Identification of bioactive compounds from *Nannochloropsis* sp.

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**Abstract.** Microalgae are known to have functional components for human life. Exploration of cholesterol-lowering active compounds from microalgae *Nannochloropsis* in Indonesia is still lacking. This research was aimed to identify bioactive compounds from *Nannochloropsis* sp that can reduce cholesterol level. Components of *Nannochloropsis* sp, including proximate, phytochemicals, beta-carotene, beta-sitosterol, stigmasterol, omega 3, and omega 6 were determined. Phytochemical test results showed that microalgae *Nannochloropsis* sp. positively contained flavonoids, tannin, glycosides, alkaloids, and saponin. Result of beta carotene was 2.74%, beta-sitosterol 7.25% stigmasterol 10.37%, beta carotene 145.26 ppm, omega 6 was 9.86% and omega 3 was 4.38% from total fatty acid.

**Keywords:** bioactive, microalgae, *Nannochloropsis* sp., phytosterol

1. **Introduction**

*Nannochloropsis* is greenish, not motile, and not flagging microalgae, and has round cells with a diameter of 2-4 μm (Wehr and Robert 2003). *Nannochloropsis* has six species, namely *Nannochloropsis gaditana, N. granulata, N. limnetica, N. oceanica, N. salina,* and *N. oculata* (Nian Ma et al 2016). *Nannochloropsis* is one type of microalgae that has been widely used as food for cultivation (Caesar 2015). *Nannochloropsis* is a microalga that has been widely cultivated and is rich in benefits, especially in terms of health. The nutrients contained in it include protein, carbohydrates, fats, some minerals such as Ca, K, Na, Mg, Zn, Fe, Mn, Cu, Ni, and Co (Rebollosio *et al* 2001), fiber (Kagana and Ray 2015) and vitamins such as tocotrienols (Durmaz 2007). Some beneficial pigments such as chlorophyll-a, zeaxanthin, canthaxanthin, astaxanthin and violaxanthin are found (Lubi’an *et al* 2000).

Cholesterol is a yellowish fat produced by the body, especially in the liver. Cholesterol has several benefits for the body, but on the other hand, it also has a negative impact on the body if the amount exceed normal level. Cholesterol attach to the surface of blood vessel and gradually harden and clog arteries. Cholesterol causes atherosclerosis, which results in cardiovascular diseases such as coronary heart disease, heart failure, hypertension and stroke (Kovala 2005).
A few research publications were found regarding the use of *Nannochloropsis* in reducing cholesterol levels. The study of Werman *et al.* (2003) that used *Nannochloropsis* sp to reduce cholesterol levels showed a positive result. They used three treatments, namely dry biomass, fat extract, and residue from *Nannochloropsis*. The results of that study indicated that the administration of 100 g/kg of dried biomass feed *Nannochloropsis* sp. can reduce cholesterol in mice.

*Nannochloropsis* is very potential as a source of bioactive compound because it has high growth rates, and easy to cultivate even in unfavorable environmental conditions (Borges *et al.* 2011). The nutrients contained in *Nannochloropsis* are also beneficial to the body, such as protein, carbohydrates, fats, minerals (Rebolloso *et al.* 2001), and vitamins (Durmaz 2007).

Remembering the dangers of excess cholesterol and the lack of further exploration of the ability of *N. oculata* especially in reducing cholesterol levels must be further investigated. So this research focuses on active ingredient in *Nannochloropsis* sp. as hipolipidemia. In this research, we tried to know the active ingredient contained in *Nannochloropsis* sp. and the relation in lowering cholesterol. Moreover, it focuses on phytosterol properties, beta carotene.

2. Materials and methods

2.1. Materials

In this research, we used fresh biomass of *Nannochloropsis* sp, ethanol, hexane, and KOH to extract crude fitosterol and reagents to analysis. The tools used were JA-14 rotor centrifuge, soxhlet extractor, oven, furnace 6,000, chiller, hotplates, reflux, sonicator TLC scanner, evaporator, and laboratory glassware.

2.2. Methods

2.2.1. Sample preparation. The fresh biomass was neutralized and centrifuged for 30 minutes by a JA-14 rotor centrifuge with a speed of 10,000 rpm and a temperature of 4°C, then the solution was removed and the supernatant was neutralized with distilled water and then centrifuged for 30 minutes by JA-14 rotor centrifuge at 10,000 rpm and 4°C. The collected supernatant was then ready to be extracted.

2.2.2. Proximate analysis. Proximate analysis was conducted according to the national standards of Indonesia consisted of moisture content (BSN 2015), protein content (BSN 2006), fat content (BSN 2017), ash content (BSN 2010) and carbohydrate analysis (Apriyantono *et al.* 1989).

2.2.3. Preparation Extract for phytosterol test. Crude phytosterol extraction was done by combining the extraction system with reflux and saponification with a modified Ham *et al.* (2000) method.

2.2.4. Test levels of phytosterol. The levels of phytosterol were analysed using thin layer chromatography scanner tool.

2.2.5. Analysis of omega 3 and omega 6. Omega 3 and 6 profiles were evaluated by GC-FID based on fatty acids methyl ester (FAME) production (O’Fallon *et al.* 2007).

3. Results and discussion

The moisture content for *Nannochloropsis* sp. powder was about 10%. The capacity to deliver microalgae powder with low water substance is critical to maintain nutrient content and prevent microbiological growth (Kent *et al.* 2015). Ash content in this study was higher than research that the
ash content only 11.32% (Kent et al 2015). The ash content was high indicating the algae can be a source of mineral for human nourishment (Sánchez-Machado et al 2004). Proximate investigation result demonstrated that Nannochloropsis sp. was a good source of protein. The level of protein was 31.68±0.37% as shown in table 1.

| Parameter                | Result                        |
|--------------------------|-------------------------------|
| Moisture content (%)     | 10.33±0.16                   |
| Ash content (%)          | 27.33±0.23                   |
| Lipid (%)                | 15.035±0.14                  |
| Protein (%)              | 31.68±0.07                   |
| Carbohydrate (%)         | 8.99±0.35                    |
| Beta carotene            | 145.26 ppm                   |
| B sitosterol             | 7.25%±0.14 per yield extract crude fitosterol |
| Stigmasterol             | 10.37%±0.46 per yield extract crude fitosterol |
| Omega-6                  | 9.86% of total fatty acid    |
| Omega-3                  | 4.38% of total fatty acid    |

Phytochemicals are a key factor in contributing to human health benefits. Nannochloropsis sp. positively contained flavonoids, tannin, glycosides, alkaloids, and saponin (table 2). Flavonoids contained in N. oculata biomass affect cholesterol levels by inhibiting cholesterol synthesis by inhibiting the synthesis of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase which is an enzyme responsible for cholesterol production in the body (Chen et al 2001).

Omega-3 content of this microalgae was 4.38%. Omega-3 contained in N. oculata can reduce cholesterol. Two of the three types of omega-3 namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are fatty acids that reduce cholesterol in the body, whereas alpha-linolenic acid (ALA) does not produce significant results in lowering cholesterol levels (Wilkinson et al 2005). Omega-3, specifically EPA and DHA can reduce triglyceride synthesis by reducing liver lipogenesis, increasing lipase beta-oxidation (Bays et al 2008).

| Parameters | Qualitative test | Description |
|------------|------------------|-------------|
| Flavonoid  | +                | Creation of intense yellow color, which becomes colorless on the addition of dilute acid |
| Tanin      | +                | Creation of white precipitate |
| Alkaloid   | +                | A yellow colored precipitate |
| Glycoside  | +                | Creation of pink to a blood-red color |
| Saponin    | +                | Foam |

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Tannin contained in *N. oculata* is one of the substances that work as an anti-hypercholesterolemia, which works by reducing cholesterol in the intestine (Tebib *et al* 1994). Tannins have the same ability as fiber in cholesterol in the body, namely by binding to bile acids that replace disrupted cholesterol micelles and increase excretion of cholesterol and bile acids in feces (Gato *et al* 2012).

*Nannochloropsis oculata* contains alkaloids which can inhibit the activity of the pancreatic lipase enzyme which works to break down fats into fatty acids and glycerol, thereby increasing fat excretion through feces. Diverting fat by the liver is blocked so that it is converted to cholesterol. Alkaloids increase the expression of low-density lipoprotein receptor (LDLR) of the liver and decrease the lesion and secretion of the LDLR modulator. The modulator converts subtilisin/Kexin type 9 (PCSK9) increases LDL-C and total cholesterol levels and growths HDL-C levels (Pirillo and Alberico 2015).

Saponins are glycosides that can reduce cholesterol. Saponins contained in *N. oculata* will form an insoluble complex with cholesterol, which inhibits the absorption of cholesterol in the intestine. Bile acid is a product of synthesis from cholesterol. Saponins form a large aggregate with bile acids in the gut which inhibits the absorption of bile acids. This increases the excretion of bile acids through feces, which causes a decrease in bile acids and cholesterol (Vinarova *et al* 2015).

The conclusion from this study showed that *Nannochloropsis* sp. had active compound that is useful to lower cholesterol and can be developed to be a functional food ingredient.

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