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Comparison of Maceration and Soxhletation Method For Flavonoid Production from *Spirulina Platensis* as a Sunscreen's Raw Material

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Abstract. *Spirulina platensis* is a blue-green microalgae (cyanobacteria) with high potential in the fields of health, cosmetics and food. Nowadays, blue-green algae are widely used as food supplements, but the use in the cosmetics field is still lacking. One of the compounds produced by *Spirulina platensis* is Flavonoid. Flavonoid is rarely used as cosmetics, but often used as food due to its antioxidant, anti-inflammatory, and antiallergic properties. However, when flavonoid is used on skin, it can absorb ultraviolet light which can be used as sunscreen. In this research, flavonoid extraction from *Spirulina platensis* was carried out with two methods, soxhletation and maceration. These two methods are chosen based on better guarantee of the acquisition of flavonoids, eventhough they are quite simple. The solvent used to extract flavonoid is ethanol. The quantitative analysis was carried out with a spectrophotometer to measure the flavonoid content and the Sun Protection Factor (SPF) level. The highest yield extract of flavonoid were obtained by the soxhletation method with a yield value of 5.26%. The value of Total Flavonoid Content obtained from soxhletation methods has higher results, that is 0.65 % (g quercetin/g biomass). The highest SPF value was obtained by the soxhletation method with the value 6.47.

INTRODUCTION

Arthrospira platensis or more commonly known as *Spirulina platensis* is a filamentous microalgae which belongs to the type of blue-green algae (Cyanobacteria). *Spirulina platensis* is rich in protein, vitamins, essential amino acids, minerals, and essential fatty acids [1]. Besides having various nutrients that are good for the body, *Spirulina platensis* also produces various secondary metabolites. One of the secondary metabolites produced by *Spirulina platensis* is Flavonoids.

Flavonoids are a group of natural substances with varying phenolic structures. Flavonoids are secondary metabolites commonly obtained from fruits, vegetables, whole grains, bark, roots, stems, flowers, and tea. Flavonoids mainly used in nutraceutical, pharmaceutical, medicinal and cosmetic applications for its antioxidant, anti-inflammatory, and antiallergic properties [2]. However, based on research conducted by Ebrahimzadeh et al. , Flavonoids also have a role in protecting against UV rays [3].

In extracting flavonoids, a polar solvent is needed to dissolve the flavonoids so that they are separated from *Spirulina platensis*. From various studies on extraction of flavonoids, there are several solvents used in extracting flavonoids such as methanol [4], ethanol [5], and water [4]. However, ethanol is chosen because it is safer for the body than methanol, and more volatile to be separated than water.

There are various methods that can be done to extract flavonoid content in *Spirulina platensis*. Examples of commonly used methods are soxhletation and maceration. Both methods are commonly used because they are quite simple to do and have high success rates in extracting flavonoids. For this reason, in this research maceration and soxhletation extraction will be carried out on *Spirulina platensis* using ethanol.

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MATERIALS AND METHODS

Instruments

Instruments used in soxhletation method are flask with soxhlet apparatus, heater, and distillator. Instrument used to measure the absorbance of the extract is Spectrophotometer UV-VIS.

Materials

The materials used to extract flavonoid from *Spirulina platensis* are fresh *Spirulina platensis* as the flavonoid's source, zarrouk medium, ethanol (99%, Merck) as extraction solvent, natrium acetate 1M (Merck), aluminium chloride 10% (Merck), methanol (Merck), filter paper, and aquadest.

Spirulina platensis Cultivation

The *Spirulina platensis* brought from Pusat Penelitian Bioteknologi LIPI, Cibinong. Then *Spirulina platensis* cultivated in Laboratorium Rekayasa Bioproses, Department of Chemical Engineering, Universitas Indonesia. The medium used in *Spirulina platensis* cultivation is Zarrouk medium with dilution (5 times) [6]. The composition of Zarrouk medium before dilution are presented in Table 1.

TABLE 1. Zarrouk medium composition

No	Substance	Concentration (gr/L)
1	$NaHCO_3$	18
2	$NaNO_3$	2.5
3	KH_2PO_4	0.5
4	K_2SO_4	1
5	NaCl	1
6	$CaCl_2$	0.04
7	EDTA	0.8
8	$MgSO_4$	0.2
9	$FeSO_4$	0.01

Cultivation was carried out by making and sterilizing Zarrouk's medium. Then Zarrouk's medium was diluted with aquadest with Zarrouk's medium ratio: aquadest = 1: 4. Then *Spirulina platensis* and diluted Zarrouk medium (*Spirulina platensis*: dilute Zarrouk medium = 1: 2) poured so that it is mixed in a small photobioreactor. This is done so that Spirulina platensis can adapt to Zarrouk's medium. *Spirulina platensis* is cultivated with continuous lighting with 23 watt LED lights. Then the lighting level was measured with lux meters to position the culture to get a lighting level between 3000-5000 lux. Furthermore, the culture is given aeration using a blower so that the absorption of carbon dioxide gas in air bubbles is more optimal.

Spirulina platensis Harvesting

Spirulina platensis is harvested using a centrifuge with a capacity of ± 2 liters. The centrifuge is run at 4000 rpm for 17 minutes considering the ability of the centrifuge to separate the *Spirulina platensis* slurry from the water and the rest of the medium. After that, the water and medium separated from the *Spirulina platensis* slurry were taken using a small hose, so that only *Spirulina platensis* slurry was left behind. *Spirulina platensis* slurry was then filtered using a screen printing cloth with a mesh size of t180. Water will flow through the screen printing cloth, so that the water content in the slurry is reduced. Then the *Spirulina platensis* slurry was collected in vial bottles of 100 ml or more.

Extraction of Flavonoid from *Spirulina platensis*

The extraction starts by mixing 2 gram of *Spirulina platensis* with 100 ml ethanol. For extraction with maceration method, mixture was left in sterile environment for 2 days. And for soxhletation method, the mixture poured into flask and was kept at 60-80 ºC for 4 hours. Then, the mixture filtered through filter paper to separate the cell debris from liquid extract. The liquid extract poured into petri dish to evaporate the ethanol from extract.

Analysis of Dry Flavonoid Extract

Estimation of Total Flavonoid Content

The estimation of total flavonoid content follows the aluminum chloride colorimetric method by Chang, et al. [7] 20 mg of dried extract was dissolved with 10 ml of methanol in vial bottle to form a sample solution with a concentration of 2000 ppm. Then take 0.5 ml of sample solution to be mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$ solution, 0.1 ml of CH3COONa, and 2.8 ml of aquadest. Then homogenize using vortex for 3 minutes and incubate for 30 minutes. Measure absorbance at a wavelength of 415 nm. The blank used was the same mixture with 10% $AlCl_3$ solution which was replaced with aquadest. The results of the absorbance measurements were compared with the standard quercetin curves that had been made.

Estimation of Sun Protection Factor

The estimation of sun protection factor follows the method carried out by Yulianti et al. [8]. 30 mg of dried extract was dissolved in 15 ml ethanol in vial bottles to form a sample solution with a concentration of 2000 ppm. Then measure the absorbance of the sample at a wavelength of 290-320 nm with a range of 5 nm.

RESULTS AND DISCUSSION

Extraction of Flavonoid from *Spirulina platensis*

The results of extracting 2 grams of Spirulina platensis slurry using 100 ml ethanol with maceration and soxhletation methods (3 repetition) are presented at Fig. 1.

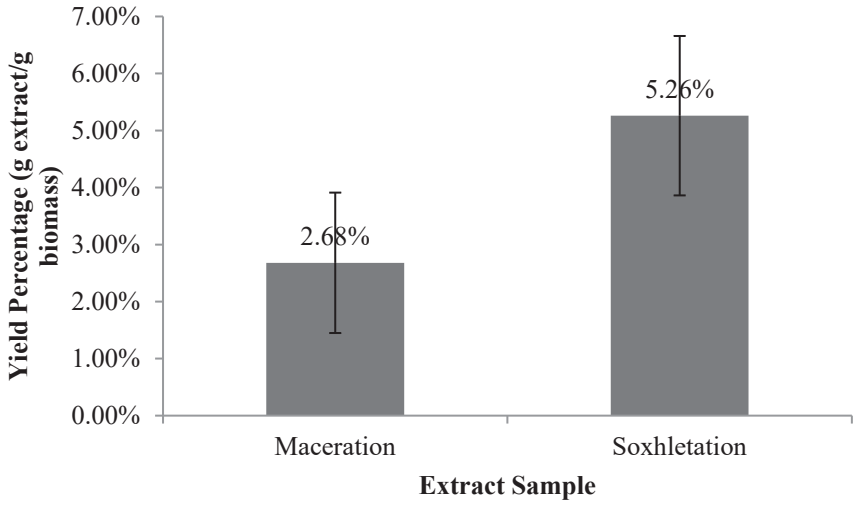

FIGURE 1. The result of Flavonoid's extraction from *Spirulina platensis*

Based on these results, it can be seen that the soxhletation method gives a higher yield than the maceration method. This is caused by the present of heating in the soxhletation method. In addition, the high water content and

solvent volume to the dry base increase the yield of the flavonoid extract. Different amounts and types of solvents also affect the amount of yield produced.

Estimation of Total Flavonoid Content

The result of estimation of total flavonoid content from dry flavonoid extract with maceration and soxhletation methods (3 repetition) are presented at Fig. 2.

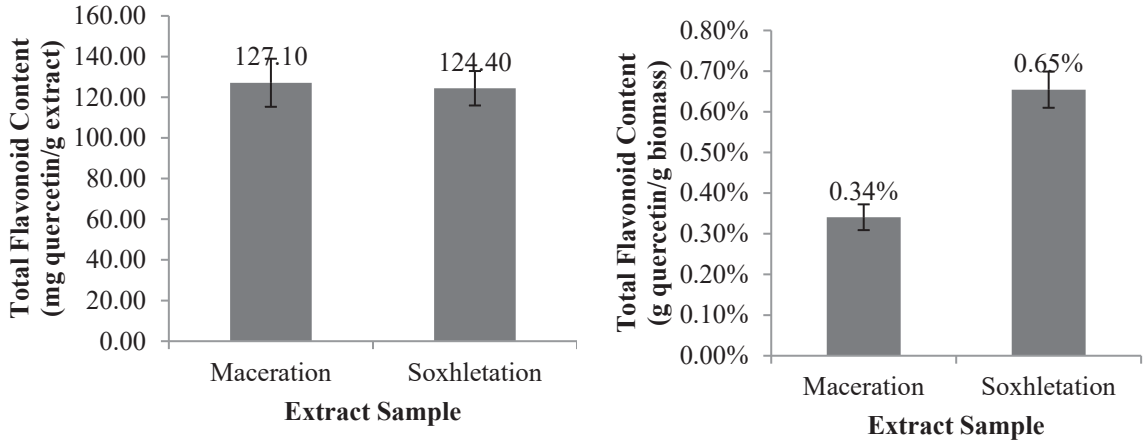

FIGURE 2. Estimation of Total Flavonoid Content

Based on these results, it can be seen that either maceration method or soxhletation method gives similar total flavonoid content result in mg quercetin/q extract unit. This shows that the quality of the flavonoid extract obtained through maceration and soxhletation methods is not very different. However, this does not change the fact that the amount of yield produced by the soxhletation method is greater than the maceration method. It can be seen when TFC using g quercetin/g biomass unit.

When compared with the results obtained by Agustini et al (2015) who compared the TFC value of fresh and dried *Spirulina platensis* extract with soxhletation and reflux extraction, when using ethanol solvents, the value of TFC in the extraction results in this study was higher. The TFC value of Agustini et al. study was 25.66 mg quercetin / g extract for samples of fresh *Spirulina platensis* with a moisture content of 90%. As for the dry sample, the TFC value obtained was 110.13 mg quercetin/g extract [9]. This is influenced by the different types of *Spirulina* used, as well as the difference in the amount of solvent used. With higher amount of solvent, the higher amount of flavonoids can be achieved. However, the optimum amount of solvent needed is still unknown.

Estimation of Sun Protection Factor

The result of estimation of sun protection factor (SPF) from dry flavonoid extract with maceration and soxhletation methods (3 repetition) are presented at Figure 3.

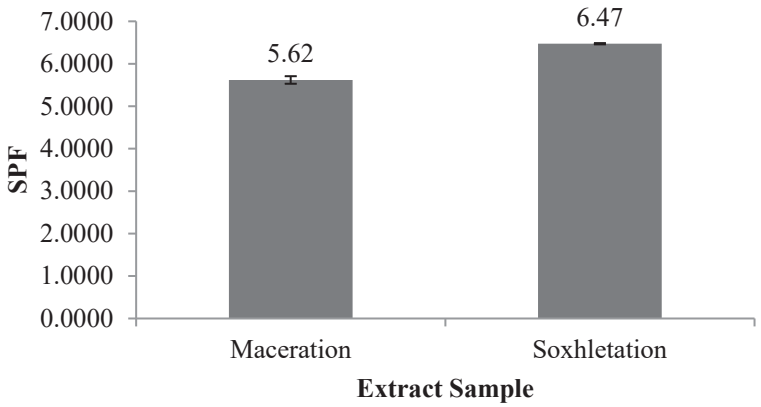

FIGURE 3. SPF's estimation of 2000 ppm flavonoid extract

Based on the data, the flavonoid extract from the soxhletation method has a slightly higher SPF value when compared to the maceration method. This shows that the heating that occurs in the soxhletation method causes substances other than flavonoids to be extracted, such as mycosporine-like amino acids (MAAs) [10], scytonemin [11], and astaxanthin [12].

SPF values are generated when using extracts at the maximum percentage (7%, data not shown) or 0.07 g extract/1 gram sunscreen is equal to 19.66 for maceration extracts and 22.65 for extracting soxhletation. This SPF value is quite high because other UV protective substances have not been added.

CONCLUSION

Based on the results of the study, it can be concluded that the soxhletation method shows good results on the yield of flavonoid extracts and on the SPF values of flavonoid extracts. The yield produced by the soxhletation and maceration methods was 5.26% and 2.68% based on the mass of *Spirulina platensis* in slurry form. On the number of equivalent flavonoid extracts, the average total flavonoid content of the flavonoid extract with the soxhletation and maceration methods was not much different, that is 124.37 mg quercetin/g extract and 127.10 mg quercetin/g extract. Even so, the value of Total Flavonoid Content obtained from soxhletation methods has higher results, that is 0.65 % (g quercetin/g biomass). While the SPF level of flavonoid extract with the soxhletation method was slightly higher when compared to the maceration method, it is 6.472 and 5,168, respectively. This is caused by the presence of UV protective substances such as MAAs, scytonemin, and astaxanthin. On the other hand, maceration method can be useful for extraction of subtance that weak of heat, like protein. In its application on a larger scale, calculations of energy, the amount of solvents, and the time period needed to produce flavonoid extracts are needed. Overall, microalgae *Spirulina platensis* extract has great potential value of flavonoids.

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