Formulation and evaluation of the Exopolysaccharide compound extract lotion from the Microalgae Spirulina sp.

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Abstract. Spirulina sp. is a type of microalgae containing exopolysaccharide compound composition. Exopolysaccharide compounds are biopolymers that have very wide applications, including as antioxidants. Antioxidant compounds have the potential to be developed as additives in cosmetics. Antioxidant compounds are useful for warding off free radicals from UV rays. This study aims to make a lotion formulation of exopolysaccharide compounds from microalgae Spirulina sp. This research was conducted by extracting exopolysaccharide compounds from Spirulina sp. using 96% ethanol. The lotion formulations were made with various concentrations of 0.5%, 1% and 1.5% exopolysaccharide compounds. The exopolysaccharide compound lotion then tested the lotion evaluation including organoleptic test, pH, homogeneity, dispersion, and irritation test. Organoleptic test results showed a white lotion, odorless, soft texture. The pH value of the exopolysaccharide compound lotion ranges from 5.75 to 6.15. Homogeneity shows no solid particles and meets the conditions. The spreadability of the lotion is between 5.8 - 6.0. The results of the irritation test using test animals showed a primary irritation index of 0, so it did not cause irritation. Based on the research results, it can be concluded that the exopolysaccharide compound extract from the microalgae Spirulina sp. can be made into a good and stable lotion formula.

Keyword: Spirulina sp., Exopolysaccharide, Lotion, Evaluation Test

1. Introduction
Microalgae are autotrophic organisms that grow through the process of photosynthesis and can live in all freshwater and marine areas. Microalgae that can be used as supplements or a source of natural medicine. One example of microalgae is Spirulina platensis [4,9].

Spirulina sp. is a microalgae that is widely distributed in nature and can be found in various types of environment, both in marine and freshwater [1]. Spirulina sp. is an autotrophic organism with a bluish green color consisting of cylindrical cells that form colonies where the cells are columned to form twisted filaments resembling a spiral [5]. Spirulina sp. produce exopolysaccharides to protect cells and as a place for microalgae to attach to a substrate to meet their nutritional needs [12]. Exopolysaccharides produced by microalgae have the prospect of being applied in a wide spectrum, such as in the food, pharmaceutical, cosmetic, and as a supplement industries [7, 10, 12].

Exopolysaccharide compounds are biopolymers that have very wide applications, including as antioxidants and antibacterials [7]. Antioxidant compounds and antibacterial compounds can be used
to protect the skin. The skin is the body's protective layer against external influences, both physical and chemical. Skin also supports one's appearance. The skin is usually irritated by touch stimuli, pain or bad influences from outside. These disorders cause the skin to become diseased.

Lotions are the most appropriate choice if you need a light moisturizer or when used for the whole body. Because it is light in shape and leaves no residue, the lotion can be used in the morning without worrying about sticking to clothes and is also used if you live in a humid climate or when the weather gets hot [14].

Based on the description above, research was carried out on the formulation and evaluation of the lotion from the microalgae exopolysaccharide compound Spirulina sp. The lotion formulation was carried out by varying the exopolysaccharide extract with the base. The results of this study were aimed at obtaining the novelty of lotions made from exopolysaccharide compounds from Spirulina sp. that qualifies for use.

2. Method

2.1. Tools and Materials
The tools used in this study are electric scales, oven, centrifuge, freeze dry, spatula, gloves, tweezers, loop needles, vials, tweezers, 250 ml erlenmeyer, beaker glas, measuring pipettes, test tubes, test tube racks, petri dishes, watch glass. The materials used were Spirulinasp.culture, 96% ethanol, steric acid, nipagin, nipasol, triethylenamine (TEA), liquid paraffin, glycerin, cetyl alcohol, distilled water.

2.2 EPS Harvesting and Extraction
Culture of Spirulina sp. separated from the biomass of Spirulina sp. using a 0.2 µm membrane filter to obtain the filtrate of Spirulina sp. Then do the separation between the biomass and the filtrate. The filtrate is then added with 96% ethanol, then centrifuged at 8000 rpm for 15 minutes at 4 oC.so that the exopolysaccharide solid is obtained, then the solid is separated. Then the drying is done using a freeze dryer [13].

2.3 Formulation and Manufacture of Exopolysaccharide Compound Lotions

| No | Name of Material     | Function     | F1 0,5% (b/b) | F2 1% (b/b) | F3 1,5% (b/b) |
|----|----------------------|--------------|---------------|-------------|---------------|
| 1  | Exopolysaccharide    | Active       | 0,2           | 0,4         | 0,6           |
|    | compounds            | substance    |               |             |               |
| 2  | Stearic acid         | Emulsifier   | 2             | 2           | 2             |
| 3  | TEA                  | Emulsifier   | 0,5           | 0,5         | 0,5           |
| 4  | Liquid Paraffin      | Moisturizer  | 5             | 5           | 5             |
| 5  | Cetyl Alcohol        | Stabilizer   | 3             | 3           | 3             |
| 6  | Glycerin             | Humectant    | 5             | 5           | 5             |
| 7  | Methyl Paraben       | Preservative | 0,1           | 0,1         | 0,1           |
| 8  | Propyl Paraben       | Preservative | 0,02          | 0,02        | 0,02          |
| 9  | Distilled water      | Solvent      | Ad 50         | Ad 50       | Ad 50         |

Weigh all ingredients according to the exopolysaccharide compound lotion preparation formulation. Combine the oil-phase ingredients (stearic acid, cetyl alcohol, and liquid paraffin) then heat it to a temperature of 70ºC using a porcelain dish. Mix the liquid phase ingredients (TEA, glycerin and distilled water) then heat using a beaker. Mix the oil phase and the liquid phase slowly using a preheated mortar and stamp until homogeneous. Add methyl paraben and propyl paraben then stir until homogeneous. Then add the extract and the remaining distilled water little by little, stirring homogeneously. Enter the lotion preparation into the lotion container. The lotion preparation is made
in 3 formulations, namely F1 with a concentration of 0.5% (w / w), F2 with a concentration of 1.0% (w / w) and F3 with a concentration of 1.5% (w / w) [2].

2.4 Evaluation Test of Spirulina sp. Exopolysaccharide Compound Lotion.

2.4.1. Homogeneity test. The active substance used is generally in the form of an extract which must be evenly distributed in the lotion preparation, so that the active substance must be homogeneously dispersed and mixed in the dispersion medium (base) in order to provide maximum effect.

2.4.2. Organoleptic test. Organoleptic tests include shape, color, smell and consistency.

2.4.3. Spreadability test. This is done by weighing 0.5 grams of lotion, placing the glass over the mass of the lotion and leaving it for 1 minute. Measuring the spread diameter of the lotion (taking the average length of the diameter from several sides) then adding 500 grams as an additional load. Each additional load was allowed to stand for 1 minute after which measure the diameter of the spread of the lotion recorded as before.

2.4.4. pH test. The pH test is carried out using a pH meter. The pH examination test is carried out using a pH meter which is inserted directly into the lotion preparation that has been dissolved with aquadest. The pH for topical preparations is usually the same as the skin pH, which is 4.5-7.

2.4.5. Skin Irritation Test. The test was carried out on healthy albino rabbits weighing 2-2.5 kg. Animals are acclimated in the bladder for 5 days. The test animal then shaved its back hair 24 hours before doing the test with an area of approximately 10x15 cm then divided into 4 areas with a size of 2x3 cm. The test is carried out on one test animal. Before being treated, the test area was cleaned with NaCl, then 0.5 mL of the preparation was applied to the back of the test animal and attached with a non-irritant plaster. Patches were made for the three exposures which were presented consecutively, patch 1 opened after 3 minutes. If no serious skin reaction is seen then the second patch is applied and the 2nd patch is removed after 1 hour. If the exposure did not cause severe irritation, and the test was judged to be continued then the third patch was carried out and the 3rd patch was opened at the 4th hour then the skin response to skin irritation was determined.

3. Results and Discussion

3.1 Harvesting and Extraction of Exopolysaccharides Compounds

Results from cultivation of the microalgae Spirulina sp. carried out separation between biomass and filtrate using a microfilter membrane filter. Filtering with a microfilter membrane aims to separate the filtrate liquid from the culture of Spirulina sp. with Spirulina sp. The filtrate results from filtering are then added with 96% ethanol as much as 2: 1, then the resulting filtrate changes color to white and a white precipitate occurs. then centrifuged at 8000 rpm for 15 minutes at 4ºC. Centrifugation is a fractionation process that aims to separate exopolysaccharide compounds from other compounds. The solid resulting from centrifugation is then freeze dry to obtain a dry crude extract of the exopolysaccharide compound.

![Figure 1. Exopolysaccharide Compound Crude Extract.](image)

3.2. Evaluation Test of Spirulina sp. Exopolysaccharide Compound Lotion.

The extract formulation of exopolysaccharide compounds from the microalgae Spirulina sp. Made in lotion dosage form. Lotion is an emulsion pharmaceutical preparation that is easy to wash with water and is less sticky than other topical preparations. In addition, its liquid form allows fast and even application to the skin.
Extracts of exopolysaccharides from microalgae Spirulina sp. has distinctive characteristics so that in the formulation it is necessary to obtain an effective base to produce lotion preparations with good stability. In this study, the lotion dosage form chosen was an oil in water (M / A) type emulsion. Emulsion type M / A is an emulsion consisting of oil droplets which are scattered or dispersed into water. The advantage of the M / A type emulsion is that it has good dispersibility properties on the skin, good release of drugs or active substances, does not inhibit the physiological function of the skin and is easy to wash so it is suitable for topical use [3]. The exopolysaccharide compound obtained, then made into a lotion preparation with the formulations F1, F2 and F3.

1. Organoleptic Test

**Table 2. Organoleptic Test.**

| Variable | Color | Smell    | Shape   | Texture |
|----------|-------|----------|---------|---------|
| F1       | White | Odorless | Semisolid | Soft   |
| F2       | White | Odorless | Semisolid | Soft   |
| F3       | White | Odorless | Semisolid | Soft   |

Based on the organoleptic test of each negative control, F 1, F 2, and F 3 showed that the lotion was white, and each lotion had no odor. This lotion has a soft texture, and does not feel sticky when applied to the skin.

2. Homogeneity, pH and Lotion Spreadability Test

**Table 3. Homogeneity, pH and Lotion Spreadability Test.**

| Formula | Homogeneity | pH   | Spreadability |
|---------|-------------|------|---------------|
| F1      | No solid particles | 6,11 | 5.8 cm       |
| F2      | No solid particles | 6,15 | 6,0 cm       |
| F3      | No solid particles | 5,75 | 6,0 cm      |

Based on the results of the homogeneity test of each formula, there are no coarse grains on the glass object, which means that this lotion has met the requirements. The results of the pH test using a pH meter showed that the lotion had met the predetermined requirements, namely between 4.5 to 8. The results of the dispersion test showed good results, namely the diameter obtained was 6 cm from all exopolysaccharide extract lotion formulas, which means that it has met conditions that have been set, namely between 5-7 cm of spreading power [11].

3. Irritation Test

**Table 4. Irritation Test.**

| Treatment | Reaction | Primary Irritation Index | Conclusion |
|-----------|----------|--------------------------|------------|
|           | Erythema | Udemad                   |            |
| K(-)      | 0        | 0                        |            |
| F1        | 0        | 0                        |            |
| F2        | 0        | 0                        | 0,0        | Not Irritating |
| F3        | 0        | 0                        |            |

Score of erythema and edema :

| Score | Degree of irritation          |
|-------|--------------------------------|
| 0     | There is no                   |
| 1     | Very Light                    |
| 2     | Light                         |
| 3     | Moderate                      |
| 4     | Weight                        |

Score of degree of irritation:

| Score | Degree of irritation          |
|-------|--------------------------------|
| 0     | Not Irritating                |
| 0.1-0.4 | Very little irritating    |
| 0.41-1.9 | Slight irritation           |
| 2.0-4.9 | Moderate Irritation         |
| 5.0-8.0 | Severe irritation            |
Based on the results of the irritation test using albino rabbits showed that each preparation was not irritating because of the absence of erythema and edema on the rabbit's skin.

4. Conclusion
Organoleptic test results showed a white lotion, odorless, soft texture. The pH value of the exopolysaccharide compound lotion ranges from 5.75 to 6.15. Homogeneity shows no solid particles and meets the conditions. The spreadability of the lotion is between 5.8 and 6.0. The results of the irritation test using test animals showed a primary irritation index of 0, so it did not cause irritation. Based on the research results, it can be concluded that the exopolysaccharide compound extract from the microalgae Spirulina sp. can be made into a good and stable lotion formula.

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