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Formulation and Antioxidant Activity Test of Phycocyanin Phytosomal Gel from Green Algae Extract (*Spirulina platensis*)

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ABSTRACT

Green algae (Spirulina platensis) contains phycocyanins are useful as natural antioxidants. Spirulina platensis extract in phytosome gel preparation intended to increase sorption efficiency, physical stability and antioxidant activity. The purpose of this study was to examine the effect of variations ratio of extract to soy lecithin on phytosome characteristics, addition of phytosomes to gel characteristics, and efficiency of sorption on the IC50 of the gel. Extraction of Spirulina platensis using maceration. Optimization of the phytosome formula was carried out with ratio of extract to lecithin, 1:1, 1:2, 1:3. Evaluation of phytosomes showed spherical vesicles, particle size 627.9 nm, polydispersity index 0.382, absorption efficiency of 94%, 95%, 96%. Physical evaluation of the gel showed good results in organoleptic, homogeneous, pH in range 6.3-6.4, viscosity in range 3896-3992 cPs, spreadability in range 6-6.5 cm, stickiness in range 5-6 seconds. Based on results of the irritation test, there was no irritation in each formula. The results of the physical stability test for 6 cycles at 4°C and 40°C showed all formulas had stable in all evaluation results except a decreasing pH. The IC50 results of Spirulina platensis extract and phytosome gel 6.53 µg/mL and 21.59 µg/mL are classified as super strength antioxidant category.

Keyword: Spirulina platensis; Phytosome; Gel; Antioxidant; Stability

ABSTRAK

Alga hijau (Spirulina platensis) mengandung fikosianin yang bermanfaat sebagai antioksidan alami. Ekstrak Spirulina platensis yang dibuat dalam sediaan gel fitosom dimaksudkan agar dapat meningkatkan efisiensi penjerapan, stabilitas fisik dan aktivitas antioksidannya. Tujuan penelitian ini yaitu mengkaji pengaruh variasi perbandingan kadar ekstrak dengan lesitin kedelai terhadap karakteristik fitosom, penambahan fitosom pada karakteristik gel, dan efisiensi penjerapan terhadap nilai IC50 gel. Ekstraksi Spirulina platensis menggunakan metode maserasi. Optimasi formula fitosom dilakukan dengan perbandingan ekstrak dengan lesitin yaitu 1:1, 1:2, dan 1:3. Evaluasi fitosom menunjukkan vesikel berbentuk sferis, ukuran partikel 627,9 nm dengan indeks polidispersitas 0,382, dan efisiensi penjerapan 94%, 95%, 96%. Evaluasi fisik gel menunjukkan hasil yang baik dari segi organoleptik dan homogen, pH pada rentang 6,3-6,4, viskositas pada rentang 3896- 3992 cps, daya sebar pada rentang 6-6,5 cm, dan daya lekat pada rentang 5-6 detik. Berdasarkan hasil uji iritasi didapatkan tidak menunjukkan adanya iritasi pada setiap formula. Hasil uji stabilitas fisik selama 6 siklus pada suhu 4°C dan 40°C menunjukkan semua formula memiliki hasil organoleptik, viskositas, daya sebar, daya lekat, dan homogenitas yang stabil namun tidak dengan pH yang semakin menurun. Hasil IC50 ekstrak Spirulina platensis dan gel fitosom sebesar 6,53 µg/mL dan 21,59 µg/mL tergolong kategori antioksidan sangat kuat.

Kata kunci: Spirulina platensis; Fitosom; Gel; Antioksidan; Stabilitas

INTRODUCTION

Premature aging is still a common skin problem in Indonesian society. Skin aging is characterized by changes in structure and skin physiology that causes the appearance of the skin to change. There are two types of factors causes of aging, namely intrinsic and extrinsic factors (Ekawati, 2021). Aging factor intrinsically, namely genetic and hormonal. Factors that can cause aging extrinsic, namely sunlight and air pollution. Sunlight is the main cause from extrinsic aging or often referred to as photoaging (Sumbayak, 2003).

Photoaging is a phenomenon of premature aging characterized by skin changes wrinkles appear due to excessive exposure to UV rays (Dampati, 2020). Deep UV A rays sunlight can cause production Reactive Oxygen Species (ROS) and reduce the amount of antioxidants in the skin resulting in aging problems (PERDOSKI, 2020).

One source of antioxidants from nature is *Spirulina platensis* with IC50 amounted to 49.59 ppm and is included in the very strong antioxidant category (Rahmawati, 2017). *Spirulina platensis* contains phycocyanin pigment which can function as an antioxidant with amounts reaching 14-20% of the dry weight (Agustina, 2018).

Phycocyanin which is hydrophilic can cause limitations to its antioxidant effectiveness when used topically because it cannot penetrate inside skin layer that tends to be lipophilic. Most antioxidants are also unstable to light and oxygen (Montenegoro, 2014). Phytosomes are one system

vesicular carriers that have been developed in drug formulations, nutraceuticals, and Cosmetics containing active natural ingredients are hydrophilic by forming complex of active compounds in phospholipids (phosphatidylcholine) so that they can penetrate better skin bilayer and increased bioavailability (Ramadon, 2016).

Deep antioxidants *Spirulina platensis* can be formulated into phytosome gel for use on skin. Based on the description above, the researcher aims to making phytosome gel preparations based on extractsSpirulina platensiswith physical properties and good antioxidant activity.

METHOD

Tools and Materials

The tools used in this research are analytical balance (ME204), paper strain, magnetic stirrer, glass slides, microscopes, vials, bandages, paper labels, tubes, aluminum foil, glassware (pyrex), spatula, stir stick, dropper pipette,rotary evaporator, turrax (IKA T25 digital ULTRA TURRAX), centrifuge (PLC SERIES centrifuges), particle size analyzer (PSA), and spectrophotometer UV-Vis (GENESYS 150 UV-Visible spectrophotometer).

The materials used in this research are dry biomass *Spirulina platensis* (PT Alga Biotechnology Indonesia), soy lecithin (Lansida), Karbopol, Glycerin, Triethanolamine (TEA), Methyl paraben, Propyl paraben,Ocean Fresh(Zenith), Gelatin, powder Mg, HCl 2 N, Mayer's reagent, Dragendroff's reagent, Liebermann-Burchard's reagent, solution 1,1-diphenyl 2-picryl-hydrazil(DPPH), albino rabbit, Ascorbic Acid, Ethanol 96%, and Aquades.

Spirulina platensis Extraction

The choice of method in this research is a modification of the previously research method by comparing the maceration extraction process, Ultrasound Assisted Extraction (UAE) and freezing extract was obtained *Spirulina platensis* with highest activity antioxidant in the maceration method (Rahmawati, 2017). First, weigh 50 grams of dry biomass *Spirulina platensis* and dissolved with 500 mL of 96% ethanol. Process soaking is carried out for 3x24 hours with solvent replacement every 24 hours. Furthermore the filtered filtrate was evaporated using solvent at a temperature of 40ºC rotary evaporator for 2 hours (Notonegoro, 2018).

Phytochemical Screening

The test solution is made by dissolving the extract *Spirulina platensis* in ethanol pa with a ratio of 1:10 then analyzed for phytochemicals.

a. Alkaloid

The test solution was mixed with Mayer's and Dragendroff's reagents and the reaction was observed happen. Alkaloids are detected if a yellow precipitate forms after being reacted with Mayer's reagent or an orange-red precipitate forms after being reacted with the reagent dragendroff (Farnsworth, 1996).

b. Flavonoid

The test solution was mixed with 0.5 g of Mg powder and 1 mL of concentrated HCl. Flavonoids detected if the color changes to red, yellow, or orange (Farnsworth, 1996).

c. Saponin

The test solution was shaken until it foamed. Saponin is detected if a stable foam forms l-10 cm high for 10 minutes, and did not disappear after adding 2N HCl (Farnsworth, 1996).

d. Tannin

The test solution was dissolved in distilled water and heated for 15 minutes filtered. The filtrate was mixed with 1% gelatin solution and shaken. Tannins are detected if a white precipitate is formed (Farnsworth, 1996).

e. Steroids and Triterpenoid

A little ether was added to the test solution and then shaken. The ether layer formed was dripped off on a drop plate and heated until dry, then 3 drops of reagent are added Liebermann-Burchard. Triterpenoids are detected if the color changes to orange, red, or yellow if a green color forms then the sample is positive for steroids (Farnsworth, 1996).

Analysis of Phycocyanin Biopigment Levels

Phycocyanin biopigment analysis was carried out using UV-Vis spectrophotometry. Extract solution *Spirulina platensis*. The absorbance was measured with a wavelength of 615 nm and 625 nm for phycocyanin pigment (Rahmawati, 2017). Phycocyanin concentration (KFS) ago calculated using the Bennet and Bogoard (1973) equation, namely:

$$KFS = \frac{(A_{615}) - 0{,}474(A_{625})}{5{,}34}$$

Information:
KFS = Phycocyanin concentration (mg/mL)
A615 = Absorbance at wavelength (λ) 615 nm
A652 = Absorbance at wavelength (λ) 625 nm

Next, the yield is calculated using the following equation:

$$Yield = \frac{KFS \times V}{BB}$$

Information:
KFS = Phycocyanin concentration (mg/mL)
V = Volume of solvent (mL)
BB = Total of dry biomass (gr)

Table 1. Formulation and Manufacturing of Phytosome

Material	Function	Concentration (g)		
		F1	F2	F3
***Spirulina platensis* ethanol extract**	Active substance	1	1	2
Soy lecithin	Vesicle forming	1	2	1

Process :

Phytosomes were prepared via solvent evaporation and thin layer hydration methods. Soy lecithin dissolved in 10 mL dichloromethane, extract *Spirulina platensis* dissolved in 10 mL of ethanol 96% in separate container. These two mixtures were put into a round bottom flask and the

solvent evaporated at a temperature of 40 ºC at a speed of 45 rpm with rotary evaporator up to layers an even thin film is formed. This thin layer is kept for 24 hours and then hydrated with 20 mL of solution buffers phosphate pH 6.4 at 45 ºC using rotary evaporator at a speed of 90 rpm for 20 minutes, then sonicated. The phytosome is then inserted into in vials and stored at 7 ºC (Amalia, 2020).

Evaluation of Phytosomes

a. Microscopic Observation of Vesicles

Phytosomes were dropped onto a glass slide and observed using a microscope magnification 1000 times. A good vesicle shape is uniform and round (spherical). evenly distributed (Indalifiany, 2021).

b.Determination of Vesicle Entrapment Efficiency

Extract *Spirulina platensis* 10 ppm (in ethanol) the absorbance was measured with UV-Vis spectrophotometry at a wavelength of 400-700 nm. Phytosome suspension centrifuged at a speed of 6000 rpm for 1 hour. The absorbance of the supernatant was measured at the maximum wavelength. Entrapment efficiency can be calculated using the following equation:

$$\%EE = \frac{(Qt-Qs)}{Qt} \times 100\%$$

Information:
EE =Entrapment Efficiency
Qt = Total drug
Qs = Over-the-counter drugs
A good adsorption efficiency is one that is closest to 100% (Akib, 2021).

c. Determination of Vesicle Size and Size Distribution

Vesicles were measured using Particle Size Analyzer (PSA). Good vesicle size ranges from 10-1000 nm. Vesicle size distribution was determined by index values polydispersity. Vesicles can be said to be evenly distributed if the IP value ranges between 0.01 – 0.7 (Akib, 2021).

Table 2. Formulation and Manufacturing of Phytosome

Material	Function	Concentration (%w/v)	
		F0	**F1**
Phytosome suspension of *Spirulina platensis*	Active substance	-	2
Carbopol 934	Gelling agent	0.5	0.5
Glycerin	Humectant	5	5
Methyl paraben	Preservative	0.1	0.1
Propyl paraben	Preservative	0.1	0.1
TEA	Alkalizing agent	qs	qs
Ocean fresh	Fragrance	qs	qs
Aquades	Solvent	Ad 100	Ad 100

Process :

Carbopol is developed in distilled water and stirred using turrax. On the glass in another beaker both types of parabens are dissolved in glycerin. The mixture put into carbopol and stir thoroughly turrax with 6000 rpm speed up to homogeneous. The pH of the preparation is measured a little by little while adding TEA while continuing stirred. The phytosome suspension was added and stirred again until homogeneous. Final, added ocean fresh and the gel can be put into a container.

Evaluation of *Spirulina platensis* Phytosome Gel

1. Organoleptic Test

Organoleptic testing of phytosome gel preparations done by directly observing changes physical characteristics of the two dosage formulas, including the shape, color and aroma produced (Tutik, 2021).

2. Homogeneity Test

Homogeneity of the phytosome gel preparation was carried out by placing 1 g. The preparation is placed on a glass object and covered with another glass object, then the coarse grains are observed preparations (Tutik, 2021). A good gel preparation is one that does not contain granules rough (homogeneous) (Yati, 2018).

3. pH Test

pH testing is carried out by dipping a calibrated pH meter into the gel phytosome and observed the detected pH. The requirement for a good skin pH is 4.5-6.5 (Tutik, 2021).

4. Viscosity Test

Viscosity testing is carried out using a viscometerBrookfieldwithspindle number 9 at a speed of 50 rpm.The sample is placed in a finite viscometer spindle sink. The viscosity that is considered good for gel preparations is the viscosity value is in the range of 2000-4000 cps (Suryani, 2019).

5. Spreadability Test

Spreadability of the phytosome gel preparation was carried out by placing 1 g prepared on a glass object and covered by another glass object and then given a load of 125 grams, measurements are made using a ruler after 1 minute. Terms of preparation semisolid is 5-7 cm (Tutik, 2021).

6. Adhesion Test

Adhesive strength of the phytosome gel preparation was carried out by placing 1 g prepared on a glass object and covered by another glass object and then given a load of 125 gram for 5 minutes, both slides were removed and the adhesion time was calculated (Yati, 2018). The requirement for good adhesion is no less than 4 seconds (Lystiyaningsih, 2018).

7. Irritation Test

The irritation test was carried out on healthy, male albino rabbits with a body weight of around 2-2.5 kg. Before testing, the fur on the back area of the test animal was shaved to the size of 10x15 cm^2, this performed 24 hours before testing. Next, the test animal's back was divided into 3 sections for negative control, positive control, and normal testing. The sample is applied on the test area and covered with a bandage. After a period of time of 4 hours, the bandage was removed, and Clean the test area using water to remove any sample residue that may still be present there is. The test area was then observed and examined at time intervals of 24, 48, and 72 hours after treatment. If a skin reaction is seen, the reaction is assessed using a scoring scale which ranges from 0 – 4. Next, the score is calculated by adding up the current values 1, 24, 48, and 72 hours after treatment then divided by 4 (BPOM, 2014). The observations then continued for 14 days after treatment for confirmation. The irritation index value is a parameter to see any irritation in the test animals (Pratimasari, 2015).

Table 3. Irritation Index Values

Index	Information
0,0 – 0,4	Very light (negligible)
0,5 – 1,9	Mild irritant (slight)
2,0 – 4,9	Moderate irritant (moderate)
5,0 – 8,0	Strong irritant (severe)

8. Physical Stability Test

The stability test was carried out by placing the gel preparation at a temperature of 4°C for 24 hours then at a temperature of 40°C for the next 24 hours. Every 1 cycle is counted every 48 hours. There are 6 cycles observed in total, and physical changes are observed gel preparation (Sani, 2021).

The preparation is considered to have good stability otherwise experiencing phase separation or syneresis (Suryani, 2019).

9. Antioxidant Activity Analysis

2.5 mg of DPPH was weighed on an analytical balance and then dissolved in 96% ethanol to 50 mL in a volumetric flask to make a 50 ppm DPPH solution. Solution 1 mL of 50 ppm DPPH standard was pipetted and put into a test tube. Next, 96% ethanol was added to a volume of 5 mL (Rahmawati, 2017).

Extract solutions are made using *Spirulina platensis* extracts a total of 10 mg was weighed then dissolved in 10 mL of 96% ethanol to form a main solution with a concentration of 1000 ppm. Test solutions were prepared with various concentrations, viz 300, 400, 500, 600, and 700 ppm of main liquor. Each concentration of the test solution then pipetted and mixed with 1 mL of 50 ppm DPPH solution and ethanol 96% until the volume reaches 5 mL then homogenized (Rahmawati, 2017).

The positive control used was Vitamin C. Vitamin C was 10 mg weighed and dissolved with 10 mL of 96% ethanol, forming a stock solution with concentration 1000 ppm. The mother solution was pipetted as 10, 20, 30, 40, and 50 µL then 96% ethanol was added to a volume of 5 mL to make a solution with 5 concentrations, namely 2, 4, 6, 8, and 10 ppm. Each solution is then mixed with the DPPH solution 50 ppm as much as 1 mL and 96% ethanol until it reaches a volume of 10 mL, then homogenize (Rahmawati, 2017).

Extract gel stock solution of *Spirulina platensis* 100 µg/mL was prepared. Series solutions were made in several concentrations, namely 300, 400, 500, 600, and 700 ppm. From the stock solution, take a certain amount and mixed with 1 mL of 50 ppm DPPH solution, as well as ethanol 96% to reach a volume of 5 mL, then homogenize (Tutik, 2021).

Determination of the maximum DPPH wavelength is done with scan solution. DPPH 50 ppm in the wavelength range 400 – 800 nm. The wavelength detected will then be used as the maximum DPPH wavelength. Blank solution, extract, vitamin C, and gel that have been mixed with the solution DPPH 50 ppm was left at room temperature for 30 minutes. These solutions then The absorbance was measured using a UV-Vis spectrophotometer at this wavelength maximum (Tutik, 2021). Next, the value IC_{50} means that the value of sample concentration that can reduce as much as 50% of DPPH radicals.

$$\% \text{ DPPH radical inhibition} = \frac{\text{serapan blanko-serapan sampel}}{\text{serapan blanko}} \text{ x } 100\%$$

After calculating using the formula above, a regression equation is then created linear. The concentration of the test sample is used as a variable on the x-axis (abscissa), meanwhile the percent DPPH inhibition value is used as a variable on the y-axis (ordinate). In this analysis, we will obtain an r value (relation coefficient) which describes the relationship between these variables.

$$Y = a + bx$$

Information:
$Y = IC_{50}$
a= intercept
b= slop
x= concentration of analyte solution
(Tutik, 2021)

Data Analysis

In this research there are 2 types of data, namely descriptive data and statistical data. Results Descriptive analysis is presented through tables, graphs or pictures. Apart from that, data too statistically analyzed using the IBM SPSS program (Product and Service Statistics Solutions). The

data were analyzed statistically including comparative data on extract concentrations and soy lecithin, physical test parameters pH, spreadability, stickiness, and viscosity were tested for normality and homogeneity. The data obtained is said to be normal and homogeneous if the values
significance > 0.05. If the data is normally distributed and homogeneous then the test is carried out parametric analysis one way ANOVA. The physical stability data of the gel was tested for normality. If the data is normally distributed then continue with the testPaired T-testand if the data not normally distributed followed by testing Wilcoxon. If value sig. (2-tailed)<0.05 then there is a significant difference between the two samples. If valuesig. (2-tailed)>0.05 then there was no significant difference between the two samples (Aminuddin, 2019).

RESULTS AND DISCUSSION

Phytochemical Screening

The extract of Spirulina platensis is made using the maceration method with soaking time of 3x24 hours using 96% ethanol as the solvent. The obtained extract of Spirulina platensis is dark green in color with a thick consistency and a strong distinctive smell. The extract yield after calculation is found to be 19.48%. This result meets the criteria for a good extract yield, which is >10% (Subaryanti, et al., 2022).

Phytochemical testing of the extract of Spirulina platensis proves that it contains flavonoids, saponins, and steroids positively, while the results of alkaloid and tannin tests show negative results. This finding is in line with previous research by Agustini (2015), which also found that ethanol extract of Spirulina platensis contains flavonoids, saponins, and steroids. The absence of alkaloid compounds is suspected because of the characteristics of Spirulina platensis as a low-level plant, whereas alkaloids are more commonly found in higher-level plants, especially in dicotyledonous plants (Notonegoro, et al., 2018).

Analysis of Phycocyanin Biopigment Levels

In Spirulina platensis, there are 2 types of phycobiliprotein pigments, namely phycocyanin and allophycocyanin. Phycocyanin analysis is performed by measuring the absorbance of phycobiliprotein pigments at wavelengths of 615 nm and 625 nm using a spectrophotometer. 96% ethanol is used as the blank.

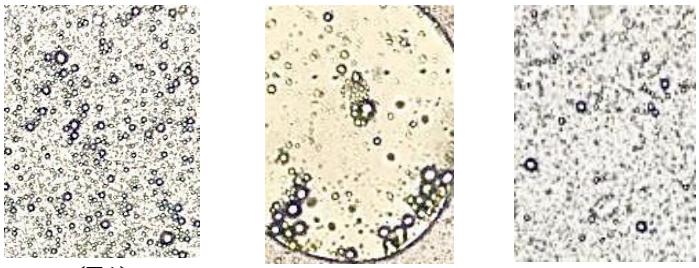
The result of the phycocyanin analysis showed a phycocyanin content of 0.694 ± 0.005 mg/mL. There are 2 types of phycocyanin, namely food-grade phycocyanin with a phycocyanin concentration (PC) > 0.4 mg/mL which can be used as food ingredients; cosmetics, and analytical phycocyanin with a phycocyanin content (PC) > 4 mg/mL which can be used as a clinical drug (Rahmawati, et al., 2017). Based on this, it is known that the phycocyanin content obtained from the ethanol extract of Spirulina platensis falls into the food-grade phycocyanin category because the concentration produced is > 0.4 mg/mL. Phycocyanin can be denatured at temperatures > 45°C (Handoyo, 2020). The obtained content is good because during extraction, a not too high temperature of 40°C was used.

The yield calculation result obtained a yield of 20.827 ± 0.160 %w/w. The yield produced differs from the research conducted by Rahmawati (2017) where the yield of Spirulina platensis extraction using the maceration method was 13.9%. This can happen because the extraction time used is different. The yield obtained from the extraction process can be influenced by various factors, such as the polarity of the solvent, the solvent concentration, and the duration of material soaking (Handoyo, 2020).

Evaluation of *Spirulina platensis* Phytosome Preparation

1. Microscopic Observation of Vesicles

Microscopic observation of vesicles is conducted to observe the formation and distribution of vesicles as a whole.



(F1) (F2) (F3)

Figure 1. Microscopic Observation Results of Vesicles

Caption:

F1: Phytosome formula with extract:lecithin ratio (1:1)

F2: Phytosome formula with extract:lecithin ratio (1:2)

F3: Phytosome formula with extract:lecithin ratio (2:1)

The appropriate shape for phytosome vesicles is spherical. A spherical shape indicates that the vesicle system has been successfully formed. The observation results show that all three formulas have spherical vesicles, but in F1 with an extract:lecithin ratio of 1:1, the vesicles have the best shape because there are more vesicles, they are more uniform, and they are evenly distributed. Meanwhile, in F2, the phytosome vesicles appear to be uneven and some are stuck together. In F3, the number of formed vesicles appears to be fewer.

2. Determination of Vesicle Entrapment Efficiency

The determination of entrapment efficiency (%EE) is conducted to determine how much the vesicles can capture active compounds. A higher %EE value indicates that the vesicles can absorb more active compounds. A good %EE value is one that is closest to 100% (Akib, et al., 2021). In this study, the determination of uptake efficiency is done using an indirect method by analyzing free drug compounds in the supernatant.

Formula	Concentration of Phytosome Components (g) (Extract : Lecithin)	Entrapment Efficiency (%)
1	1 : 1	96%
2	1 : 2	95%
3	2 : 1	94%

Caption:

F1: Phytosome formula with extract:lecithin ratio (1:1)

F2: Phytosome formula with extract:lecithin ratio (1:2)

F3: Phytosome formula with extract:lecithin ratio (2:1)

The Kruskall-Wallis test results indicate that the significance value is $0.025 < 0.05$. Therefore, it can be concluded that there is a significant difference between the three phytosome formulas with different extract-lecithin ratios. The results show that F1 with an extract:lecithin ratio of 1:1 is the formula with the best uptake efficiency among the other two formulas. This is in line with the literature where, in many cases, the extract to lecithin ratio of 1:1 is considered the most optimal formula because the choline part of phosphatidylcholine plays a role in binding the extract; when the amounts are proportional, the extract will be completely absorbed into the vesicles and the uptake efficiency will increase (Apriliani, et al., 2021).

2. Particle Size Determination

Particle size determination aims to determine the size of the formed vesicles. Particle sizes that are too small have low absorption abilities, while particle sizes that are too large will absorb more

active substances but make it difficult for the active substances to penetrate the skin. An ideal nanoparticle size is between 10-1000 nm (Akib, et al., 2021). Based on the PSA testing results, phytosome F1 has a size of 627.9 nm.

3. Polydispersity Index Determination

The polydispersity index value can describe the uniformity of globule distribution in nanovesicles. A good polydispersity index value is between 0.01 - 0.7 (Akib, et al., 2021). The polydispersity index result obtained is 0.382. This is within the good range and indicates that the vesicle size distribution is even and stable.

Evaluation of *Spirulina platensis* Phytosomal Gel Preparation

1. Organoleptic Test

The organoleptic test results for gel F0 show a gel with a thick texture, a stronger ocean fresh scent, and a slightly cloudy color. Gel F1 (2%) has a slightly less thick texture than F0, a softer ocean fresh scent, and a slightly yellowish color. The difference between these two formulas is due to the addition of phytosome suspension in F1 by 2%, making the phytosome which is liquid yellow in color and has a distinctive lecithin smell affecting the organoleptic results of gel F1.

2. Homogeneity Test

The homogeneity of a preparation is indicated by a preparation that is evenly dispersed and does not contain particle clumping. The homogeneity test results show that the phytosome gel preparations are homogeneous in all formulas, which is in line with the requirements of a good gel preparation (Tutik, et al., 2021).

3. pH Test

The pH test is used to determine the pH value in the gel preparation, as the pH in topical preparations must be suitable for the skin, ranging from 4.5 - 6.5 (Rosida, et al., 2018). The pH test results range from 6.3-6.4, so all formulas meet the skin pH standard. This finding is also supported by irritation testing results which indicate that the phytosome gel preparation does not cause irritation to the skin.

The influence of the formula on the pH of the preparation was tested using ANOVA and a significance of 0.041 (sig <0.05) was obtained, proving that there is a significant difference between the two formulas. This is because of the addition of phytosomes to gel F1, causing the pH between gel F0 and F1 to differ.

4. Spreadability Test

The results of the spreadability test for all preparation formulas meet the criteria for good gel spreadability, ranging from 5 to 7 cm. There is a difference in spreadability values between F0 and F1, where F0 has a smaller spreadability compared to F1. This difference is due to viscosity, which is inversely proportional to spreadability. Increased viscosity will result in decreased spreadability, and vice versa. The spreadability value is important to know because the larger the spreadability value, the more evenly the preparation will spread on the skin, providing comfort and optimal effects (Tutik, et al., 2021).

The influence of the formula on the spreadability of the preparation was tested using ANOVA and a significance of 0.374 (sig> 0.05) was obtained, proving that there is no significant difference between the two formulas.

5. Adhesion Test

The higher the adhesion, the more optimal the absorption of active substances because the preparation can remain in contact with the skin for a long time. Good adhesion quality in gel preparations is measured by the adhesion time of not less than 4 seconds. The results of the adhesion test for all preparation formulas meet the criteria for good adhesion. There is a difference in adhesion values between F0 and F1, where F0 has a higher adhesion than F1. This is because adhesion is inversely proportional to spreadability but directly proportional to viscosity. A higher viscosity value will result in a smaller spreadability, while the adhesion of the preparation will be longer, and vice versa (Iskandar, et al., 2021).

The influence of the formula on the adhesion of the preparation was tested using ANOVA and a significance of 0.046 (sig <0.05) was obtained, proving that there is a significant difference between the two formulas. This is because of the addition of phytosomes to gel F1, causing the adhesion between gel F0 and F1 to differ.

6. Viscosity Test

Viscosity was measured using a Brookfield viscometer with spindle number 9. The results of the viscosity test for all formulas meet the standard viscosity for good gel, ranging from 2000 - 4000 cps. There is a difference in viscosity values between F0 and F1, where F1 has a lower viscosity value than F0. This is because of the addition of phytosomes to F1, which has a liquid property, thus causing the viscosity to be lower (Sukmawati, et al., 2022).

The influence of the formula on the viscosity of the preparation was tested using ANOVA and a significance of 0.000 (sig <0.05) was obtained, proving that there is a significant difference between the two formulas. This is because of the addition of phytosomes to gel F1, causing the viscosity between gel F0 and F1 to differ.

Ethical Clearance

The animal code of ethics test is conducted at the Research Ethical Commission, Jendral Achmad Yani Yogyakarta University by filling out a formula and sending a research proposal online. The code of ethics test was carried out before the research took place. This research has received research ethics approval from Jendral Achmad Yani Yogyakarta University, with number Skep/217/KEP/V/2023.

Irritation Test

The back hair of albino rabbits was shaved and divided into 3 parts: for normal conditions (skin without gel application), negative control (area for F0 gel application, gel without phytosome), and positive control (area for F1 gel application, gel with the addition of 2% Spirulina platensis extract phytosome). Observations were made at intervals of 24, 48, and 72 hours after treatment.

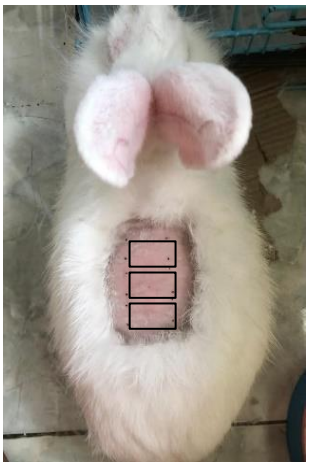


Figure 2. Results of Irritation Test in the back hair of Albino Rabbits

The research results show that the phytosome gel of *Spirulina platensis* extract does not cause irritation (BPOM RI, 2014). This is because the ingredients used in the phytosome gel formula of Spirulina platensis extract are known not to induce skin irritation, and the concentrations used in the formula are within safe limits, thus not inducing irritation on the skin.

Antioxidant Activity Analysis

The antioxidant activity test was carried out using the DPPH method. The basic principle of this method is that compounds with antioxidant activity will reduce the DPPH free radical by transferring hydrogen atoms. The characteristic sign of a sample with antioxidant activity is a color change of the DPPH solution from purple to yellow when the DPPH solution is mixed with the sample. This change occurs because DPPH is reduced due to the hydrogen atom transfer reaction from the antioxidant compound (Tristantini, et al., 2016).

The parameter used to measure the antioxidant activity of a compound is the IC50 value, or Inhibition Concentration 50. IC50 is the sample concentration that is able to reduce 50% of the DPPH radicals. The IC50 value is divided into four categories: <50 µg/mL, which is classified as very strong antioxidant; 50-100 µg/mL, which is classified as strong antioxidant; 100-150 µg/mL, which is classified as moderate antioxidant; and 150-200 µg/mL, which is classified as weak antioxidant. The IC50 value is inversely related to antioxidant activity, meaning that if the IC50 value of a sample is smaller, the antioxidant activity is stronger, and vice versa (Tutik, et al., 2021).

The antioxidant activity test using the DPPH method is carried out by measuring absorbance at a wavelength of 517 nm using a UV-Vis spectrophotometer. The measured absorbance is then used to calculate the antioxidant inhibitory power or % inhibition. Pearson correlation statistical tests in this study produced significance values for vitamin C, Spirulina platensis extract, gel F0, and gel F1, respectively, were 0.01; 0.024; 0.001; 0.012. These values indicate that the significance value is <0.05, which proves the correlation between concentration and % inhibition level. The Pearson correlation coefficients obtained were 0.959; 0.926; 0.993; 0.954, which means there is a strong positive correlation.

Sample	IC50 (µg/mL)	Antioxidant Activity Category
Vitamin C	3,08	Very Strong
Spirulina platensis Extract	6,53	Very Strong
Gel F0	169,04	Weak
Gel F1	21,59	Very Strong

Based on the results, it was found that vitamin C, Spirulina platensis extract, and gel F1 containing Spirulina platensis extract phytosome have antioxidant activity and are classified as very strong antioxidants because the IC50 values are less than 50 µg/mL.

Physical Stability Test

The physical stability test was conducted to evaluate the ability of the preparation to maintain its physical properties. This stability test is an accelerated stability test that lasts for 14 days or equivalent to 6 cycles, where each cycle consists of the preparation being stored at 4 °C for 24 hours and then at 40 °C for 24 hours. The physical stability test in this study included organoleptic evaluation, pH, viscosity, spreadability, adhesion, and homogeneity of the preparation (Lestari, 2017).

1. Organoleptic Test

After undergoing stability testing for 6 cycles, the results showed that there were no observable differences in the phytosome gel preparation from cycle 0 to cycle 6. This confirms that the phytosome gel preparation remained physically stable.

2. pH Test

The pH test results for the phytosome gel preparation in each cycle were in the range of 5.19 to 6.3, thus meeting the pH requirements suitable for the skin. Paired T-test results for gel F0 and F1 showed a significance of 0.00. This indicates that all gel formulas, both before and after stability testing, have a significance value (2-tailed) <0.05, proving a significant difference in gel pH before and after stability testing. Based on the pH testing of the gel preparation, it can be seen that the pH tends to decrease until reaching cycle 6. This decrease is caused by the acidic nature of the gelling

agent used, which is carbopol. This reaction between the carboxyl groups in carbopol and water forms H30+, making the gel preparation more acidic during storage. Despite the decrease, the pH of the phytosome gel preparation remains within the appropriate range, making it safe for use on the skin.

3. Viscosity Test

The viscosity test results for the phytosome gel preparation in each cycle were in the range of 3008 to 3928 cps, thus meeting the standard for good gel viscosity. Paired T-test results for gel F0 showed a significance of 0.008, while gel F1 had a significance of 0.012. These results indicate that all gel formulations, both before and after stability testing, have a significance value (2-tailed) >0.05. Therefore, it can be concluded that there is no significant difference in gel viscosity before and after stability testing. This result indicates that the viscosity of the phytosome gel preparation is relatively stable.

4. Spreadability Test

The spreadability test results for the phytosome gel preparation showed results between 5.4 to 6.8 cm in each cycle, which is in line with the standard for good spreadability. Paired T-test results for gel F0 showed a significance of 0.225, while gel F1 had a significance of 0.184. These results indicate that all gel formulations, both before and after stability testing, have a significance value (2-tailed) >0.05. Therefore, it can be concluded that there is no significant difference in gel spreadability before and after stability testing. This result indicates that the spreadability of the phytosome gel preparation is relatively stable.

5. Adhesion Test

The adhesion test results for the phytosome gel preparation showed values ranging from 4 to 7 seconds in each cycle, which is in line with the standard for good adhesion. Paired T-test results for gel F0 showed a significance of 0.968, while gel F1 had a significance of 0.111. These results indicate that all gel formulations, both before and after stability testing, have a significance value (2-tailed) >0.05. Therefore, it can be concluded that there is no significant difference in gel adhesion before and after stability testing. This result indicates that the adhesion of the phytosome gel preparation is relatively stable.

6. Homogeneity Test

The homogeneity test results for the phytosome gel preparation showed that each cycle was homogeneous and there was no change from cycle 1 to cycle 6, thus meeting the requirement for good homogeneity.

CONCLUSIONS AND SUGGESTIONS

The difference in the concentration ratio of extract:lecithin in the phytosome formula can affect the characteristics of the phytosome. The phytosome formula F1 with an extract:lecithin ratio of 1:1 resulted in the best preparation. Based on phytosome evaluation, F1 had the best microscopic results with the highest absorption efficiency of 96% and particle size within the nano preparation range at 627.9 nm.

The addition of phytosome suspension by 2% to the gel formula F1 resulted in differences in the obtained gel. Gel F1 had organoleptic characteristics that were yellow in color with lower viscosity compared to gel F0 without the addition of phytosome suspension. However, both gel formulas had evaluation results within the range of good gel preparations and did not cause irritation.

The result of entrapment efficiency from phytosome F1 affected the antioxidant activity of the gel, as seen from the IC50 value of gel F1 containing phytosome F1. The calculated IC50 value of gel F1 was 21.59 µg/mL. This proves that the gel containing phytosome F1 has very strong antioxidant activity.

ETHICAL CONSIDERATIONS

This research has received ethical permission from the Research Ethical Commission, Jendral Achmad Yani Yogyakarta University, with number Skep/217/KEP/V/2023, obtained before conducting the research.

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