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Original Article

FORMULATION AND CHARACTERIZATION OF FACIAL SERUM FROM ASTAXANTHIN-BETA CAROTENE NANOEMULSION AS AN ANTIOXIDANT

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ABSTRACT

Objective: The purpose of this study is to make facial serum from astaxanthin-beta carotene nanoemulsion as an antioxidant.

Methods: Nanoemulsions were prepared using Spontaneous Nanoemulsion (SNE) method with a ratio of 1:8:1 for (Sunflower oil): surfactant (Kolliphor® RH40): and co-surfactant (PEG 400). Physical nanoemulsion characterization includes globule size, polydispersity index, zeta potential, visual appearance, pH and entrapment efficiency test. The best results from nanoemulsion were then combined into serum preparations which were then tested for evaluation of the preparations, including organoleptic, homogeneity, viscosity, pH, adhesion and stability test (cycling test).

Results: The results showed that the nanoemulsion of astaxanthin and beta-carotene combination that had been developed had a globule size of<50 nm (with a normal globule size distribution curve), polydispersity index value was less than 0.5, zeta potential was greater than-20 mV and entrapment efficiency was ranging from 80-85%. The results of the preparation evaluation showed that serum astaxanthin-beta carotene nanoemulsion had good results in physical, chemical and stability test during storage. The best serum formula is formula 3 when viewed from the results of the evaluation of the dosage form with an IC_{50} value of 8.562 ppm with a very strong category as an antioxidant.

Conclusion: Facial serum from astaxanthin-beta carotene is the potential to be an antioxidant.

Keywords: Serum, Astaxanthin-beta carotene combination, Antoxidant, Carotenoids

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INTRODUCTION

Astaxanthin is a group of xanthophyll carotenoids that are found in various microorganisms and marine animals that have characteristics as strong antioxidants [1-4]. Astaxanthin has a conjugated double bond, a hydroxyl group and a keto-so it has both lipophilic and hydrophilic properties. The bioavailability of some lipophilic carotenoids is very low, causing low dissolution availability in gastrointestinal fluids [5, 6]. Antioxidant compounds are known to counteract free radicals; antioxidant compounds are believed to make skin youthful or prevent aging [1], There are many types of antioxidant compounds, one of which is β-carotene which can eliminate excessive reactive oxygen species produced in the body. So that β-carotene has the potential to be used in the pharmaceutical, food, and cosmetic industries [2]. β-carotene has advantages such as being able to deliver active ingredients into the skin because the stratum corneum has lipoid characteristics. β-carotene has hydrophobic properties so it has a better chance of penetrating lipids than water-soluble components [7].

Astaxanthin and β-carotene have some limitations in terms of their solubility because they are lipophilic, so their bioavailability is very low [8-9]. One of the efforts that have been made to increase its hydrophilicity is by offering nanotechnology by developing astaxanthin and β-carotene nanoemulsions which are aimed for increasing stability and solubility in the preparation and also in the future to develop new delivery pathways in the use of antioxidants, through the topical route for drug use, so it can be optimized. Some of the advantages of nanoemulsions are increasing bioavailability through increased drug solubility, drug protection against hydrolysis enzymes, increasing the specific surface area of globules so that the drug is widely distributed in the gastrointestinal tract and changes in permeability induced by surfactants.

There are many benefits of consuming astaxanthin and β-carotene as antioxidants, one of which is the protection of the skin against oxidative stress which plays an important role in the aging process of the skin and damage to the dermal layer in humans. The intrinsic (chronological) and extrinsic (photo-) mechanisms of aging include the emergence of reactive oxygen species (ROS) through oxidative metabolism and exposure to ultraviolet (UV) light from the sun. The formation of ROS can trigger skin aging [10-12]. One of the clinical manifestations that can occur due to skin aging is occurrence of wrinkles on the skin, the appearance of dark spots due to sunlight (skin pigmentation) [13].

The development of a skin-lightening serum (radiance serum) containing a combination of astaxanthin and β-carotene nanoemulsion for topical use is one of the strategies used to increase the efficacy of the use of these carotenoid antioxidants. Through the administration of the topical route, it can eliminate the deficiencies that occur due to oral use. Then, by packing the drug into a nanoemulsion, it maximizes its absorption into the dermal layer.

MATERIALS AND METHODS

Materials

The materials used was astaxanthin and β-carotene (Sigma-Aldrich®). Sunflower oil was purchased from Jan Dekker International (Netherland). Polyoxy-35-castor oil (Kolliphor® RH40) was purchased from BASF (Indonesia). Polyethylene Glycol 400 (PEG 400) was purchased from Merck (Indonesia), ascorbic acid, propylene glycol, 2,2-diphenyl-1-picrylhydrazyl (dpph), sodium carboxymethyl cellulose (Na-CMC), demineralization water, dimethylol-5-5-dimethylhydantoin (DMDM hydantoin), sodium metabisulfite, and methanol p. a (Germany Merck).

Methods

Preparation of astaxanthin-β-carotene nanoemulsion

Nanaoemulsions are made using the self-nanoemulsifying (SNE) method by optimizing the ratio of the oil, surfactant and co-surfactant phases. The best ratio used was the result of a previous study (14) with a ratio of 1:8:1 for (Sunflower oil): surfactant (Kolliphor® RH40): and co-surfactant (PEG 400). The mixture was stirred at 100 rpm for 30 min using a magnetic stirrer (IKA® C-MAG HS7), then sonicated for an hour and nanoemulsions were formed after adding deionized water with slow stirring.

Physical and chemical characterization of zeaxanthin-β-carotene nanoemulsion

This characterization includes organoleptic test, pH, globule size, particle size distribution, visual appearance, zeta potential, and entrapment

efficiency. Characterization of size, PDI and zeta potential using particle size analyzer from Malvern by taking a sample of about 2-3 ml inserted into a cuvette which was then measured. Determination of entrapment efficiency in nanoemulsion based on the difference between the amount active substances used in the manufacture of nanoemulsions with the amount of active substance that is entangled by means of centrifugation at 3000 rpm for 15 min. Free sample will precipitate, so that the entangled active substance can be analyzed using UV-Visible spectrophotometer at wavelength 470 nm.

Preparation of radiance serum naoemulsion zeaxanthin-β-carotene

Radiance serum was made in the form of a gel using various concentrations of astaxanthin and beta-carotene nanoemulsion. The gelling agent used is HPMC which is added with other additives such as propylene glycol, DMDM hydantoin, and sodium metabisulfite to form a transparent gel mass.

Physical and chemical characterization of radiance serum astaxanthin-β-carotene nanoemulsion

Organoleptic and pH test

Organoleptic testing was carried out by visually observing the radiance of the serum astaxanthin-β-carotene nanoemulsion produced, including color, odor and clarity. Then, the pH test of the serum radiance was carried out by measuring the pH using a calibrated pH meter.

Determination of viscosity dan spreadability test

The radiance viscosity of serum astaxanthin-β-carotene nanoemulsion was determined using a Brookfield Viscometer with a suitable spindle type and at a shear rate. The spreadability test was carried out by placing a sample of±0.5 grams on a 20x20 cm glass, then the glass was covered with plastic and given a weight of ±50 grams. After 1 min, the diameter of the distribution formed was measured.

Antioxidant activity of zeaxanthin-β-carotene

The antioxidant activity of the sample was determined by adding 1 ml of the sample solution to 2 ml of DPPH solution (0.005%, w/v), then the mixture was incubated for 5 min. Then, the absorbance of the mixture was measured using a UV-Visible Spectrophotometer with an absorbance range of 400-800 nm. Absorbance measurements were carried out until a stable absorbance was obtained.

RESULTS

Astaxanthin-β-carotene nanoemulsion was prepared using the spontaneous nanoemulsification method in which an isotropic mixture of oil, surfactant, and drug will spontaneously form a nanoemulsion (oil in water) when it encounters the aqueous phase under mild agitation conditions. This mixture can form an emulsion spontaneously if the change in entropy of the dispersion system is greater than the energy required to increase the surface area of the dispersion. SNE also requires cosurfactants to facilitate the nanoemulsification process or enhance drug incorporation in the nanoemulsion [15]. The results showed that nanoemulsion of astaxanthin and beta-carotene combination that had been developed had a globule size of<50 nm (with a normal globule size distribution curve), polydispersity index value was less than 0.5, zeta potential was greater than-20 mV and entrapment efficiency was ranging from 80-85%.

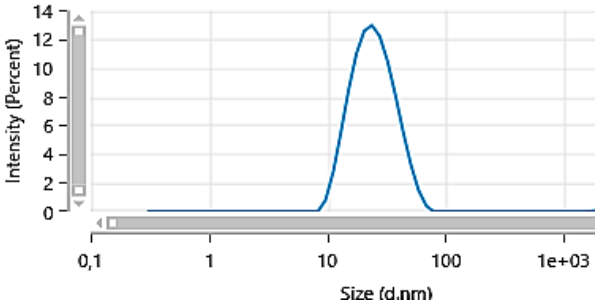

Fig. 1: Astaxanthin-β-carotene nanoemulsion globule size distribution curve

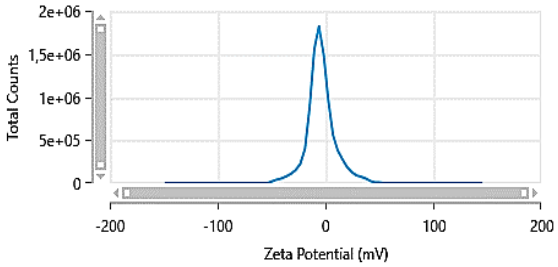

Fig. 2: Zeta potential value curve of astaxanthin-β-carotene nanoemulsion

Table 1: pH of astaxanthin-β-carotene nanoemulsion

Sample	Replication	pH	Average±SD
Formula 1	I	6.00	6.00±0.00
	II	6.00	
	III	6.00	
Formula 2	I	5.98	5.98±0.00
	II	5.98	
	III	5.98	
Formula 3	I	5.97	5.97±0.00
	II	5.97	
	III	5.97	

Table 2: Physical and chemical properties of radiance serum nanoemulsion astaxanthin-β-carotene

Parameters	F1	F2	F3
Visual properties serum	Clear red	Clear red	Clear red
pH of serum	5.90±0.023	5.96±0.0264	5.98±0.02
Viscosity value (cps)	1777±5.86	1983±11.86	1383±4.86
Homogeneity Test	Homogeneous	Homogeneous	Homogeneous
Spreadability test (cm)	5.65±0.34	5.450±0.12	5.23±0.104

Values are given as mean±standard deviation (n=3). The results of the antioxidant activity test of astaxanthin using DPPH with vitamin C as a comparison showed a very strong activity of astaxanthin with IC_{50} values of 8.62 ppm and 2.231 ppm for vitamin C.

Table 3: Antioxidant activity of vitamin c and radiance serum from astaxanthin-β-carotene nanoemulsions

Sample	IC_{50}	Intensity value of IC_{50}
Vitamin C	2.231	Very Strong
Sample	8.62	Very Strong

DISCUSSION

Self-nano emulsifying dosage forms were anhydrous homogenous liquid mixtures consisting of oil, surfactant, drug, and co-surfactant, which spontaneously form oil-in-water nanoemulsion upon dilution water under gentle stirring. Adding surfactant and co-surfactant into systems enhances drug dissolution and formulation dispersibility during dilution with an aqueous medium of GIT. During dilution with water, active substance dissolves in oil phase and/or surfactant, which forms a film between oil and water phase [14-17].

The results of observations on the astaxanthin-β-carotene nanoemulsion made were transparent red in color, had no odor, and were clear. The formation of nanoemulsions was characterized by a clear and transparent mixture due to the homogeneous and nano-sized dispersion of oil globules with the drug in the solution [14]. In this study, the oil phase ratio: surfactant: optimum co-surfactant was used from previous studies [14] with a ratio of 1:8:1 for (Sunflower oil): surfactant (Kolliphor® RH40): and co-surfactant (PEG 400). From the results of the study, the globule size ranged from 23.68 nm (with a normal globule size distribution curve), polydispersity index value of 0.2136, zeta potential greater than (-4.218) mV and entrapment efficiency ranging from 80-85%. This polydispersity index provides information about the physical stability of a dispersion system that is formed which is more stable in the long term [18-19].

The zeta potential value can indicate the stability of a system containing dispersed globules through the repulsion between particles of the same charge when they are close together. A zeta potential value greater than (+30) mV or less than (-30) mV will be electrostatically stable, while a zeta potential value greater than (+20) mV or less than (-20) mV will be stable. sterically [14]. Astaxanthin-β-carotene nanoemulsion with a zeta potential value close to±20 mV was sterically stabilized by the presence of non-ionic surfactant polymer chains in micelles [20].

The value of the entrapment efficiency of the active substance is an important parameter in the formulation related to the active substance that can be entangled in the nanoemulsion dosage formula. The entrapment efficiency values of all formulas are around 80-85%. This indicates that the entrapment of the combination of astaxanthin-β-carotene in the nanoemulsion globules was quite efficient.

The results of the organoleptic evaluation showed that the three radiance serum formulas of astaxanthin-β-carotene nanoemulsion had a thick, odorless and bright red color due to the combination of red from astaxanthin and yellow from-carotene. Homogeneity observations show that the three formulas that have been made have high homogeneity because the results of the visual test by dripping the preparation on the surface of the prepared glass and then covering it with another prepared glass do not show any lumps and coarse grains.

Based on the results of the viscosity test, it can be seen that the three formulas meet the viscosity requirements as a gel which ranges from 800-3000 cps [21]. The viscosity factor greatly affects the spreadability of the preparation and the ease of pouring the preparation from the packaging. pH testing aims to determine and evaluate the pH value of the preparation to match the pH value of the skin, so that skin irritation does not occur and ensures the safety of the preparation. In this pH test, F1, F2 and F3 meet the requirements, namely pH 6 is in the skin pH range (4.5-6.5) [22]; because the pH of the preparation is in the skin pH range, the preparation is safe to be applied to the skin. If the pH exceeds this range, it can cause damage to the skin.

Adhesion test aims to see how long it takes the preparation to stick to the skin surface by using adhesive test equipment not in animal or human skin, this test is carried out 3 times a repetition for 10 seconds for each formula. The results of the adhesion test for all formulas showed the ability of the preparation to adhere to the skin surface for more than 10 seconds. This proves that the preparation in each formula has a good ability to adhere to the skin.

The antioxidant activity test was carried out using the DPPH (1,1-diphenyl-2-pycrilihydrazyl) method, where DPPH is a fairly stable free radical compound. In addition, the DPPH method has the advantage that the analysis is simple, fast, easy and sensitive to samples with small concentrations. Based on the results of the antioxidant activity test, it was stated that astaxanthin and β-carotene were included in the very strong antioxidant group because they were in the IC_{50}<50 ppm range, namely 8.562 ppm [23].

CONCLUSION

In this study, a nanoemulsion-based radiance serum formulation for topical administration was successfully developed to deliver lipophilic agent such as astaxanthin and beta carotene. The astaxanthin-beta carotene nanoemulsions had good physical and chemical characteristics and its antioxidant activity is very strong and with an IC_{50} value of less than 50 ppm. the results of the evaluation of the astaxanthin-beta carotene nanoemulsion serum radiance preparation meet the test requirements.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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