Extraction of High Economic Potential of Lipids from Heterotrophic Cultivation of Indigenous Aurantiochytrium Microalgae Strain

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ABSTRACT

Microalgae of the genus Aurantiochytrium have been recognized for a long time as oleaginous microalgae due to their capacity to produce a high lipids content such as omega-3 docosahexaenoic acid. Therefore, the biomass from the Aurantiochytrium microalgae can be used as raw materials for food, cosmetics, and pharmaceuticals. The extraction procedure must be optimized to maximize the cultivation process's biomass utilization. Therefore, this study presents the optimization of lipids extracted from microalgae of the genus Aurantiochytrium. This pure isolate was obtained from the Indonesian mangrove forest of Bunaken, located in North Sulawesi. The cultivation procedure consists of three steps: standing culture (SC; 48 hours), pre-culture (PC; 48 hours), and primary culture (MC; 120 hours). The cultivation occurred in an Erlenmeyer flask using an orbital agitator at 220 rpm at room temperature and pressure. The average biomass concentration was 9.4 grams per liter. In addition to the cultivation procedure, a recent paper describes an extraction technique employing organic solvents such as methanol, chloroform, acetone, ethyl acetate, and n-hexane. The lipids fraction can be extracted with a minimum of 7% and a maximum of 47%. The maximum fraction of lipids was extracted from Aurantiochytrium biomass using acetone-chloroform-ethyl acetate solvents, according to the results of this study. Since there has never been a publication discussing the extraction procedure of the native Indonesian strain of Aurantiochytrium microalgae, this paper will serve as a valuable foundation for future studies in this field.

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1. Introduction

Omega-3 docosahexaenoic acid (DHA) is an important polyunsaturated fatty acid for human health [1], [2]. Unfortunately, the human body cannot produce this high economic value of lipids, and hence, the daily requirement is fulfilled by dietary supplements. Previous research affirms that consuming omega-3 docosahexaenoic acid (DHA) lowers the risk of cardiovascular disease and cancer, especially breast cancer [3]. They are also an energy source and help to lower blood pressure, blood fat, and the risk of blood clots, stroke, and sudden cardiac death [4], [5]. Children are suggested to consume omega-3 DHA due to its vital role in brain and retina development [6], [7].

Industrial production of omega-3 DHA mainly comes from fish products, with fish caught by marine fish preferred. The aquaculture industry is facing an upward trend as an alternative source of omega-3 DHA, even though the quantity of omega-3 DHA from the aquaculture industry is less than caught marine fish. However, the limited catch of marine fish due to the impact of pollution, higher operational costs in the aquaculture industry, mainly due to increased feed cost and the impact of

global warming, there have been research previously to seek alternative sources of omega-3 raw material from microalgae [8], [9]. In addition, there is an increase in awareness of the consumption omega-3 from non-fish products due to environmental problems causing high contaminants in fish bodies.

Therefore, various studies have emerged to replace the source of omega-3 raw materials, one of which is made from Aurantiochytrium microalgae. Fig. 1. illustrates the production life cycle and the search for alternative technologies for sustainable omega-3 production. Non-fish omega-3 has comparative advantages in value-added components with competitive characteristics, including a high content of omega-3 DHA, suitable for vegan consumers, and no heavy metals. In addition, omega-3 DHA from Aurantiochytrium microalgae can be produced with a scalable capacity for commercial use and, finally, is safe to use (as herbs, cosmetics, and health supplements).

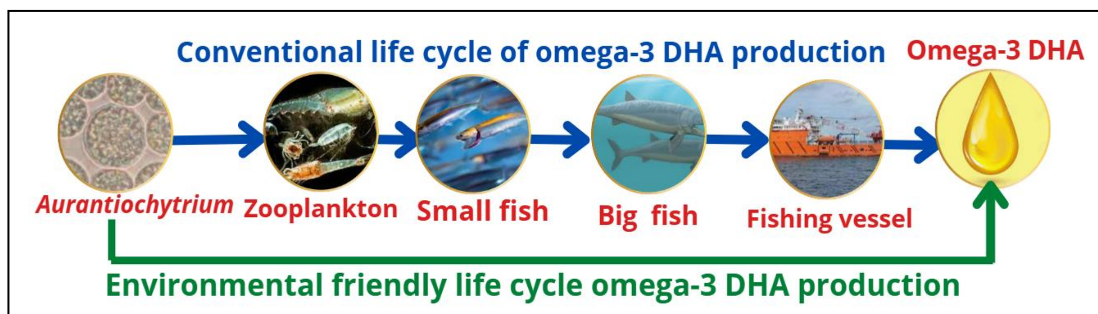

Fig. 1. Narrative of alternative technologies made from omega-3-producing microalgae

Aurantiochytrium microalgae is widespread in marine ecosystems, especially in mangrove forests [10]. Aurantiochytrium microalgae is a species of oleaginous microalgae whose meaning is synonymous with the ability to produce large amounts of lipids in its life cycle. One of the important ingredients in such lipids is omega-3 (DHA, EPA), which has been used as a substitute for conventional fish oil sources [11].

Even though Indonesia is known as the country with the most extensive mangrove forests in the world, the use of Aurantiochytrium sp microalgae in Indonesia has not been carried out optimally. Several review studies have been initiated to reveal some preliminary research on the potential of Aurantiochytrium microalgae [12], [13]. Learning videos of research activities on the isolation of Aurantiochytrium microalgae from Indonesian mangroves have been shown previously [14], [15].

The research theme on lipid extraction methodology from biomass from the cultivation of Aurantiochytrium microalgae from Indonesian mangroves has never been done before. The lipid extraction stage is important in producing the active components of the Aurantiochytrium microalgae biomass products, one of which is omega-3 DHA. Based on these considerations, this study wants to contribute to determining the lipid extraction method from biomass produced from the cultivation of Aurantiochytrium microalgae. The extraction techniques used are limited to the use of common solvent variations. The results of this study are expected to obtain the optimum method used for lipid extraction of the microalgae Aurantiochytrium isolated from one of the mangrove forests in Indonesia.

2. Research Methodology

The procedures for producing microalgae biomass and their processing are depicted in the following Fig. 2. The process entails isolated preparation (Fig. 2A), culture medium preparation followed by inoculation (Fig. 2B), centrifugation of produced biomass, sonication (Fig. 2C) and then followed by evaporation using rotary evaporation (Fig. 2D)and distillation (Fig. 2E).

The materials needed in this study include pure isolates of Aurantiochytrium microalgae, aquadest, yeast extract, glucose, reef salt, methanol, chloroform, ethyl acetate, acetone, and n-hexane. Using a direct plating method, the isolates used in this study are isolated from Bunaken Island, North Sulawesi. An educational video on the sampling method of mangrove leaves from several mangrove forests in Indonesia has been shown by Suhendra [15].

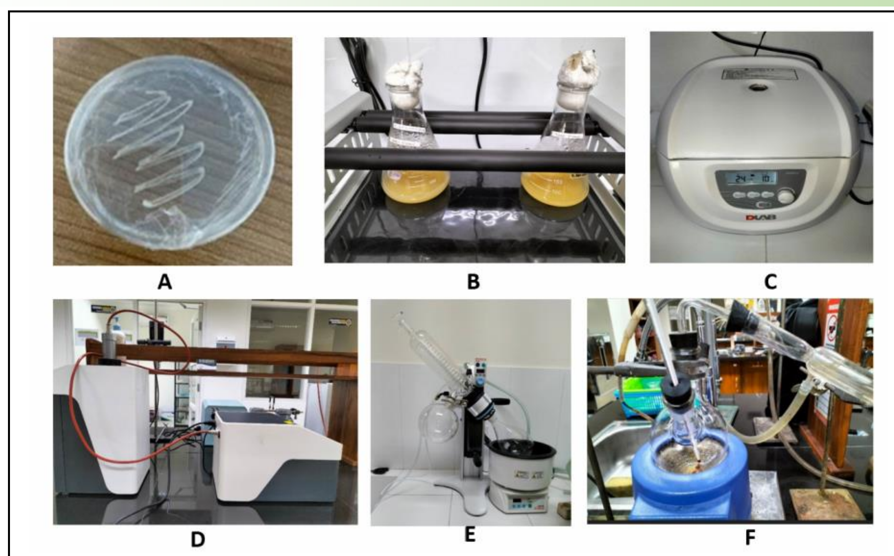

Fig. 2. The steps of this experiment

Equipment used in the study were orbital shaker, Erlenmeyer 100 ml, 250 ml, and 2000 ml, laminar air flow, microscope and digital camera, personal computer (PC), stirring glass, pro pipette, micropipette, plastic bottle, petri dish, bunsen, aluminum foil, cotton, gauze, ose needle, centrifuge, oven, digital balance sheet, vacuum evaporator, sonicator, beaker glass 100 and 250 ml, distillation device, tube/vial, label paper.

2.1. Cultivation process

Aurantiochytrium microalgae was cultivated in the bioprocess laboratory of the Chemical Engineering study program of UAD. The cultivation is carried out in three stages, namely, standing culture (SC), pre-culture (PC), and primary culture (MC) stages. The formula and duration of the three stages of cultivation are shown in Table 1. All stages of cultivation are carried out using orbital shakers at a speed of 220 rpm.

Table 1. Operating Parameters of SC, PC, and MC

Parameters	Cultivation Stages		
	SC	***PC***	***MC***
Glucose (mg)	0.25	4	20
Yeast Extract	0.75	12	80
Reef Salt	0.36	2.88	5.76
Total Volume	60	400	800
Duration (hours)	48	48	120

2.2. Extraction of lipids from biomass

First of all, the resulting microalgae biomass is centrifuged. Then, the solid biomass is separated and then dried in the oven at 40 °C to a constant mass. This study used universal solvents (methanol, chloroform) and non-polar organic solvents (N-hexane, acetone, ethyl acetate) to extract lipids from dry biomass. The dried biomass was put into a beaker glass, and methanol solvent was added in a ratio of 1:2. The solution was then sonicated with 85% power for 30 min and then continued with evaporation at a temperature above the boiling point of methanol until all the methanol were evaporated. The dried biomass was put into a beaker glass and then diluted with chloroform solvent in a ratio of 1: 2. Then the process continued with sonication and evaporation.

2.3. Purification of lipid fractions

The resulting biomass lipid extraction in the evaporation process of extracted biomass was added with a variation of 2 organic solvents. The first variable was acetone-ethyl acetate solvent, and the second variation was acetate-n-hexane. Both variations were set at a ratio of 1:1. The mixture was then distilled at a temperature of 62 °C for 30 minutes. The remaining sample residues in the distillation flask were collected, and the remaining liquids continued into the stage 2 distillation

process with the same time and temperature settings as the first distillation. The distillation results are put into the tube/vial, and the volume obtained is so that the lipid fraction of each sample can be calculated. The calculation of the lipid fraction using equation 1 :

$$\text{lipid fractions (\%)} = \frac{\text{final volume of distillations result (ml)}}{\text{initial mass of the sample (gr)}} \times 100 \quad (1)$$

3. Results

3.1. Results of cultivation process

Fig. 3 shows the results of the cultivation process of Aurantiochytrium microalgae originating from Bunaken Island, north Sulawesi. The products reveal a brownish-yellow color, and their aroma is fishy, smelling like fish Fig. 3. (a). Observations of microalgae cells under a microscope showed features of spherical shape, yellowish-white cell color, and intra-cell-containing pockets of lipids body. The results of measuring cell diameter range from 13 – 14 µm Fig. 3. (b) at a magnification of 10x100 times. The cell used in this experiment is bigger than the cell of Aurantiochytrium microalgae used in the previous experiment from Chauhan et al., which has a diameter ranging from 2 to 4 µm [16]. The resulting wet and dried biomass after the centrifugation and drying process are presented in Fig. 3. (c) and Fig. 3. (d). The average mass of dried biomass obtained was 9.4 g/L.

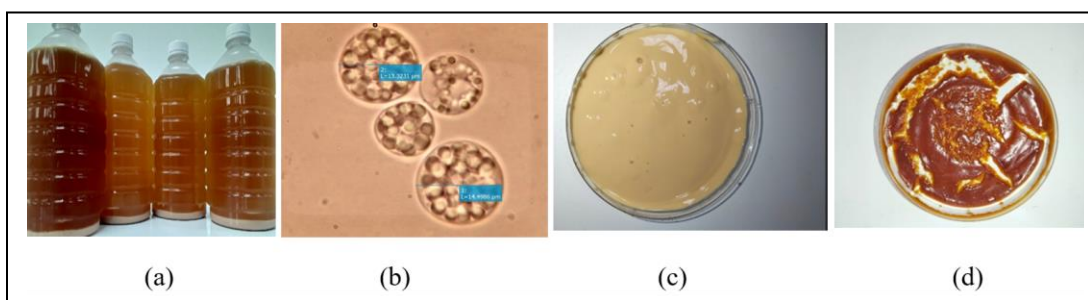

Fig. 3. The results of the cultivation process of Aurantiochytrium microalgae (a) Results of the cultivation of Aurantiochytrium microalgae (b) Mircrograpf pf Aurantiochytrium microalgae cells and their sizes (c) Filtered biomass (d) Dried biomass

3.2. Results of extraction and purification processes

Fig. 4 shows the results of lipid extraction using universal and polar solvents. Fig. 4. (a) shows the results of extracted lipids utilizing a combination of acetone-ethyl acetate (left) and n-hexane-ethyl acetate (right) solvents after extraction using methanol. Fig. 4. (b) shows a result of extracted lipids utilizing a combination of acetone-n-hexane solvents after methanol extraction. Fig. 4. (c) shows the results of extracted lipids using a combination of acetone-ethyl acetate and n-hexane-ethyl acetate solvents after chloroform extraction. The volume of extracted lipids is listed in Table 2. The combination of chloroform acetone-ethyl acetate solvents produced the highest volume fraction of extracted lipids, 47%, compared to other solvent blends.

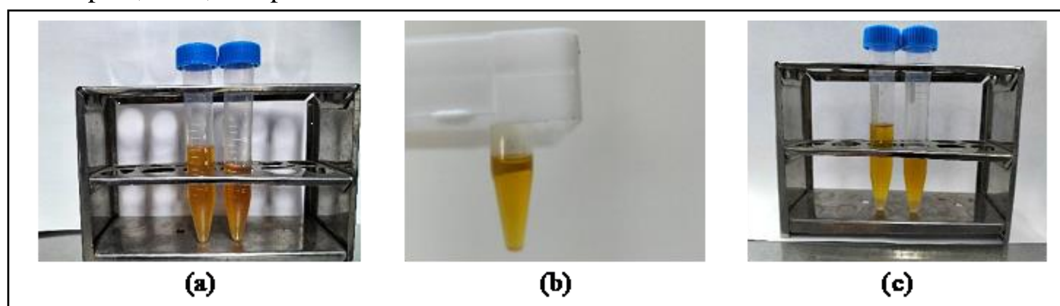

Fig. 4. The results of lipid extraction using universal and polar solvents (a) Lipid extraction results of a combination of acetone-ethyl acetate and n-hexane-ethyl acetate solvents methanol (b) a combination of acetone-n-hexane solvents methanol (c) a combination of acetone-ethyl acetate and n-hexane-ethyl acetate chloroform solvents

Table 2. Results of Lipid Extraction from the Biomass of Aurantiochytrium Microalgae using Different Solvents

Mass (gr)	**Universal Solvents**		**Organic Solvents**			**Volume (ml)**	**Lipid Fraction (%)**
	Methanol (ml)	***Chloroform (ml)***	***Acetone (ml)***	***N-Hexane (ml)***	***Ethyl Acetate (ml)***		
12	24		24		24	4	33
12	24		24	24		0.8	7
12	24			24	24	5.5	46
11		22	22		22	5.2	47
11		22		22	22	3	27

3. Discussion

The extraction process of Aurantiochytrium microalgae is a critical aspect of harnessing its bioactive compounds for various applications. Our study employed two solvent extraction methods, yielding variability of lipid fraction results. Compared to previous studies [17], [18], our findings align with the general trend observed in the literature. However, variations in extraction efficiency highlight the influence of factors such as strain variability and cultivation conditions.

The choice of extraction methods significantly impacted the efficiency of the process [19]. The combined solvent extraction using combined polar and non-polar solvents demonstrated promising efficacy in extracting expected lipids fraction, leading to a percentage increase in overall yield compared to alternative methods. This suggests that our selected methods are suitable for maximizing the extraction of valuable compounds from Aurantiochytrium microalgae. Lipids in microalgae include polar lipids such as phospholipids, glycolipids, and natural lipids such as triacylglycerols and unsaturated fatty acids [20]. The volume of lipids successfully extracted depends on the solvent used. Previously, methanol and chloroform were often used in the extraction process [21], [22]. In addition, Lee et al. mentioned that the most effective method for breaking down cells and extracting lipids is the bead-beater method, followed by chloroform-methanol extraction (2:1) [23].

In this study, methanol was used, which is polar, so that it is easy to extract lipids of Aurantiochytrium microalgae. Lipid extraction efficiency can be optimized if the cell membrane can be broken down to facilitate the entry of solvents into the cell [24]–[26]. In this study, the sonication process served to break down microalgae cells. The intensity and frequency of sonication will affect the lipid extraction rate [27]. Lipid extracts produced from the evaporation and distillation process are golden yellow Fig. 4.

In general, variables such as solvent type, extraction time, and temperature played crucial roles in the extraction process [19], [28], [29]. For instance, optimum selection of extraction tie and temperature, a solvent-to-biomass ratio, and mechanical disruption, emphasizing the optimal conditions that resulted in the highest yield and quality of extracted compounds. This understanding contributes to the optimization of future extraction protocols.

The analysis of the extracted compounds revealed a rich biochemical composition in Aurantiochytrium microalgae. The high content of valuable fatty acids in Aurantiochytrium microalgae biomass is particularly promising for applications in biofuel production, nutraceuticals, animal feed, and pharmaceuticals [30], [31]. The diverse composition underscores the potential of Aurantiochytrium microalgae as a valuable source of bioactive compounds.

Considering the extraction methods' scalability is crucial for potential industrial applications. While our laboratory-scale processes have shown promising results, further research is needed to assess the feasibility and cost-effectiveness of upscaling the extraction process for large-scale production.

The findings of this study have significant implications for various applications. The lipids extracted from the cultivation process of Aurantiochytrium microalgae contain several valuable lipids such as C14:0 (myristic acid), C15:0 (pentadecilic acid), C16:0 (palmitic acid), C18:0 (octadecanoic acid), C20:0 (arachidic acid), C20:5n3 (Eicosapentaenoic acid/EPA), C22:5n6 (docosapentaenoic acid/ DPA), and C22:6n3 (docosahexaenoic acid/ DHA) [32]–[34]. A smell-fish aroma and the yellow-beige color might indicate an existing omega-3 DHA content in the lipid [35]. These applications align with the growing demand for sustainable and bio-based alternatives.

Despite the promising results, certain limitations should be acknowledged. Using a specific strain and controlled laboratory conditions may not fully represent the variability that could be encountered in real-world applications. Additionally, the study focused on lipid extraction, and further research may be needed to explore the full spectrum of Aurantiochytrium microalgae's bioactive compounds. To build on the current findings, future research should explore [potential improvements or optimizations], investigate the scalability of the extraction process, and delve into applications that extend beyond the scope of this study. This will contribute to a more comprehensive understanding of Aurantiochytrium microalgae's potential in various industries.

For further research, it is essential to understand the biosynthetic analysis in the cultivation of biomass from native Indonesian Aurantiochytrium microalgae. It was known that lipid accumulation increases optimized conditions such as nutrition, airflow, and starving conditions [36]–[38]. Several studies have been conducted to identify critical parameters in optimum extraction processes [39]–[42]. However, the lipids extraction method from Indonesian Aurantiochytrium has never been published before. This experiment shows an optimum solvent combination to attain an optimum yield of valuable lipids in Aurantiochytrium biomass.

4. Conclusion

Aurantiochytrium microalgae is a promising raw material source of omega-3 DHA to replace a conventional source from fish raw materials. Producing valuable lipids from Aurantiochytrium microalgae is a challenging technology for future sustainable omega-3 production. This research showed the formula and stages of cultivation of native-local strain of Aurantiochytrium microalgae biomass isolated from Bunaken mangrove forest, North Sulawesi. Aurantiochytrium microalgae biomass was successfully cultivated, and the resulting lipids can be extracted using several variations of solvents. The most optimal solvent combination to extract lipids was acetone-chloroform and ethyl acetate. As a bioactive component, lipids from microalgae contain omega-3, DHA, and EPA which have several beneficial functions for human health. Since the extraction process of Aurantiochytrium microalgae isolated from Indonesian mangrove forests has never been published before, it is hoped that this paper will be a fundamental basis for further research on this topic.

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