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Total content of Alcohol and other chemical phytoos from Microalga from Rawa River, Sungai Apit, Siak District

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ABSTRACT

This study aims to determine the phytochemical content, total alcohol, of the microalgae *Nannochloropsis* sp. *Chlorella* sp., *Trachelomonas* Sp. *Bacillaria paxillifera*, and *Cyclotellacryptica*. Microalgae were which extracted by single extraction using ethanol. Phytochemical screening was carried out qualitatively. Total alcohol and other biochemical analyzes were performed spectrophotometric ally by the Folin-Ciocalteu method. Phytochemical screening showed the presence of carbohydrate, glucose, protein and fat compounds, and alkaloids in the ethanol extract of the four types of superior microalgae. Meanwhile, most alcohol compounds were found in the *Trachelomonas* Sp. (7, 00 %). Meanwhile, of the 5 types of superior microalgae, it was known that the highest content of other biochemical compounds was found for glucose compounds of 32.30% in *Trachelomonas* Sp., carbohydrates 294.88% in *Nannochloropsis* sp, and 292.75% in *Cyclotellacryptica*. For the highest protein and fat content, respectively 412, 16 % and 26.43% were found in *Bacillaria paxillifera*. From the results of this study the types of *Trachelomonas* Sp. very suitable as a source of raw materials for bioethanol.

Key words : *Biomass, Superior microalgae, Phytochemical test, Total alcohol content*

Introduction

Many people believe that products labeled "natural" are safer and better for the body. The World Health Organization (WHO) estimates that 80% of the world's population uses herbal medicine to maintain their primary health. Exploration of biological materials and their potential as herbal medicine is currently being carried out. One of the commodities that have not been widely explored in Indonesia and have high potential in the development of herbal medicines is microalgae. Microalgae are rich sources of carbohydrates, protein, enzymes, and fiber. Also, microalgae contain vitamins and minerals such as vitamins A, C, B1, B2, B6, niacin, iodine, potassium, iron, magnesium, and calcium (Amin, 2009; Biller and Ross, 2011).

Microalgae are a type of seaweed or algae that are microscopic. In the food cycle in waters, microalgae play a role as the main producer. It is estimated that 40% of photosynthesis globally is carried out by microalgae (Sialve *et al*., 2009). Microalgae can be an alternative source of continuous and reliable natural products because microalgae can be cultivated in bioreactors on a large scale (Hoseini *et al*., 2013). Besides, the condition of microalgae cells can be controlled, using clean media for growth, so that they are not contaminated with herbicides, pesticides, and other toxic substances (Daranas *et al.,* 2001). Microalgae have been known as a source of various valuable pigments, namely chlorophyll a, zeaxanthin, canthaxanthin, and astaxanthin (Arifah *et al.,* 2019; Djunaedi, Suryono and Sardjito, 2017). Currently, several microalgae species have been produced on a large scale, namely *Bacillaria* sp. and *Chlorella* sp. for the consumption of human multivitamins and *Nannochloropsis* sp. for fish feed (Guedes *et al.*, 2015; Hemaiswarya *et al*., 2011; Shah *et al*., 2018).

Bacillaria sp. is a spiral microalga which due to its high nutritional properties and the presence of bioactive compounds such as phycocyanin is one of the most studied microalgae (Clifton *et al*., 2009; García-Casal *et al*., 2007). *Chlorella* sp. (Henley *et al.,* 2004; Reisser, 1984) categorized into the green algae group which has a genus number of about 450 and some species more than 7500. *Nannochloropsis* sp. is spherical unicellular green algae about 2–5 m in diameter, class Eustigmatophyceae. *Nannochloropsis* sp. plays an important role in the food chain system, generally used as feed and is widely cultivated for fish and shrimp farming (Gong *et al*., 2018; Jad-Allah and Nabris, 2012; Rodolfi *et al.*, 2003).

Phytochemical screening is a simple, fast, and highly selective method that can be used to identify groups of compounds and determine the presence of biologically active compounds distributed in plant tissue (Quideau, *et al.,* 2011; Santner, *et al.,* 2009). According to (Aiyelaagbe and Osamudiamen, 2009; Devi, *et al*., 2012; Mahajan and Badgujar, 2008) the results of the phytochemical screening test on the microalgae *Tetrachelmischui*, show that the ethanol extract of *Tetraselmischui* contains phytochemical compounds of the alkaloid, flavonoids and glycoside flavonoids. Many of the studies are interested in reviewing these microalgae phytochemical content, because of this phytochemical content required in various industries. Researchers who have conducted studies on these phytochemicals include (Chiu *et al*., 2017; De Mello-Sampayo *et al*., 2017; de Melo *et al*., 2019; Grumezescu and Holban, 2018; Hamouda Ali and Doumandji, 2017; Hussein *et al*., 2019; Rigobello-Masini and Masini, 2013; Salem *et al*., 2014; Singh, *et al*., 2019; Vicky *et al*., 2016; Yadavalli *et al*., 2018). To find sources of bioethanol as raw material, as well as protein, carbohydrate, glucose, and fat from microalgae, research was carried out on five superior microalgae species from Sungai Rawa, Sungai Apit District, Siak Regency which are easy to cultivate. This study aims to study the phytochemical content, total alcohol content, and biomass of the five microalgae species.

Materials and Methods

Material

The materials used in this study were pure cultures of microalgae *Nannochloropsis* sp. *Chlorella* sp., *Trachelomonas* Sp. *BacillariaPaxillifera*, and *Cyclotellacryptica*. Ethanol pa (Merck), methanol pa (Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), ciocialteufolin, 2,4,6-tri-pyridyl-s-triazine (TPTZ) (Sigma-Aldrich), Dragendroff reagent, and Mayer reagent, NPK and EDTA fertilizers.

Method

Cultivation and harvesting of microalgae type *Nannochloropsis* sp. *Chlorella* sp., *Trachelomonas* Sp. *Bacillaria paxillifera*, and *Cyclotellacryptica*.

In sterile seawater media, the microalgae culture of *Nannochloropsis* sp. *Chlorella* sp., *Trachelomonas* sp. *Bacillaria Paxillifera*, and *Cyclotellacryptica* with initial densities of 1×10^4 cells/ml each, then added with Conway fertilizer (Vicky *et al*., 2016; Yadavalli *et al*., 2018). Microalgae cultivation is carried out for 5-7 days. Harvesting was carried out at the age of Bacillaria sp. 5–7 days of maintenance that is in the exponential phase (growth phase). Microalgae biomass was harvested using a centrifuge for *Nannochloropsis* sp. *Chlorella* sp., *Trachelomonas* sp. *Bacillaria paxillifera* and *Cyclotellacryptica*, and filtering microalgae by using paper millipore. The biomass is then washed with water with a ratio of 1: 20 (biomass: water) and centrifuged using a centrifuge at a speed of 2455 G, temperature 10 °C, for 10 minutes. The supernatant was removed and the biomass sediment was washed again with water. This washing was repeated until the biomass had a pH of 7. The washed biomass was then dried using a freeze dryer to obtain microalgae powder.

Microalgae extraction

Microalgae were extracted by adding ethanol with a ratio of biomass and ethanol 1: 10, macerated for one day, and centrifuged at a speed of 2455 G, and a temperature of 10 °C, for 10 minutes. The concentrated supernatant was collected and then concentrated using a vacuum rotary evaporator (Buchi R-

205 and Buchi, R-215) until no solvent remained. Then the extract which dries in a rotary evaporator flask is put into a dark bottle and dried with the help of nitrogen gas. This extraction process was repeated 3 times.

Phytochemical test

Preparation of test solutions for phytochemical screening (carbohydrates, glucose, protein, and lipid) was carried out by dissolving the microalgae extracts of *Bacillaria* sp., *Chlorella* sp. and *Nannochloropsis* sp. in ethanol pa with a ratio of 1: 10. Alkaloid examination was carried out by reacting to the test solution with Dragendroff reagent and the test solution with Mayer's reagent. The formation of orange deposits in the reaction with Dragendroff reagent and yellow precipitate in the reaction with Meyer reagent indicates the presence of phytochemicals (Fithriani *et al.,* 2015; Firdiyani and Agustini, 2015; Nasrul Sani *et al.*, 2014).

Total alcohol test

The total alcohol test of microalgae extract was tested based on the method of Chatatikun *et al*. (2013). The microalgae extract was dissolved in distilled water and made at a concentration of 10,000 ppm. A total of 50 µl of the extract was added with 50 µl of distilled water. The solution was then added with 50 µl of the 10% foline solution and 50 l of the bicarbonate solution (60 g/l). The microplates were then incubated at room temperature for 60 minutes. The absorbance was read with a spectrophotometer at a wavelength of 750 nm. Gallic acid standard was made by dissolving gallic acid in distilled water and made at a concentration of 3.125; 6.25; 12.5; 25; 50; 100 (g/ml). Aquades are also used as blanks. Standards and blanks are tested in the same way as samples. Total alcohol is calibrated against the Gallic acid standard and expressed as mg gallic acid equivalent (GAE) g-1D.W.

Analysis

The data from this research are tabulated and drawn in graphic form. Furthermore, it is discussed descriptively.

Results and Discussion

Identify types of microalgae

From the identification results of microalgae samples from the Rawa river, it is known that there are 29 species, including the Bacillariophyceae Class (19 species), the Dinophyceae class (9 species), Chlorophyceae (2 species), and Cyanophyceae (1 species) (Table 1). Microalgae from the class Bacillariophyceae (Diatom) are the most common species found. This type of Bacillariophyceae (Diatom), which is mostly in the RawaRiver, is thought to be because the microalgae included in this class have high adaptability and survival in various water conditions including extreme conditions. According to (Blanco *et al.,* 2008; Torres *et al*., 2008), the number of Bacillariophyceae (Diatom) classes in waters is caused by their ability to adapt to the environment, are cosmopolitan, resistant to extreme conditions, and have high reproductive power. (Kaczmarska *et al.,* 2013; Miralto *et al.,* 1999) stated that when there was an increase in nutrient concentration, diatoms were able to reproduce three times in 24 hours, while dinoflagellates were only able to do it once in 24 hours under nutrient conditions. the same (Armbrust and Galindo, 2001; Chepurnov, *et al*., 2004; Vargas *et al.,* 2006).

Table 1. Identification and abundance of 5 microalgae species from the waters of Sungai Rawa, Mekar Jaya Village, Sungai Apit District, Siak Regency

No	Spesies	Total (cell/l)	Average (cell/l)
1	*Nannochloropsis sp.*	307.8	34.2
2	*Chlorella sp.*	664.2	73.8
3	*Trachelomonas Sp.*	243	27
4	*Bacillaria Paxillifera*	121.5	24.3
5	*Cyclotellacryptica*	162	18

Microalgae abundance and biomass

Based on the predetermined stations, each station 1-9 the number of microalgae found, which is greater than 63%, comes from the Bacillariophyceae (Diatom) class. However, there is a difference at stations 5-7, namely that there is no Cyanophyceae class. Meanwhile, the other classes Dinophyceae and Chlorophyceae were found with the same percentage respectively 13.5%. The Bacillariophyceae (Diatom) class is abundant in water by 73%, higher than stations 1, 3, 6, and 9.

The abundance of *Bacillariophyceae* sp (Diatom) is thought to be due to the high fluctuation in the station area, apart from being an area that is busy with shipping activities, this area also gets a lot of nutrient input from river flows. According to (Dickenson *et al.,* 2011; Dittmar and Kattner, 2003) microalgae from the Bacillariophyceae (Diatom) class have a very fast response to nutrient additions and can adapt to the environment in which they live compared to genera from other classes (Robles -Molina *et al*., 2014). Furthermore, based on the results of the identification, there were 5 types of microalgae, namely *Nannochloropsis* sp. *Chlorella* sp., *Trachelomonas* Sp. *Bacillaria paxillifera*, and *Cyclotellacryptica* and these five types can be found in all stations and become the most abundant among other types, and are therefore called superior types. For the calculation of biomass for 5 superior microalgae species, it is shown in Table 1.

From the calculation of biomass for these 5 types of microalgae, it is known that the types of *Trachelomonas* Sp. It was the highest species (5.02 grams/l) at the beginning of culture, but after being cultured for 15 days, the *Nannochloropsis* sp species showed the highest biomass value (5.16 g/l). From Table 2 shows that *Nannochloropsis* sp, *Trachelomonas* sp and *Bacillaria Paxillifera* are 3 types of microalgae that are suitable to be cultivated and developed in scale bulk for greater interest and industry.

Microalgae phytochemical content

Phytochemical test results from the microalgae biomass of *Bacillaria* sp., *Nannochloropsis* sp. and *Chlorella* sp. showed that *Bacillaria* sp., *Nannochloropsis* sp., and *Chlorella* sp. contains carbohydrates, glucose, protein, alcohol, and fat. However, saponins were only detected in *Bacillaria* sp. and *Chlorella* sp. while triterpenoids were not detected in the three types of microalgae. Furthermore, based on the results of the phytochemical analysis in the laboratory, the results of the concentration of fat, carbohydrate, protein, water, and alcohol content were obtained as presented in Table 3.

From the results of laboratory analysis of the biochemical content of microalgae from the Rawa Mekar Jaya river, after being cultured for 15 days, the results showed that the best ethanol content was in the species *Trachelomonas* Sp. Meanwhile, other species that have the potential to be used as raw material for ethanol are Synedraamphycephala and *Bacillaria paxillifera*. The three species of microalgae show that the ethanol content they contain is indeed quite high compared to other species. Besides that, these three species can develop well in culture media. This also allows this species to be able to increase its ethanol content under the conditions of the culture media. This is possible because these three species have good adaptability to the culture media given to them (Xin *et al*., 2010; Xin *et al*., 2011). However, it is said that the optimal value is not yet known to support the development of this species.

Then based on the biochemical analysis in the laboratory, it is known that the five microalgae species also contain important compounds and have the potential to be developed as future industrial products. The other biochemical compounds that are also contained by these five microalgae are alcohol, glucose, fat, carbohydrates, and protein. The highest biochemical content of these five species was (1) the highest alcohol content was found in *Trachelomonas* sp species, (2) the highest glucose content was found in *Trachelomonas* sp species (3) the highest fat content was found in the *Choloecaetesp* species, (4) the highest carbohydrate content was found in the species *Synedraamphycephala* and *Trachelomonas s*p, and (5) the highest protein content was found in the *Choloecaete* sp.

Based on the results of these biochemical tests, it shows that the five species have the potential to be developed as a source of raw materials for future industrial interests. The biochemical test results show that the biochemical content of the five species is not the same, and each species has its own advantages. However, from the five species, it shows that the *Trachelomonas* species is very potential to be de-

Table 2. The most biomass of 5 microalgae species comes from Sungai Rawa, Mekar Jaya Village, Kec. Sungai Apit, Siak District cultured on a laboratory scale

No	Microalgae species	Microalgae on Day 1 (gram)			Microalgae on Day 15 (gram)		
		Gross weight	Dry Weight	Biomass	Gross weight	Dry Weight	Biomass
1	*Nannochloropsis sp.*	3.55	0.23	3.33	8.11	2.95	5.16
2	*Chlorella sp.*	3.93	0.17	3.76	3.61	0.85	2.76
3	*Trachelomonas Sp.*	5.99	0.97	5.02	6.59	1.78	4.81
4	*BacillariaPaxillifera*	3.01	0.13	2.88	4.99	0.03	4.96
5	*Cyclotellacryptica*	2.57	0.06	2.51	33.21	1.30	31.91

veloped (Franklin *et al.,* 2004; Linton *et al*., 2010). This is possible because this species contains several strategic biochemicals in its cells (de Farias Silva and Bertucco, 2016; Torres *et al*., 2008) . In addition, this species is a species that is fast growing and developing, and this is a pointing characteristic in preparing raw material sources later. From the aspect of developing biochemical quality, it shows that this species can be increased its biochemical content through the management of culture media that supports its growth. It is estimated that this species growth can be triggered through the provision of fertilizers and the correct dosage. Where the content of phytochemical compounds is influenced by many factors such as species, varieties, growing conditions, seasonal variations, meth ode processing, and storage (Pasquet *et al*., 2011; Turon *et al*., 2016).

Carbohydrates are food reserves for microalgae and function to help their metabolic processes (Bach Knudsen *et al*., 2012; Jeukendrup, 2010). Li[pid is an important compound for microalgae and play a role in circulating material feeding to other organs (Abumrad and Davidson, 2012). Protein also a food reserve for microalgae function helps the reproductive process of microalgae (Leidy *et al*., 2015). G glucose is the same as carbohydrates and plays a role in the process of growth and photosynthesis of microalgae (Machado, *et al*., 2019; "Mecanismosmolecularesqueintervienen en el transporte de la glucosa," 2007). While alcohol has an important role in microalgae life (Simanjuntak, 2009; Zorcand Grga, 1999). The chemical compound is produced from the fermentation of chemical compounds contained in the microalgae cells (Pandey, 1992; Pandey *et al*., 2000). The results of phytochemical tests showed that the five types of superior microalgae could be used as raw material for bioethanol, but the concentrations in each of these species varied. This is because the phytochemical content in cells also varies according to the type. Also, note chemical compounds that are part of instead of sugar from saponin turned out to have antioxidant activity which has benefits such as lowering the risk of cancer and heart disease.

The results of the total alcohol content test for the 5 types of superior microalgae showed that the total alcohol varied between three and seven percent. On the microalgae *Trachelomonas* sp. The content of the aloe value reaches 7%. While the total alcohol produced by microalgae is other relatively low more as found on *Nannochloropsis* sp. and *Chlorella* sp. Only 4%, *Bacillaria paxillifera* 3.5%, and the lowest is *Cyclotellacryptica* 3%. The difference in total alcohol in the same species is possible because total alcohol is influenced by several factors, including stress. According to (Masojídek, Torzillo, andKoblízek, 2013) sunlight is a form of stressor that can increase the biosynthesis of alcohol content in plant tissues. Furthermore, sunlight plays an important role in the anthocyanin biosynthetic pathway. The total alcohol content in plants is influenced by several factors, namely genetic, environmental, and technology applied after the harvesting process (Barros *et al*., 2015; Varela *et al*., 2015).

The alcohol content is lower, can because cell walls of microalgae that are likely to be more pow-

Table 3. Phytochemical Content of microalgae were cultured on a laboratory scale for 15 days.

Phytochemical Contents	*Nannochloropsis sp.*	*Chlorella sp.*	*Trachelomonas Sp.*	*BacillariaPaxillifera*	*Cyclotellacryptica*
Alcohol (%)	Fermentation Time (Hours)	120	120	120	120	120
	Alcohol (%)	**4**	**4**	**7**	3.5	3
Glucose (gram)	Time (Hour)	120	120	120	120	16.457
	Absorbance	0.926	0.1876	0.2495	0.1082	120
	Concentration (ppm)	10.813	25.358	**32.298**	16.457	0.1187
Fat (%)	Wet	0,2155	0,2004	0,0694	0,2903	0,0244
	Dry	16,585	12,632	0,4766	**26,430**	0,3035
Carbohydrate (%)	Wet	38,318	38,142	41,703	26,796	23,518
	Dry	**294,878**	240,440	**286,348**	243,921	**292,746**
Protein (%)	Wet	23,748	47,241	32,996	45,266	17,284
	Dry	177,497	297,780	226,516	**412,158**	214,602
Water Content (%)	Weight spl	23,664	18,464	92,195	18,943	61,517
	Carbon	0,5412	0,5197	15,647	0,4303	0,7193
	Water weight	20,489	15,535	78,768	16,862	56,570
	Water (%)	865,830	841,367	854,363	890,144	919,583

erful than the kind that has a higher alcohol content as *Trachelomonas* sp. so that the extraction process does not optimally remove all active substances of microalgae such. Some of the microalgae cell walls contain cellulose and some species have additional tri-laminar sheat (TLS) containing algae, a substance known to have resistance to degradation (Moreno-Garrido, 2008). In this study, when viewed from the alcohol content test value (Table 3), the tested microalgae is quite potential if used as a source of raw materials for producing alcohol because the value is quite high (> 3%). When compared with other microalgae species whose alcohol content is only <3%.

Conclusion

The research identified 29 species of microalgae from the Rawa river, and the highest abundance was found in 5 superior microalgae species, namely *Nannochloropsis* sp., *Chlorella* sp., *Trachelomonas* sp., *Bacillaria paxillifera* and *Cyclotellacryptica*. Based on the biomass calculation, each obtained *Nannochloropsis* sp., a total of 5.16 grams / l, *Chlorella* sp. amount of 2.76 g/L, *Trachelomonas* Sp. 4.81 g /L, *Bacillaria Paxillifera* 4.96 g /L and *Cyclotellacryptica* 31.91 g/L. While the results of phytochemical screening showed the presence of carbohydrates, glucose, protein, alcohol, and fat in superior microalgae starch. Meanwhile, the highest alcohol content was only detected in *Trachelomonas* sp. (7%), and this species can be used as raw material for bioethanol. Meanwhile, other potential phytochemicals for carbohydrate raw materials are the type of *Nannochloropsis* sp, *Trachelomonas* sp. and *Cyclotellacryptica*, glucose is the kind *Trachelomonas* Sp. , fat and protein are types of *Bacillaria paxillifera*.

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