Effect of fucoidan concentration from \textit{Sargassum} sp. on skin lotion antioxidant activities

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Abstract. Fucoidan is a polysaccharide with complex sulfate groups that are found in the cell walls of brown seaweed, one is \textit{Sargassum} sp. This study aimed to determine the effect of addition fucoidan from \textit{Sargassum} sp. on the antioxidant activity of skin lotion and to determine antioxidant activity of skin lotion with the addition of antioxidants after storage. The method used was experimental with completely randomized design (RAL) with four treatments and five replications. The treatments used are: P1 (fucoidan 1 X IC$_{50}$), P2 (fucoidan 2 X IC$_{50}$), P3 (fucoidan 3X IC$_{50}$) and Control (Skin lotion with green tea extract commercial). The antioxidant activity test is done with DPPH method. The results showed that the IC$_{50}$ value of fucoidan extract were 1407.667 ppm. In the result of adding fucoidan on skin lotion produce result IC$_{50}$ values P1, P2, P3 and Control in a row was 1709, 816, 645 and 314 ppm. While the IC$_{50}$ value of skin lotion 30th day in a row was 1723, 825, 699 and 323 ppm. Based on these results, the skin lotion with fucoidan has a weak antioxidant activity because it had IC$_{50}$ values above 200 ppm, while storage for 30 days can increase the value of IC$_{50}$.

1. Introduction

The skin is one of the most important parts of the body because it functions as the widest body protector. In order for the skin to be maintained and function as it should be, skin care cosmetics are needed [1]. The process of skin damage marked by the appearance of wrinkles, scales, dryness and cracking is caused more by free radicals. Besides looking dull and wrinkled, the skin becomes older faster and black spots appear [2]. Ultraviolet light exposure also gives bad effects on the skin such as premature aging to skin cancer. Consumption of antioxidants in the skin is needed by the skin to fight free radicals [3].

Cosmetics consist of the oil phase and the water phase, where some of the oil components contain unsaturated bonds. The oil or fat component in cosmetics is easily oxidized, which causes bad aroma compounds or causes safety problems such as skin irritation. The addition of antioxidants to cosmetics aims to prevent changes in the quality of cosmetics [4]. Many studies show that fucoidan has significant antioxidant activity in vitro research. Natural antioxidants from fucoidan have the potential to be agents of free radical prevention [5]. Fucoidan is a polysaccharide with complex sulfate groups that are found in brown seaweed cell walls [6], one of which is \textit{Sargassum} sp. [7].
2. Materials and methods

This research has been conducted in Education Laboratory, Faculty of Fisheries and Marine Resources, University of Airlangga Surabaya in March-May 2016.

2.1. Tools and materials

The tools needed in this research is a thermometer, a water bath, a glass stirrer, a cool box, pipettes, glass beaker, centrifuge, Fourier Transformed Infrared Spectrometer (FTIR), UV-VIS spectrometer, a water bath, analytical balance, measuring cups, pumpkin measuring, dark bottles, oven, vortex mixer, a sterile plastic, scissors and knives.

Materials used in this study is the brown alga *Sargassum* sp., Distilled water, citric acid, Na2HPO4, CaCl2.2H2O, ethanol, methanol, commercial fucoidan, a solution of DPPH (1,1-diphenyl-2-picrylhydrazyl), stearic acid, alcohol cetil, liquid paraffin, glycerin, nipagin (methyl paraben), Tri Ethanol Amines (TEA), dimeticone and perfume. Brown algae *Sargassum* sp. used came from Mandangin Island waters, Sampang. Fucoidan used commercially branded "Fucoidan 100".

2.2. Research procedure

The first stage carried out in this study was fukoidan extraction. The extraction carried out refers to the research of [9] to get fukoidan from *Sargassum* sp. This research was conducted in several stages. In the first step, the crushed seaweed was treated maceration at 60° C with a McIlvaine buffer buffer pH 4 with a ratio of 1:10 (w / v) for three hours. The extract was separated from the algal residue by filtration. In the second step, the extracted filtrate was added with a solution of 3% CaCl2 at a ratio of 1: 1 (v / v) and allowed to stand for more than 12 hours at 4 ° C. Then do the separation again with vacuum filtration. The third stage, the resulting filtrate was added with ethanol in a ratio of 1: 2 (v / v). The mixture is allowed to stand for more than 12 hours at 4 ° C. Then ethanol was separated to get fukoidan by 6000 rpm centrifugation for 15 minutes. Then it is carried out drying at 50° C, softened, the yield value is calculated and stored for analysis.

Fukoidan that has been obtained from the extraction was identified by using FTIR spectrophotometry compared with commercial fukoidan at wave numbers 4000-400 cm-1. The wave number is a medium infrared region which is related to the transition of vibrational energy from molecules that provide information about the groups of groups function in these molecules [10]. Fukoidan IC50 values were calculated by the DPPH method (1,1-diphenyl-2-picrylhydrazyl). Fukoidan solution samples were made in several concentrations, namely 0.1 ppm, 1 ppm, 10 ppm 100 ppm and 500 ppm. DPPH solution was made with a concentration of 50 ppm then a maximum wavelength of absorption was 517 nm determined by UV-Vis spectrophotometer [11]. Antioxidant tests were carried out by the DPPH method with a test using UV-Vis spectrophotometer. Absorption or absorbance of the test solution is measured at maximum wavelength. From the absorbance data obtained then calculated% inhibition of extract against DPPH free radicals by the equation [11]. After obtaining the% inhibition data from each concentration, then the IC50 value was calculated using a linear regression equation y = a + bx by entering the value 50 as y [12].

The fukoidan IC50 value is used as part of the dose of fukoidan in skin lotion. Making skin lotion is done by separating the ingredients into two parts, namely oil-soluble material (oil phase or preparation 1) and water-soluble material (water phase or preparation 2). Ingredients including oil phase include stearic acid, cetyl alcohol and liquid paraffin put into the beaker glass. Ingredients include water phases such as glycerin, TEA and water [13]. Preparations 1 and 2 are heated and stirred at 70-75° C separately until homogeneous. Homogeneous preparations are mixed and stirred. The process of mixing the two different preparations was carried out at a temperature of 70° C. The stirring process is carried out until the two homogeneous preparations are mixed and reaches a temperature of
40° C (preparation 3) [12]. The foidane used is first dissolved into several parts of water and heated at 35-40° C before mixing into preparation 3. Mixing is done when preparation 3 reaches a temperature of 40° C. At 37° C, methyl paraben was put into preparation 3, then a deodorizer was added at 35 ° C. After the addition of fragrance, stirring continues until the skin lotion is formed [14].

Safety testing is available with an irritation test on 10 volunteers. The technique used is an open patch test (Patch Test), which is carried out by applying a formula to the back of the right hand voluntary area of 2.5 cm². Safety tests were carried out at the same place for 3 consecutive days after manufacture and on the last day of storage for each preparation. The symptoms that arise are observed. Generally, irritation will be immediately shown by the reaction of the skin shortly after sticking or touching the skin. Such irritation is called primary irritation with a + sign, but if this reaction occurs a few hours after touching or gluing the skin, then this irritation is called secondary irritation and is given a ++ sign. If skin irritation does not occur then it is marked - [15].

Skin lotions were stored for 30 days at room temperature of 25° C [16]. Testing the antioxidant activity of skin lotion preparations with fukoidan was done on the 0th day and the last day of storage by DPPH method. Then the data will be obtained IC50 value of skin lotion preparations during storage [12].

2.3 Data analysis

This study uses an experimental laboratory method in the form of making skin lotion with 4 treatments and 5 replications with one distinguishing factor, namely fukoidan concentration. The reading of the antioxidant activity of skin lotion was carried out on the 0th day of storage and 30th day of storage. The data obtained from the results of this study were IC50 value charts. The data will then be analyzed statistically descriptive to determine the trend of the results of research experiments, whether included in the category of low, medium or high [17]. The irritation test data was also analyzed statistically descriptive to determine the tendency of the nature of the skin lotion, whether primary, secondary or negative [17].

3. Results and discussion

3.1. The yield

Based on the fukoidan extraction that has been done, the result of dry fukoidan extract was 39.34 grams from 200 grams of *Sargassum* sp. dry. So the yield of fukoidan produced was 19.67%. Based on the extraction results that have been carried out, it was found that the yield of fukoidan was 19.67%. These results are greater than previous studies, namely fukoidan extraction from *Sargassum crassifolium* of 1.46% [18], *Sargassum polycystums* by 7.15% [19] and *Sargassum binderi* by 7.5% [20] with different methods.

3.2. Fourier transformed infrared (FTIR) spectrophotometric analysis

FTIR analysis aims to identify the results of fukoidan extraction from the study. The results of FTIR obtained from fukoidan extract were compared with FTIR from commercial fukoidan based on the standard based on literature. The results of the FTIR fukoidan extract and commercial fukoidan can be seen in Figure 1.
The peak of fucoidan extract at 1641 cm\(^{-1}\) and 1589 cm\(^{-1}\) in commercial fucoidan showed the absorbance of uronic acid, as research by [21], that the peak around 1633 cm\(^{-1}\) indicated the presence of uronic acid. Wave numbers 1408 cm\(^{-1}\) in fucoidan extracts and 1406 cm\(^{-1}\) in commercial fucoidan show variations of CH vibrations from carbohydrates, as explained by [19], wave numbers between 1420 - 1384 cm\(^{-1}\) show variations in CH vibrations from carbohydrates containing D-glucose, D-mannose, D-xylosa, and galacturonic acid. Peak 1132 cm\(^{-1}\) in fucoidan extract and 1122 cm\(^{-1}\) in commercial fucoidan showed asymmetric stretching of S = O and confirmed the presence of sulfate groups. This is in accordance with the research of [19], which obtained peaks at 1139 cm\(^{-1}\) and 1118 cm\(^{-1}\), namely stretching CH vibrations from the phucosa and axial S = O bonds at C-4 position. Peak 864 cm\(^{-1}\) in fucoidan extract and 877 cm\(^{-1}\) in commercial fucoidan showed C-O-S sulfate groups. The peak is not too different from the research of [19], which obtained peaks of 850 cm\(^{-1}\) and 820 cm\(^{-1}\) which are C-O-S sulfate groups. The existence of a peak that shows sulfate content plays an important role because it is an indicator of antioxidant activity in fucoidan [5].

3.3. Determination of the fucoidan IC50 value
Antioxidant activity was measured by looking at the ability of the sample to inhibit DPPH free radical activity. DPPH free radicals are stable free radicals in aqueous solutions or solutions in methanol and in an oxidized form having a strong absorption at 517 nm wavelength [22].

Based on the value of% inhibition obtained, the regression equation \( y = 0.027x + 11.993 \) with \( R^2 = 0.9767 \). The regression equation is used to calculate the IC50 value by entering a value of 50 as Y. So as to obtain the IC50 value of fucoidan extract of 1407,667 ppm. The IC50 value is used as a reference for the treatment dose in making skin lotion. R2 or R-Square is a coefficient of determination that explains the alignment of the regression model. The higher the value of R2, the better the value with a maximum value is 1. Based on the results of \( R^2 = 0.9767 \), the linearity relationship% inhibition is influenced by the absorbance obtained by 97.67% while the remaining 2.33% is influenced by other variables that do not exist in the linear regression model [23].
3.4. Antioxidant activity of skin lotion

Skin lotion made with the addition of fukoidan has been analyzed its antioxidant activity by DPPH method on day 0 of storage and day 30 of storage. The antioxidant activity of skin lotion can be seen in table 1.

| Table 1. The antioxidant activity of skin lotion |
|------------------------------------------------|
| **Treatment** | **Day-0 storage** | **The 30th day of storage** |
|               | **IC50**          | **The antioxidant activity** | **IC50** | **The antioxidant activity** |
| P11           | 0.708            | 17.947                       | Weak     | .9536 | 22.742 | Weak     |
| P12           | .8065            | 19.95                        | Weak     | .7346 | 02.721 | Weak     |
| P13           | .9137            | 09.043                       | Weak     | .8755 | 02.108 | Weak     |
| P14           | .2175            | 61.163                       | Weak     | .8065 | 41.772 | Weak     |
| P15           | .4567            | 58.456                       | Weak     | .565  | 25.987 | Weak     |
| P21           | .6532            | 24.646                       | Weak     | .7057 | 4.785 | Weak     |
| P22           | .8262            | 6.2887                       | Weak     | .8966 | 5.520 | Weak     |
| P23           | .839             | 68.292                       | Weak     | .6103 | 0.740 | Weak     |
| P24           | 0.9457           | 5.7876                       | Weak     | .5375 | 7.025 | Weak     |
| P25           | .8099            | 4.6053                       | Weak     | .8661 | 7.522 | Weak     |
| P31           | .9108            | 4.7743                       | Weak     | .8553 | 4.176 | Weak     |
| P32           | 0.83             | 2.0137                       | Weak     | .7861 | 5.359 | Weak     |
| P33           | .7314            | 90.449                       | Weak     | .9447 | 9.050 | Weak     |
| P34           | .6532            | 8.2367                       | Weak     | .8315 | 8.323 | Weak     |
| P35           | .8631            | 1.2762                       | Weak     | .5597 | 0.651 | Weak     |
| K1            | 0.8022           | 1.022                         | Weak     | .8622 | 5.881 | Weak     |
| K2            | .8161            | 4.843                         | Weak     | .9015 | 3.445 | Weak     |
| K3            | .9036            | 4.2456                       | Weak     | .789  | 5.108 | Weak     |
| K4            | .7848            | 9.9084                       | Weak     | .7449 | 5.211 | Weak     |
| K5            | .871             | 3.0151                       | Weak     | .8793 | 3.294 | Weak     |

DPPH analysis is done using the same dilution concentration by diluting the IC50 analysis of fucoidan at 0.1, 1, 10, 100 and 500 ppm. According to the table 1 R2 and IC50 values obtained are different, so the only treatment of skin lotion with the highest R2 value of each treatment will be discussed. Making the best treatment based on the value of R2 highest of all treatments because R2 is a determination that explains alignment koefien regression model, ie the higher the value of R2, the better alignment [23]. Best skin treatment lotion on day 0 storage are P13, P24, P31 and K1, whereas
the best treatment of skin lotion on the 30th day of storage is P11, P22, P33 and K2. Skin lotion with the best treatment is presented in Table 2 and chart the best treatment IC50 values are presented in Figure 2.

**Table 2.** The antioxidant activity of skin lotion best treatment

| Treatment | IC50 (ppm) Early | IC50 (ppm) End | Antioxidant activity |
|-----------|-----------------|----------------|---------------------|
| P1        | 1709.043        | 1722.742       | Weak                |
| P2        | 815.7867        | 825.5202       | Weak                |
| P3        | 644.7743        | 699.0504       | Weak                |
| K         | 314.2456        | 323.4451       | Weak                |

Figure 2. Graph values IC50 best skin treatment lotions,
Information:  
K = Skin commercial lotion with green tea extract  
P1 = Skin lotion Extra fucoidan concentration of 1 X IC50  
P2 = Skin lotion Extra fucoidan concentration of 2 X IC50  
P3 = Skin lotion Extra fucoidan concentration of 3 X IC50

Fukoidan substitution in the manufacture of skin lotion affects the antioxidant activity of fukoidan itself. The previous fukoidan IC50 value was 1407.667 ppm to 1709.043 ppm in skin lotions with fukoidan concentration of 1xIC50. The antioxidant activity is lower because when making skin lotion, fukoidan undergoes a reheating process to a temperature of 40° C so that it can result in reduced antioxidant activity. Based on Figure 2, the higher the concentration of fukoidan in skin lotion the higher antioxidant activity is based on the lower IC50 value. The IC50 skin lotion value for the 0th day of storage (P1, P2, P3 and control) was 1709, 816, 645 and 314 ppm, while the IC50 skin lotion value for the 30th day was 1723, 825, 699 and 323 ppm. Based on IC50 skin lotion values obtained, it is known that storage time affects the antioxidant activity of skin lotion. The antioxidant activity of the skin lotion decreases during storage as seen from the higher IC50 value. This can occur because the antioxidants in skin lotion play a role in protecting the skin lotion itself from oxidation [24].

Skin lotion is an emulsion product containing fat or oil which is easily oxidized which causes the quality of the product will decrease, so the addition of antioxidants will reduce the speed of the oxidation process [25]. The oxidation reaction consists of three stages: initiation, propagation and termination. At the initiation stage, fatty acid radicals form, which are unstable and reactive fatty acid-derived compounds resulting from the loss of one hydrogen atom. In the propagation stage, fatty acid
radicals will react with oxygen to form peroxy radicals. At the termination stage, peroxy radicals will then attack fatty acids to produce hydroperoxides and new fatty acid radicals [26]. The hydroperoxide that is formed is unstable and will be further degraded to produce short chain carbonyl compounds such as aldehydes and ketones that cause rancidity in fats [25]. A good antioxidant will react with fatty acid radicals as soon as the compound is formed [26]. The antioxidant activity of skin lotion with fukoidan is still lower than the control of skin lotion with green tea extract. In a study conducted by [27], green tea extract had an IC50 value of 4.773 ppm so that its antioxidant activity was included in the very strong antioxidant category.

3.5 Irritation test
Skin irritation in general is a skin reaction resulting from touching an irritant on the skin. Symptoms that arise are generally in the form of redness of the skin, itching or burning sensation that arises shortly after touching or after several hours after touching the skin. The irritation test is carried out on volunteers with normal skin without any injuries. Volunteers consisted of 5 men and 5 women with an average age of 22 years because this age group is the age group that uses a lot of cosmetics [28]. The results of irritation tests that have been done show that all skin lotion formulas do not cause irritation both on day 0 of storage and day 30 of storage as shown in table 3. Thus, skin lotion with the addition of fukoidan is safe as a topical preparation.

4. Conclusions
Addition of fukoidan from Sargassum sp. in different concentrations gives effect to the antioxidant activity of skin lotions. The higher the concentration of fukoidan in the skin lotion, the antioxidant activity of the skin lotion also increased as evidenced by the smaller IC50 value. In contrast to the antioxidant activity of skin lotion after storage because it has decreased. All skin lotion treatments namely P1, P2, P3 and positive control decreased antioxidant activity as evidenced by the increased IC50 value of each skin lotion treatment. It is recommended in future studies to use other fukoidan extraction methods which allow to get high sulfate levels. Proximate analysis is also needed from fukoidan including sulfate levels produced and other polysaccharides that can affect antioxidant activity.

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