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Formulation and evaluation of astaxanthin lotions as natural antioxidants for the skin

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Abstract. Antioxidants are compounds used to inhibit free radical activity. Antioxidants derived from natural ingredients are astaxanthin.This study aims to determine the value of IC50 astaxanthin, knowing the effect of adding astaxanthin concentration in antioxidant lotion preparations and determining good lotion formula based on physics, chemical, moisture, stability and antioxidant test results. Antioxidant activity testing was performed using DPPH. The lotion is made by the variation of astaxanthin concentration. Results of antioxidant activity Astaxanthin has a very strong antioxidant activity with IC50 value of 30.45 ppm. The antioxidant activity test of the astaxanthin lotion preparation showed that all formulas had strong antioxidant activity with Formula 1 (1%) 98,961 ppm, Formula 2 (3%) 88,921 ppm and Formula 3 (5%) 87,571 ppm. With the difference in concentration of astaxanthin the higher the concentration the stronger the antioxidant activity. The formula that has the best antioxidant activity is Formula 3 with IC50 87,571 ppm. Physical test results, chemistry, stability test during 28 days storage of astaxanthin lotion preparation showed that the four formulas met the requirements. While the humidity test showed that the preparation of astaxanthin lotion which has the highest percentage of moisture increase is Formula 3.

Keywords: Antioxidant, DPPH (1,1-diphenyl-2-picrilhidrazil), Losio, Astaxanthin.

1. Introduction

The development of science and increasing public awareness of the importance of skin health care is one of the factors driving the increasing demand for cosmetic products for skin care. The use of skin care cosmetics is shown as one of the protection of direct exposure to sunlight or ultraviolet light continuously against the skin [14]. Dry skin is one common problem found in tropical regions such as Indonesia. Exposure to ultraviolet light hurts the skin such as premature ageing. Consumption of antioxidants in the skin is needed by the surface to fight free radicals from UV rays [6].

Free radicals are one form of reactive compounds and unstable molecules, which are generally known as compounds that have unpaired electrons. The existence of unpaired electrons can cause these compounds to be very reactive looking for a partner by binding to the particles around them so that they can trigger disease [28].

Antioxidants are compounds that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules so that cell damage will be inhibited. Some antioxidants can be produced naturally, both from land and waters such as crabs, shrimp and lobster. One of the antioxidants derived from natural ingredients is astaxanthin.

Astaxanthin is a powerful antioxidant derived from carotenoids which are xanthophyll groups (Xanthophylls) but do not have activities like vitamin A. Astaxanthin is synthetised by plants and

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some types of algae which are fat soluble oxygen [11].

Astaxanthin is a fat-soluble orange-red pigment. Astaxanthin is said to be the best source of antioxidants because it has the strength of 50-100 times stronger than vitamin E and helps vitamin C in its activity as an antioxidant [29].To prevent the effects of free radicals that can damage skin cells, it is necessary to design a cosmetic preparation formulation containing astaxanthin which has excellent antioxidant activity. Antioxidants can be formulated as cosmetic preparations in the form of creams, gels and lotions. Regarding practicality and convenience to use, the lotion is made (9).

A lotion is a liquid preparation intended for external use on the skin. Most lotions contain fine powder ingredients which are not soluble in dispersion media and are suspended using suspending substances and dispersing agents [2]. Forms of lotion are good as anti-ageing cosmetics because they have several advantages, namely their ability to maintain skin moisture, soften skin, and evenly and quickly use on a broad skin surface compared to other semi-solid preparations [3].

Based on this, astaxanthin is made as a product, lotion as a natural antioxidant. A lotion is a cosmetic preparation in the form of emulsions containing more water than oil and has the properties as a moisturiser for the skin, soft and easy to apply. The lotion dosage form is chosen, because it can be spread thinly compared to cream or ointment preparations and can cover large areas of skin. [23]. Based on the description above, a study entitled the formulation and evaluation of Astaxanthin lotion preparations were carried out as an antioxidant using DPPH method (1.1 Diphenyl-2-Pikrilhidrazine).

2. Methodology

2.1. Material

The tools used in this study are analytical balance sheets (Shimadzu UX620H and Mettler Toledo AG245), UV-Vis Spectrophotometers (Genesys 10S UV-Vis), water heaters, porcelain cups, mortars, stampers, glassware (Pyrex®), hotplate magnetic stirrers (IKA C-MAG HS-7, Germany), test tube, measuring cup 10 mL and 100 mL, beaker, measuring flask, volume pipette, pipette pump, cuvette and Skin Moisture Analyzer (FCM-1).

The materials used in this study are Astaxanthin (Sigma Aldrich), Sutil Alcohol (PT.Brataco®), Cremophor RH 40 (PT.Brataco®), Carbomer (PT.Brataco®), Triethanolamine (TEA) (PT. Brataco®) , Propylene glycol (PT.Brataco®), DMDM Hydantoin (PT. Brataco®), Oleum appel (PT. Brataco®), Sunflower oil (Sigma Aldrick®), DPPH (Sigma Aldrick®), Vitamin C (PT. Brataco®) and Methanol pa (PT.Brataco®).

2.2. Method

Astaxanthin lotion formula is made in 4 variations of the method with the same excipient composition but different concentrations of the active substance.

Table 1. Formulation of lotion astaxanthin

Ingredient	Function	F0 (0%) b/v	FI (1%) b/v	FII (3%) b/v	FIII (5%) b/v
Astaxanthin	API	0	1	3	5
Cethyl alcohol	Viscosity agent	0.5	0.5	0.5	0.5
Stearic acid	emulsifier	0.5	0.5	0.5	0.5
Cremophor RH 40	Surfactant	0.039	0.039	0.039	0.039
carbomer	Viscosity agent	2	2	2	2
Triethanolamin	emulsifier	0.5	0.5	0.5	0.5
Propylene glicol	Humectant	2	2	2	2
DMDM Hydantoin	preservatif	0.5	0.5	0.5	0.5
Oleum lyly	odour	q.s	q.s	q.s	q.s
Sunflower oil	Solvent	q.s	q.s	q.s	q.s
Aquadest	solvent	ad 150	ad 150	ad 150	ad 150

The lotion is made by first dissolving astaxanthin in sunflower oil. Each oil phase (cetyl alcohol) and water phase (Cremophor RH 40, DMDM Hydantoin) were melted on a water bath at 50oC. After all the dissolved aspects, the aqueous phase is mixed into the oil phase in hot conditions little by a little while stirring until an emulsion is formed until it is homogeneous. The carbomer which was previously dissolved with aqua Deion then adds triethanolamine while mixing and add propylene glycol stir until similar. The mixture is then put into a mortar that has been heated and crushed until it is homogeneous. After the base is cold, then the

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mixture is added to the astaxanthin and stirred until it is comparable. A distillation is added and stirred until it is similar and the distilled water is added to 150 grams. Lotion preparations are put into containers and evaluation of qualifications.

2.2.1 Physical Evaluation of Lotion

1. Organoleptic Test
Observations were carried out every week for four weeks of storage, and the samples were evaluated visually covering views of changes in shape, colour, and odour that occur at any given time span [21]

2. Homogeneity Test
Homogeneity testing of lotions was carried out using a small sample of lotion formula preparations then placed between the two objects. Observed the composition of coarse particles or non-homogeneity [15]. Tests are carried out every week for four weeks of storage.

3. pH test
pH checks were measured using a pH meter; then the electrodes were dipped in lotion until the pH meter showed a fixed reading and the pH value was recorded at pH meters [24].

4. Spread Power Test
The weighed preparations as much as 0.5 grams were then placed in the middle between 2 glass plates, then weighed 50 g, 100 g, 200 g and 500 g and left for 1 minute later the exact area was measured [14].

5. Viscosity Testing
Determination of viscosity was carried out using the Brookfield Viscometer. Measurements are made for each preparation when the preparation is finished and every week for four weeks of storage [5].

6. Cycling Test
This test is carried out by storing preparations from each formula placed in a transparent glass container. The development was stored at 4oC for 24 hours, then transferred to room temperature, and in an oven with a temperature of 40^{o}C for 24 hours (one cycle). The test was carried out in six periods and seen whether there were changes that occurred in each preparation [5].

7. Moisture Test
The moisture test is used using the Skin Moisture Analyzer tool to test volunteers who have previously checked the moisture of their skin. Lotion applied to the surface let stand for 5 minutes and observe the results of its moisture concentration. Testing is done on day 0 then after two weeks (after 14 days) and after four weeks (after 28 days).

2.2.2 Antioxidant Activity Testing with DPPH Method

1. Making DPPH Solution
DPPH 50 mg powder was dissolved with methanol p.a then put into a 100 mL volumetric flask, and the volume was filled with methanol p.a to the boundary mark so that a concentration of 500 ppm was obtained.

2. Determination of DPPH Maximum Wavelength
Dilution of DPP 500 ppm solution to 50 ppm then as much as two mL was inserted into the brown vial, then poured into the cuvette. The wavelength was then determined using a UV-Vis spectrophotometer at a wavelength of 400-800 [16].

3. Operating Time
Add 2 mL of DPP 50 ppm solution into the brown vial and add 1 ml of 50 ppm sample solution, incubated and measured every five minutes so that a stable time span is obtained, at the maximum DPPH wavelength that has been obtained which is then used as a reference for the measurement time antioxidants.

4. Preparation of Blank Solution
Two mL of 50 ppm DPPH solution was put into a brown vial, added 1 mL of methanol p. a, incubated in a dark room with time according to the operating time obtained. Uptake is measured at a wavelength of 400-800 nm and determines its maximum wavelength [16].

5. Preparation of Comparative Solution for Vitamin C
Vitamin C as a comparison solution was weighed as much as 50 mg, dissolved with methanol p.a then put into a 100 mL volumetric flask. The volume was supplemented with methanol p.a until the boundary sign so that a concentration of 500 ppm was obtained. The primary solution of vitamin C is then made a series of vitamin C at various levels.

6. Measurement of the Antioxidant Activity of Comparative Vitamin C
Make a vitamin C solution with different concentrations and then take one mL and add two mL of DPPH solution to be shaken until homogeneous and incubated in a dark room at the time obtained on operating time. Then the absorption is measured at the maximum DPPH wavelength that has been set.

7. Measurement of Astaxanthin Antioxidant Activity and Lotion Preparation

a. Making a mother liquor concentration of 500 ppm
Astaxanthin and preparations of finished lotions weighed as much as 50 mg, dissolved with methanol p.a then put into a 100 mL volumetric flask, the volume was filled with methanol p.a to the boundary markings.

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b. Preparation of series test solutions of various concentrations
The astaxanthin mother liquor and each lotion are made on multiple levels.

c. Absorption measurements using UV-Vis spectrophotometer
Astaxanthin test solution and one mL lotion preparation put into a test tube, DPPH solution was added which has been diluted 50 ml by two mL, incubated in a dark space the time obtained from the results of operating time. Then absorption is measured at the maximum wavelength.

8. Determination of Percent Inhibition

Radical antidote activity is expressed as per cent inhibition which can be calculated by the following formula:

% inhibition = ((Absorption of DPPH-Absorbance of the sample) / (Absorbance DPPH)) x 100 (1)

Determination of IC50 Value (Inhibitory Concentration)

Sample concentrations and per cent inhibition samples were plotted respectively on the x and y-axes in the linear regression equation. The equation is used to determine the IC50 ¬ value of each sample expressed with a y value of 50 and the amount of x to be obtained as an IC50 value [18].

3. Results and Discussion

3.1 Formulation of Astaxanthin Lotion

The formulation of this lotion preparation was carried out with variations in astaxanthin concentration. The concentration used in astaxanthin lotions is 1, 3 and 5%. From the results of the reformulation study, the ingredients used in the manufacture of astaxanthin lotion were found as active substances which functioned as antioxidants, cetyl alcohol and stearic acid as an oil phase which performed as an emulsifying agent. Cremophor RH 40, DMDM hydantoin as a water phase, carbomer as a thickener, triethanolamine (TEA) which works as an alkalizing agent [22]. Also, triethanolamine also works as an emulsifier or to increase pH in a lotion.

Triethanolamine has a concentration limit of 2-4% [22]. Another additive is propylene glycol which is used as a humectant with a concentration limit of using propylene glycol as a humectant is less than 15% (22). Aquades used as solvents, and sunflower oil are used to dissolve astaxanthin because the solubility of astaxanthin in sunflower oil is excellent.

3.2 Evaluation of Astaxanthin Lotion

3.2.1 Organoleptic Observation

Organoleptic observations were made from lotion preparations which aimed to determine changes in physical appearance of development including colour, odour, and shape for 28 days of storage carried out at room temperature.

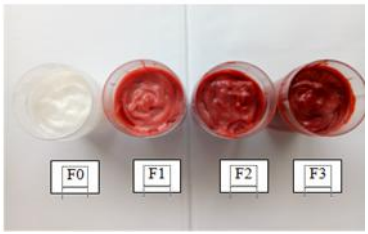

Fig 1. Observation of Organoleptics

The results obtained in the four formulations of the dosage form received in the form of semisolid, white as a blank and pink, dark red and red with astaxanthin colour and the resulting odour are smelled of lily essential oil. The concentration of astaxanthin influences the tone produced from lotion preparations used, while the resulting aroma depends on the addition of lily essential oil added. Based on the results of organoleptic tests on formulas 0, 1, 2 and three which were carried out for 28 days at room temperature astaxanthin lotion was not showing changes in colour, odour and dosage form.

In organoleptic observation, the higher the concentration of astaxanthin, the colour produced will get older.

3.2.2 Observation of Homogeneity

The homogeneity test aims to see and find out whether preparation is mixed or not evenly distributed which is seen using a microscope [1]. The results of homogeneity observation showed that formula 0, 1, 2 and 3 had good homogeneity for 28 days of storage.

3.2.3 Spread Power Test

Spread power measurement is done to determine the speed of spread or even distribution of lotions.

Table 2. Results of the scattered power test for lotion

day	Diameter (cm)			
	F0	F1	F2	F3
1	5.9	5.7	5.7	5.7
7	5.9	5.7	5.5	5.5
14	5.5	5.4	5.3	5.3
21	5.5	5.5	5.4	5.4
28	5.4	5.4	5.4	5.4

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The results of the dispersion power test for 28 days storage formula 0,1,2 and 3 met the requirements where the optimum range of dispersion according to SNI 16-4399-1996 ranged between 4.5-8 cm. The dispersion surface produced by increasing the load is intended to describe a dispersing power characteristic, where the resulting surface area is directly proportional to the increase in the added pressure. The higher the added load, the more the dispersion power in the lotion increases [20].

3.2.4 PH measurement

The pH measurement aims to determine the acidity of the lotion preparation when using so as not to irritate the skin. Developments that are too acidic will irritate the skin while events that are too alkaline will make the skin dry and itchy [25].

The preparation of astaxanthin lotion on formulas 0 and 1 has a pH of 6.5 while methods 2 and 3 have a pH of 6.6 which means that there is a range of pH in the skin and meet the SNI requirements. 4.0-8.0.

3.2.5 Viscosity Testing

Viscosity is a measure of the thickness of a fluid which expresses the size of the friction in the liquid. The higher the fluid viscosity, the more difficult the fluid to flow and also shows the more difficult a moving object in the liquid [4]. Viscosity is done to determine the thickness of a preparation. Viscosity testing of lotion preparations was carried out using the Brookfield Viscometer using spindle number 7 and speed of 100 rpm.

Table 3. Viscosity Test Results of Lotion Preparations

day	Viscosity (cp)			
	F0	F1	F2	F3
1	5840	5840	5840	6333
7	5000	5880	6160	6226
14	5000	5866	6182	6173
21	4986	5440	4733	5306
28	4533	4973	4120	4066

Viscosity results during 28 days storage showed a decrease in viscosity from the four formulas. This is because the preparation of lotion can undergo an autooxidation process during storage. The requirements for viscosity values according to SNI 16-4399-1996 are in the range of viscosity values of 2000-50000 cP.

3.2.6 Cycling Test

Cycling test is carried out to obtain an overview of the physical stability of the preparation with temperature variations during storage which is indicated by the presence or absence of separation between the water phase and the oil phase. Cycling test is a test preparation that involves changes in temperature at specific time intervals. This test is carried out in 6 cycles because an emulsion must withstand at least 6-8 heating or cooling between the refrigerator temperature and 40oC temperature [13].

Observations on the four formulas showed that the formula 0, 1, 2, and three were physically stable during the storage temperature of 4oC, room temperature and temperature of 40oC. From the results of the observation, there is no phase separation during storage.

3.2.7 Moisture Testing

Moisture tests are carried out to see the moisture from a lotion preparation to the skin.

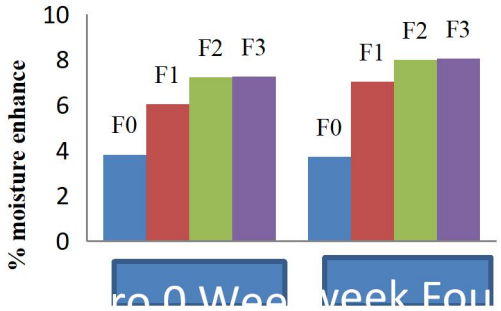

Fig 2. percentage of moisturization

The results of the humidity test at week 0 to week 4 showed that all formulas experienced an increase in the percentage of humidity. From these results, it was found that the rate of humidity increase was the best in Formula 3.To determine the effect of astaxanthin concentration on skin moisture, the humidity test data were analysed statistically using the T-Test. Based on the results of the T-test the significance values obtained from all formulas were less than 0.05 (0.000 <0.05) with a 95% confidence level meaning that astaxanthin lotion affected the skin moisture.

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3.3 Antioxidant Activity Testing

Testing of antioxidant activity was carried out using the DPPH method. This method is used because DPPH method is a method commonly used to test antioxidant activity in vitro, besides that it is a simple, fast method and chemicals and only a few samples are used [8]. The testing phase of antioxidant activity begins with determining the maximum wavelength of DPPH and operating time (OT). The maximum wavelength is a wavelength that can provide maximum absorbance at the time of measurement.

The maximum DPPH wavelength used is the maximum wavelength of DPPH which does not react with antioxidant compounds. Testing the antioxidant activity of astaxanthin and the standard of vitamin C begins with determining the maximum wavelength. The determination of the maximum wavelength was carried out using 50 ppm DPPH solution and the blank used was methanol p. A using UV-Vis spectrophotometry. The resulting wavelength is 517 nm with an absorbance of 0.647.

After obtaining the maximum wavelength, then the operating time is determined for the standard of vitamin C and astaxanthin. Operating time (OT) is carried out to get the measurement time when the reaction has run optimally which is indicated by obtaining a stable absorbance value over a specified period [19]. Based on experiments that have been carried out operating time for vitamin C and astaxanthin that is at 30 minutes. After incubation, then the absorbance is seen at a wavelength of 517 nm.

Positive control used is vitamin C, which is a comparison that is more often used because the antioxidant activity is powerful [7]. The use of positive control on antioxidant activity testing to find out how strong antioxidant potential is in astaxanthin compared to vitamin C. If the IC50 value of the sample is the same or close to the IC50 positive control then it can be said that the example has the potential as one of the compelling antioxidant alternatives [7].

Based on the results of testing the antioxidant activity that has been carried out the IC50 value for vitamin C is equal to 5.8 ppm while astaxanthin is equal to 30.45 ppm. So it can be said that vitamin C and astaxanthin are in the range <50 ppm which means it has the potential as the potent antioxidant activity.

Results of Testing the Activity of Astaxanthin Lotion Antioxidants

Antioxidants are formulated in the form of topical preparations which are expected to protect the skin from free radicals caused by sunlight, then made in the form of a lotion. The lotion is a liquid preparation that is intended for external use on the skin.

Testing of antioxidant activity for the preparation of astaxanthin lotion begins with determining the maximum wavelength. Determination of maximum wavelength was carried out using 50 ppm DPPH solution using UV-Vis spectrophotometry. The wavelength obtained is 517 nm. After receiving the maximum wavelength, then determine the operating time for the preparation of astaxanthin lotion.

Based on the measurement of operating time for astaxanthin lotion preparation, stable absorbance obtained was formula 1 in the 20th minute for method 2 in the 35th minute and formula 3 in the 25th minute. After incubation, then the absorbance is seen at a wavelength of 517 nm.

In this method, DPPH solution which acts as free radicals will react with antioxidant compounds so DPPH will form 1.1, -diphenyl-2-pycrilhydrazine which is non-radical [16].

The results of testing the antioxidant activity of the available astaxanthin lotions in this study were able to reduce DPPH with values ranging from 50-100 ppm. This shows that the preparation of astaxanthin lotions is categorized as having strong antioxidant activity in reducing free radicals.

The amount of antioxidant activity is expressed in IC50 (Inhibitory Concentration 50) value, which is the concentration needed to inhibit DPPH activity by 50%. The smaller IC50 ¬ value is owned by a compound, the stronger the antioxidant activity of the compound. A mixture is said to have potent antioxidant activity if IC50 is less than 50 ppm, antioxidants are strong if IC50 is worth 50-100 ppm, antioxidants are moderate if IC50 is worth 100-150 ppm, and antioxidants are weak if IC50 is 151-200 ppm [16].

Based on the results of testing the antioxidant activity that has been carried out the IC50 value for Formula 1 is 98.961 ppm, Formula 2 is 88.921 ppm, and Formula 3 is 87.571 ppm. So it can be said that the preparation of astaxanthin lotions is in the range of 50-100 ppm which means it has the potential as a powerful antioxidant. So with the difference in the concentration of astaxanthin, the higher the concentration, the stronger the antioxidant activity, where the formula that has the best antioxidant activity is Formula 3 with a Formula 3 value of 87.571 ppm.

Conclusion

Astaxanthin is a natural antioxidant with strong potential which can increase good moisture in the skin

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after formulated in lotion preparations with good physical and chemical stability in storage for 28 days.

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