International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 13, Special Issue 4, 2021

Original Article

FORMULATION AND EFFECTIVITY OF THE ANTIOXIDANT GEL PREPARATION CONTAINING ZEAXANTHIN AS ANTI AGING

FAJAR SETIAWAN*, LUSI NURDIANTI, NENG SRI

Pharmacy Department, STIKes Bakti Tunas Husada, Jl. Cilolohan 36 Tasikmalaya, West Java, Indonesia, 46115
Email: fajarsetiawan@stikes-bth.ac.id

Received: 18 Aug 2021, Revised and Accepted: 25 Sep 2021

ABSTRACT

Objective: This study was conducted to determine the IC50 value of zeaxanthin, to know the formula that meets the requirements of the results of physical evaluation of gel preparations, and to know the effectiveness of zeaxanthin gel as an anti-aging on the skin.

Methods: The preparation of this gel is made using zeaxanthin as an active substance with concentrations of 5%; 7.5%; and 10%. Tests conducted are organoleptic test, homogeneity test, pH, spreadability, viscosity, irritation test and cycling test. Tests conducted are organoleptic test, homogeneity test, pH, spreadability, viscosity, irritation test and cycling test. Tests on the effectiveness of zeaxanthin gel preparations against the backs of volunteers' hands were divided into 4 groups as well as testing conducted over 28 d.

Results: The results showed a value of IC50 zeaxanthin of 9.044 µg/ml, all gel preparations met the requirements of physical evaluation results except in cycling test and test results of the effectiveness of zeaxanthin gel preparations on the backs of volunteer hands there was an increase in humidity with an average increase of 33.17%±11.867 and wrinkles obedience with an average decrease of 47.466%±7.115.

Conclusion: Zeaxanthin can be formulated as gel anti-aging.

Keywords: Antioxidant, Zeaxanthin, Anti-aging, Gel

DOI: https://dx.doi.org/10.22159/ijap.2021.v13s4.43846 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Aging process is a sure thing that will happen to everyone, but the occurrence of premature aging is something to avoid [1]. Premature aging is the process of aging the skin faster than the time that should happen to anyone, especially in Indonesia with a tropical climate [2]. The process of premature aging is characterized by wrinkles, black spots, fine lines, dry and rough skin [3].

Free radical components (reactive oxygen or ROS) or lipid peroxide (LPO) are involved in the pathogenesis and accelerated development of skin aging in the event of prolonged oxidative stress [4], so antioxidants are indispensable for the body to overcome and prevent oxidative stress [5]. One of the substances that has the potential as an antioxidant is zeaxanthin. Zeaxanthin has antioxidant activity that belongs to a very powerful group of antioxidants. According to (Sindhu *et al.*, 2010), zeaxanthin has an IC50 value of 10 µg/ml where antioxidants belong to the strong group [6].

Oral administration of zeaxanthin may improve acetic acid-induced colitis in rats through antioxidant effects and modulation of pro-inflammatory cytokines and mediator activity [7]. In addition, zeaxanthin includes carotenoids that can protect the skin from oxidation caused by sun exposure [8]. Lutein and zeaxanthin have been reported to reduce lipid peroxidation and increase moisture in the skin. These results suggest that lutein and zeaxanthin may exhibit protective effects against oxidative light in humans [9].

The route of the drug through topical administration is widely chosen to improve the effectiveness and ease in the application of drugs, one of which is gel preparations. Gel is a semi-solid or semi-solid system of at least two constituents consisting of a tight fence-like mass and covered by liquid [10]. The reason made gel is because gel preparations are much in demand in the drug and cosmetic industry because it has advantages over other preparations that are good spread on the skin, the presence of cold effects when applied to the skin, the release of good drugs, easy to wash [11], does not cause skin marks, and easy in its use [12].

MATERIALS AND METHODS

Materials

Zeaxanthin was purchased from Fuji Chemical Industries (Japan). DPPH was purchased from Sigma Aldrich, Polyethylene Glycol 400 (PEG 400) was purchased from Merck (Indonesia). Carbomer was purchased from Brataco, methylparaben and propylparaben (PT. Bratachem), TEA (PT. Bratachem), ascorbic acid p. a was purchased from Merck (Indonesia). All other chemicals used were of pharmaceutical grade.

Test antioxidant activity with DPPH method

Determination of maximum wavelength of DPPH

Before the test of antioxidant activity, the determination of maximum wavelength, as much as 5 ml of DPPH solution, is observed absorption at wavelengths of 400-800 nm [12].

Zeaxanthin antioxidant activity test

Zeaxanthin is made with a concentration of 1000 ppm. From the main solution is made a series concentration of 2 to 10 ppm. Each test solution of 1 ml is inserted into the vial. Added DPPH solution 25 ppm as much as 2 ml, then incubated for 30 min and measured absorbance at wavelength 516.5 nm [12].

Vitamin C antioxidant activity test

Vitamin C is made with a concentration of 1000 ppm. From the main solution is made a series of concentrations of 0.5 to 5 ppm. Each test solution of 1 ml is inserted into the vial. Added DPPH solution 25 ppm as much as 2 ml, then incubated for 30 min and measured absorbance at wavelength 516.5 nm [12].

IC 50 determination

IC50 value determination of antioxidant activity is obtained from the measurement of absorbance of the series of concentrations that have been made so as to produce % inhibition that can be calculated based on the formula as follows: [12]

$$\text{Inhibition [\%]} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of Blank}} \text{x} 100\%$$

Preparation of zeaxanthin gel preparation

First, first, dissolve the zeaxanthin with the addition of surfactant and cosurfactant for 30 min until soluble zeaxanthin is formed, which is characterized by a clear solution [13]. After that, expand the carbopol with warm water±50 °C for 30 min until it expands. Then,

add triethanolamine little by little until a gel base is formed. Then dissolve methylparaben and propylparaben with glycerin, add to the gel base. Then add zeaxanthin to the gel base. After that, add little by little aqua deion [14].

Preparation formula

Table 1: Composition of gel zeaxanthin

Material	Formula (% w/v)				Function
	F0	F1	F2	F3	
Zeaxanthin	-	5	7.5	10	Active substance
Carbopol 940	0.5	0.5	0.5	0.5	Gel bases
Glycerin	10	10	10	10	Humectants
Methyl Paraben	0.18	0.18	0.18	0.18	Antimicrobial
Propyl Paraben	0.02	0.02	0.02	0.02	Antimicrobial
Triethanolamine	7 gtt	7 gtt	7 gtt	7 gtt	Alkalizing agent
deionized water ad	100	100	100	100	Solvent

Visual observation and pH determination

Visual observation includes observation of color, odor, and clarity of gel zeaxanthin. A pH of zeaxanthin gel was determined by using a calibrated pH meter (Mettler® Toledo) [15, 16]

Homogeneity test

The homogeneity test is carried out by applying the sample to a piece of glass or other suitable transparent material, the preparation must show a homogeneous arrangement and there are no visible coarse grains [16].

Viscosity test

Viscosity test was carried out using a Brookfield Viscometer. The preparation is put into a beaker. Then the spindle is installed 4. The spindle must be submerged in the test preparation. The viscometer is turned on and it is ensured that the rotor can rotate at a speed of 50 rpm. Observed the needle guide from the viscometer that leads to the viscosity scale and then recorded and multiplied by a factor of 100 [17].

Spreadability test

The preparation was weighed as much as 0.5 grams and then placed in the middle between 2 glass plates, then given a load of 50 g, 100 g, 200 g and left for 1 minute and then the actual area was measured [18].

Cycling test

The cycling test was carried out for 6 cycles. The gel preparation was stored at a cold temperature of±4 °C for 24 h and then removed and then stored at room temperature (25 °C) for 24 h and placed at a temperature of±40 °C, this process was counted as 1 cycle [19].

***Anti-aging* effectiveness test**

The skin condition of the back of the volunteer's hand before the treatment is checked first with test parameters including moisture and skin wrinkles using the moisturizer analyzer and dino-lite Dermatoscopy. Testing of anti-aging activities was conducted on 20 volunteers divided into 4 groups, namely:

Group I: 5 volunteers for blank formula (without active substance)

Group II: 5 volunteers for gel formula with zeaxanthin concentration 5%

Group III: 5 volunteers for gel formula with zeaxanthin concentration of 7.5%

Group IV: 5 volunteers for gel formula with zeaxanthin concentration of 10%.

RESULTS

Antioxidant activity test is conducted using DPPH method (1,1–difenil-2-pikrilhidrazil). Ic50 value is obtained from linear regression calculation between percent (%) inhibition with sample concentration. The smaller the IC50, the better its antioxidant activity.

Table 2: IC $_{50}$ value of vitamin c and zeaxanthin

Sample	IC $_{50}$	IC$_{50}$ rated intensity
Vitamin C	2.467	Very strong
Zeaxanthin	9.440	Very strong

Table 3: Organoleptic observations results on weeks 0 to 4

Preparation	Week	Color	Smell	Form
Formula 0	0	Clear	No smell	Gel
(base)	1	Clear	No smell	Gel
	2	Clear	No smell	Gel
	3	Clear	No smell	Gel
	4	Clear	No smell	Gel
Formula I	0	Yellow	Typical zeaxanthin	Gel
	1	Yellow	Typical zeaxanthin	Gel
	2	Yellow	Typical zeaxanthin	Gel
	3	Yellow	Typical zeaxanthin	Gel
	4	Yellow	Typical zeaxanthin	Gel
Formula II	0	Yellow-Orange	Typical zeaxanthin	Gel
	1	Yellow-Orange	Typical zeaxanthin	Gel
	2	Yellow-Orange	Typical zeaxanthin	Gel
	3	Yellow-Orange	Typical zeaxanthin	Gel
	4	Yellow-Orange	Typical zeaxanthin	Gel
Formula III	0	Orange	Typical zeaxanthin	Gel
	1	Orange	Typical zeaxanthin	Gel
	2	Orange	Typical zeaxanthin	Gel
	3	Orange	Typical zeaxanthin	Gel
	4	Orange	Typical zeaxanthin	Gel

Based on the results obtained, zeaxanthin has activity as an antioxidant and is very powerful. This is in accordance with the literature where a substance is said to have an antioxidant activity group is very strong if the ic50 value is less than 50 ppm, the IC50 strong group is between 50-10 ppm, the moderate group if the IC50 value is between 101-150 ppm, the group is weak if the IC50 value is between 150-200 ppm, and the group is very weak when the IC50 value is more than 200 ppm [20].

Organoleptic gel tests are conducted by visually observing the shape, color and smell of the gel. Organoleptic results of the four gel preparation formulas can be seen in table 3 where color differences are obtained due to differences in zeaxanthin concentration.

Homogeneity test aims to see and know the mixing of preparations. Gel preparations should be homogeneous and evenly distributed so as not to cause irritation when applied to the skin. This test is done by placing gel preparations between two glass objects. The results showed that all preparation formulas for 4 w can be said to be homogeneous because when viewed under a microscope, there are no visible fine particles.

pH testing aimed to determine the acidity or wetness of a preparation. Where when a preparation is too acidic will cause irritation to the skin and will cause heat on the skin or smeared areas and when the preparation is too alkaline can cause the skin to become dry and itchy. Based on the results listed in table 4 for 4 w shows that all formulas are in a good pH range of 4-7 [21].

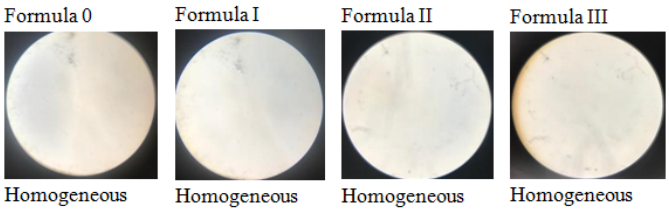

Fig. 1: Homogeneity test results

Table 4: Observations of pH measurements in weeks 0 to 4

Formula	Observation duration (Week)				
	0	1	2	3	4
F0	6	5	5	5	5
FI	5	5	5	5	5
FII	5	5	5	5	5
FIII	6	5	5	5	5

Spreadability testing aims to determine the speed of spread or equalization of the gel. From the data table 5 can be seen the spread value in all four formulas has entered the range of requirements, although, during the 4 w of testing, there is a shift in the value of the spread but still in a good range of spreadability. The good spread value of gel is in the range of 5-7 cm [21].

Table 5: Observation results of the spread test in week 0 to 4

Formula	Observation duration (Week)				
	0	1	2	3	4
F0	6.0 cm	6.0 cm	6.4 cm	5.8 cm	5.6 cm
FI	5.2 cm	5.4 cm	5.4 cm	5.7 cm	5.2 cm
FII	5.5 cm	7.0 cm	6.0 cm	5.5 cm	5.2 cm
FIII	5.0 cm	5.5 cm	5.1 cm	5.0 cm	5.4 cm

Viscosity test aims to see the viscosity of a preparation. The results of the measurement of the viscosity of gel preparations can be seen in fig. 2 where the viscosity of the gel preparations produced shows that the higher the concentration of zeaxanthin viscosity the higher. Although for 28 d there is an increase and decrease, but it can still be tolerated because it is still in a good viscosity range. The good viscosity range is 2000-4000 cps [22].

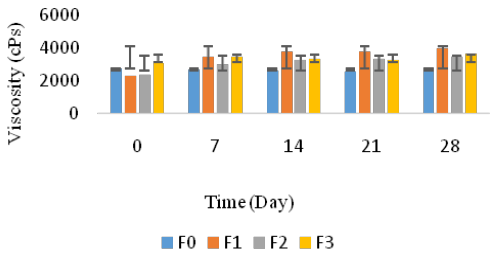

Fig. 2: Viscosity observation results on days 0 to 28

Cycling test aims to determine the physical stability of dosage with the influence of temperature variations. Cycling test evaluation is conducted for 6 cycles. Where in 1 cycle into two stages that are placed in the refrigerator at a temperature of 4 °C for 24 h, and the next 24 h placed in the oven at a temperature of 40 °C. Cycling test results can be seen in table 5. Based on the data obtained shows that only formula II and III are more stable compared to other formulas based on the parameters of pH, color and also smell.

Testing anti-aging activities using skin moisture analyzer (FCM-1) and Dermatoscopy, test parameters include moisture and skin wrinkles. Measurement of anti-aging effectiveness begins by measuring the initial condition of the skin in the area of the back of the hand that has been marked. Then zeaxanthin gel is applied topically every morning and evening. Once a week, measured the change, up to 4 w of use.

Moisture Measurement is done using a skin moisture analyzer tool. The results showed that the moisture on the skin of each group's back before the use of zeaxanthin gel was not all in normal condition. The normal range for humidity is 30-50% [23]. However, after four weeks of use of zeaxanthin gel there was an increase,

although the increase was unstable. This could be due to the non-compliance of volunteers in the use of zeaxanthin gel, resulting in reduced therapeutic effects. Based on the results of observations showed that during the four weeks of use of zeaxanthin gel, moisture in the skin of volunteers increased especially in formula III with an average percent increase of 33.17 percent±11.86

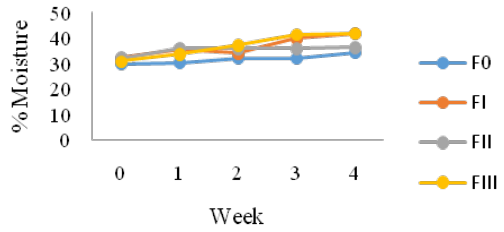

Fig. 3: Results average moisture increase

Wrinkles measurement using Dermatoscopy tool that is by using a 50-fold magnification lens. The results of the wrinkle measurement can be seen in the picture showing that the skin of the back of the volunteer group's hands before using zeaxanthin gel and after using for 4 w there was a decrease in wrinkles.

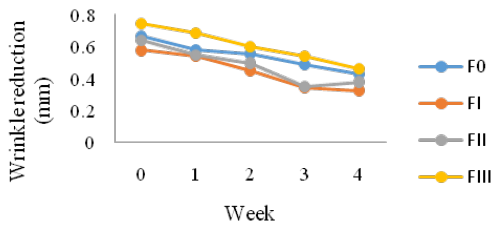

Fig. 4: Average wrinkle reduction results

DISCUSSION

Antioxidant activity test is conducted using DPPH method (1,1–diphenyl-2-pckrylhydrazyl where DPPH method has the advantage of the simple, fast, easy and sensitive analysis method to samples with small concentration [24]. Zeaxanthin and vitamin C solution is measured at a wavelength of 516.5 nm. The antioxidant potential of a compound can be determined by calculating its IC50 nilia (Inhibitory Concentration). The result of IC50 zeaxanthin is 9.044 ppm is in a very strong range because it is in the range of 0-50 ppm.

In the manufacture of zeaxanthin gel preparations used variations in concentrations in each formula namely FI 5%, FII 7.5%, and FIII 10% b/v using a gel base that is carbomer which will form a mass of gel when pH 6-8, so to form a clear gel base need to be added TEA that is alkaline. TEA is a compound that will increase the mass pH of the gel, where a good and clear gel mass is formed so that it is expected not to irritate the skin. Another additional ingredient used is glycerin. Glycerin serves as a humectant and emollient to prevent the occurrence of syneresis and as a moisturizer on the gel.

The results of the evaluation test of zeaxanthin gel preparations obtained organoleptic observations of orange-yellow color with a distinctive smell of zeaxanthin that can form gels with transparent color and provide a cool effect that is easy to wash with water. Homogeneity test results show that all preparation formulas for 4 w can be said to be homogeneous because when viewed under a microscope, there are no visible fine particles. The dosage pH test shows the acidity level of the preparation is within the good pH range of 4-7 [21]. The results of the spread of preparations show the ability to spread the preparation when applied to the skin. The good spread value of gel is in the range of 5-7 cm [21]. When a preparation has a high spread value means the greater the spread area that causes the active substances in the preparation is spread evenly and more effective in producing therapeutic effects. This spreadability is influenced by viscosity, the greater the viscosity value of gel preparations, the spread value will decrease and vice versa. When the gel preparation has a low viscosity value, the spread value will be greater. With a large spread, it will make it easier when applying on the skin or make it easier to contact with active substances so that it will soon be absorbed [25]. Cycling test results aim to determine the physical stability of a dosage with the influence of temperature variations. The data obtained shows that zeaxanthin gel formula has good stability during storage at room temperature and temperature 40C is characterized by the absence of changes of the pH parameters, color and also smell.

Testing anti-aging activities using skin moisture analyzer (FCM-1) and Dermatoscopy, test parameters include moisture and skin wrinkles. The data showed that there was a significant difference ($P<0.05$) between formulas either from increased humidity or from a percent decrease in wrinkle diameter. Increased levels of zeaxanthin in gel formulas showed an increase in better anti-aging activity. It is appropriate to hypothesize that zeaxanthin as a very powerful natural antioxidant, is able to protect the skin against oxidative stress. Many benefits are obtained from the consumption of zeaxanthin as an antioxidant, one of which is the protection of the skin against oxidative stress. Oxidative stress plays an important role in the aging process of the skin and the destruction of the dermal layer in humans. Intrinsic (chronological) and extrinsic (photo-) aging mechanisms occur each, including the emergence of reactive oxygen speci (ROS) through oxidative metabolism and due to exposure to ultraviolet (UV) light from the sun. The formation of ROS can trigger the occurrence of skin aging [26-28]. One of the clinical manifestations that can occur due to skin aging is the appearance of wrinkles on the skin, the appearance of dark spots due to sunlight (skin pigmentation) [29].

CONCLUSION

Zeaxanthin has an IC50 value of 9.044 µg/ml. Gel zeaxanthin meets the requirements of physical evaluation of gel preparations except in cycling test and has the effect of being anti-aging as shown by the presence of a percent increase in average humidity of 33.17%±11.867 and also a percent decrease in the average of 47.466%±7.115.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

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