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SCREENING PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF MICROALGAE STRAIN *Scenedesmus sp.* AUMA-020

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ABSTRACT
This study aimed to identify active substances that can use as antibacterial agents. To reach this target, different solvent extracts (n-hexane and methanol) of microalgae strain Scenedesmus sp. AUMA-020 were examined. Phytochemical screening of n-hexane extract from microalgae strains Scenedesmus sp. AUMA-020 showed the presence of terpenoids and saponins, while methanol extracts contained flavonoids, phenolics, steroids, terpenoids and saponins. N-hexane extract has vigorous antibacterial activity compared to methanol extract. Both extracts isolates showed no antibacterial activity against Escherichia coli bacteria. In conclusion, the studied microalgae strain Scenedesmus sp. AUMA-020 can be considered a potential
Keywords: Phytochemicals; Antibacterial Activity; Microalgae; Scenedesmus sp.

INTRODUCTION

The plant is one of the natural ingredients that are potent against various diseases caused by bacterial infections. Using natural ingredients is much more effective in reducing the side effects of drug use chemicals, either synthetic or semi-synthetic (Cowan, 1999). One of the natural ingredients that need to be developed is microalgae.

Microalgae produce bioactive compounds that can utilize in developing pharmaceutical fields such as antibacterial, antiviral, antifungal and anti-microalgae (Amaro et al., 2011), anticancer, and antioxidants (Laungsuwon & Chulalaksananukul, 2013). Furthermore, microalgae can also be a source of food supplements (Capelli & Cysewski, 2010), and food colouring (M. Bishop & M. Zubeck, 2012). Composition of bioactive secondary metabolites successfully isolated from freshwater of Thamirabarani River, Tamil Nadu, South India, namely acrylic acids, fatty acids, terpenoid, sterol, carbohydrate, acetogenin, and phenol (Prakash et al., 2011).

In previous research, Zainul et al. (Chaidir et al., 2016) have managed to isolate and identify the type of microalgae *Scenedesmus sp*. from lake Maninjau West Sumatra, Indonesia. The isolate was given the code *Scenedesmus sp.* AUMA-020. Based on these studies, the researchers determined the composition of secondary metabolites and the antibacterial activity of the isolate *Scenedesmus sp*. AUMA-020.

METHODOLOGY

Material: Microalgae *Scenedesmus sp*. AUMA-020 (collection laboratory Biochemistry Universitas Andalas), *Bold's Basal Medium* (BBM) ($NaNO_3$, NaCl, $MgSO_4.7H_2O$, K_2HPO_4, $CaCl_2.2H_2O$, KH_2PO_4, H_3BO_3, KOH, EDTA, $MnCl_2.4H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, MoO_3, $Co(NO_3)_2.6H_2O$, $CuSO_4.5H_2O$) Merck, n-hexane, a sterile distilled water, methanol, chloroform, ethanol, physiological saline, nutrient agar (NA), nutrient broth (NB), Muller- Hilton Media (MHM), the indicator bacteria *Escherichia coli,*

Staphylococcus aureus, Bacillus cereus and Bacillus subtilis.

Instrument: Spectrophotometer, autoclave, sonicator, centrifuges, laminar flow, incubator, Vortex, DensiChek (Vitek, Biomerieux) and tools glass,

Preparation of Sample Extracts

Microalgae growth curve

Growth curve determination is done by using a spectrophotometer, Microalgae *Scenedesmus sp.* AUMA-020 has purely grown using a medium BBM ratio of 1: 9 (v / v). Every day is calculated absorbance at maximum wavelength calibration using a spectrophotometer containing distilled water at wavelengths that have been obtained.

Microalgae Cultivation

Microalgae *Scenedesmus sp.* AUMA-020 cultivated in bottles of 500 mL in medium BBM 1: 9 (v/v). Culture is harvested at the beginning of the death phase. The process of harvesting is done by a deposition method. Biomass obtained dried and weighed dry weight to be used later.

Microalgae Biomass Extraction

Microalgae biomass was extracted using the maceration method with organic solvents, namely n-hexane and methanol, with a ratio of 1:10 (g/mL). Furthermore, in sonication for 40 minutes, then leave overnight. The solution extract dried, and the results so obtained crude extracts of microalgae.

Preliminary Phytochemical Screening

Microalgae the dried and extracted with methanol with a ratio of 1: 5 (w/v), then sonicated for 15 minutes. The extract obtained was added chloroform and distilled water in a ratio of 1:1 (v/v), shaken and left to form two layers, namely the chloroform layer below and above the water layer. The water layer was transferred to another test tube for testing of flavonoids and phenolics, while the chloroform layer was for examination of steroids and terpenoids (Dantas et al., 2019).

Test for Flavonoids (Sianidin Tests)

Flavonoid examination is done by taking some parts of the water layer into a test tube and then adding concentrated hydrochloric acid and some magnesium powder if the orange-to-red colouration indicates flavonoid compounds.

Test for Phenolic

The phenolic examination is done by taking some parts of the water layer into a test tube and adding 2-3 drops of 5% iron (III) chloride reagent. If a green-to-blue colour develops, this indicates the presence of phenols.

Test for Steroids and terpenoids (Liebermann Burchard)

Steroid and terpenoid tests are carried out by placing three drops of a layer of chloroform into the drip plate hole. Hole 1 as a control, hole 2 added one drop of acetic anhydride and sulfuric acid, and hole 3 added sulfuric acid p.a. the appearance of a green or blue ring in hole 2 indicates the presence of steroids and a red or purple ring in hole three indicates triterpenoid.

Test for Alkaloids

Alkaloid compound examination was conducted by the extract dissolved in H_2SO_4 2 N, then added reagent Dragendroff, when precipitation of red to orange indicates positive alkaloid compounds.

Antibacterial Susceptibility Test

Indicator bacteria

The media used is nutrient agar (NA). Weigh NA 2 grams and dissolve in distilled water to 100 mL and heat to dissolve completely. Then put into a test tube that has been sterilized, then the tube is tilted and cooled until solid. Someone ose stock of *Escherichia coli, Staphylococcus aureus, Bacillus cereus and Bacillus subtilis* respectively inoculated into the above medium and then incubated at 370C for 18-24 hours.

Bacterial culture indicator

Bacteria (*E. coli, S. aureus, B. cereus and B. subtilis*) were freshly inoculated in 1 ose into NB media, then incubated overnight at 37^0C. Each of the test bacteria was inoculated as much as 1% into 5 mL of physiological NaCl and covered with gauze. Then vortex with a low speed. Then the bacterial growth was observed until the turbidity

reached 0.5 standards McFarland using the DensiCheck.

Examination of antibacterial activity

Before testing, 10 mg of crude extract of microalgae *Scenedesmus sp.* AUMA-020 was weighed and dissolved with DMSO solvent (Najdenski et al., 2013) with a final concentration of 10% solvent to obtain a concentration of 10 mg/ml.

Antibacterial activity testing was performed using the disc diffusion method with an MHA medium. Bacterial liquid culture of the pipette of as much as 300 µL was spread into the liquid MHA medium on a Petri dish, then homogenized and wait for it to freeze. 75 µL of microalgae extract at 10 mg/mL concentration was taken and then eluted on a paper disc. The positive control treatment uses tetracycline antibiotics with a concentration of 30 µg, while the negative control uses a 10% DMSO solvent. The paper disc is placed into a frozen MHA medium and incubated at 37^{0}C for 24 hours. Measurement of clear zones formed is done using callipers (mm).

Results and Discussion

Isolates Growth Curve Microalgae

Growth can be defined as an increase in cell mass coupled with its size by synthesizing macromolecules to produce new structures. As with other organisms, microalgae growth is influenced by the intensity of light, temperature, pH, and nutrients contained in a growth medium (Duerr et al., 1998). Isolates microalgae *Scenedesmus sp.* AUMA-020 were grown in media *Bold Bassal's Medium* (BBM), and absorbance was measured every day at a maximum wavelength that is *Optical Density* (OD) 680 nm. The growth of microalgae isolates on medium BBM-based absorbance. The growth curve of microalgae isolates. *Scenedesmus sp* AUMA-020 can be seen in Figure 1.

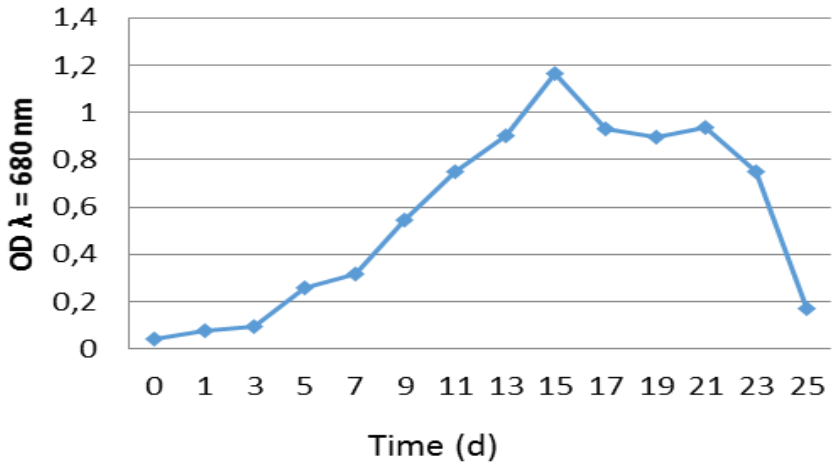

Figure 1. The growth curve strain *Scenedesmus sp*. AUMA-020 Isolates.

Based on the growth pattern of the microalgae isolate above, it was seen that *Scenedesmus sp.* AUMA-020 isolates did not pass through the lag phase. This happens because the isolates *Scenedesmus sp.* AUMA-020 in the inoculation of cultures in the exponential phase did not experience a lag phase. In addition, it isolates *Scenedesmus sp.* AUMA-020 had been grown in the same medium and, thus, no longer had time for adaptation—*Scenedesmus sp.* AUMA-020 experienced a death phase on day 21. Phase towards death indicates the number of cells that die more number of living cells. This is because the nutrients in the growth medium have run out while the surviving cells are not able to grow and survive only.

Microalgae Isolates Phytochemicals

Phytochemical compounds are metabolites produced as products of the secondary metabolic

processes of micro-organisms that are beneficial to humans and other creatures because many of them are drugs (Wawrosch & Zotchev, 2021), pigments, vitamins or hormones. Phytochemical test microalgae *Scenedesmus sp*. AUMA-020 was performed using two organic solvents, n-hexane and methanol. The results of the phytochemical test are presented in Table 1.

Table 1. Chemical content of microalgae strain *Scenedesmus sp*. AUMA-020

Chemical Compound	Solvent	AUMA-020 Isolate
Alkaloids	Hexane methanol	- -
Flavonoids	Hexane methanol	- +
Phenolic	hexane methanol	- +
Steroids	hexane methanol	- +
Terpenoids	Hexane methanol	++ +
Saponin	Hexane Methanol	++ -

Keterangan: + : presence, ++ : More presence, - : Absence

The above table shows that microalgae *Scenedesmus sp* AUMA-020 does not contain the alkaloid compounds in hexane and methanol. Phytochemical test strains of microalgae *Scenedesmus sp.* AUMA-020 with n-hexane solvent positively to terpenoids and saponins and methanol positively to the flavonoids, phenolic, steroids and terpenoids. The presence of phytochemical compounds is expected in microalgae *Scenedesmus sp* AUMA-020 has the potential for antibacterial activity. Benzene and ethyl acetate extracts of *Chlorococcum humicola* contain bioactive compounds such as carotenoids, alkaloids, flavonoids, fatty acids, saponins, amino acids, chlorophyll which effectively gives 80% inhibit microbial growth (Bhagavathy et al., 2011). The methanol extract of microalgae Chlorosarcinopsis sp revealed the presence of Alkaloids, Anthraquinones, Cardiac glycosides, Flavonoids, reducing sugars, Saponins and Terpenoids (Angayarkanni, 2013).

Antibacterial Activity Isolates Microalgae

Test results extract for antibacterial activity of microalgae strain *Scenedesmus sp*. AUMA-020 are presented in Table 2.

The magnitude of the inhibitory region which is formed adapted to CLSI criteria (2014) that if the area resistor formed ≥ 19 mm is said to be sensitive, and if the area resistor ≤ 14 mm, it is said to be resistant, while between 14 mm - 19 mm is said to be intermediate to the concentrations of tetracycline were dripped into the *paper discs* of 30 g (Franklin R. Cockerill, III & Jean B. Patel, PhD, 2015).

The amount of antibacterial activity is indicated by the clear zone around the *paper* disc. Based on table 2, each microalga extract of *Scenedesmus sp*. AUMA-020 only showed the presence of antibacterial activity against Gram-positive bacteria, namely *Staphylococcus aureus, Bacillus cereus*, and *Bacillus subtilis* and the absence of antibacterial activity against Gram-negative bacteria, namely *Escherichia coli*. It is equally generated in research by Salem et al. (Salem et al., 2014) where *Scenedesmus sp.* has no inhibition on Gram-negative bacteria *Klebsiella pneumonia* in methanol and acetone extracts.

Table 2. The results of the antibacterial activity test strain *Scenedesmus sp*.AUMA-020.

Micro-organisms	Zone of Inhibition in mm		
	Positive Control	*Scenedesmus sp.* AUMA-020 Isolate	
		Methanol Extract	n-hexane Extract
Staphilococcus aureus	20	11	25
Bacillus cereus	29.5	11	20
Bacillus subtilis	23	12	21
Escherichia coli	-	-	-

In this study, hexane extract in microalgae *Scenedesmus sp*. AUMA-020 showed solid antibacterial activity in inhibiting the growth of *Staphylococcus aureus* (25 mm), *Bacillus cereus* (20 mm) and *Bacillus subtilis* (21 mm). At the same time, the antibacterial activity of small methanol solvents in inhibiting bacterial growth are *Staphylococcus aureus* (11 mm), *Bacillus cereus* (11 mm) and *Bacillus subtilis* (12 mm). Given the magnitude of the inhibition zone, hexane, compared to methanol, can be assumed to be many secondary metabolites that are dissolved in non-polar solvents compared with polar solvents. Studies by Shannon et al. (Shannon & Abu-Ghannam, 2016) reported the effects of the extraction method and the use of solvents to affect the potency of antibacterial activity. Incubation temperature, pH of the culture medium, prolonged incubation, light intensity and nutrient content of the medium is other factors that influence the antibacterial activity (Noaman et al., 2004).

Conclusion

Screening phytochemicals from *Scenedesmus sp*. AUMA-020 isolate with n-hexane solvent has a chemical content of terpenoids and saponins compound, while methanol contains flavonoids, phenolics, terpenoids and steroids. N-hexane extract of microalgae *Scenedesmus sp.* AUMA-020 provides a powerful antibacterial activity in inhibiting the growth of gram-positive bacteria compared to the methanol extract. The presence of antibacterial activity is due to the content of secondary metabolites owned by the microalgae *Scenedesmus sp*. AUMA-020.

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