Antioxidant Activity and Lipid Content of *Aurantiochytrium* sp. from Raja Ampat and Kepulauan Seribu

Hardi Astuti Witasari[1,2,6], Kintoko[2], Nurkhasanah Mahfudh[3]*, Suhendra[4], Ahmad Faizal Rangkuti[5],vNisrien Ilmia[2], Blegoh Iwan Santoso[2], Andri Hutari[6],

ABSTRACT

Raja Ampat (Southwest Papua Province) and Kepulauan Seribu (Jakarta Raya Province) are part of Indonesia's territory, which includes the mangrove forest area. This habitat supports a diverse array of animals, plants, and microalgae, including *Aurantiochytrium* sp. It has antioxidant activity related to lipid content. This study aims to identify the lipid content and the antioxidant effects of the microalgae *Aurantiochytrium* sp.'s extract isolated from the mangrove forest areas of Raja Ampat and Kepulauan Seribu. The extraction process was conducted using the Folch method, which involves a 2:1 (v/v) chloroform-methanol solvent ratio. The microalgae *Aurantiochytrium* sp. extract of Raja Ampat (MAERA) and microalgae *Aurantiochytrium* sp. extract of Kepulauan Seribu (MAEKS) were analyzed using GC-MS, antioxidant potential tested *in silico* by target proteins (5ZLG, 2A1X, 3VLN, and 1EEM) using Autodock Tools, and *in vitro* by DPPH and ABTS radicals and the carotenoid bleaching method. The results showed that total squalene and fatty acids in MAERA and MAEKS were at different levels. Squalene as a ligand had the lowest binding energy (-9.4, -6.6, -7.7, and -6.5) compared to the native ligand (-5.6, -4.4, -5.2, and -5.2) and ascorbic acid (-5.6, -4.8, -5.3, and -4.9) by *in silico* antioxidant activity. The *in vitro* antioxidant activities by DPPH, ABTS, and ß-caroteen bleaching were 103.00 ± 3.75, 216.39 ± 367, 214.62 ± 7,07 (MAERA); and 131.85 ± 23.48, 225.50 ± 3.36, 251.69 ± 5.39 (MAEKS) were not significantly different ($p>0.05$) by all methods. These findings showed that the *Aurantiochytrium* sp. strains Raja Ampat and Kepulauan Seribu contained diverse levels of fatty acids and squalene. In the *in silico* study, squalene was evaluated and *in vitro*, the MAERA and MAEKS extracts showed moderate antioxidant activity.

Keywords:GC-MS; IC_{50}; *In silico; In vitro*; Microalgae

OPEN ACCESS

[1] Doctoral Program in Pharmaceutical Science, Universitas Ahmad Dahlan, Yogyakarta, 55164, Indonesia
[2] Department of Pharmaceutical Biology, Universitas Ahmad Dahlan, Yogyakarta, 55164, Indonesia
[3] Department of Analytical Pharmacy and Medicinal Chemistry, Universitas Ahmad Dahlan, Yogyakarta, 55164, Indonesia
[4] Department of Chemical Engineering, Universitas Ahmad Dahlan, Yogyakarta, 55164, Indonesia
[5] Department of Public Health, Universitas Ahmad Dahlan, Yogyakarta, 55164, Indonesia
[6] Department of Biology Education, Muhammadiyah University of Prof. Dr. Hamka, Jakarta, 13830, Indonesia, Jl. Prof. Dr. Soepomo Janturan Yogyakarta Indonesia

*Corresponding Author:
nurkhasanah@pharm.uad.ac.id

Received: 4 December 2024
Accepted: 17 September 2025
Published: 22 October 2025
Academic Editor: Dr. Tatty Yuniarti, ST, M.Si

 Accreditation Number:169/E/KPT/2024. ISSN: 2089-5690, e-ISSN: 2406-9272. https://doi.org/ 10.15578/squalen.980

Introduction

Antioxidants could protect cells against damage to lipids, proteins, cell membranes, and nucleic acids caused by reactive oxygen species (ROS) and free radicals (Jauffrais, 2021). Cell damage was one of the factors causing non-communicable diseases (NCDs) (Agregán *et al.*, 2018). The World Health Organization (WHO) states that worldwide, every year, around 73% of deaths are caused by non-communicable diseases (NCDs) (Allen *et al.*, 2018). The four diseases that cause the most deaths are cardiovascular disease (17.9 million deaths annually), cancer (9.0 million), respiratory disease (3.9 million), and diabetes (1.6 million) (Budreviciute *et al*., 2020). Synthetic antioxidants have many side effects (Karima *et al.*, 2020). Therefore, exploration continues to be carried out to find sources of natural antioxidants (Batubara *et al.*, 2020; Marino *et al.*, 2020), including those derived from the diversity of marine biota (Joshi *et al*., 2018).

Marine ecosystems cover 70% of the Earth's surface and are the largest ecosystems in the biosphere (Gupta *et al*., 2016). The marine mangrove forest areas are home to microorganisms such as *Aurantiochytrium* sp. (Morabito *et al*., 2019) which are rich in polyunsaturated fatty acids (PUFAs) and have antioxidant, acetylcholine esterase inhibitory, and neuroprotective activities (Hoang *et al.,* 2022). *Aurantiochytrium* sp. is beneficial as an ingredient for nutraceuticals containing docosahexaenoic acid

(DHA) and squalene (Patel *et al.,* 2019). The genus Aurantiochytrium belongs to the order Labyrinthulales and the family Thraustochytriaceae (Rodjaroen *et al*., 2024), a unicellular, heterotrophic marine protist, abundant in seawater, that can grow on various carbon sources (Du *et al*., 2021; B. Liu et al., 2016) explored from mangrove forest areas along the world's coasts and cultivated under certain conditions (Hoang *et al*., 2022; Kalidasan *et al.*, 2021; Lee Chang *et al*., 2013; Nakazawa *et al.*, 2012; Suhendra *et al*., 2019).

Exploration of *Aurantiochytrium* sp. had been carried out in mangrove forest areas along the coast in Raja Ampat Regency, Southwest Papua Province, and Kepulauan Seribu Regency, Special Capital Region of Jakarta Raya Province, Indonesia (Hutari *et al.*, 2022; Suhendra *et al*., 2022). Raja Ampat Regency is an area rich in coral reefs because, it is located in the heart of the world's Coral Triangle (White *et al*., 2022). Raja Ampat was a mangrove forest area along the coast affected by pollution (Aji *et al*., 2023). The marine biota ecosystem in Raja Ampat was a protected and guarded area (Gustiarini *et al.*, 2023; Maas *et al*., 2020). This aquatic ecosystem and the mangrove forest area were located opposite the Kepulauan Seribu, approximately 30 km from the Tanjung Priok Port in Jakarta. It was reported that the mangrove forest in Kepulauan Seribu had decreased by 31.46% over the past 7 years. Coastal ecosystems and mangrove forest areas, which serve as the primary habitat of Aurantiochytrium sp., have been destroyed (Sirirak *et al*., 2020). Therefore, it was important to research the effect of the chemical component contained in the microalgae (Molino *et al.*, 2020; Su & Li, 2020; Hoang *et al.*, 2022). This study aims to identify the lipid content and the antioxidant effects of the microalgae *Aurantiochytrium* sp.'s extract isolated from the mangrove forest areas of Raja Ampat and Kepulauan Seribu.

Materials and Methods

Chemicals and Reagents

Ascorbic acid (CAS number: 50-81-7), glucose anhydrous (CAS number: 50-99-7), chloroform (CAS number: 67-66-3), methanol (CAS number: 67-56-1), 2,2-diphenyl-1-picrylhydrazyl (DPPH)(CAS number: 1898-66-4), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (CAS number: 30931-67-0), linoleic acid(CAS number: 60-33-3), yeast extract (CAS number: 8013-01-2), and ethanol 96% (CAS number: 64-17-5), were obtained from Sigma-Aldrich.

Microalgae Isolation

A sample of *Aurantiochytrium* sp. from Raja Ampat as isolated from its natural habitat under mangrove plants in Waisei District, Raja Ampat Regency, Southwest Papua Province, Indonesia (coordinates: 0°25'52.8"S 130°48'30.2"E) in October 2023 (**Figure 1**). Direct plating isolation was performed using the method described by Saengwong *et al*. (2018) and Suhendra *et al.* (2022), following the same procedure as previously outlined. The mangrove leaf samples were brought to the laboratory in seawater. Each leaf sample was directly plated on 15 g/L bacteriological agar (Oxoid), 20 g/L glucose (Sigma-Aldrich), 5 g/L yeast extract (Sigma-Aldrich), 50% distilled water, and 50% seawater. The plates were then covered with Parafilm (M) and incubated at room temperature for 2 days. Afterward, a pure streaking method was used to obtain pure isolates from each colony. Pure isolates were obtained after single colonies from the pure streaking stage of each sample were observed under a microscope (Yazumi XSZ-107BN). The colonies used for purifying the isolates were those whose microscopic morphological observations were similar to the image of *Aurantiuchytrium* sp. (*Schizochytrium* sp.) reported by Honda *et al*. (1998). The results of the microscope observations were saved to a computer using a USB microscope camera. The microalgae *Aurantiochytrium* sp. strain of the Kepulauan Seribu was isolated from its original habitat in the mangrove forest area of Pulau Harapan Village, Kepulauan Seribu Regency, DKI Jakarta Province, Indonesia (coordinates 05° 39'10" S and 106° 34'41" E) in October 2023 (**Figure 1**). Both samples have the same isolation method.

Single colonies of pure isolates of *Aurantiochytrium* sp. strains of Raja Ampat and Kepulauan Seribu were stored in sterile disposable dishes measuring 90x15 mm (Labware Charuzu) containing approximately 17 mL of agar medium (containing glucose, yeast extract, and revsalt) and covered with Parafilm (Malawet et al., 2019). The colonies of each pure isolate were streaked onto a new agar medium approximately once every two weeks to maintain the isolate. All processes were conducted in the Laboratorium Biomedic Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia, and the isolates were saved there.

A: Raja Ampat Regency, Southwest Papua Province, Indonesia.
B: Kepulauan Seribu Regency, Special Capital Region of Jakarta Raya Province, Indonesia

Figure 1. The original habitat location of the microalgae *Aurantiochytrium* sp. Raja Ampat and Kepulauan Seribu strain (Google map, 2025)

Cultivation of microalgae Aurantiochytrium *sp. and the extraction method of biomass*

Microalgae isolates, specifically the *Aurantiochytrium* strains Raja Ampat and Kepulauan Seribu, were cultivated using modified methods based on those described by Chandrasekaran *et al.* (2018). The standard nutrient components for laboratory-scale cultivation included 80 g of anhydrous glucose (Sigma-Aldrich®), 18 g of yeast extract (Sigma-Aldrich®), 7.2 g of revsalt, and distilled water to a total volume of 1000 mL. Biomass production was conducted in a 1000 mL Erlenmeyer flask (Pyrex®), filled to a maximum capacity of half. The flask was placed on a shaker (Scilogex® SCI-033-Pro) operating at 200 rpm. The cultivation process consisted of three stages: a 48-hour standing culture, a 48-hour pre-culture, and a primary culture lasting 120 hours. All procedures were conducted in a well-regulated, air-conditioned environment at a comfortable 25°C. The lighting was provided by fluorescent lamps (Philips 23W/220V, white light), ensuring consistency during the process without the need for specialized light or dark cycles. The duration of the main culture was optimized for maximal squalene concentration in the biomass. After cultivation, the biomass of the *Aurantiochytrium* sp. strains Raja Ampat and Kepulauan Seribu was separated from the liquid and dried using a freeze-dryer (Sihuandonggan® LGJ-12A) (Chandrasekaran *et al*., 2018).

The extraction process was conducted using the Folch method, which involves a 2:1 (v/v) chloroform-methanol solvent ratio. To optimize the extraction, the dry biomass was carefully combined with the solvent at a 1:5 ratio, and the extraction was facilitated with the assistance of a sonicator (Elmasonic® S 100 H) for 15 minutes at a temperature of 25°C. Three replications were performed (María Señoráns, 2020; Saini et al., 2021). After extraction, the solvent was filtered to separate it from the precipitate and then evaporated using a rotary evaporator (Heidolph®) at 60°C until it thickened. The microalgae *Aurantiochytrium* sp. extracts from the Raja Ampat (MAERA) and the microalgae *Aurantiochytrium* sp. extracts from Kepulauan Seribu (MAEKS) were weighed, and their lipid content was examined.

Analysis methods of MAERA and MAEKS

Each sample of MAERA and MAEKS was analyzed to determine lipid content using Gas Chromatography-Mass Spectrometry (GC-MS). Additionally, antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical, and â-carotene bleaching analysis.

GC-MS instrument (Shimadzu-QP2010 SE) with capillary column conditions (stationary phase) RTX-5MS, injector temperature with the split method set at 275°C, oven temperature 125°C for 3 minutes then the temperature increased by 15°C/minute to reach 250°C for 10 minutes then the temperature increased by 10°C/minute, a temperature of 300°C and maintained for 7 minutes with a pressure of 61.5 kPa. The carrier gas is helium with a flow rate of 0.78 mL/min. MAERA and MAEKS samples of 0.2 ìL each were injected into the injector on the GC-MS instrument (Abdel-Wahab et al., 2021).

Antioxidant activity was tested by inhibiting free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Matilda et al., 2021). About DPPH 0.1 mM was made by dissolving 9.858 mg of DPPH powder in 250 mL of ethanol p.a. The 0.1 mM DPPH solution was stored in a dark room. The sample in each concentration was taken at as much as 1.0 mL, then added with DPPH solution at as much as 1.0 mL, homogenized, and incubated for 60 minutes in a dark and closed

place. Absorbance was measured using a UV-Vis spectrophotometer (Shimadzu® UV 1800) with a wavelength of 517 nm. The results show the percentage of inhibition of DPPH free radicals and are calculated using the following formula:

$$\%\ \text{Free radical scavenging} = \frac{\text{Abs control DPPH} - \text{abs sample}}{\text{Abs control DPPH}} \text{x } 100\%$$

The experiment was replicated three times. The concentration of the test compound was then processed using linear regression analysis to obtain a radical scavenging concentration of 50% (IC_{50}). The antioxidant test using the DPPH method is presented in the IC_{50} parameter.

Antioxidant activity was tested by inhibiting ABTS free radicals according to previous research *(Ma et al.*, 2021) With modification. The ABTS solution was prepared by mixing 5 ml of 7 mM ABTS stock solution and 5 ml of 2.45 mM potassium persulfate solution, and the mixture was incubated for 16 hours at room temperature and in the dark. The ABTS solution was added with phosphate buffer (pH 7.4) until an absorbance value of 0.7 ± 0.02 was obtained at a wavelength of 734 nm. MAERA and MAEKS samples and ascorbic acid standards were diluted using 96% ethanol in a concentration series. About 0.1 mL of the extract or standard solution was mixed with 9.9 mL of ABTS solution. The mixture was incubated in the dark for 6 minutes, then the absorbance was measured at a wavelength of 734 nm with a spectrophotometer (Shimadzu® UV 1800). The percentage value of free radical scavenging is calculated using the following formula:

$$\%\ \text{Free radical scavenging} = \frac{\text{Abs control ABTS} - \text{abs sample}}{\text{Abs control ABTS}} \text{x } 100\%$$

The concentration of the test compound was then processed using linear regression analysis to obtain a radical scavenging concentration of 50% (IC_{50}). The antioxidant test using the ABTS method is presented in the IC_{50} parameter.

Rouy *et al.* (2020) conducted â-carotene bleaching using a modified method (Jianu et al., 2020). The stock solution was obtained by dissolving 1 mg of â-carotene (1 mg/mL in chloroform), 30 mg of linoleic acid, and 200 mg of Tween–80. The mixture was homogenized to form an emulsion, then dissolved in 60 mL of distilled water and vortexed with an ultrasonicator (ElmaSonic® S 100 H) for 4 minutes. A total of 1.0 mL of sample solution (200 ppm) was added with 2.0 mL of â-carotene linoleic acid emulsion and 2.0 mL of distilled water, then incubated at 50°C. The control was made by mixing 5.0 mg of linoleic acid and 33.33 mg of Tween-80. The mixture was then homogenized to form an emulsion and dissolved in 10 mL of distilled water. Negative control was made by mixing 2.0 mL of â-carotene linoleic acid emulsion, 2.0 mL of distilled water, and 1.0 mL of 96% ethanol. Absorbance was measured at ë 459.13 nm with a spectrophotometer (Shimadzu UV-1800). Absorbance was measured at 15-minute intervals until the â-carotene's color faded (120 minutes). The experiment was carried out three times. The percentage value of antioxidant activity is calculated using the following formula:

$$\%\ \text{Antioxidant Activity} = (1 - \left(\frac{(\text{Abs sample 0} - \text{Abs sample 120})}{(\text{Abs control 0} - \text{Abs control 120})}\right)) \text{x } 100\%$$

Abs sample 0: Absorbance sample at 0 minutes
Abs sample 120: Absorbance sample at 120 minutes
Abs control 0: Absorbance control at 0 minutes
Abs control 120: Absorbance control at 120 minutes

The % antioxidant activity was then processed using linear regression analysis to obtain a % antioxidant activity of 50% (IC_{50}). The antioxidant test using the ß-caroten bleaching method was presented in the IC_{50} parameter.

Molecular Docking of Bioactive Compounds and **In Silico *Prediction of Bioactivity***

The 3D crystal structure data for the receptor used for molecular docking analysis were obtained from the Protein Data Bank (PDB) at http://rcsb.org/pdb/ following the research of Matilda *et al*. (2021) and presented in **Table 1.**

Table 1. Molecular Docking Parameters and Protein Targets.

Protein	PDB ID	Grid Box Center Coordinates	Grid Box Size
Oxidoreductase	5ZLG	center_x = 18.2822 center_y = 35.2926 center_z = 9.6867	size_x = 41.8897 size_y =65.1072 size_z = 30.6890
Oxidoreductase	2A1X	center_x = 16.2936 center_y = 17.3690 center_z = 35.7062	size_x = 45.2936 size_y =43.6957 size_z = 52.0517
Glutathione Transferase	3VLN	center_x = 22.6713 center_y = -10498 center_z = 20.0052	size_x = 33.4684 size_y =54.2071 size_z = 56.9128
Glutathione Transferase	1EEM	center_x = 20.3402 center_y = 14.4748 center_z = 43.4856	size_x = 53.3341 size_y = 35.1311 size_z = 56.9648

PDB: Protein Data Bank

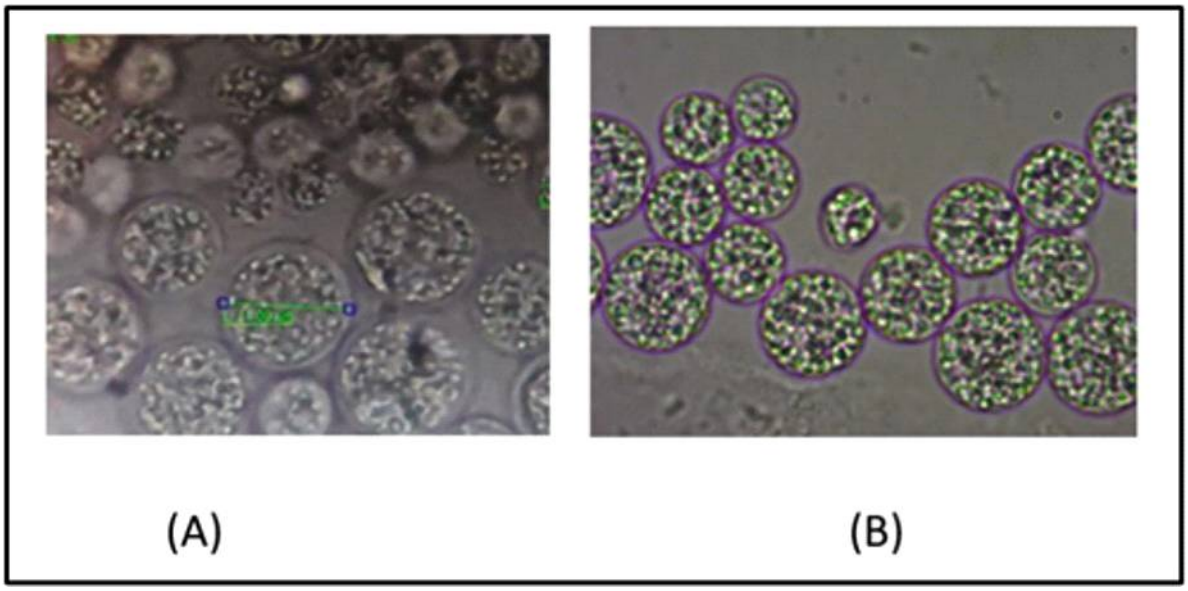
The receptor was characterized as a vitamin C transporter coupled with phosphorylation. The docked protein was isolated from other molecules, including water and its native ligand. Preparation of the native ligand involved separating it from the surrounding molecules—such as water, cofactors, and the receptor—using Biovia Discovery Studio software in PDB file format. Molecular docking of the native and test ligand was carried out using AutoDock 4 software. The docking results, specifically the best pose, were determined by selecting the ligand conformation with the lowest binding energy. Visualization of the docking results, including the best pose and the bond interactions, was performed in 2D format using Biovia Discovery Studio software (Dassault Systems Biovia, San Diego, CA, USA). The parameters analyzed included binding energy (ÄG), inhibition constant (IC), amino acid residues, and the number of binding interactions formed. Additionally, PyMOL software was utilized to visualize the test ligand's surface area within the protein in 3D format.

Data Analysis

The independent tests, including triplicate analysis, were performed for each sample, and the means and standard deviations were determined using IBM SPSS 25.0 software (SPSS Inc., Wacker Drive, Chicago, IL, USA). The antioxidant activity assessments (at the 5% level) and significant values were obtained using the post-hoc test (Tukey).

Result and Discussion

The microalgae *Aurantiochytrium* sp. isolated from the Raja Ampat strain and the Kepulauan Seribu, exhibit similar cell shapes when compared to *Aurantiochytrium* sp. (SY-03), which was isolated from the Syhat mangroves in the Arabian Gulf, Saudi Arabia (Abdel-Wahab *et al*., 2021), and another *Aurantiochytrium* sp. SC145 strain from Sand Cay (Son Ca) Island, Vietnam (Hien *et al.*, 2022). **Figure 2** shows the cell shapes of these two strains.



A) *Aurantiochytrium* sp. strain Raja Ampat
B) *Aurantiochytrium* sp. strain Kepulauan Seribu

Figure 2. The shape of Microalgae *Aurantiochytrium* sp. strain Raja Ampat and Kepulauan Seribu

The extraction of microalgae, specifically *Aurantiochytrium* sp., was conducted using the Folch method. The yield from MAERA indicated a lipid content of 42.96% derived from the dry biomass of the *Aurantiochytrium* sp. strain from Raja Ampat. In comparison, the yield from MAEKS showed a higher lipid content of 56.80% from the dry biomass of the *Aurantiochytrium* sp. strain isolated from the Kepulauan Seribu. Analysis of chemical compounds using GC-MS was chosen because this method can selectively analyze nonpolar compounds, such as fatty acids and terpenoids (X. Lu et al., 2018), and has high sensitivity because it can analyze and separate mixtures of compounds in small concentrations and volumes (Surani *et al.*, 2023). The chromatogram was translated based on the Similarity Index (SI) between the mass spectrum pattern of the sample compared to the mass spectrum in the Wiley9 library contained in the GC-MS program, and are shown in **Figures 3** and **4**.

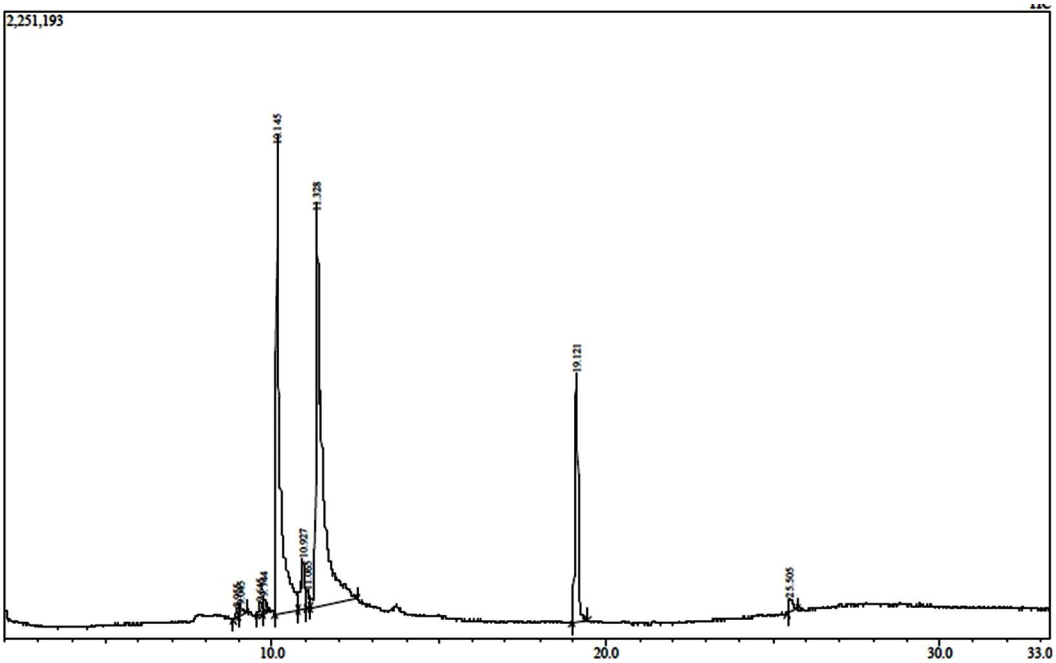


Figure 3. Microalgae *Aurantiochytrium* sp. Extract strain Raja Ampat (MAERA) gas chromatography spectra

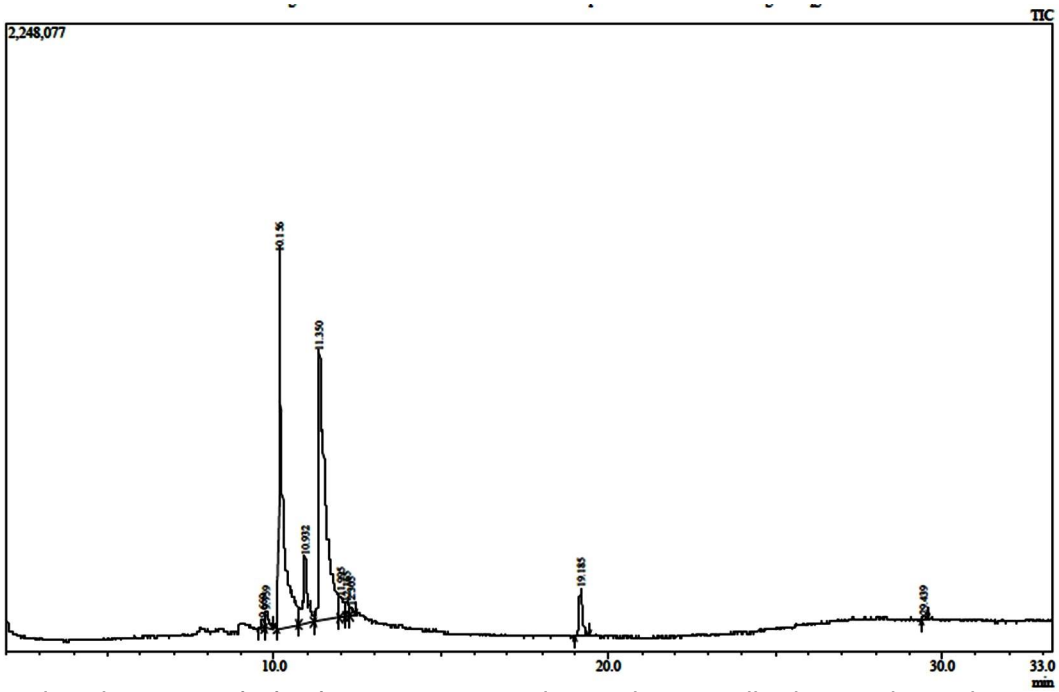


Figure 4. Microalgae *Aurantiochytrium* sp. Extract strain Kepulauan Seribu (MAEKS) gas chromatography spectra

The top 10 areas detected by GC-MS analysis of MAERA and MAEKS are shown in **Tables 3** and **4.** The result of SI > 89% indicates a very high similarity between the mass spectrum of the analyzed compound and the mass spectrum of the compound in the library. Furthermore, SI results < 89% had a low similarity between the sample and the library, so they could not be considered (Tahya *et al*., 2021).

Table 2. The Chemical Compounds of Microalgae *Aurantiochytrium* sp. Extract strain Raja Ampat (MAERA)

R.Time	Compound	SI (%)	Area (%)
8.955	Tetradecanoic acid	74*	0.47
9.045	Pentadecanoic acid, methyl ester	80*	0.56
9.645	9-Octadecenoic acid	82*	0.57
9.744	Hexadecanoic acid, methyl ester (methyl palmitate)	90	0.78
10.145	Hexadecanoic acid (palmitic acid)	94	31.10[2]
10.927	9-Octadecenoic acid (Z)-, methyl ester	92	3.73[4]
11.065	Octadecanoic acid, methyl ester	93	0.75
11.328	Octadec-9-enoic acid (oleic acid)	94	46.26[1]
19.121	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (Squalene)	94	14.57[3]
25.505	Cholest-5-en-3-ol (3.beta.)- (Cholesterol)	87*	1.21
	Total		100

RTime: Retention Time;
SI; Similarity Index;
*) SI<89

Table 3. Chemical Compounds of Microalgae *Aurantiochytrium* sp. Extract strain Kepulauan Seribu (MAEKS)

R.Time	Compound	SI (%)	Area (%)
9.669	9-Octadecenoic acid (Oleic acid)	94	0.56
9.759	Hexadecanoic acid, methyl ester (methyl palmitate)	92	1.55
10.156	Hexadecanoic acid (palmitic acid)	92	35.94[2]
10.932	9-Octadecenoic acid, methyl ester (methyl oleic)	93	7.04[3]
11.350	9-Hexadecanoic acid (palmitoleic acid)	94	46.86[1]
11.995	Octadecanoic acid (stearic acid)	94	1.94[5]
12.165	Cyclohexanol	65*	1.24
12.305	Cyclohexanepropanoic acid, 3,4-dihydroxy-	55*	0.60
19.185	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene (Squalene)	94	4.01[4]
29.439	Oxirane, tetradecyl-	69*	0.26
	Total		100

RT: Retention Time;
SI; Similarity Index;
*) SI<89

The dominant compounds (SI>89%) in MAERA were oleic acid (40.26%), palmitic acid (30.10%), squalene (14.57%), 9-Octadecenoic acid (Z)-, methyl ester (3.73%), and Cholesterol (1.21%) however in MAEKS were palmitoleic acid (46.86%), palmitic acid (35.94%), methyl oleate (7.04%), squalene (4.01%), and stearic acid (1.94%). Meanwhile, the same cultivation and extraction methods of *Aurantiochytrium* sp. strain Raja Ampat and Kepulauan Seribu, MAERA and MAEKS showed varying results. MAERA and MAEKS samples had different quantities of fatty acids and squalene (Tables 1 and 2).

The lipid content detected by the GC-MS instrument in MAERA and MAEKS includes saturated and unsaturated fatty acids. In MAERA, the highest lipid content was oleic acid, which constitutes 40.26% of the total, and is an unsaturated fatty acid. This was followed by palmitic acid, which comprised 30.10% of the total, and was a saturated fatty acid. In contrast, MAEKS had a different profile, with palmitoleic acid being the predominant fatty acid at 46.86%, also an unsaturated fatty acid, followed by palmitic acid at 35.94%, a saturated fatty acid. Several studies have investigated the factors influencing fatty acid production, such as the nitrogen content in the media and the supply of oxygen. However, further research was necessary to understand the molecular mechanisms that link nitrogen deficiency to the accumulation of intracellular fatty acids in *Aurantiochytrium* sp.

The other difference was that MAERA contains steroids in the form of cholesterol, while in MAEKS, no sterol compounds were detected. Differences were also shown by microalgae *Aurantiochytrium* sp cultivated from its natural habitat in the coastal area of Sagar Vihar (19.0762æ% N, 72.9881æ% E), Vashi, Navi, Mumbai, with a dominance of steroid compounds because squalene and fatty acids were only in small amounts (Bagul & Annapure, 2021). The sterol was synthesized from squalene in the metabolic pathway; as a result, the amount of squalene was reduced (Hanif et al., 2019; L. Liu et al., 2020; Vyas et al., 2022).

The lipid content of MAERA is primarily composed of oleic acid (C:18-9), an unsaturated fatty acid. Numerous studies have explored the benefits of oleic acid. While the most commonly known source is olive oil, oleic acid plays a significant role in developing and progressing chronic diseases. In the cardiovascular system, oleic acid is advantageous as

it reduces the incidence of myocardial infarction, decreases platelet aggregation, and helps reduce systolic blood pressure. Additionally, oleic acid intake is associated with lower LDL cholesterol levels. Furthermore, research has shown that oleic acid can inhibit the action of the HER-2/neu oncogene, which is linked to breast cancer. Consequently, the oleic acid content in MAERA can be utilized as an ingredient in health foods, nutraceuticals, pharmaceuticals, and cosmetic products (Karacor & Cam, 2015).

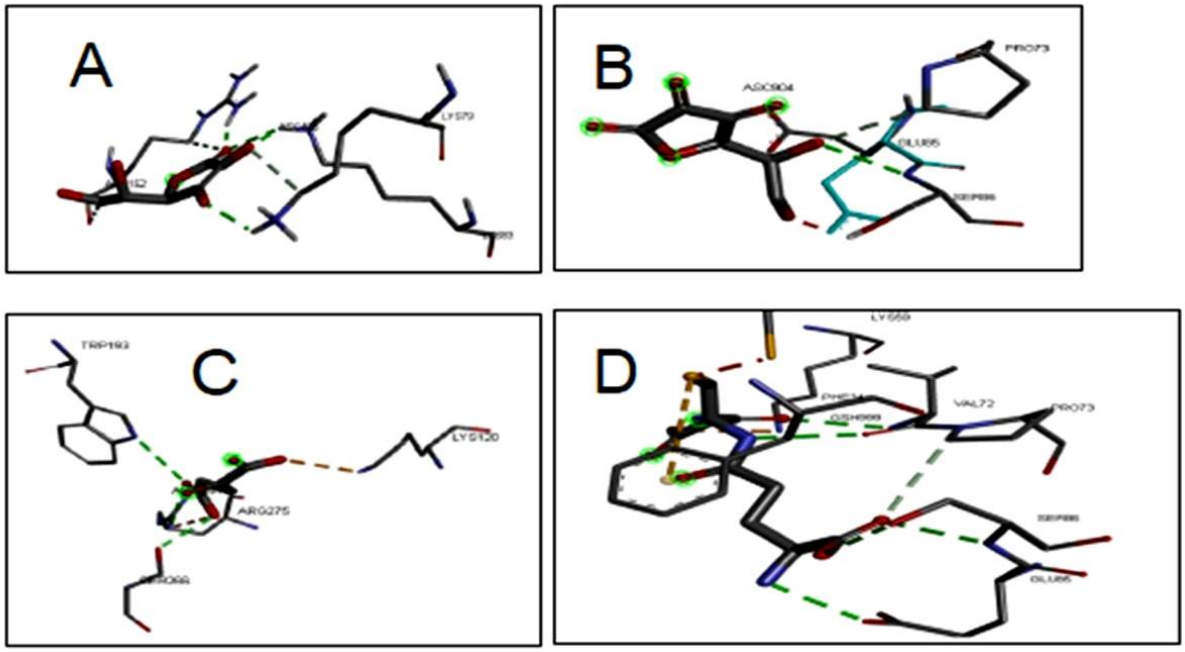
The predominant fatty acid in MAEKS was palmitoleic, a monounsaturated fatty acid. Palmitoleic acid was secreted by human adipose tissue and was linked to decreased insulin resistance in peripheral tissues. Research indicated that palmitoleic acid also lowers the expression of pro-inflammatory cytokines in cultured macrophages. Additionally, it showed promise as an anti-inflammatory agent by promoting a suppressive effect on lymphocyte proliferation (Passos et al., 2016).

Lipids accumulated by *Aurantiochytrium* sp. were made of palmitic acid (C16:0) and DHA (C22:6) (Morabito et al., 2019), with the total amount of lipids and their profiles varying (Marchan *et al.*, 2018). In this study, MAERA and MAEKS had a main content of fatty acids with several C atoms between 14-18 and not a long chain above 22, like DHA (Table 1 and Table 2). This was in line with previous studies with *Aurantiochytrium* sp. isolated from several selected areas of Paknakhon, Pakphanang, and Thasala mangrove forests (Malawet et al., 2019) that long-chain PUFAs (>18:3), including DPA and DHA, were absent in total lipids, which may be due to insufficient amounts of desaturase and elongase at low substrate levels (Patel et al., 2020). There were two distinct pathways for PUFA synthesis, namely the oxygen-dependent aerobic fatty acid synthase (FAS) pathway, also known as the elongase–desaturase pathway, and the oxygen-independent anaerobic polyketide synthase (PKS) pathway, also referred to as the PUFA synthase pathway (Qiu *et al.,* 2020). The starting point for PUFA synthesis (FAS pathway) is C18:3–9,12,15, which is converted to C18:4–6,9,12,15 by desaturase D6 and to C20:4–8,11,14,17 by elongase. The subsequent process by desaturase D5 is converted to C20:5–5,8,11,14,17, followed by elongation to 22:5–7,10,13,16,19, and, finally, by desaturase D4 is converted to DHA (22:6–4,7,10,13,16,19) (Sun *et al.*, 2024). Therefore, in the FAS pathway, an adequate supply of C18:3 substrate dramatically affects the success of PUFA synthesis. Deficiency of C18:3 leads to failure or low DHA production (Qiu *et al*., 2020; Xie *et al.*, 2020). This probably occurred in the MAERA and MAEKS biomass. A deficiency of C18:3 results in reduced or absent DHA production, which leads to the identification of lipid content primarily within the C14-C18 range.

The results of GC-MS identification showed in the library (SI > 89%) as squalene compound ($C_{30}H_{50}$) with retention time 19.121 (MAERA) and 19.185 (MAEKS). Squalene is an unsaturated fatty acid consisting of six isoprene units (Popa *et al.*, 2015) which were included in the terpenoid group of compounds, consisting of six unconjugated double bonds. The squalene content in MAERA was higher than MAEKS's (Tables 2 and 3). Squalene production from *Aurantiochytrium* sp. showed varying results (Gao & Ma, 2022), influenced by genetic aspects, nutritional, and environmental conditions (Du *et al*., 2021; Liu *et al*., 2020; Liu *et al.*, 2022).

Squalene (triterpene) is an intermediate for the biosynthesis of phytosterol or cholesterol in plants, animals, and humans (Malawet *et al*., 2019; Popa *et al*., 2015) and works in various biological processes (Herrera-Marcos *et al.*, 2023). Squalene was used in the pharmaceutical, cosmetic, and food industries due to its emollient, antioxidant, antitumor, and nutritional properties (Kim & Karadeniz, 2012; Lou-Bonafonte *et al.*, 2018). Squalene is mainly isolated from shark liver and olive oil for commercial use (Popa *et al.*, 2015; Ghimiand *et al.*, 2016). Nowadays, marine resources that can be utilized as a source of squalene have been discovered, including *Aurantiochytrium* (Lu *et al.*, 2003; Saengwong *et a*l., 2018; Gohil *et al.*, 2019; Vyas *et al.*, 2022; Koopmann *et al.,* 2023).

The potential chemical components of MAERA and MAEKS could be predicted using molecular docking analysis. Advanced computational techniques could predict possible biological effects with increasing predictive capacity (Andrei *et al.*, 2021; Tao *et al*., 2019). These computational methods have been widely used in various stages of modern drug discovery research, helping scientists in their ongoing quest to develop potent, therapeutically active compounds (Andrei *et al.*, 2021). The protein target was determined from the antioxidant receptor obtained from the PDB (Figure 5). A native ligand was chosen as the standard for the bond between the target protein and the ligand. The target protein-native ligand binding was visualized in **Figure 5**.



(A) Native ligand (ASC405)-target protein (5ZLG)
(B) Native ligand (ASC 904)- target protein (3VLN)
(C) Native ligand (AKG451)- target protein (2A1X)
(D) Native ligand (GSH999)- target protein (1EEM)

Figure 5. Native ligand and target protein binding in 2D visualization

Molecular docking was a valuable technique that could help further understand the plausible mechanisms of action exhibited by biologically active molecules *in vitro* (Nayab et al., 2020). The ligands were obtained from the PubChem National Library of Medicine (https://pubchem.ncbi.nlm.nih.gov/) to identify the compounds contained in MAERA and MAEKS. Furthermore, molecular docking was performed against the selected target proteins from the PDB, as presented in **Table** 1. The results of the binding activity of protein targets and ligands are shown in **Table 4**.

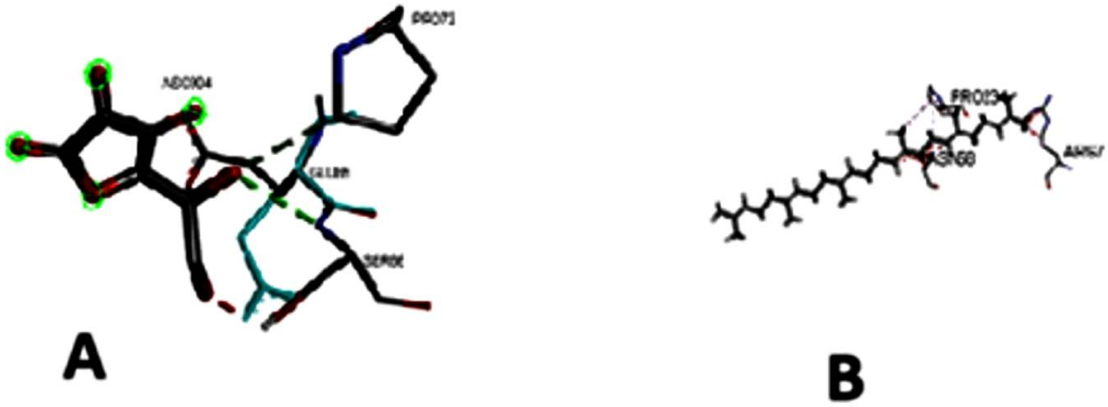
Table 4. Binding Free Energy (kcal/mol)

Ligand	Protein PDB ID			
	5ZLG	2A1X	3VLN	1EEM
Hexadecanoic acid, methyl ester (methyl palmitate)	-6.5	-4.2	-5.9	-4.9
Hexadecanoic acid (palmitic acid)	-5.7	-4.7	-5.6	-4.6
9-Hexadecanoic acid (palmitoleic acid)	-5.6	-4.4	-6.4	-5.0
9-Octadecenoic acid (Z)-, methyl ester (methyl oleic)	-5.8	-4.7	-6.0	-5.6
Octadec-9-enoic acid (oleic acid)	-6.3	-5.1	-6.1	-5.9
Octadecanoic acid (stearic acid)	-5.7	-4.0	-5.3	-5.1
Squalene	-9.4*	-6.6**	-7.7'	-6.5"
Ascorbic acid	-5.6	-4.8	-5.3	-4.9
Native Ligan	-5.6	-4.4	-5.2	-5.2

PDB: Protein Data Bank
*) lowest binding energy of receptor oxidoreductase (5ZLG)
**) lowest binding energy of receptor oxidoreductase (2A1X)
¹) lowest binding energy of receptor Glutathione transferase (3VLN)
º) lowest binding energy of receptor Glutathione transferase (1EEM)

The binding energy of the protein targets and ligand is compared to that of the receptor and native ligand. The fatty acids were ligands with binding energy higher than the native ligand by all protein targets. It means binding the antioxidant receptor oxidoreductase protein (5ZLG, 2A1X) and Glutathione transferase (3VLN, 1EEM) against fatty acids was weaker than the native ligand. Some fatty acids had binding energies equivalent to ascorbic acid, which had become the standard for antioxidants. Table 5 shows that squalene as a ligand had the lowest binding energy, which means it was the most potent candidate compound and better than the standard (ascorbic acid) and native ligands.

The lowest binding energy of each protein target is the antioxidant receptor oxidoreductase (5ZLG, 2A1X) and Glutathione transferase (3VLN, 1EEM) with the ligand squalene. **Figure 6** exhibits the binding of the target protein (3VLN) and squalene as a ligand.



(A) The binding of target protein (3VLN)-native ligand (ASC 904)
(B) The binding of target protein (3VLN)-squalene as ligand ($C_{20}H_{50}$)

Figure 6. The target protein-squalene as a ligand binding in 2D visualization

Low binding energy means that the bond between the protein and the ligand is strong (Nayab et al., 2020). Squalene ($C_{30}H_{50}$) was a long-chain molecule with conjugated C atoms that had the potential to capture reactive electrons so that it could be a potent antioxidant candidate (Vyas et al., 2022). Squalene has been used in pharmaceuticals, cosmetics, nutraceuticals, and even today as a vaccine adjuvant (Rani *et al*., 2018; Tran *et al.*, 2020). The most widely used source of squalene was shark liver oil, whose exploration had caused environmental conflict (Venugopal et al., 2016). The squalene content in MAERA and MAEKS can be developed as an environmentally friendly source of squalene (Ghimire *at al*., 2016).

Oxidoreductase enzymes are redox enzymes that selectively donate electrons. These enzymes include cytochrome P450 (CYP), which controls the metabolism of drugs, steroids, and xenobiotics in humans and the biosynthesis of natural products in plants (Jensen et al., 2021). Nevertheless, glutathione transferase (GST) is an enzyme that has the same ability to catalyze conjugation reactions, which occur spontaneously to protect cells against damage caused by harmful molecules (Nicolaï et al., 2022). Glutathione transferase (GST) forms a group of multifunctional enzymes involved in phase II of cellular detoxification mechanisms. It is associated with increased susceptibility to cancer development and resistance to anticancer drugs (Bodourian et al., 2022). As a fundamental activity in the body, antioxidants have numerous uses, as they can inhibit or delay undesired oxidation reactions, thereby preventing oxidative stress. Furthermore, the effects of antioxidants were studied *in vitro*.

The results were the mean SD of triplicate assays in **Table 5**. In the DPPH assay, the ability of MAERA and MAEKS to act as donors for hydrogen atoms or electrons in the transformation of DPPH into its reduced form DPPH-H was measured spectrophotometrically. MAERA and MAEKS could reduce DPPH radicals into stable yellow DPPH-H, achieving a 50% reduction with IC50 values of 103.00±3.75 and 131.85±23.48 µg/mL. In contrast, the ability to capture free radicals by ascorbic acid (14.88±2.42 µg/mL) was significantly ($p < 0.05$) smaller than that of MAERA and MAEKS. The ABTS staining method was excellent for determining the antioxidant activity of various substances, such as hydrogen donor antioxidants or water-phase radical scavengers, chain-breaking antioxidants, or lipid peroxyl radical scavengers (Matilda *et al.*, 2021). In

the ABTS radical scavenging method, MAERA and MAEKS exhibited less potent antioxidant activity, with IC50 values of 216.39±3.67 µg/mL and 225.50±3.36 µg/mL, respectively (Table 5). This difference was significant ($p < 0.05$) compared to that of ascorbic acid, which had an IC_{50} value of 2.90±0.32 µg/mL. This means that MAERA and MAEKS had weak antioxidant activity.

Table 5. Antioxidant activities of Microalgae *Aurantiochytrium* sp. Extractstrain Raja Ampat and strain Kepulauan Seribu

Sample	Parameter		
	DPPH, IC_{50} (µg/mL)	ABTS, IC_{50} (µg/mL)	Carotene/Linoleic Acid, IC_{50}(µg/mL)
MAERA	103.00 ± 3,75	216.39 ± 3,67	214.62 ± 7,07
MAEKS	131.85 ± 23,48	225.50 ± 3,36	251.69 ± 5,39
Ascorbic Acid	14.88 ± 2,42	2.90 ± 0,32	13.39 ± 0,08

MAERA: Microalgae *Aurantiochytrium* sp. Extract strain Raja Ampat
MAEKS: Microalgae *Aurantiochytrium* sp. Extract strain Kepulauan Seribu

The capacity of extract to scavenge free radicals such as DPPH or ABTS reflects its capacity to act essentially in the presence of ROS at the cellular level. (Thiyagarasaiyar *et al.*, 2020; Martinello *et al.*, 2021; Sami *et al.*, 2021). DPPH or ABTS methods are widely used in research and have shown a clear correlation between the ability of natural products to scavenge free radicals, as assessed by these methods, and the ability of the same products to reduce ROS production in vitro (Santos *et al*., 2020; Ma *et al.*, 2021; Munteanu *et al.*, 2021). However, this correlation may be challenging to consider due to the very complex content of active compounds in the extract. This correlation has also been reported in cases where the antioxidant activity of a single chemical compound has been determined. Considering the results, we can conclude that MAERA and MAEKS have weak in vitro antioxidant potential. Generally, the results are exceptionally heterogeneous, depending on the microalgae species considered and the measure utilized to assess the antioxidant action. Measure conventions shift from consider to consider, counting the extraction strategy, the dissolvable utilized, the response time, and the concentration tested (Jauffrais, 2021).

The antioxidant activities of MAERA and MAEKS were evaluated by three *in vitro* assays: DPPH, ABTS, and â-carotene bleaching assays. The most common measures to examine antioxidant movement were the DPPH and ABTS tests, which are based on and appropriately named after an electron-deficient radical. As a free steady radical, DPPH did not have to be arranged crisply like the ABTS radical cation. Be that as it may, the ABTS test was less prone to interference from colored tests due to the higher wavelength utilized. Both were manufactured and nonphysiologically significant radicals; only direct reactions of the radical with the antioxidant compound under investigation were measured. Both assays were based on the ability of bioactive compounds to stabilize free radicals by donating protons (Rumpf & Schulze, 2023). At the same time, the â-carotene test allows both lipophilic and hydrophilic samples to be analyzed with high reproducibility (García-Nicolás *et al*., 2023).

Antioxidant activity testing, which significantly inhibits or delays lipid oxidation, can be carried out by scavenging â-carotene/linoleic acid (Olszowy *et al*., 2016). This assay uses a model lipid substrate, which is recognized as a good model for membrane-based lipid peroxidation. In the â-carotene-linoleic acid assay, the antioxidant activity of MAERA and MAEKS was significantly ($p<0.05$) less potent than ascorbic acid. The current study demonstrated that the antioxidant activity of natural extracts derived from plants was often assessed using established methods. The results indicate that MAERA and MAEKS had minimal effectiveness in donating protons to detain free radicals, as measured by the DPPH and ABTS tests. Additionally, MAERA and MAEKS showed limited ability to slow down lipid oxidation. Similarly, minimal antioxidant effects were observed in the antioxidant activity test of *Aurantiochytrium* sp. SC145 isolated from its native habitat on Son Ca Island, Vietnam (Hien *et al*., 2022).

According to molecular docking, chemical compounds in MAERA and MAEKS have potential antioxidant properties. The binding energy of the target protein, the receptor, and ligand of the compounds contained in MAERA and MAEK was lower than that of the standard antioxidant compounds, ascorbic acid and its native ligand. Molecular docking and antioxidant activity measurements, including DPPH, ABTS, and â-carotene bleaching, were inconsistent. These were because, first, MAERA and MAEKS contain crude extracts that had not been purified, so many impurity compounds may not have antioxidant effects. This could be overcome by purifying the target

compound according to the molecular docking study, through the isolation of squalene. Second, the extraction method was not precise enough for squalene-masly. Third, the squalene levels in MAERA and MAEKS were too low, making the results of in silico and in vitro studies less accurate.

In this study, the squalene content of MAERA was significantly higher than that of MAEKS, with values of 14.57% and 4.01%, respectively. Additionally, the antioxidant activity (IC_{50}) of MAERA was higher, while MAEKS showed no significant differences. As detailed in Table 5, squalene was identified as the compound responsible for this antioxidant activity, as confirmed by previous research (Yarkent & Oncel, 2022; P. Zhang *et al*., 2023). Despite the higher squalene content in MAERA compared to MAEKS, it remains significantly lower than the levels reported in other studies. This discrepancy can be attributed to the fact that biomass production in this study was conducted without the squalene-increasing modifications implemented in other research (Chen *et al*., 2010; Nakazawa *et al.*, 2012; A. Zhang *et al.*, 2019).

The *Aurantiocytrium* sp. cultivation was carried out under controlled conditions in the Laboratory. Continuous research is carried out to modify nutrition, treatment, and the environment to produce secondary metabolites from the biosynthesis of *Aurantiochytrium* sp. The strain of microalgae and these factors can influence which are high in PUFAs, DHA, squalene, or other secondary metabolites. A few *Aurantiochytrium* strains can produce high quantities of squalene in cultivation (Lu *et al*., 2003; Nakazawa *et al.*, 2012). The *Aurantiochytrium* sp. 18W-13a strain can produce a maximum yield of approximately 171 mg/g of dry weight and 0.9 g/L, which was higher than others.. These optimal conditions are achieved at a temperature of 25 °C, a seawater concentration of 25% to 50%, and a glucose concentration of 2% to 6%. The experimental design suggested that glucose concentration, yeast extract, peptone, and shaking speed were significant factorsi influencing squalene production (Saengwong *et al*., 2018; Zhang *et al.*, 2019). Another experiment found that monosodium glutamate, yeast extract, and tryptone were required nitrogen sources by *Aurantiochytrium* sp., and were at their optimal levels for cell growth, then the production of squalene was significantly enhanced (Chen *et al.*, 2010; Auma *et al*, 2018). Optimum results were obtained when monosodium glutamate was 6.61 g/L, yeast extract was 6.13 g/L, and tryptone was 4.50 g/L. Adding these ingredients to the original media significantly increased the yield and squalene content by 10.1% and 26.3%, respectively (Chen *et al*., 2010). Jasmonic acid (growth hormone) and terbinafine (sterol formation inhibitor) increase squalene production by a specifically described mechanism (Wai *et al*, 2010; Elmataeeshy *et al.*, 2018; Paliwal & Kaur, 2019).

Conclusion

The microalgae *Aurantiochytrium* sp. extract Raja Ampat strain (MAERA) and the microalgae *Aurantiochytrium* sp. extract Kepulauan Seribu strain (MAEKS) did not show significantly different lipid content. In an *in silico* study, squalene was identified as the ligand with the lowest binding energy among all target molecules, suggesting a strong bond and indicating significant potential for antioxidant activity. However, *in vitro* studies revealed that neither MAERA nor MAEKS demonstrated high antioxidant activity. This was primarily due to the squalene level. MAERA contained 14.57 % squalene, while MAEKS contained only 4.01 % of the total lipid content. Therefore, further research is recommended to modify the cultivation process to enhance the production of squalene-rich biomass.

Acknowledgment

The authors would like to thank the Ministry of Research, Technology, and Higher Education for providing the budget through the Regular Fundamental Research Scheme number: 107/E5/PG.02.00.PL/2024

References

Abdel-Wahab, M. A., El-Samawaty, A. E. R. M. A., Elgorban, A. M., & Bahkali, A. H. (2021). Fatty acid production of thraustochytrids from Saudi Arabian mangroves. *Saudi Journal of Biological Sciences*, *28*(1), 855–864. https://doi.org/10.1016/j.sjbs.2020.11.024

Agregán, R., Munekata, P., Franco, D., Carballo, J., Barba, F., & Lorenzo, J. (2018). Antioxidant Potential of Extracts Obtained from Macro- (Ascophyllum nodosum, Fucus vesiculosus and Bifurcaria bifurcata) and Micro-Algae (Chlorella vulgaris and Spirulina platensis) Assisted by Ultrasound. *Medicines*, *5*(2), 33. https://doi.org/10.3390/medicines5020033

Aji, L. P., Goud, J., van der Steeg, S., Tapilatu, R., Maas, D.L., & Becking, L.E. (2023). The diversity of molluscan faunas in marine lakes of Raja Ampat, West Papua, Indonesia. *Contributions to Zoology 92*, 391–430. https://doi.org/10.1163/18759866-bja10047

Allen, L. N., Nicholson, B. D., Yeung, B. Y. T., & Goianada-silva, F. (2018). Articles Implementation of non-communicable disease policies/ : a geopolitical analysis of 151 countries. *The Lancet Global Health*, *8*(1), e50–e58. https://doi.org/10.1016/S2214-109X(19)30446-2

Andrei, P., Rodica, S., Delia, O., Felix, C., & Serban, G. (2021). Molecular Docking Studies on Phenolic

Constituents of Anethum graveolens L . Seed Extracts. *Journal of Pharmaceutical Research International*, *33*(56B), 119–128. https://doi.org/10.9734/JPRI/2021/v33i56B33939

Auma, K., Hamid, A. A., & Yusoff, W. M. W. (2018). Effect of nitrogen sources on biomass, lipid and docosahexanoic acid production by Aurantiochytrium sp. SW1. *AIP Conference Proceedings*, *1940*(April), 1–6. https://doi.org/10.1063/1.5027980

Ayu Shabrina, Kintoko, Sapto Yuliani, A. G. (2019). Effect of Spirulina platensis suspension on quality and quantity of sperm and expression of caspase-3 of testis in diabetic wistar rats induced by streptozotocin. *Pharmaciana*, *9*(1), 71–78. https://doi.org/10.12928/pharmaciana.v

Bagul, V. P., & Annapure, U. S. (2021). Isolation and characterization of docosahexaenoic acid-producing novel strain Aurantiochytrium sp. ICTFD5: A sterol with vitamin D-cholecalciferol, and cellulase and lipase producing thraustochytrid. *Bioresource Technology Reports*, *14*(January), 100688. https://doi.org/10.1016/j.biteb.2021.100688

Bartosova, Z., Ertesvåg, H., Nyfløt, E. L., Kämpe, K., Aasen, I. M., & Bruheim, P. (2021). Combined metabolome and lipidome analyses for in-depth characterization of lipid accumulation in the DHA producing aurantiochytrium sp. t66. *Metabolites*, *11*(3), 1–18. https://doi.org/10.3390/metabo11030135

Batubara, R., Hanum, T. I., Handika, A., & Affandi, O. (2020). *The screening of phytochemical and antioxidant activity of agarwood leaves (Aquilaria malaccensis) from two sites in North Sumatra , Indonesia*. *21*(4), 1588–1596. https://doi.org/10.13057/biodiv/d210440

Bodourian, C. S., Poudel, N., Papageorgiou, A. C., Antoniadi, M., Georgakis, N. D., Abe, H., & Labrou, N. E. (2022). Ligandability Assessment of Human Glutathione Transferase M1-1 Using Pesticides as Chemical Probes. *Molecular Science*, *23*(3606).

Budreviciute, A., Damiati, S., Sabir, D. K., & Onder, K. (2020). *Management and Prevention Strategies for Non-communicable Diseases (NCDs) and Their Risk Factors*. *8*(November), 1–11. https://doi.org/10.3389/fpubh.2020.574111

Chandrasekaran, K., Roy, R. K., & Chadha, A. (2018). Docosahexaenoic acid production by a novel high yielding strain of Thraustochytrium sp. of Indian origin: Isolation and bioprocess optimization studies. *Algal Research*, *32*(October 2017), 93–100. https://doi.org/10.1016/j.algal.2018.03.011

Chen, G., Fan, K. W., Lu, F. P., Li, Q., Aki, T., Chen, F., & Jiang, Y. (2010). Optimization of nitrogen source for enhanced production of squalene from thraustochytrid Aurantiochytrium sp. *New Biotechnology*, *27*(4), 382–389. https://doi.org/10.1016/j.nbt.2010.04.005

Du, F., Wang, Y. Z., Xu, Y. S., Shi, T. Q., Liu, W. Z., Sun, X. M., & Huang, H. (2021). Biotechnological production of lipid and terpenoid from thraustochytrids. *Biotechnology Advances*, *48*(March), 107725. https://doi.org/10.1016/j.biotechadv.2021.107725

Elmataeeshy, M. E., Sokar, M. S., Bahey-El-Din, M., & Shaker, D. S. (2018). Enhanced transdermal permeability of Terbinafine through novel nanoemulgel formulation; Development, in vitro and in vivo characterization. *Future Journal of Pharmaceutical Sciences*, *4*(1), 18–28. https://doi.org/10.1016/j.fjps.2017.07.003

Gao, Y., & Ma, X. (2022). Dietary supplementation of squalene increases the growth performance of early-weaned piglets by improving gut microbiota , intestinal barrier , and blood antioxidant capacity. *Frontiers in Veterinary Science*.

García-Nicolás, M., Ledesma-Escobar, C. A. (2023). Spatial Distribution and Antioxidant Activity of Extracts from Citrus Fruits. *Antioxidans*, *12*(781), 1–12.

Ghimire, G. P., Thuan, N. H., Koirala, N., & Sohng, J. K. (2016). Advances in Biochemistry and Microbial Production of Squalene and Its Derivatives. *Journal Microbiology Biotechnology*, *26*(3), 441–451.

Gohil, N., Bhattacharjee, G., Khambhati, K., Braddick, D., & Singh, V. (2019). Engineering strategies in microorganisms for the enhanced production of squalene: Advances, challenges and opportunities. *Frontiers in Bioengineering and Biotechnology*, *7*(MAR). https://doi.org/10.3389/fbioe.2019.00050

Gupta, A., Singh, D., Byreddy, A. R., Thyagarajan, T., Sonkar, S. P., Mathur, A. S., Tuli, D. K., Barrow, C. J., & Puri, M. (2016). Exploring omega-3 fatty acids , enzymes and biodiesel producing thraustochytrids from Australian and Indian marine biodiversity. *Biotechnology Journal*, *11*, 345–355. https://doi.org/10.1002/biot.201500279

Gustiarini, A., Tampubolon, N., Saranga, N., & Ampat, R. (2023). Kriteria Eco Resort Pariwisata Berkelanjutan Kawasan Perairan Waigeo Selatan Kepulauan Raja Ampat. *Jurnal Sumberdaya Akuatik Indopasifik*, *7*(3), 273–282.

Hanif, N., Murni, A., Tanaka, C., & Tanaka, J. (2019). Marine Natural Products from Indonesian Waters. *Marine Drugs*, *17*(364).

Herrera-Marcos, L.V., Martínez-Beamonte, R., Arnal, C., Barranquero, C., Puente-Lanzarote, J. J., Herrero-Continente, T., Lou-Bonafonte, J. M., Gonzalo-Romeo, G., Mocciaro, G., Jenkins, B., Surra, J. C., Rodríguez-Yoldi, M. J., Carlos, J., Lasheras, R., García-Gil, A., Güemes, A., Koulman, A., & Osada, J. (2023). Dietary squalene supplementation decreases triglyceride species and modifies phospholipid lipidomic profile in the liver of a porcine model of non-alcoholic steatohepatitis. *The Journal of Nutritional Biochemistry*, *112*, 109207. https://doi.org/10.1016/j.jnutbio.2022.109207

Hien, H. T. M., Thom, L. T., Ha, N. C., Tam, L. T., Thu, N. T. H., Nguyen, T. Van, Loan, V. T., Dan, N. T., & Hong, D. D. (2022). Characterization and Optimization of Culture Conditions for Aurantiochytrium sp. SC145 Isolated from Sand Cay (Son Ca) Island, Vietnam, and Antioxidative and Neuroprotective Activities of Its Polyunsaturated Fatty Acid Mixture. *Marine Drugs*, *20*(12). https://doi.org/10.3390/md20120780

Hoang, Hien, M., Thom, L. T., Ha, N. C., Tam, L. T., Thi, N., Thu, H., Nguyen, T. Van, Loan, V. T., Dan, N. T., & Hong, D. D. (2022). Characterization and Optimization of Culture Conditions for Activities of Its Polyunsaturated Fatty Acid Mixture. *Mareine Drugs*, *20*(270).

Hutari, A., Nisaa, R. A., Agustin, Y., & Ayunda, K. A. (2022). Eksplorasi Mikroalga Bernilai Ekonomi Tinggi Di Kawasan Mangrove Pulau Pari, Kepulauan Seribu, Jakarta. *Biotechnology Research*, *8*(November), 1–6.

Jauffrais, T. (2021). Antioxidant Compounds from Microalgae/ : A Review. *Marine Drugs*, *19*(549).

Jensen, S. B., Thodberg, S., Parween, S., Moses, M. E., Hansen, C. C., Thomsen, J., Sletfjerding, M. B., Knudsen, C., Giudice, R. Del, Lund, P. M., Castaño, P. R., Bustamante, Y. G., Natalia, M., Velazquez, R., Jørgensen, F. S., Pandey, A. V, Laursen, T., Møller, B. L., & Hatzakis, N. S. (2021). Biased cytochrome P450-mediated metabolism via small-molecule ligands binding P450 oxidoreductase. *Nature Communications*, *12*(2260), 1–14. https://doi.org/10.1038/s41467-021-22562-w

Jianu, C., Ionut, Golet, Stoin, D., Cocan, I., & Lukinich-Gruia, A. T. (2020). Antioxidant Activity of Pastinaca sativa L. ssp. sylvestris [Mill.] Rouy and Camus Essential Oil. *Molecules*, *25*(869).

Joshi, S., Roshani Kumari, V. N. U. (2018). Applications of Algae in Cosmetics: An Overview. *International Journal of Innovative Research in Science*, *7*(2). https://doi.org/10.15680/IJIRSET.2018.0702038

Kalidasan, K., Vinithkumar, N. V., Peter, D. M., Dharani, G., & Dufossé, L. (2021). Thraustochytrids of mangrove habitats from andaman islands: Species diversity, PUFA profiles and biotechnological potential. *Marine Drugs*, *19*(10). https://doi.org/10.3390/md19100571

Karacor, K., & Cam, M. (2015). Effects of oleic acid. *Medical Science and Discovery*, *2*(2), 125–132. https://doi.org/10.17546/msd.25609

Kim, S. K., & Karadeniz, F. (2012). Biological Importance and Applications of Squalene and Squalane. In *Advances in Food and Nutrition Research* (1st ed., Vol. 65). Elsevier Inc. https://doi.org/10.1016/B978-0-12-416003-3.00014-7

Kintoko, K., Witasari, H. A., Kusumo, D. W., Kapri, H., Muldiyana, T. Y. A., & Wahyono, S. (2018). Effectiveness And Mechanism Of Physician Herbs From Kali Putih Batur Banjarnegara Central Java Against Diabetic Nephropathy. *Asian J Pharm Clin Res*, *11*(12).

Koopmann, I. K., Müller, B. A., & Labes, A. (2023). Screening of a Thraustochytrid Strain Collection for Carotenoid and Squalene Production Characterized by Cluster Analysis, Comparison of 18S rRNA Gene Sequences, Growth Behavior, and Morphology. *Marine Drugs*, *21*(4). https://doi.org/10.3390/md21040204

Lee Chang, K. J., Dumsday, G., Nichols, P. D., Dunstan, G. A., Blackburn, S. I., & Koutoulis, A. (2013). High cell density cultivation of a novel Aurantiochytrium sp. strain TC 20 in a fed-batch system using glycerol to produce feedstock for biodiesel and omega-3 oils. *Applied Microbiology and Biotechnology*, *97*(15), 6907–6918. https://doi.org/10.1007/s00253-013-4965-z

Liu, B., Ertesvåg, H., Aasen, I. M., Vadstein, O., Brautaset, T., & Heggeset, T. M. B. (2016). Draft genome sequence of the docosahexaenoic acid producing thraustochytrid Aurantiochytrium sp. T66. *Genomics Data*, *8*, 115–116. https://doi.org/10.1016/j.gdata.2016.04.013

Liu, L., Hu, Z., Li, S., Yang, H., Li, S., Lv, C., Zaynab, M., Cheng, C. H. K., Chen, H., & Yang, X. (2020). Comparative transcriptomic analysis uncovers genes responsible for the DHA enhancement in the mutant aurantiochytrium sp. *Microorganisms*, *8*(4), 1–19. https://doi.org/10.3390/microorganisms8040529

Liu, Y., Liu, X., Cui, Y., & Yuan, W. (2022). Ultrasound for microalgal cell disruption and product extraction: A review. *Ultrasonics Sonochemistry*, *87*(May), 106054. https://doi.org/10.1016/j.ultsonch.2022.106054

Lou-Bonafonte, J. M., Martínez-Beamonte, R., Sanclemente, T., Surra, J. C., Herrera-Marcos, L. V, & Sanchez-Marco, J. (2018). Current insights into the biological action of squalene. *Molecular Nutrition*, *62*(15), 1–59. https://doi.org/10.1002/mnfr.201800136

Loucif, K., Benabdallah, H., Benchikh, F., Mehlous, S., Souici, C. Ben, & Amira, S. (2020). Total Phenolic Contents, DPPH Radical Scavenging and â-Carotene Bleaching Activities of Aqueous Extract from Ammoides atlantica.pdf. *Journal of Drug Delivery & Therapeutics*, *10*(3s), 196–198.

Lu, H. T., Jiang, Y., & Chen, F. (2003). Preparative separation and purification of squalene from the microalga Thraustochytrium ATCC 26185 by high-speed counter-current chromatography. *Journal of Chromatography A*, *994*(1–2), 37–43. https://doi.org/10.1016/S0021-9673(03)00454-0

Lu, X., Ma, S., Chen, Y., Yangzom, D., & Jiang, H. (2018). Squalene found in alpine grassland soils under a harsh environment in the Tibetan Plateau, China. *Biomolecules*, *8*(4), 1–12. https://doi.org/10.3390/biom8040154

Ma, Q., Cai, S., Liu, X., Shi, J., & Yi, J. (2021). Characterization of phytochemical components and identification of main antioxidants in Crateva unilocalaris Buch . shoots by. *Food Research International*, *143*(March), 110264. https://doi.org/10.1016/j.foodres.2021.110264

Maas, D. L., Capriati, A., Ahmad, A., Erdmann, M. V, Lamers, M., Leeuw, C. A. De, Prins, L., Putri, A. P., Tapilatu, R. F., & Becking, L. E. (2020). Recognizing peripheral ecosystems in marine protected areas/ : A case study of golden jellyfish lakes in Raja Ampat , Indonesia. *Marine Pollution Bulletin*, *151*(July 2019), 110700. https://doi.org/10.1016/j.marpolbul.2019.110700

Mahfudh, N., Sulistyani, N., Khoirot, A. F., Safira, T. I., Othman, F., & Zakaria, Z. A. (2022). *Sweet Potato (*

Ipomoea batatas L .) Leaves Ethanol Extract Increases Endogenous Antioxidant Activities in Hyperlipidemic Rats. *51*(9), 2873–2883.

Malawet, T., Bangrak, P., Peerapornpisal, Y., & Kamlangdee, N. (2019). *Newly Isolated High Squalene Producing Thraustochytrid Strain Aurantochytrium sp . P5 / 2 from Mangrove Habitats in Nakhon Si Thammarat Province , Thailand*. *17*(Ldl), 212–221.

Marchan, F., Chang, L. L., Nichols, K. J., Mitchell, P. D., J., W., Polglase, J., L., A., & Gutierrez, T. (2018). Taxonomy, ecology and biotechnological applications of thraustochytrids: A review. *Biotechnology Advances*, *36*(1), 26–46. https://doi.org/10.1016/j.biotechadv.2017.09.003

María Señoráns, N. C. and F. J. S. (2020). Advanced Extraction of Lipids with DHA from Isochrysis galbana with Enzymatic Pre-Treatment Combined with Pressurized Liquids and Ultrasound Assisted Extractions. *Molecules*, *25*(3310).

Marino, T., National, I., Casella, P., Chianese, S., & Larocca, V. (2020). From Haematococcus Pluvialis Microalgae a Powerful Antioxidant for Cosmetic Applications. *CHEMICAL ENGINEERING TRANSACTIONS*, *79*(April). https://doi.org/10.3303/CET2079046

Martinello, M., & Mutinelli, F. (2021). Antioxidant activity in bee products: A review. *Antioxidants*, *10*(1), 1–42. https://doi.org/10.3390/antiox10010071

Matilda, R., Jianu, C., Lukinich-gruia, A. T., Mioc, M., Mioc, A., & Stana, L. G. (2021). Chemical Composition , In Vitro and In Silico Antioxidant Potential of Melissa officinalis subsp . officinalis Essential Oil. *Antioxidants*, *10*(1081), 1–13.

Molino, A., Mehariya, S., Di, G., Larocca, V., Martino, M., Paolo, G., Marino, T., Chianese, S., Balducchi, R., & Musmarra, D. (2020). Recent developments in supercritical fl uid extraction of bioactive compounds from microalgae/ : Role of key parameters , technological achievements and challenges. *Journal of CO2 Utilization*, *36*(November 2019), 196–209. https://doi.org/10.1016/j.jcou.2019.11.014

Morabito, C., Bournaud, C., Maës, C., Schuler, M., Aiese Cigliano, R., Dellero, Y., Maréchal, E., Amato, A., & Rébeillé, F. (2019). The lipid metabolism in thraustochytrids. *Progress in Lipid Research*, *76*(August), 101007. https://doi.org/10.1016/j.plipres.2019.101007

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, *22*(7). https://doi.org/10.3390/ijms22073380

Nakazawa, A., Matsuura, H., Kose, R., Kato, S., Honda, D., Inouye, I., Kaya, K., & Watanabe, M. M. (2012). Optimization of culture conditions of the thraustochytrid Aurantiochytrium sp. strain 18W-13a for squalene production. *Bioresource Technology*, *109*, 287–291. https://doi.org/10.1016/j.biortech.2011.09.127

Nayab, R. S., Maddila, S., Krishna, M. P., Titinchi, J. J., Thaslim, B. S., Chintha, V., Wudayagiri, R., Nagam, V., Tartte, V., Chinnam, S., & Raju, N. (2020). In silico molecular docking and in vitro antioxidant activity studies of novel á -aminophosphonates bearing 6-amino-1 , 3-dimethyl uracil. *Journal of Receptors and Signal Transduction*, *0*(0), 1–7. https://doi.org/10.1080/10799893.2020.1722166

Nicolaï, A., Petiot, N., Grassein, P., Delarue, P., & Neiers, F. (2022). Free-Energy Landscape Analysis of Protein-Ligand Binding/ : The Case of Human Glutathione Transferase A1. *Applied Sciences*, *12*(16), 1–17.

Nurkhasanah, Nanik, S., & Noorlina. (2019). In vitro activity of immunomodulator of n-hexane fraction of bangle (Zingiber cassumunar roxb.) ethanol extract. *Research Journal of Chemistry and Environment*, *23*(8), 62–64.

Olszowy, M., & Dawidowicz, A. L. (2016). Essential oils as antioxidants/ : their evaluation by DPPH , ABTS , FRAP , CUPRAC , and b -carotene bleaching methods. *Monatshefte Für Chemie - Chemical Monthly*. https://doi.org/10.1007/s00706-016-1837-0

Paliwal, S., & Kaur, G. (2019). Formulation and Characterization of Topical Nano Emulgel of Terbinafine. *Universal Journal of Pharmaceutical Research*, *March*. https://doi.org/10.22270/ujpr.v3i6.223

Passos, M. E. P., Alves, H. H. O., Momesso, C. M., Faria, F. G., Murata, G., & Hatanaka, E. (2016). Differential effects of palmitoleic acid on human lymphocyte proliferation and function. *Lipids in Health and Disease*, *15*(217), 1–11. https://doi.org/10.1186/s12944-016-0385-2

Patel, A., Rova, U., Christakopoulos, P., & Matsakas, L. (2019). Simultaneous production of DHA and squalene from Aurantiochytrium sp. grown on forest biomass hydrolysates. *Biotechnology for Biofuels*, *12*(1), 1–12. https://doi.org/10.1186/s13068-019-1593-6

Patel, A., Rova, U., Christakopoulos, P., & Matsakas, L. (2020). Assessment of fatty acids profile and omega-3 polyunsaturated fatty acid production by the oleaginous marine thraustochytrid aurantiochytrium sp. T66 cultivated on volatile fatty acids. *Biomolecules*, *10*(5). https://doi.org/10.3390/biom10050694

Popa, O., BYbeanu, N. E., Popa, I., NiþY, S., & Dinu-Pârvu, C. E. (2015). Methods for obtaining and determination of squalene from natural sources. *BioMed Research International*, *2015*. https://doi.org/10.1155/2015/367202

Qiu, X., Xie, X., & Meesapyodsuk, D. (2020). Progress in Lipid Research Molecular mechanisms for biosynthesis and assembly of nutritionally important very long chain polyunsaturated fatty acids in microorganisms. *Progress in Lipid Research*, *79*(May), 101047. https://doi.org/10.1016/j.plipres.2020.101047

Rani, A., Meghana, R., & Kush, A. (2018). Squalene production in the cell suspension cultures of Indian sandalwood (Santalum album L.) in shake flasks and air lift bioreactor. *Plant Cell, Tissue and Organ Culture*, *135*(1), 155–167. https://doi.org/10.1007/s11240-018-1452-3

Rodjaroen, S., Chittapun, S., Charoenrat, T., & Nuntapong, N. (2024). Dietary supplementation of Aurantiochytrium sp . significantly improved growth , feed utilization , skin lightness , muscle protein synthesis capacity , and whole-body protein in juvenile African sharptooth catfish (Clarias gariepinus). *Aquaculture Reports*, *35*, 101986. https://doi.org/10.1016/j.aqrep.2024.101986

Rumpf, J., & Schulze, M. (2023). International Journal of Biological Macromolecules Statistical evaluation of DPPH , ABTS , FRAP , and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. *International Journal of Biological Macromolecules*, *233*(February). https://doi.org/10.1016/j.ijbiomac.2023.123470

Saengwong, A., Yongmanitchai, W., & Chonudomkul, D. (2018). Screening and optimization of squalene production from microalgae Aurantiochytrium sp. *Chiang Mai Journal of Science*, *45*(2), 680–691.

Saini, R. K., Prasad, P., Shang, X., & Keum, Y. (2021). Advances in Lipid Extraction Methods — A Review. *Molecular Science*, *22*(13643), 1–19.

Sami, F. J., Soekamto, N. H., Firdaus, & Latip, J. (2021). Bioactivity profile of three types of seaweed as an antioxidant, uv-protection as sunscreen and their correlation activity. *Food Research*, *5*(1), 441–447. https://doi.org/10.26656/fr.2017.5(1).389

Santos, C. M. M., & Silva, A. M. S. (2020). The antioxidant activity of prenylflavonoids. *Molecules*, *25*(3). https://doi.org/10.3390/molecules25030696

Sirirak, K., Suanjit, S., Powtongsook, S., & Jaritkhuan, S. (2020). Characterization and PUFA production of Aurantiochytrium limacinum BUCHAXM 122 isolated from fallen mangrove leaves. *Science Asia*, *20*, 1–9.

Sridhar, K., & Charles, A. L. (2019). In vitro antioxidant activity of Kyoho grape extracts in DPPH [rad] and ABTS [rad] assays: Estimation methods for EC 50 using advanced statistical programs. *Food Chemistry*, *275*, 41–49. https://doi.org/10.1016/j.foodchem.2018.09.040

Su, Y., & Li, L. (2020). Structural characterization and antioxidant activity of polysaccharide from four auriculariales. *Carbohydrate Polymers*, *229*, 115407. https://doi.org/10.1016/j.carbpol.2019.115407

Suhendra, E., S., H., Z., & A, H. (2019). Kajian Singkat Rancang Bangun Pabrik Docohexanoic Acid dari Mikroalga Species Aurantiochytrium dari Hutan Bakau Indonesia. *Konversi*, *8*(1), 33–44.

Suhendra, Septianingsih, L., Ariandi, T. R., Husna, M., Laksana, Z. A., Yuniasih, D., & Hutari, A. (2022). Isolasi mikroalga Aurantiochytrium dari Raja Ampat dan potensinya pada industri bahan baku adjuvant vaksin. *Jurnal Rekayasa Proses*, *16*, 34–44. https://doi.org/10.22146/jrekpros.72045

Sun, K., Meesapyodsuk, D., & Qiu, X. (2024). Biosynthetic mechanisms of omega-3 polyunsaturated fatty acids in microalgae. *Journal of Biological Chemistry*, *300*(9), 107699. https://doi.org/10.1016/j.jbc.2024.107699

Surani, Cahyo, P., & Asep, K. (2023). Penentuan Suhu Terprogram Optimum pada Analisis Asam Lemak Hasil Ekstrak Mikroalga Chlorella Menggunakan Instrument GCMS. *UNESA Journal of Chemistry*, *12*(1).

Tahya, C. Y., & Arnelasatri. (2021). Gas Chromatography-Mass SpectrometryAnalysis and á-GlucosidaseInhibitory Activity of n-Hexane Extract of Bilajang Bulu (MerremiaVitifolia)Leaves.pdf. *Walisongo Journal of Chemistry*, *4*(2), 162–172.

Tao, X., Huang, Y., Chen, X., Wang, C., Chen, F., Yang, L., Ling, L., & Che, Z. (2019). Review Recent developments in molecular docking technology applied in food science/ : a review. *International Journal of Food Science Technology*, 1–13. https://doi.org/10.1111/ijfs.14325

Thiyagarasaiyar, K., Goh, B. H., Jeon, Y. J., & Yow, Y. Y. (2020). Algae metabolites in cosmeceutical: An overview of current applications and challenges. *Marine Drugs*, *18*(6). https://doi.org/10.3390/md18060323

Tran, T. L. N., Miranda, A. F., Gupta, A., Puri, M., Ball, A. S., Adhikari, B., & Mouradov, A. (2020). The nutritional and pharmacological potential of new australian thraustochytrids isolated from mangrove sediments. *Marine Drugs*, *18*(3), 1–17. https://doi.org/10.3390/md18030151

Venugopal, V., Kumaran, A. K., Sekhar Chatterjee, N., Kumar, S., Kavilakath, S., Nair, J. R., & Mathew, S. (2016). Biochemical Characterization of Liver Oil of Echinorhinus brucus (Bramble Shark) and Its Cytotoxic Evaluation on Neuroblastoma Cell Lines (SHSY-5Y). *Scientifica*, *2016*. https://doi.org/10.1155/2016/6294030

Vyas, S., Bettiga, M., Rova, U., Christakopoulos, P., Matsakas, L., & Patel, A. (2022). Structural and Molecular Characterization of Squalene Synthase Belonging to the Marine Thraustochytrid Species Aurantiochytrium limacinum Using Bioinformatics Approach. *Marine Drugs*, *20*(3). https://doi.org/10.3390/md20030180

Wai, K., Tsunehiro, F., & Feng, A. (2010). Enhanced production of squalene in the thraustochytrid Aurantiochytrium mangrovei by medium optimization and treatment with terbinafine. *World J Microbiol Biotechnol*, *26*, 1303–1309. https://doi.org/10.1007/s11274-009-0301-2

White, C. M., Mangubhai, S., Rumetna, L., & Brooks, C. M. (2022). The bridging role of non-governmental organizations in the planning , adoption , and management of the marine protected area network in Raja Ampat , Indonesia. *Marine Policy*, *141*(May), 105095. https://doi.org/10.1016/j.marpol.2022.105095

Xie, X., Suna, K., Dauenpen Meesapyodsuka, Y. M., & Qiua, X. (2020). Distinct Functions of two FabA-like

Dehydratase Domains of Polyunsaturated Fatty Acid Synthase in the Biosynthesis of Very Long Chain Polyunsaturated Fatty Acids. *Environmental Microbiology*, *22*(9). https://doi.org/10.1111/1462-2920.15149

Yarkent, Ç., & Oncel, S. S. (2022). Recent Progress in Microalgal Squalene Production and Its Cosmetic Application. *Biotechnology and Bioprocess Engineering*, *27*(3), 295–305. https://doi.org/10.1007/s12257-021-0355-z

Zhang, A., Xie, Y., He, Y., Wang, W., Sen, B., & Wang, G. (2019). Bioresource Technology Bio-based squalene production by Aurantiochytrium sp . through optimization of culture conditions , and elucidation of the putative biosynthetic pathway genes. *Bioresource Technology*, *287*(May), 121415. https://doi.org/10.1016/j.biortech.2019.121415

Zhang, P., Liu, N., Xue, M., Zhang, M., Xiao, Z., Xu, C., Fan, Y., Liu, W., Qiu, J., Zhang, Q., & Zhou, Y. (2023). Anti-Inflammatory and Antioxidant Properties of Squalene in Copper Sulfate-Induced Inflammation in Zebrafish (Danio rerio). *Moleculae Science*, *24*(8518), 1–16.