CHEMICAL COMPOSITION, PHENOLIC, TANNIN AND ANTIOXIDANT ACTIVITY IN WATER AND METHANOL EXTRACT FROM MARINE MICROALGAE *Oscillatoria sp.*

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ABSTRACT

The *Oscillatoria sp* microalgae have the potential for functional food because it has a high antioxidant activity, phenolic content, and tannin content. This experiment used the extract of microalgae in water and methanol solvent. The structure of chemical composition using FTIR analysis resulted in the functional group of O-H, C=C, C-H, C-O-P, O-CH₃, -N=O, S=O, C-N, and C-O-O-C. The peak amount in water extract is more than methanol extract. The water extract analysis gave the antioxidant activity with IC₅₀ of 0,16 µg/mL, phenolic content of 232 mg/g, and tannin content of 479.23 mg/L. The methanol extract analysis gave the antioxidant activity with IC₅₀ of 0.85 µg/mL, phenolic content of 201 mg/g and tannin content of 319.45 mg/L. The water extract has a high potential for functional food than methanol extract because in water extract antioxidant activity, phenolic content, and tannin were more than methanol extract.

Keywords: *Oscillatoria sp*, Antioxidant, Chemical Composition.

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INTRODUCTION

Blue-green algae cyanobacteria is a type of algae that can live in seawater and freshwater. One of the cyanobacteria microalgae is *Oscillatoria sp*. It has the potential to produce beneficial ingredients such as polysaccharides, hormones, vitamins, minerals, protein, and bioactive compounds.^{1,2} This group of algae has potential as an antioxidant³ and anti-inflammatory⁴ and it can be used for nutraceutical and functional food.⁵

Most of the natural antioxidants are derived from terrestrial plants but they can also find in unicellular microalgae as an alternative source of natural antioxidants. Microalgae is a novel source of natural antioxidants and used as additives in the food industry.⁶ The microalgae can prevent the accumulation of free radicals.⁷ The important class of antioxidants in microalgae is phenolic compounds. The antioxidant of microalgae correspondent to phenolic compounds. The fraction that has rich in phenolic compounds also had a high antioxidant capacity. Phenolic compounds are important antioxidants. They can donate an electron or a hydrogen atom to form the stable radical.⁸

Many researches has been reported on the antioxidant activity in microalgae⁹ such as *Spirulina plantesi*¹⁰, *Haematococcus pluvialis*¹¹, *Synechococcus*¹², *Brotyococcus braunii*¹³, and *Phorpridium sp.*¹⁴ The group of cyanobacteria and potential for anti-cancer, anti-bacteria, and antioxidant.¹⁵⁻¹⁷ The potential of *Oscillatoria sp* as an antioxidant is limited to reported by the researcher. The chemical composition included functional group influences of the characteristic of microalgae as bioactive which act as antioxidants and other functions. It is necessary to the identification of the chemical composition of microalgae.

In this study, we investigated the chemical composition and contributed to phenolic substance to antioxidant activity from extract *Oscillatoria sp* in water and methanol solvent. The method to measure antioxidant activity was 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging and the phenolic content was estimated by the folin-ciocalteu procedure. The potential for functional food by analyzed of tannin in this extract.

EXPERIMENTAL

Extraction

Oscillatoria sp extraction was done using water and methanol extract. 2 grams of samples was dissolved in 250 mL water and 250 mL methanol. The sample was stirred at 150 rpm for 24 hours. The extract was filtered and residues were extracted until a colorless filtrate. The solvent was evaporated and used for analysis.

Antioxidant Activity by DPPH Method

Antioxidant activity was measured by the DPPH method. It is calculated as IC₅₀. 250 mg extract was dissolved in 50 mL of methanol. The volume of solution 0.1, 0.25, 0.5, 1.0, 2.0 ml added of 5 mL methanol. A blank solution was made from 11 mL of DPPH solution into a test tube and added 5 mL of methanol. All samples and blanks were stored in 37°C for 30 minutes and it was measured at 517 nm wavelength using spectrophotometer UV-Vis. The % inhibition was calculated and a linear equation was determined between the concentration of sample and % inhibition.¹⁸

Determination of Phenolic Content

Analysis of phenol content measured by the spectrophotometric method using Folin-Ciocalteu reagents. The concentration of the extract was 10 ppm and a volume of 0.1 mL was added folin-ciocalteu reagent in the ratio of 1:2 (reagent: aqua distillation) added 1 mL of Na₂CO₃ 7% and incubated for 30 minutes. Absorbance was measured at a wavelength of 750 nm. The standard solution used gallic acid with concentrations of 0, 20, 40, 60, and 80 mg/L.¹⁹

Determination of Tannin Content

0.05 g of extract was added to 50 mL of hot water and incubated for 20 minutes. The solution was filtered and 2 mL of supernatant added 150 mL aqua distillation, 5 mL indigo carmine and titration with KMnO₄ 0.1N with the color changed from blue to yellow. 10 mL filtrate added 10 mL NaCl-acid, 5 mL gelatin and 1 g kaolin (*a* mL). The solution was filtered, and 5 mL of filtrate added 150 mL aqua distillation and 5 mL indigo carmine and titration with KMnO₄ 0,1 N (*b* mL).¹

$$\text{Tannin Content} = \frac{(a - b) \times N \text{ KMnO}_4 / 0,1 \times 4,16 \times 1000}{\text{Volume of Sample (mL)}}$$

Where,

a = Sample volume for titration (mL)

b = Blank volume for titration (mL)

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition identified using FTIR analysis for detected of functional group in extracts of *Oscillatoria sp*.

Fig.-1: Spectrum of Infrared from *Oscillatoria Sp* (a) Methanol Extract, (b) Water Extract

Table-1: The Wavenumber and Chemical Composition of *Oscillatoria sp* Extracts

Methanol Extract		
Wavenumber (cm ⁻¹)	Functional Group	Chemical Group
3346	O-H	Hydroxyl group
1633	C=C	Alkenyl, aromatic ring stretch
1440	C-H	Alkana
1107	C-H aromatic	Alkana aromatic
603	C-O-P S-S C-I	Phosphate compounds disulphides Aliphatic iodo compounds
Water Extract		
Wavenumber cm ⁻¹	Functional Group	Chemical Group
3278	C-H aromatic	Alkana aromatic
2920	C-H	Methyl
2851	O-CH ₃	Metoxy, methyl ether
1628	C=C	Alkenyl
1537	N=O	Aliphatic nitro compounds
1415	S=O	Organic sulphate
1239	P-O-C	Organic phosphate
1041	C-N Si-O-Si	Primary amine Organic siloxane or silicon
853	C-O-O-C	Peroxides

Infrared spectroscopy can be used for the identification of organic and inorganic compounds. The fundamental concept of infrared activity is based on the molecule vibration or the functional group of compounds.²⁰ The C-H vibrations for methyl are the most characteristic of aliphatic fragment. Saturated (C=C-H) and or aromatic rings were found between 3150 and 300 cm⁻¹.

The C-H stretching was observed at about 3000 cm⁻¹. The vibration peaks between 4000-2500 cm⁻¹ related to C-H, N-H, and O-H. the peaks of O-H are the most important in infrared spectroscopy and it was observed at 3700-3600 cm⁻¹. The peaks for N-H stretching were observed between 3400 and 3300 cm⁻¹.

The microalgae contain a biological value, such as amino acids, protein, fatty acids, and antioxidants². Based on the research, the natural antioxidant can be found in microalgae.²¹

Antioxidant Activity

Antioxidants are the compounds that inhibiting the initiator or propagation of oxidizing chain reaction. Natural antioxidants gave biological effects such as antibacterial, anti-inflammatory, and, antiallergic.²²

Fig.-2: The Correlation between Concentration and Absorbance (a) Methanol Extract (b) Water Extract

Table-2: The Antioxidant Activity, Phenolic, and Tannin Content of *Oscillatoria sp* Extract

Extract	IC ₅₀ µg/mL	Phenolic (mg/g)	Tannin (mg/L)
Water	0.16	232	479.23
Methanol	0.85	201	319.48

Fig.-3: The % Inhibition of Extract in Methanol and Water

The antioxidant activity with the DDPH method showed a decrease in absorbance with the increase of inhibition. The higher of sample concentration, the higher of inhibition value. Based on Figure 2, the resulting equation for methanol extract was $y = 40.617x + 15.447$ with IC₅₀ was 0.85 µg/mL. It meaning that the sample has a strong antioxidant activity. In water extract gave an equation was $y = 43.66x + 42.955$ and IC₅₀ was 0.16 µg/mL. Based on the experiment showed the *Oscillatoria sp* has very strong antioxidant activity. The antioxidant activity from *Oscillatoria sp* was strong in water extract than methanol extract.

Phenolic Content

This experiment used the method of Folin-Ciocalteu for the analysis of phenolic content. The phenolic acts as an antioxidant because of its ability to eliminate free radicals and effective in inhibiting lipid oxidation.^{23,24}

Fig.-4: Correlation between Gallic Acid and Phenolic Content

Based on this research showed that *Oscillatoria sp* in water extract has phenolic content is higher than methanol extract. Phenolic content from water extract was 232 mg GAE/g and methanol extract were 201 mg GAE/g. It correlated to the antioxidant activity in water extract is higher than methanol extract. Phenolic compounds can function as antioxidants because of their ability to stabilize free radicals by giving hydrogen atoms to free radicals.²⁵ The experiment showed an increase in phenolic content, an increase in antioxidant activity. Phenolic acid is a secondary metabolite widely distributed in the plant. Polyphenol is plant metabolites characterized by the presence of several phenol groups.²⁶

Tannin Content

Based on the experiment resulted in the tannin content in water extract was higher than methanol extract. The water extract gave the tannin content was 479,23 mg/mL and methanol extract were 319,48 mg/mL. Tannin can decrease feed intake and net metabolizable energy. Tannic acids are the polyphenol which found and shown to possess antioxidant.^{27, 28}

CONCLUSION

The *Oscillatoria sp* in water and methanol extract has potential in antioxidant activity, phenolic content, and tannin content. The chemical structure with FTIR analysis showed the functional group in water extract has more than methanol extract. The functional group in chemical structure plays a role in antioxidant activity. The water extract has potential for functional food because they have antioxidant activity, phenolic and tannin content was more than methanol extract.

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