*ORIGINAL ARTICLE*

A C G publications

*Rec. Nat. Prod.* 19:6 (2025) 680-687

records of natural products

# Saganhaematenones: Novel Bioactive Natural Products, Derived from *Haematococcus* (Chlorophyceae) dSgH-K1 Strain, that Inhibit Angiotensin-Converting Enzyme

**Mikihide Demura [1,2], Toshiyasu Inuzuka [3] and Yoshinori Kawazoe [4*]**

[1]*Institute of Ocean Energy, Saga University, 1 Honjo, Saga 840-8502, Japan*
[2]*Saga Algal Ingustry R&D Center in Regional Innovation Center, Saga University, 1 Honjo, Saga 840-8502, Japan*
[3]*Life Science Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
[4]*Center for Bioresource Education and Research, Saga University, 152-1 Shonan-cho, Karatsu, Saga 847-0021, Japan*

*(Received July 11, 2025; Revised August 20, 2025; Accepted September 01, 2025)*

**Abstract:** It is believed that microalgae biomass can be used in a variety of ways. However, their use is currently limited to specific fields, and there are a few applications of microalgae biomass in drug discovery. Numerous microalgae inhabit the world, and they likely produce unknown substances that contribute to human health. In this study, therefore, we used *Haematococcus* as a material to search for substances that were beneficial to human health. We tried to identify them using their blood pressure-lowering effect as an indicator, and then we succeeded in isolating two novel substances, saganhaematenone A and B. The structural analysis revealed that they belong to glyceroglycolipid and were analogues with a stereoisomeric relationship. Our findings suggest that microalgae biomass may also have beneficial effects on human health.

**Keywords:** *Haematococcus*; microalgae; angiotensin-converting enzyme; bioactive molecules; glyceroglycolipids; Saganhaematenone 

## 1. Introduction

Microalgae biomass has various uses, including industrial materials and fuels [1-3]. Optimization of microalgae biomass production is progressing, but it is still very costly, and high-value-added final products are needed [3]. Therefore, the pharmaceutical use of microalgae biomass is the best way. Based on this background, we decided to search for biologically active substances using microalgae.

Among a range of pharmacological effects, we focused on the anti-hypertension effect because the number of patients with hypertension is increasing with the increase in the elderly population [4]. The blood pressure level is controlled by several ways, and the renin-angiotensin system is one of the most-characterized ones [5, 6]. Angiotensin II, a peptide hormone that raises blood pressure level, is synthesized from an inactive precursor, angiotensinogen. The precursor is digested by the protease, renin, to produce angiotensin I, which is then cleaved by the protease, angiotensin-converting enzyme (ACE), to form an active version, angiotensin II. Therefore, it is expected that inhibiting the protease

* Corresponding author: E-Mail: ykawazoe@cc.saga-u.ac.jp; Phone:+81-955-77-4484 *Fax:*+61-955-77-4486

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products November-December 2025 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.550.2507.3585
Available online: September 9, 2025

will block the production of angiotensin II and prevent an increase in blood pressure. [7, 8]. Indeed, one of the two proteases, ACE, is a promising drug target for the treatment of hypertension, and several prescription drugs were brought to the market, including captopril, enalapril, and lisinopril [9, 10]. In this study, we tried to look for the substances that inhibit ACE activity using *Haematococcus lacustris* (Gir.-Chantr.) Rostaf. (a.k.a. *Haematococcus pluvialis* Flot.) Our efforts successfully identified two novel compounds, saganhaematenone A and B (enone moiety-containing compounds derived from the *Haemat*ococcus strain established at Saga city).

## 2. Materials and Methods

### *2.1. Strain and Culturing Conditions*

The *H. lacustris* dSgH-K1strain used in the study was an axenic clonal culture isolated on December 3, 2017, from a small puddle in Kita-Kawasoe Town, Saga City, Saga Pref., Japan. We established more than 50 strains at that time, and the strain showed the highest growth rate. This culture strain has been maintained in a polycarbonate bottle (10 L, 2251-0020, Thermo Fisher Scientific) in 8L AF6 medium [11] at ca. 25 °C under a 12-h light/12-h dark cycle with white, fluorescent illumination (approximately 100 µmol photons $m^{-2}s^{-1}$). The culture was aerated with 5% $CO_2$ at 1 $Lmin^{-1}$. Every 2 weeks, half of the culture (4 L) was harvested and stored in a refrigerator (ca. 10 °C) for spontaneous sedimentation of K1 strain cells. Dry cell weight at harvest was approximately 0.2 $gL^{-1}$. Microscopic examination showed no accumulation of astaxanthin in the cells. After the cells spontaneously sedimented, the supernatant was discarded, and a cell concentrate was prepared and used as a sample for the next extraction step. In total, five harvesting and concentration operations were conducted.

### *2.2. Extraction and Isolation of ACE Inhibitors*

The harvested dSgH-K1 strain cells were pooled together and extracted with aqueous methanol for two weeks with sonication once a week, then the residue was removed by filtration. The aqueous methanol extract was concentrated, then partitioned between water and ethyl acetate. The ethyl acetate fraction was partitioned again between hexane and 80% aqueous methanol. These three fractions (water, hexane, and 80% methanol) were examined to determine whether they could inhibit ACE activity or not, and it was found that the 80% methanol fraction could. Then, the 80% methanol fraction was fractionated with reverse-phase open column chromatography (50 I.D. x 150 mm, COSMOSIL 75$C_{18}$-OPN, Nacalai Tesque) with aqueous methanol. Next, the active fraction was subjected to reverse phase open column chromatography again (30 I.D. x 150 mm), followed by preparative thin-layer chromatography (PLC, 10 x 10 cm silica gel 60 $F_{254}$, 0.5 mm, Merck) with a 1:4 mixture of hexane and ethyl acetate as a solvent. A reverse-phase high-performance liquid chromatography (HPLC, COSMOSIL C18-MS-II, 4.6 I.D. x 250 mm, Nacalai Tesque) with a 43% acetonitrile in water as an eluent was used at the final step of purification.

### *2.3. Evaluation of ACE Inhibitory Activity*

ACE activity was examined by means of using the ACE kit-WST (DOJINDO). The microalgae extracts were dissolved in methanol or dimethyl sulfoxide, followed by dilution with $H_2O$, and then used to test their effect against ACE activity. The ACE activity was evaluated per the manufacturer's instructions by measuring the absorbance at 450 nm of the pigment after cleavage of the substrate peptide using a plate reader (SpectraMax iD3, Molecular Devices). The activity of saganhaematenones was evaluated by performing ACE activity evaluation in the presence of various ACE inhibitor concentrations, then the ACE activity rate was calculated using the following formula: ($A_S$-$A_N$) / ($A_P$-$A_N$) x 100%, where $A_N$: the absorbance in the negative control without enzyme, ACE, $A_P$: the absorbance in the positive control without algae sample, and $A_S$: the absorbance in the presence of ACE inhibitors.

*2.3. Docking Simulation Study*

The three-dimensional structure of ACE was retrieved from the Protein Data Bank (PDB accession no. 1UZF). The protein was prepared for docking by removing water and ligand molecules, adding polar hydrogen atoms, and energy minimization using UCSF Chimera (version 1.19) [12]. Energy minimization of saganhaematenones was carried out by OpenBabel software (version 3.1.1) [13]. Molecular docking simulations were performed using AutoDock Vina (version 1.1.2) [14]. A grid box was maximized throughout the protein; the size of the grid box was approximately 50 Å × 50 Å × 50 Å. The exhaustiveness of the search was set to 100. The docking results were analyzed, and the pose with the lowest binding affinity ($\Delta G_{binding}$, kcal/mol) was selected for further analysis. The interaction between ACE and saganhaematenonen was visualized and analyzed using PyMOL (version 3.1.0) and PoseView[15] in the Protein Plus Web server. The 2D diagram generated by PoseView highlighted key interactions, including hydrogen bonds and hydrophobic interactions between the ligand and the amino acid residues of the ACE protein.

## 3. Results and Discussion

*3.1. Purification of Saganhaematenones*

The 80% methanol fraction was divided into 5 parts (#31-#35) with reversed-phase open column chromatography and examined which one that was effective in inhibiting ACE activity. As a result, all fractions except #34 inhibited ACE activity and identified two compounds from fractions #31 and #32, respectively [16]. In this study, we focused on the fraction #35. Sequential fractionation of the #35 with Octa Decyl Silyl (ODS) open column, PLC, and HPLC successfully purified two compounds, saganhaematenone A (0.25 mg) and saganhaematenone B (0.23 mg), as colorless solids.

*3.2. Structure Elucidation*

Then we tried to elucidate their chemical structures. Analyzing HRMS data (Figure S1) for saganhaematenone A indicated a molecular ion of *m/z* 527.2816 for $[M + Na]^+$, providing a molecular formula of $C_{25}H_{44}O_{10}$. The $^1$H-NMR spectrum (Figure S2) of saganhaematenone A showed one triplet methyl (0.89 ppm), some methylenes (1.3-1.8 ppm), and one olefin next to the carbonyl group (6.13 ppm and 6.91ppm). $^{13}$C-NMR and HMBC were not even when the measurement time was extended, probably because of low quantity. COSY and TOCSY correlations (Figures S3 and S4) revealed six partial structures, C2-C7, C9-C10, C15-C16, C1'-C3', C1''-C-2'', and C1'''-C4''' (Figure 1). From the molecular formula and 1D and 2D NMR spectra and HMBC correlations, H9-C8 and H16-C14, it was estimated that some methylenes were present between C10 and C15, and a carbonyl group was present between C7 and C9. From the correlation, H2-C1, another carbonyl group was presented next to C2. The geometry of the olefin was determined to be *E* by the coupling constant of H7-H8 (15.8 Hz). Although having elucidated these three partial structures, we were not able to link them together because of obtaining few valid correlations in the HMBC spectrum.

Analyzing HRMS data for saganhaematenone B (Figure S1) indicated a molecular ion of *m/z* 527.2815 for $[M + Na]^+$, providing a molecular formula of $C_{25}H_{44}O_{10}$. The $^1$H-NMR spectrum (Figure S6) of saganhaematenone B showed one triplet methyl (0.90 ppm), some methylenes (1.3–1.8 ppm), and one olefin (6.13 ppm and 6.91 ppm). COSY and TOCSY correlations (Figures S7 and S8) revealed six partial structures, C2-C7, C9-C10, C15-C16, C1'-C3', C1''-C-2'', and C3''-C6'' (Figure 2). HMBC (Figure S10) correlations, H6-C8, H7-C8, H9-C8 and H10-C8, connected C7 and C9, and HMBC correlation, H16-C14, connected C14 and C15. HMBC correlations, H2''-C3'' and H4''-C2'', connected

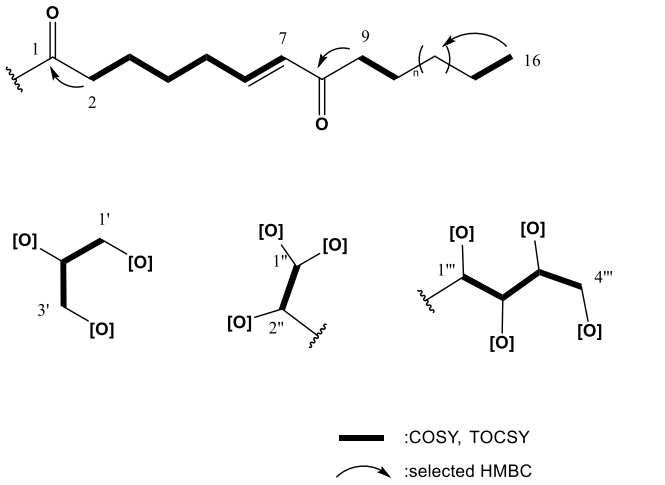


**Figure 1**. Partial structures of saganhaematenones

C2‟-C3‟, and another correlation, H5‟-C1‟, indicated the presence of a hexose. From the molecular formula and 1D and 2D NMR spectra, it was estimated that three methylenes are present between C10 and C14. HMBC correlations, H‟1-C1 and H2-C1, indicated that a fatty acid was present at C1‟, and another correlation, H3‟-C1‟, indicated that a sugar moiety was present at C3’. The geometry of the olefin was determined to be *E* by the coupling constant of H7-H8 (15.8 Hz). Based on these results, saganhaematenone B was determined to have a planar structure as shown in Figure 2.

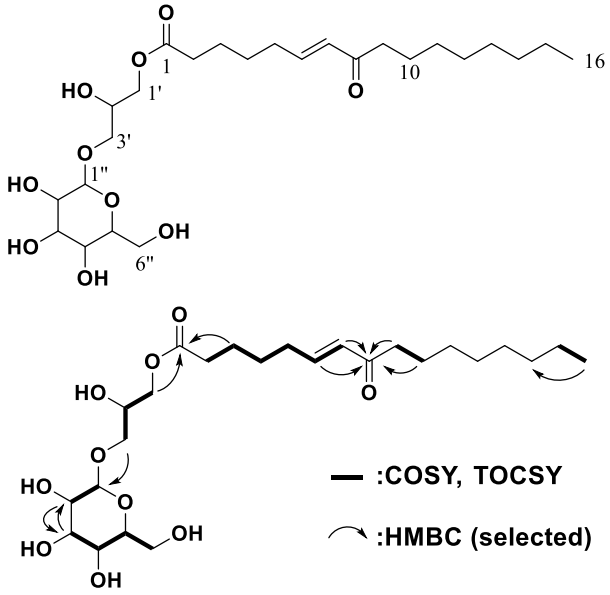


**Figure 2**. Planer structure of saganhaematenones

Saganhaematenones A and B had the same molecular formula, but their $^{1}$H-NMR spectra did not coincide, although they were similar. All elucidated partial structures of saganhaematenone A were present in the structure of saganhaematenone B. Considering these results, it was thought that saganhaematenones A and B were stereoisomers. Table 1 summarizes the $^{1}$H-NMR and assigned $^{13}$C-NMR data.

**Table 1.** NMR data for saganhaematenones A and B in $CD_3OD$

| Saganhaematenone A | | | | Saganhaematenone B | | | |
|---|---|---|---|---|---|---|---|
| **position** | **$^{13}C^a$** | **$^{1}H^b$** | **Coupling$^c$** | **position** | **$^{13}C^a$** | **$^{1}H^b$** | **Coupling$^c$** |
| 1 | 175.8 | | | 1 | 175.4 | | |
| 2 | 33.7 | 2.39 | t (7.4) | 2 | 35.0 | 2.36 | t (7.4) |
| 3 | 25.6 | 1.66 | m | 3 | 26.0 | 1.63 | m |
| 4 | 25.6 | 1.52 | m | 4 | 29.4 | 1.48 | m |
| 5 | 33.3 | 2.26 | dt (6.6,6.6) | 5 | 33.9 | 2.24 | dt (6.6, 6.6) |
| 6 | 149.4 | 6.91 | dt (15.8, 6.6) | 6 | 149.7 | 6.91 | dt (15.8, 6.6) |
| 7 | 129.9 | 6.13 | d (15.8) | 7 | 131.5 | 6.13 | d (15.8) |
| 8 | 204.2 | | | 8 | 204.0 | | |
| 9 | 38.7 | 2.57 | t (7.3) | 9 | 40.6 | 2.60 | t (7.3) |
| 10 | 23.2 | 1.56 | m | 10 | 25.2 | 1.60 | m |
| 11-15 | 24.2-33.3 | 1.30-1.33 | | 11-15 | 23.9-33.0 | 1.30-1.33 | |
| 16 | 14.9 | 0.89 | t (6.9) | 16 | 14.6 | 0.90 | t (6.9) |
| | | | | | | | |
| 1' | 66.8 | 4.15 | m | 1' | 66.8 | 4.15 | m |
| 2' | 69.9 | 3.98 | m | 2' | 69.8 | 3.98 | m |
| 3'a | 72.2 | 3.91 | dd (10.7, 4.7) | 3'a | 72.2 | 3.91 | dd (10.1, 4.3) |
| 3'b | | 3.64 | dd (10.7, 4.6) | 3'b | | 3.65 | dd (10.1, 4.6) |
| | | | | | | | |
| 1" | 105.7 | 4.22 | d (7.7) | 1" | 105.5 | 4.22 | d (7.0) |
| 2" | 72.7 | 3.52 | m | 2" | 72.8 | 3.53 | m |
| 3" | 74.9 | 3.45 | dd (9.5, 3.1) | 3" | 75.1 | 3.47 | dd (9.8, 3.2) |
| 4" | 70.6 | 3.81 | brd (3.2) | 4" | 70.5 | 3.81 | brd 3.0 |
| 5" | 77.2 | 3.52 | m | 5" | 77.0 | 3.51 | m |
| 6"a | 62.8 | 3.75 | m | 6"a | 62.7 | 3.75 | dd (11.4,7.5) |
| 6"b | | 3.70 | m | 6"b | | 3.70 | dd (11.0, 5.0) |

[a]Chemical shift values were based on the HMBC and HMQC spectra. [b]Recorded at 600 MHz. [c]Coupling constants (Hz) are in parentheses

*3.3 ACE Inhibitory Activities of Saganhaematenones*

Finally, we evaluated the ACE inhibitory effects of these compounds. ACE activities were measured in the presence of several concentrations or absence of saganhaematenones to determine $IC_{50}$ values. Interestingly, the dose response curves of saganhaematenones were almost the same (Figure 3). ACE activities were inhibited by 61% and 63%, and $IC_{50}$ values were calculated 159.3 and 184.0 μM, for saganhaemtenone A and B, respectively.

To confirm the specific binding between ACE and saganhaematenones, we performed a *in silico* docking study. Since the binding mode between ACE and the saganhaematenones was unknown, a docking simulation was performed using the grid as the entire ACE. Interestingly, the results suggested that saganhaematenone may bind to the same pocket as the antihypertensive drug captopril (Figure 4) with a binding energy ($\Delta G_{binding}$ ) of -7.6 kcl/mol.

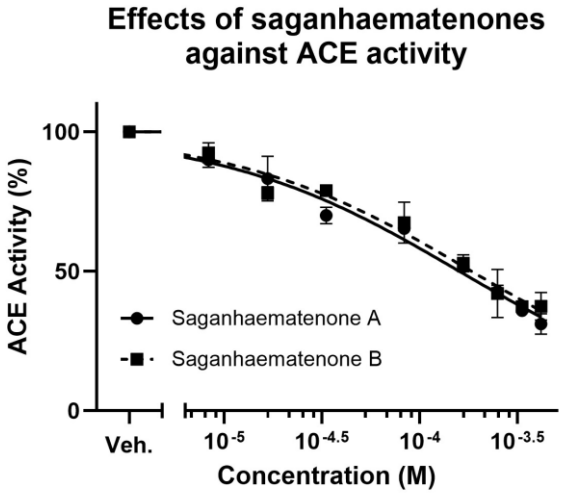


**Figure 3.** Inhibitory effects of saganhaematenones against ACE. ACE activities were measured under various concentrations of saganhaematenones A and B. Each value is the mean ± standard error of the mean of triplicate determinations.

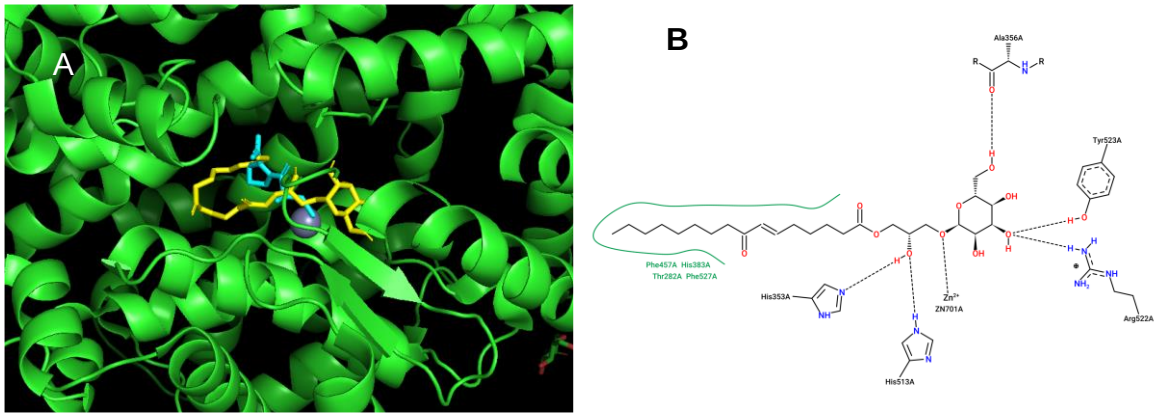


**Figure 4**. $^{1}$H-$^{1}$H COSY Visualization of comparison between captopril (yellow) and docking results position of saganhaematenone (yellow) (A), and interaction between residues of ACE and saganhaematenones, where dashed lines represent hydrogen bonds and green line does hydrophobic ionteravtions.

In this study, using the *Haematococcus lacustris* dSgH-K1 strain established in Saga City, Saga Prefecture, two natural products, saganhaematenone A and B, were identified as potential antihypertensive substances with ACE inhibitory activity. They belong to glyceroglycolipid, a conjugate composed of glycerol, sugar, and fatty acid, and are stereoisomeric structures of each other. It is known that glyceroglycolipids are abundant in the thylakoid membranes of chloroplasts of plants or cyanobacteria [17]. It is unclear why *Haematococcus* synthesizes saganhaematenones, but possibly, they may play a role in photosynthesis in chloroplasts like other glyceroglycolipids.

However, against animal cells, glyceroglycolipids have been shown to have physiological activities such as cancer cell proliferation inhibition, angiogenesis inhibition, and anti-inflammatory activity [18]. The anti-inflammatory activity is thought to be related to the suppression of reactive oxygen species production in phagocytes such as macrophages [19]. To the best of our knowledge, however, this is the first example of glyceroglycolipids that exhibit ACE inhibitory activity.

Docking simulations revealed that saganhaematenones bind to the same site as captopril, suggesting that they competitively inhibit ACE. The binding energy of captopril to ACE is estimated to

be approximately -6.1 kcal/mol [20], while the predicted binding energies of saganhaematenones and ACE are nearly identical at -7.6 kcal/mol. However, the $IC_{50}$ values are significantly different (Saganhaematenones: approximately 180 μM; captopril: 12 nM [21]). This is likely due to the difference in physical properties between them; saganhaematenones are larger molecules and have a long fatty acid chain.

It is unclear what the structural differences between the two inhibitors are because the purified amounts were too small to determine the absolute configuration. However, both compounds inhibited ACE activity in a concentration-dependent manner and showed almost equal $IC_{50}$ values even though they were isomers, suggesting that their structural differences did not affect ACE activity. This finding is thought to be important for future structure-activity relationship studies.

In conclusion, we successfully identified ACE inhibitors, saganhaematenones A and B,f using the *Haematococcus* dSgH-K1 strain. Despite limited past utilization of microalgae in natural product discovery, a systematic study holds the potential to reveal new bioactive compounds.

## Acknowledgments

This work was partially supported by Saga City. We are grateful to Saga City and Saga Algae Biomass Council for their continuous support.

## Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

**ORCID**
Mikihide Demura: 0009-0008-2682-9729
Toshiyasu Inuzuka: 0000-0001-6976-6993
Yoshinori Kawazoe: 0000-0001-7925-7113

## References

[1] H. Al-Jabri, P. Das, S. Khan, M. Thaher and M. AbdulQuadir (2021), Treatment of wastewaters by microalgae and the potential applications of the produced biomass—A review, *Water* **13**(***1***), 27.

[2] K. Chandrasekhar, T. Raj, S. V. Ramanaiah, G. Kumar, J. R. Banu, S. Varjani, P. Sharma, A. Pandey, S. Kumar and S. -H. Kim (2022) Algae biorefinery: A promising approach to promote microalgae industry and waste utilization, *J. Biotechnol.* **345**, 1-16.

[3] A. T. Ubando, S. Anderson, E. Ng, W. -H. Chen, A. B. Culaba and E. E. Kwon (2022). Life cycle assessment of microalgal biorefinery: A state-of-the-art review, *Bioresour. Technol.* **360**, 127615.

[4] R. F. C. NCD (2021). Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants, *The Lancet* **398**(**10304**), 957-980.

[5] S. Patel, A. Rauf, H. Khan and T. Abu-Izneid (2017). Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies, *Biomed. Pharmacother.* **94**, 317-325.

[6] E. Takimoto-Ohnishi and K. Murakami (2019) Renin–angiotensin system research: from molecules to the whole body, *J. Physiol.Sci.* **69**(**4**), 581-587.

[7] C. Bavishi, S. Bangalore and F. H. Messerli (2016). Renin angiotensin aldosterone system inhibitors in hypertension: is there evidence for benefit independent of blood pressure reduction? *Prog. Cardiovasc. Dis.* **59**(**3**), 253-261.

[8] M. G. Nicholls, C. J. Charles, I. G. Crozier, E. A. Espiner, H. Ikram, M. J. Rademaker, A. M. Richards and T. G. Yandle (1994). Blockade of the renin-angiotensin system, *J. Hypertens. Suppl* **12**(**10**), S95-103.

[9] G. L. Plosker and D. McTavish (1995). Captopril. A review of its pharmacology and therapeutic efficacy after myocardial infarction and in ischaemic heart disease, *Drugs Aging.* **7**(**3**), 226-253.

[10] C. Rosendorff (1982). Captopril-an overview, *S. Afr. Med. J.* **62**(**17**), 593-599.

[11] F. Kasai, M. Kawauchi, M. Erata and M. M. Watanabe (2004). *NIES-Collection, list of strains : Microalgae and Protozoa*. 7th ed.; National Institute for Environmental Studies: Vol. **182**, p v, 257 p.

[12] E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin (2004). UCSF Chimera--a visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**(**13**), 1605-1612.

[13] N. M. O'Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch and G. R. Hutchison (2011). Open babel: An open chemical toolbox, *J. Cheminform.* **3**(**1**), 33 doi:10.1186/1758-2946-3-33

[14] O. Trott and A. J. Olson (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, *J. Comput. Chem.* **31**(**2**) 455-461.

[15] K. Stierand, P. C. Maaß and M. Rarey (2006). Molecular complexes at a glance: automated generation of two-dimensional complex diagrams, *Bioinformatics* **22**(**14**), 1710-1716.

[16] M. Demura, T. Inuzuka and Y. Kawazoe (2025). Isolation of angiotensin-converting enzyme inhibitors from the *Haematococcus* (Chlorophyceae, Chlorophyta) dSgH-K1, *Jpn. J. Phycolo. (Sôrui)* **73**, 93-100.

[17] K. Kobayashi (2016). Role of membrane glycerolipids in photosynthesis, thylakoid biogenesis and chloroplast development, *J. Plant Res.* **129**(**4**), 565-580.

[18] N. Maeda, K. Matsubara, H. Yoshida and Y. Mizushina (2011). Anti-cancer effect of spinach glycoglycerolipids as angiogenesis inhibitors based on the selective inhibition of DNA polymerase activity. *Mini-Rev. Med. Chem.* **11**(**1**), 32-38.

[19] M. Takahashi, Y. Sugiyama, K. Kawabata, Y. Takahashi, K. Irie, A. Murakami, Y. Kubo, K. Kobayashi and H. Ohigashi (2011). 1,2-Di-O-α-linolenoyl-3-O-β-galactosyl-sn-glycerol as a Superoxide Generation Inhibitor from Perilla frutescens var. crispa, *Biosci., Biotechnol. Biochem.* **75**(**11**), 2240-2242.

[20] S. Wisnasari, M. Rohman and M. Lukitasari (2016). In silico binding affinity study of lisinopril and captopril to I/D intron 16 variant of angiotensin converting enzyme protein, *Int. J. Pharm. Clin. Res.* **8** 1132-1134

[21] A. K. Lund, A. Adsersen and U. Nyman (1998). Biological activities of benzoquinones from Badula barthesia and *Embelia angustifolia*, *Phytomedicine* **5**(**3**), 199-203.