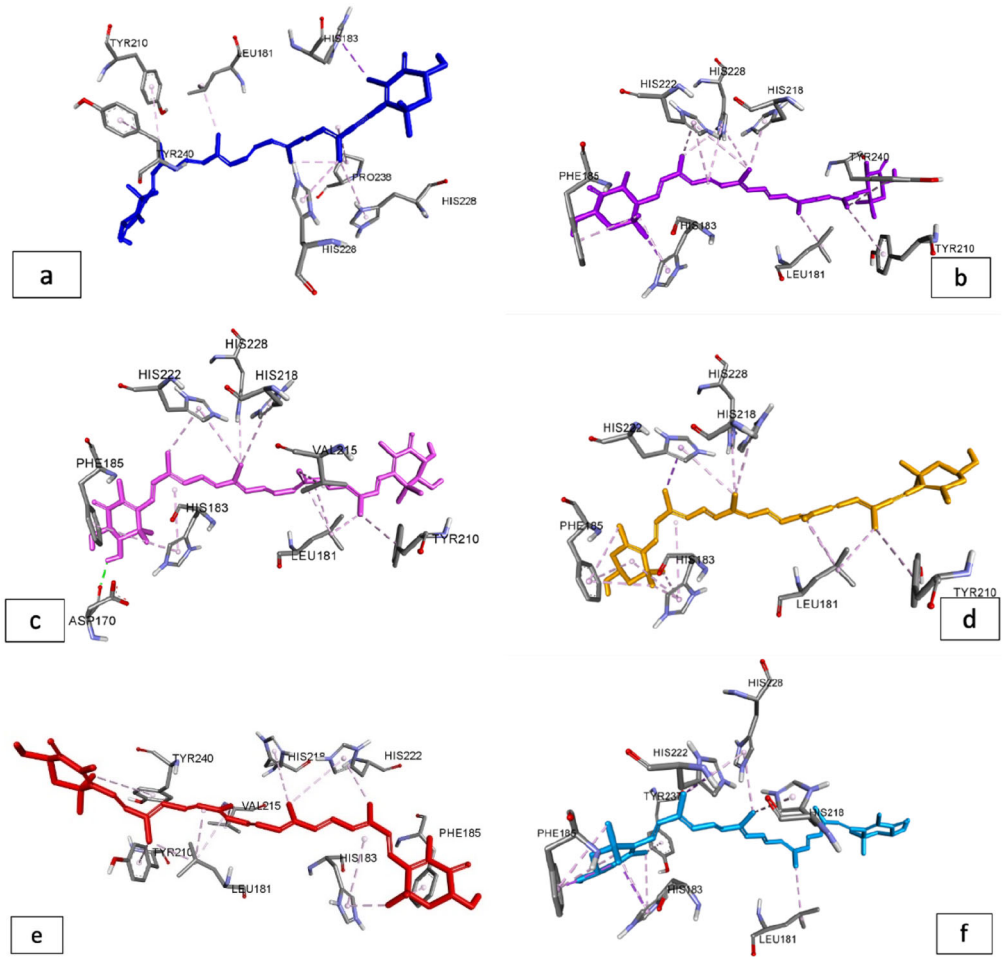
Berdasarkan hasil HPLC dari karapas betinaG. albolineatus,ditemukan beberapa jenis karotenoid, antara lain didehydrodiastaxanthin, tetrahydroastaxanthin, dihydroastaxanthin, diatoxanthin, astaxanthin, dan adonixanthin. Identifikasi karotenoid dari betinaG. albolineatusadalah hal baru.

Karotenoid memberikan perlindungan antioksidan yang efektif terhadap radikal peroksil selama proses foto-oksidasi. Karotenoid memiliki panjang gelombang maksimum 450 nm karena struktur senyawanya.[11,31]Beberapa penelitian menunjukkan bahwa orang dengan kadar karotenoid tinggi di kulitnya memiliki kulit yang terlihat lebih muda dari usianya, sedangkan orang dengan kadar karotenoid rendah memiliki kulit yang terlihat lebih tua.[32]Ini mungkin karena penuaan kulit terkait dengan radiasi UV, dan karotenoid dianggap pemulung radikal yang efektif.[33]

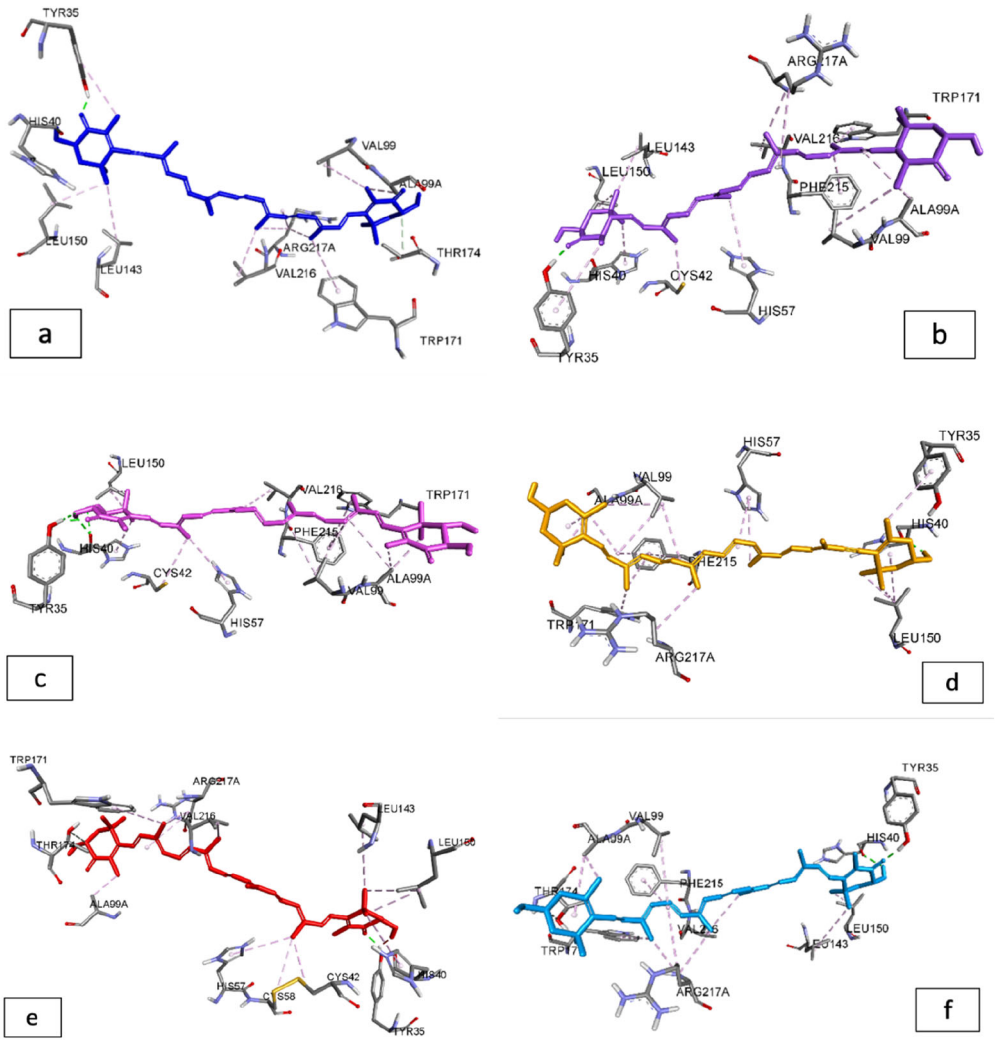
Eksposisi kulit terhadap radikal bebas tingkat tinggi dapat mengaktifkan enzim kolagenase, elastase, dan hyaluronidase yang menyebabkan penurunan kolagen, elastin, dan asam hialuronat sehingga menyebabkan penuaan pada kulit. Studi docking molekuler telah digunakan untuk mengetahui bagaimana karotenoid menekan enzim kolagenase, elastase, dan hyaluronidase, danTabel 5 menunjukkan energi ikat masing-masing komponen.Angka 1-3menunjukkan representasi gambar dari ikatan yang terjadi. Inhibitor asli memiliki energi pengikatan yang lebih tinggi dalam protein kolagenase (1cgl) daripada turunan karotenoidG. albolineatus.Ini karena inhibitor asli berinteraksi dengan residu asam amino His222, His218, dan His228 melalui interaksi hidrogen.[29,34]

Tabel 4.Estimasi energi ikat, konstanta inhibisi, dan tipe interaksi karotenoid terhadap enzim kolagenase.

Senyawa	Mengikat energi (kkal/mol)	Inhibisi konstan (mM)	Hidrogen ikatan	Ikatan hidrofobik
Didehydroastaxanthin	- 4,97	225,61	-	Leu181; Pro238; His228; Tyr210; Tyr240; Nya183
Tetrahydroxanthin	- 3.30	3790	-	Tyr210, Tyr240, Leu181, Phe185, His183, His218, His222, His228
Dihidroksiastaxantin	- 3.66	2070	Asp170	Phe185, His183, His222, Tyr210; Leu181; Val215; His218; Nya228
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
Warga asli	- 7.83	1,81	Pro238; His218; His222; Nya228	Gly179; Asp175



Gambar 2.Hasil visualisasi inhibitor enzim karotenoid sascollagenase (1CGL): (a) didehydroastaxanthin; (b) tetrahidroksantin; (c) dihidroksiastaxantin; (d) diatoksantin; (e)astaxanthin; (f) adonixanthin.

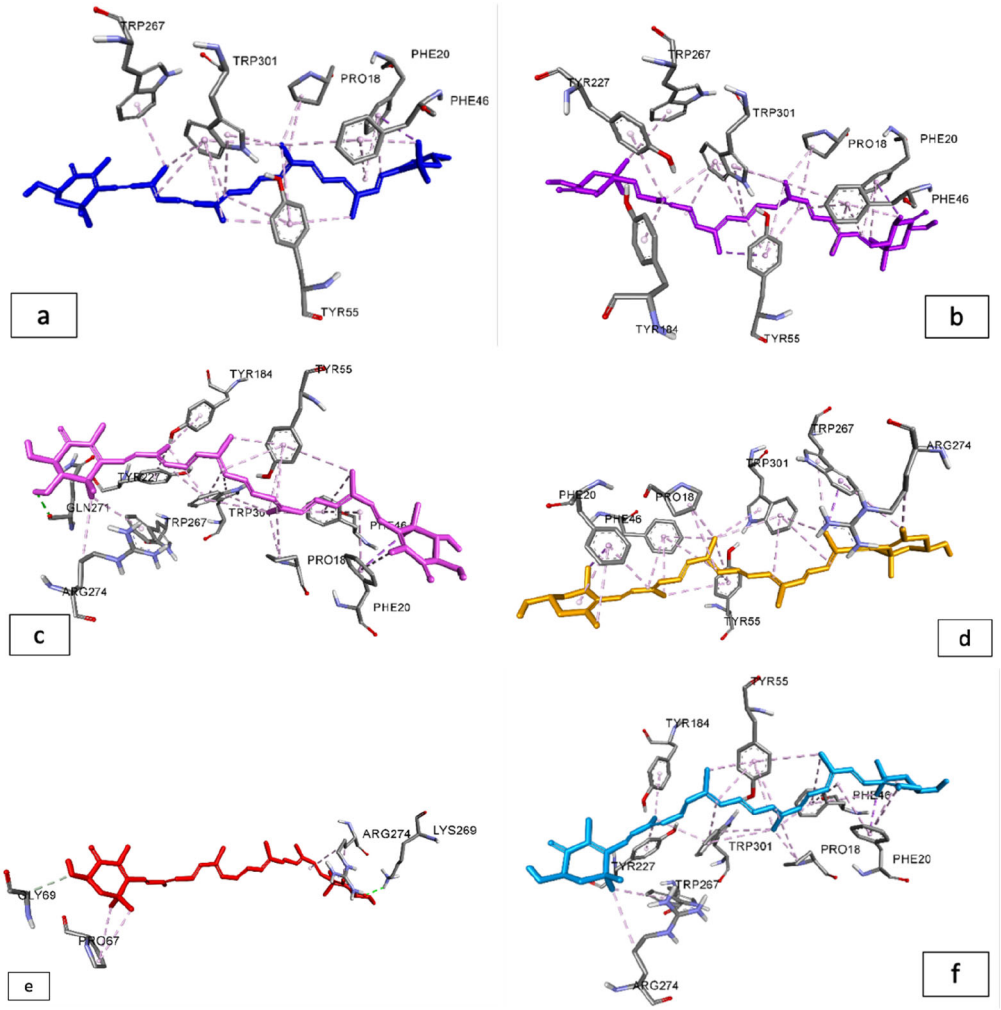


Gambar 3.Hasil visualisasi penghambat enzim carotenoidsas collagenase (1CGL): (a) didehydroastaxanthin; (b) tetrahidroksantin; (c) dihidroksiastaxantin; (d) diatoksantin; (e)astaxanthin; (f) adonixanthin.

Sedangkan interaksi antar residu tersebut pada interaksi antara senyawa karotenoid dengan reseptor kolagenase hanya terdapat pada interaksi hidrofobik. Energi torsi adalah faktor lain yang mempengaruhi energi ikat. Nilai positif untuk energi puntir antara interaksi karotenoid yang terlalu besar diduga berpengaruh pada kecilnya energi ikat yang dihasilkan. Namun, adonixanthin (Tabel 4) memiliki energi ikat yang relatif tinggi dan nilai penghambatan 14,23 mM, yang menentukan bioavailabilitas komponen ini untuk digunakan sebagai obat[29](Gambar 2dan3).

Menurut literatur, elastase adalah protease serin yang fungsi utamanya adalah pemutusan ikatan peptida dari banyak protein, termasuk elastin, yang bertanggung jawab atas elastisitas jaringan ikat dan terutama ditemukan di paru-paru, arteri, dan ligamen.[35,36]Telah diidentifikasi bahwa pengikatan Tyr35, His40, dan Val216 memiliki pengaruh yang signifikan terhadap kekuatan energi pengikatan antara ligan dan reseptor target dalam interaksi docking molekuler antara karotenoid dan elastase. Hasil docking mengungkapkan bahwa astaxanthin dan adonixanthin memiliki energi pengikatan tertinggi,yaitu -9,01 dan -9,03 kkal/mol. Berdasarkan hasil energi ikat, senyawa karotenoid dari G. albolineatusdiTabel 6dapat digunakan sebagai inhibitor elastase bila dibandingkan dengan inhibitor asli (Gambar 3).

Pada interaksi yang terjadi antara kompleks ligan dengan protein hyaluronidase, dari hasil bila dibandingkan dengan native inhibitor, residu Tyr227, Phe46, dan Pro18 diduga berpengaruh terhadap peningkatan energi ikat kompleks yang terbentuk, dan ikatan hidrofobik lebih disukai. . Tidak adanya interaksi antara native dan Pro18 memperkuat bahwa interaksi yang terjadi berpengaruh besar terhadap energi ikatan yang dihasilkan. Selain itu, astaxanthin saja



Gambar 4.Hasil visualisasi karotenoid sebagai inhibitor enzim kolagenase (1CGL): (a). didehidroastaxantin; (b) tetrahidroksantin; (c) dihidroksiastaxantin; (d) diatoksantin; (e) astaksantin; (f) adonixanthin.

Tabel 5.Estimasi energi ikat, konstanta inhibisi, dan tipe interaksi karotenoid terhadap enzim elastase.

Senyawa	Mengikat energi (Kkal/mol)	Inhibisi konstan (mM)	Hidrogen ikatan	Ikatan hidrofobik
Didehydroastaxanthin	- 8.42	0,673	Tyr35; Nya40	Leu150; Leu143; Val216; Arg217; Trp171; Ala99; Val99; Thr174
Tetrahydroxanthin	- 8.31	0,86	Tyr35	Cys42; Leu143; Nya40; Leu150; Nya57; Val216; Arg217; Phe215; Trp171; Ala99; Val99
Dihydroastaxanthin	- 7.58	2,78	Nya40; Tyr35	Ala99; Trp171;Phe215; Val99; Nya57; Cys42; Leu150
Diatoxanthin	- 7.81	1,78	Nya40	Leu150; Trp171; Arg217; Ala99; Val99; Phe215; Nya57; Cys42; Leu150
Astaxanthin	- 9,01	0,248	Tyr35	Nya40; Leu143; Trp171; Arg217; Ala99; Val216; Cys58; Nya57; Cys42
Adonixanthin	- 9,03	0,239	His40, Tyr35	Leu143, Ala99, Leu150, Val216, Val99, Arg217, Trp171, Phe215
Warga asli	- 5,55	85,31	Cys220; Gln192; Gly193; Ser195	Val216

Tabel 6.Estimasi energi ikat, konstanta inhibisi, dan tipe interaksi karotenoid terhadap enzim elastase.

Senyawa	Mengikat energi (kkal/mol)	Inhibisi konstan (mM)	Hidrogen ikatan	Ikatan hidrofobik
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	21,79x10$_3$	Lys269	Arg274; Gly69; Pro67
Adonixanthin	- 7.45	3,46	-	Phe20, Pro18, Tyr55, Phe46, Trp305, Tyr227, Tyr184, Trp267, Arg274
Warga asli	- 4,17	875,19	Tyr227; Asp111; Ser304; Asp305; Glu113	Tyr184; trp301; Phe46; Arg47; Ser303

menghasilkan energi ikat sebesar -2,27 kkal/mol. Sebagai hasil dari penemuan ini, dapat disimpulkan bahwa astaxanthin memiliki konstanta penghambatan yang terlalu besar, sehingga menghasilkan nilai bioavailabilitas yang rendah.[9]Sementara itu, karotenoid lain dengan energi ikat tinggi, seperti didehydroastaxanthin, tetrahydroastaxanthin, diatoxanthin, dan adonixanthin berpotensi untuk digunakan sebagai inhibitor alami protein hyaluronidase. Karena interaksi ligan uji berlabuh di lokasi yang sama dengan situs aktif, senyawa ini dapat digunakan sebagai penghambat kompetitif protein kolagenase, elastase, dan hyaluronidase.

Kesimpulan

Kandungan pigmen karapas adalah 4,46 mg berdasarkan pengukuran kuantitatif dari total ekstraksi pigmen pada betinaG. albolineatus.Secara kualitatif, karapas diketahui mengandung pigmen karotenoid seperti didehydroastaxanthin, tetrahydroastaxanthin, dihydroastaxanthin, diatoxanthin, dan astaxanthin berdasarkan HPLC. Karotenoid adalah antioksidan alami yang memainkan peran penting dalam regenerasi kulit. Menurut hasil docking molekuler, senyawa karotenoid dariG. albolineatusadalah inhibitor yang lebih kuat daripada inhibitor asli, yang secara kompetitif berikatan dengan residu katalitik yang sama pada protein elastase dan hyaluronidase. Namun,in vivodanin vitrostudi diperlukan untuk mengkonfirmasi pendekatan ini sehingga data ini dapat digunakan sebagai pesaing prospek pengobatan, khususnya di bidang kosmetik.

Ketersediaan data

Data yang mendasari

Figshare: Karotenoid Berpotensi sebagai Anti-aging dari Grapsus albolineatus Betina,https://doi.org/10.6084/m9.figshare.19947758[37]

Proyek ini berisi data dasar berikut:

- 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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