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Original Article

PHYSICAL AND CHEMICAL EVALUATIONS OF TOPICAL RADIANCE SERUM CONTAINING NANOEMULSION COMBINATION OF ASTAXANTHIN AND ZEAXANTHIN: DESIGNED AS ANTI-WRINKLE AND SKIN-BRIGHTENING SERUM

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ABSTRACT

Objective: The present study was conducted to formulate and evaluate the radiance serum containing the combination of astaxanthin and zeaxanthin nanoemulsion designed for anti-wrinkle and skin brightening serum by topical route of administration.

Methods: The nanoemulsion containing astaxanthin and zeaxanthin was prepared using the self-nano emulsifying method, followed by incorporation into the radiance serum by the conventional mixing method. The quantity and ratio of surfactant, co-surfactant, and oil phase have been optimized in the previous study, as well as the radiance serum formula. The evaluation of the nanoemulsion and radiance serum was carried out by physical and chemical characterization. At the end of the study, an antioxidant activity of the serum containing nanoemulsion of astaxanthin and zeaxanthin was performed by DPPH method and the antioxidant activity was compared to its pure forms. The evaluation of the *ex vivo* permeation study was carried out to evaluate its possibility as an anti-wrinkle and skin brightener.

Results: An astaxanthin and zeaxanthin nanoemulsion had a good physical properties with a globule size around of 20 nm (narrow particle size distribution), an entrapment efficiency range greater than 85%, and had a spherical morphology. The radiance serum had a good organoleptic and spreadability with the semifluid characteristic. Based on the result of antioxidant activity, the radiance serum had a highly active antioxidant activity. The radiance serum contained of astaxanthin and zeaxanthin nanoemulsion of 1% concentration, astaxanthin had a 2-6 times cumulative released compared than zeaxanthin ($p<0.05$) and all of the formulations exhibited a high skin permeation significantly.

Conclusion: A formulation of nanoemulsion-based serum containing astaxanthin and zeaxanthin for topical delivery has been successfully developed. Based on the results of physical evaluation and especially from the permeation study, it seems that radiance serum containing astaxanthin and zeaxanthin nanoemulsion was potential to be used as an anti-wrinkle and skin brightening, however this function must be proven in further research.

Keywords: Astaxanthin, Zeaxanthin, Nanoemulsion, Radiance serum

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INTRODUCTION

Astaxanthin and zeaxanthin are a group of xanthophyll with lipophilic characteristics and high antioxidant activity [1-3]. Bioavailability of several lipophilic carotenoids was extremely low, between 10 to 50%. Limitation of dissolution in the gastrointestinal fluid was one of the causes of this low bioavailability. Another contributing factor was the saturation of carotenoids to penetrate the micelles formed by the biliary salts into the gastrointestinal tract at high doses [4, 5]. Zeaxanthin is hydrolyzed by carboxyl ester lipase in the lumen of the small intestine; afterwards, it will be pumped out from the enterocytes of small intestine and secreted into the spleen or portal vein. Zeaxanthin may undergo a first-pass metabolism in the liver following oral administration [6-8].

There are many benefits of consuming astaxanthin and zeaxanthin as antioxidants, one of the benefits is the protection of skin against stress oxidative which plays an important role in the process of skin aging and dermal layer damage in human. The intrinsic and extrinsic mechanisms of photoaging occur because the formation of reactive oxygen species (ROS) by oxidative metabolism and due to exposure ultraviolet radiation [9-11]. One of the clinical manifestations of skin aging is the appearance of wrinkles and dark spots (due to skin pigmentation) [12].

This research aimed to develop a radiance serum containing a combined nanoemulsion of astaxanthin and zeaxanthin (AZ-NE) for topical administration to optimize the efficiency of these carotenoids. This research was further research on the development of astaxanthin nanoemulsion that was successfully developed in a previous study [13]. In this study, AZ-NE was prepared using the self-nanoemulsifying method, and afterwards AZ-NE was formulated in radiance serum (AZ-NES). Physical and chemical evaluations of AZ-NE were performed by organoleptic, pH, globule size and distribution, zeta potential, morphology, and entrapment efficiency test. A physical evaluations of AZ-NES were performed, including organoleptic, pH, viscosity, and spreadability. At the end of the study, the antioxidant activity of astaxanthin and zeaxanthin nanoemulsion in the radiance serum was carried out by the DPPH test and compared with its pure forms. The efficacy and potential function of radiance serum containing astaxanthin and zeaxanthin nanoemulsion as anti-wrinkle and skin brightening was performed by an *ex vivo* permeation test.

MATERIALS AND METHODS

Materials

Astaxanthin (Astareal®L10) was purchased from Fuji Chemical Industries (Japan). Zeaxanthin was purchased from Inner Mongolia (China). 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). Niacinamide, disodium EDTA, PEG-12 dimethicone, sodium hyaluronate were purchased from Thornhill (Canada). Glycerin was purchased from Kemiko Indonesia (Indonesia). Xanthan gum was purchased from Deosen Biochemical (Ordos) (China). Dl-alpha tocopheryl acetate was purchased from Zhejiang Medicine (China). *Python reticulatus* skin was obtained from Bandung Zoo (Indonesia). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). All other chemicals uses in the study were of analytical reagent grade.

Preparation of astaxanthin and zeaxanthin nanoemulsion (AZ-NE)

The AZ-NE was prepared by using the self-nanoemulsifying (SNE) method that was optimized on the previous study [13-15]. The ratio and quantity of astaxanthin and zeaxanthin was 1:1 (in total 4 mg), 2:1 (in total 6 mg), and 3:1 (in total 8 mg). The mixture was dissolved in 1 gram of oil phase containing sunflower oil, kolliphor®RH40, and PEG 400 with a ratio of 1:8:1, respectively. The mixture was then mixed at 100 rpm for 30 min using a magnetic stirrer (IKA®C-MAG HS7), followed by a one-hour sonication (Krisbow®). Nanoemulsion will be formed after addition deionized water until 20 gram of solution.

Physical and chemical characterization of AZ-NE

Organoleptic and pH determination

The organoleptic test includes the observation of the color, odor, and clarity of AZ-NE. A pH of AZ-NE (1 g solution in 5 ml deionized water) was determined with a calibrated pH meter (Mettler Toledo) [13-15].

Globule size, polydispersity index, and zeta potential analysis

Globule size and polydispersity index analysis of the AZ-NE was determined by photon correlation spectroscopy that analyzes fluctuations in light scattering due to Brownian motion of the particles, using a Zetasizer version 7.11. (Malvern Instruments, Canada). The formulation (1 gram) was dispersed in 5 ml of deionized water, mixed thoroughly and the light scattering was performed at an angle of 90° at 25 °C [13-15].

Zeta potential analysis of the AZ-NE was determined by using a Zetasizer version 7.11. (Malvern Instruments, Canada), which calculates zeta potential using the principle of electrophoresis. The formulation (1 gram) was dispersed in 5 ml of deionized water, mixed thoroughly and the zeta potential was measured at 25 °C [13-15].

Morphology

The morphology of AZ-NE was examined by using transmission electron microscopy (TEM). Approximately 10 µl of AZ-NE solution was dropped on standard carbon-coated copper grids (400-mesh). After 1 minute, approximately 10 µl of uranyl acetate was dropped into the grid and air-dried for approximately 30 min. The TEM images were obtained using a TEM JEOL JEM 1400 (Japan) operating at 120.0 kV [13-15].

Entrapment efficiency

The entrapment efficiency was obtained by measurement of free amount of astaxanthin and zeaxanthin in the solution. One gram of AZ-NE was dispersed with 5 ml deionized water in an eppendorf tube (Vivaspin® 500), and then solution was centrifuged at a speed 11,000 rpm for 180 min (Thermo Scientific MicroCL 21). Free astaxanthin and zeaxanthin will precipitate and separate at the top of solution. A supernatant was collected and was dissolved in methanol and analyzed with a UV-visible spectrophotometer at a wavelengths of 488 nm and 428 nm, respectively [13-15].

Preparation of radiance serum of astaxanthin and zeaxanthin nanoemulsion (AZ-NES)

A concentration of 1%, 3%, and 5% (w/w) of AZ-NE was added slowly into the serum mixture that was optimized in the previous study [15]. The final mixing was done by using a magnetic stirrer (IKA®C-MAG HS7) in at 100 rpm for 30 min. The formulas are presented on the table 1 below.

Table 1: Radiance serum formulation containing AZ-NE

Materials	Material compositions		
	Formula 1	Formula 2	Formula 3
AZ-NE	1%	3%	5%
Niacinamide	0.5%	0.5%	0.5%
Disodium EDTA	0.2%	0.2%	0.2%
PEG-12 dimethicone	2.0%	2.0%	2.0%
Rose hip oil (*Rosa moschata seed oil*)	1.5%	1.5%	1.5%
PEG 400 caprylic/capric glycerides	5.0%	5.0%	5.0%
DMDM hydantoin	0.1%	0.1%	0.1%
Sodium hyaluronate	1.0%	1.0%	1.0%
Glycerin	3.0%	3.0%	3.0%
Xanthan gum	0.1%	0.1%	0.1%
dl-alpha tocopheryl acetate	1.0%	1.0%	1.0%
Natural rose fragrance	0.05%	0.05%	0.05%
Purified water	Ad 100%	Ad 100%	Ad 100%

Physical and chemical characterization of AZ-NES

Organoleptic and pH determination

Organoleptic includes the color, odor, and clarity of AZ-NES were observed. A pH of AZ-NES was determined by using a calibrated pH meter (Mettler Toledo) [15].

Viscosity and spreadability tests

The viscosity test of AZ-NES was performed by using a Viscometer (Brookfield®) with a typical spindle of 7 and a shear rate of 100 rpm (rotation per minute). Spreadability test was performed by putting±0.5 grams on the 20x20 cm glass, then covered with mica plastic and given a weight of 100 grams. After 1 minute, the spread diameter was measured [15].

Antioxidant activity test

An antioxidant activity test of AZ-NES was determined by adding 1 ml sampel solution (0.005 %, w/v) in a 2 ml DPPH solution (0.005 %, w/v), then mixed solution was incubated for 5 min. The absorbance of the mixed solution was measured using the UV-Visible Spectrophotometer (Genesys 10S) in an absorbance range of 400-600 nm. Absorbance measurements were carried out until a stable absorbance was obtained and an antioxidant activity (IC_{50}) was measured [15].

***Ex Vivo* permeation test**

An *ex vivo* permeation test was conducted using a Franz diffusion cell with *Python reticulatus* skin as a membrane barrier. Zero point five (0.5) gram of AZ-NES was putted onto the±2.8 cm^2 of skin as a donor compartment. The receptor compartment contains of 50 ml of phosphate buffer pH 7.4. During the operation of the Franz diffusion cell, the temperature was adjusted to 37 °C±0.5 °C with the water jacket. One (1) ml of blank media was collected prior to the operation. Then sampling was carried out of 1 ml from the receptor compartment at 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min using a micropipette (Socorex) and immediately replaced with the same volume of phosphate buffer pH 7.4. The sample was added in a 5 ml volumetric flask and the volume was adjusted to 5 ml and shaken homogeneously. Then, the absorbance was measured by UV-Visible Spectrophotometer (Genesys 10S) for astaxanthin and zeaxanthin at the maximum wavelengths of 488 and 428 nm, respectively.

Astaxanthin and zeaxanthin levels which penetrated to the receptor fluid every time sampling was calculated [15].

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant intergroup differences in each parameter. A p-value<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Self-nano emulsifying dosage forms are anhydrous homogenous liquid mixtures consisting of oil, surfactant, drug, and co-surfactant, which spontaneously form oil-in-water nanoemulsion after dilution with water by gently stirring. During dilution with water, the active substance dissolves in the oil phase and/or surfactant, which forms a film between oil and water phase [16, 17]. The appropriate type and ratio of oil phase, surfactant and co-surfactant are critical parameters in the formation of nanoemulsion. In this study, the nanoemulsion system included the composition of the oil, surfactant, and co-surfactant as well as the preparation method were referred to the previous study [13-15]. The nanoemulsion properties containing astaxanthin and zeaxanthin combination are presented in table 2.

Table 2: Physical properties of AZ-NE

Compositions		Parameters	Results
Astaxanthin	Zeaxanthin		
2 mg	2 mg	Organoleptic	
		- Color	- Red
		- Odor	- Odorless
		- Clarity	- Clear
		pH	4.87±0.11
		Globule size (nm)	21.04±0.13
		Polydispersity index	0.098±0.027
		Zeta Potential (mV)	-6.75±0.761
4 mg	2 mg	Organoleptic	
		- Color	- Red
		- Odor	- Odorless
		- Clarity	- Clear
		pH	4.69±0.06
		Globule size (nm)	20.92±0.08
		Polydispersity index	0.113±0.007
		Zeta Potential (mV)	-7.48±1.515
6 mg	2 mg	Organoleptic	
		- Color	- Red
		- Odor	- Odorless
		- Clarity	- Clear
		pH	4.41±0.02
		Globule size (nm)	20.94±0.05
		Polydispersity index	0.106±0.019
		Zeta Potential (mV)	-7.12±3.551

*Values are given as the mean±standard deviation (n=3).

The AZ-NE droplet size was in the nano-range (about 20 nm) with an entrapment efficiency value range greater than 85% (table 3 below) and a pH of about 4. A Polydispersity index (PI) of the nanoparticle system describes a particle size distribution. Based on table 1 above, all formulas had a PI of approximately 0.1 and meets the requirements of an ideal PI range between 0 (narrow particle size distribution)–0.5 (wider particle size distribution). A PI indicates the physical stability of the dispersion system, whereas a lower PI indicates that the dispersion system is more stable than a higher PI [18]. A Zeta potential describes a characteristic of the surface charge of the nanoparticles system. The Zeta potential value demonstrated the stability of the system containing of particles dispersed by repulsion forces among the same charge particles. The AZ-NE had a zeta potential value of about-7 mV that is considered to be approximately neutral [19].

Table 3: An entrapment efficiency of astaxanthin and zeaxanthin in AZ-NE

Ratio of astaxanthin and zeaxanthin	Entrapment efficiency (%)	
	Astaxanthin	Zeaxanthin
1:1	88.3±0.22	91.4±0.39
2:1	93,6±0.25	93.9±0.31
3:1	95,8±0.19	95.7±0.27

*Values are given as the mean±standard deviation (n=3).

The morphology of AZ-NE containing a 3:1 astaxanthin and zeaxanthin ratio was performed by TEM and fig. 1 showed that AZ-NE habit was relatively spherical.

The three (3) of radiance serum formulations were prepared by using AZ-NE with a 3:1 astaxanthin and zeaxanthin ratio, while the AZ-NES properties are presented in table 4.

According to the results in table 4, AZ-NES had good physical properties with a pH of approximately 5. The spreadability test was carried out using a parallel-plate method to determine the ability of the serum to spread on the skin topically. A preferred serum can easily spread over the skin for convenient use. Formula 1 had a spread diameter of>50 mm but<70 mm that the serum was classified to the semifluid characteristic, while formula 2 and formula 3 had a spread diameter of≤ 50 mm, then were classified to semistiff characteristics [20]. The decrease of spread diameter of the radiance serum was associated with an increase in the concentration of the AZ-NE. As well as spread diameter, the viscosity was correlated with an AZ-NE concentration. Formula 3 had a higher consistency than formula 1 and 2, due to the concentration of AZ-NE. The viscosity of the serum may have an impact on skin retention and skin penetration, as well as reflects the consistency.

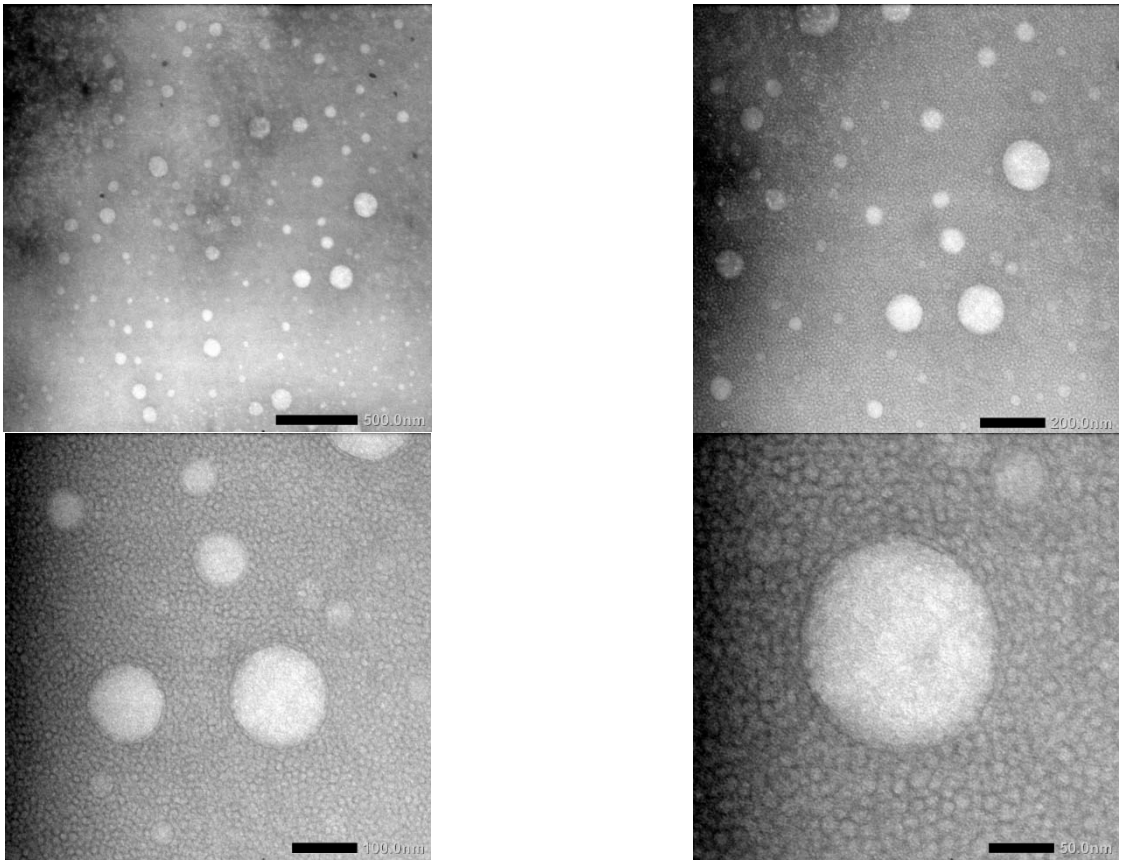

Fig. 1: Morphology of AZ-NE on the scale of 500 nm (top left); 200 nm (top right); 100 nm (bottom left); and 50 nm (bottom right)

Table 4: Physical properties of AZ-NES

Formula	Organoleptic	pH	Viscosity (cPs)	Spread diameter (mm)
1	Transparent liquid with a red color and rose odor	5.25±0.02	3,429±12.22	51.3±2.1
2	Transparent liquid with a red color and rose odor	5.09±0.03	3,720±16.00	50.0±1.0
3	Transparent liquid with a red color and rose odor	5.13±0.02	3,784±8.00	46.7±1.5

*Values are given as the mean±standard deviation (n=3).

Table 5: Antioxidant activity test of AZ-NES

Formula	Antioxidant activity (IC_{50} values) (µg/ml)	Antioxidant activity category
1	45.55±1.57	Very active
2	47.03±2.30	Very active
3	47.03±0.37	Very active

*Values are given as the mean±standard deviation (n=3).

The results for antioxidant activity are presented in table 5. The IC_{50} value is defined as the concentration of the substrate that causes a 50% loss of the DPPH activity (color). The DPPH method is an antioxidant assay based on electron transfer that produces a purple solution in ethanol [21]. IC_{50} describes the antioxidant activity, which is the lower of IC_{50} value, the higher of the antioxidant activity [22, 23]. Antioxidant activity categories are classified into 4 categories, namely IC_{50} less than 50 µg/ml, which is categorized as very active, 50-100 µg/ml, which is categorized as active, 100-200 µg/ml, which is categorized as quite active and more than 200 µg/ml which is categorized as inactive. Based on the results above, it can be seen that all of the formulas of AZ-NES are categorized as highly active antioxidant.

The cumulative release profiles of astaxanthin and zeaxanthin from AZ-NES matrix system from *ex vivo* permeation test are shown in fig. 2. Based on the graph above, astaxanthin had a higher released (about 2-6 times) compared than zeaxanthin from the nanocarrier system ($p<0.05$). We hypothesized that this was because astaxanthin was more bioactive compared than zeaxanthin due to the presence of a keto-group and a hydroxyl group at each end of its molecule [24]. The results of this study assessed the function of astaxanthin which has beneficial to skin health. All formulations showed significantly greater permeation compared to pure forms after 120 min. The difference of the fluxes of astaxanthin and zeaxanthin on penetrating to the skin compared its pure forms were due to its nanoemulsion properties which has ability to penetrate through bilayer structures of the intracellular lipids in the stratum corneum and supported by the existences of surfactant and co-surfactant in the dosage form [25]. However, an amount of astaxanthin which was formulated in the serum was affected in its release profile, while zeaxanthin was unaffected.

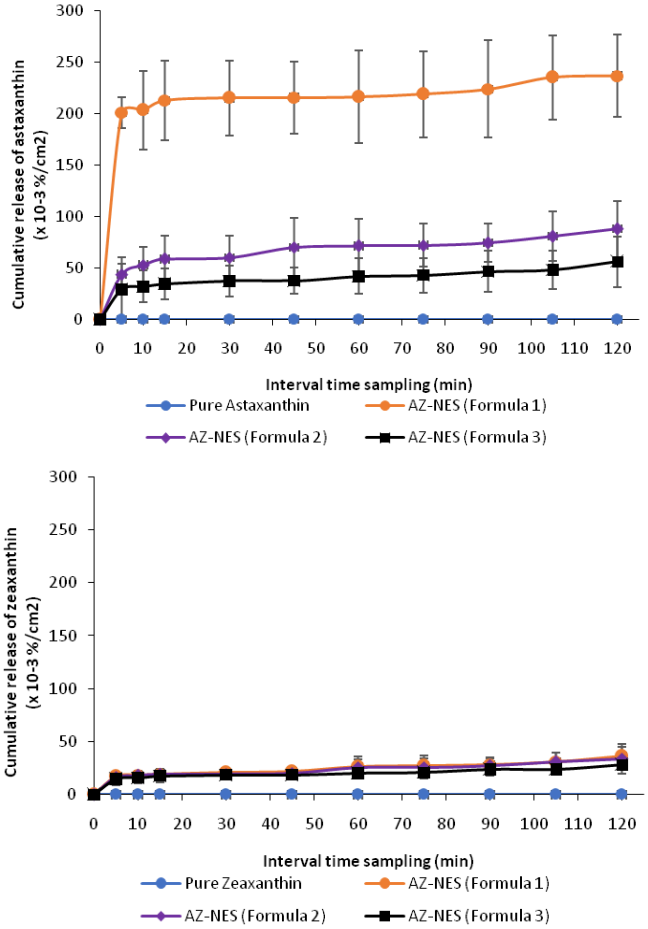

Fig. 2: A cumulative release profiles of astaxanthin (up) and zeaxanthin (down) from AZ-NES (n=3)

CONCLUSION

In summary, a formulation of nanoemulsion-based serum containing astaxanthin and zeaxanthin for topical delivery has been successfully developed. The radiance serum containing astaxanthin and zeaxanthin nanoemulsion had good physical characteristics, was highly active as an antioxidant, and had a high skin permeation compared to its pure forms. These properties supported the target of the serum function as an anti-wrinkle and skin-brightening agent that should be proven in further research.

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Nil

AUTHORS CONTRIBUTIONS

All authors contributed equally to this manuscript

CONFLICT OF INTERESTS

Declared none

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