International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 1, 2022

Original Article

THE USE OF CARROT SEED OIL (DAUCUS CAROTA L.) TO FORMULATE NANOEMULGELS AS AN EFFECTIVE NATURAL SUNSCREEN AND SKIN ANTI-AGING

ANAYANTI ARIANTO*, HAKIM BANGUN, SUMAIYAH, CHRISTY NATASYA DWI YANTI PUTRI SIREGAR

Faculty of Pharmacy, Center of Excellence Nanomedicine Innovation, Universitas Sumatera Utara, Medan 20155, Indonesia
***Email: anayanti@usu.ac.id**

Received: 29 Oct 2021, Revised and Accepted: 06 Dec 2021

ABSTRACT

Objective: The purpose of this study was to develop a nanoemulgel containing vegetable oil of carrot seed oil as an effective natural sunscreen and skin anti-aging.

Methods: Nanoemulgels containing 4% carrot seed oil were formulated in three formulas with different ratios of Tween 80 and Sorbitol and prepared by using the high-energy emulsification method. The nanoemulgels were determined for the organoleptic characteristic, globule size, pH, physical stability during storage for 12 w at three different temperatures (room, high and low temperature), centrifugation, and cycling test. The Sun Protection Factor (SPF) value was determined by UV spectrophotometric method and the effectiveness of anti-aging was evaluated by using a skin analyzer and the results were compared with sunscreen emulgel.

Results: Nanoemulgel containing 4% carrot seed oil with a ratio of Tween 80 as surfactant and Sorbitol as co-surfactant 40 and 20 resulted in the smallest mean droplet size of 338.34 nm and the sizes were increased during 12 w of storage at room temperature but still in the nano size and this nanoemulgel did not show phase separation or still stable. These nanoemulgels were also stable after the centrifugation and cycling test. The emulgel preparation was not stable or showed phase separation after the centrifugation test. The SPF value obtained from the nanoemulgel was 20.28±0.22 and these values were higher than the sunscreen emulgel (13.94±0.27). The pore size, spot, and wrinkles of the volunteer skin were reduced after using the nanoemulgel containing 4% carrot seed.

Conclusion: The sunscreen and skin anti-aging activity of nanoemulgel preparation containing 4% carrot seed oil with a ratio of surfactant Tween 80 and co-surfactant Sorbitol 40 and 20 were more effective compare with emulgel preparation.

Keywords: Carrot seed oil, Nanoemulgel, Sunscreen, Skin anti-aging

DOI: https://dx.doi.org/10.22159/ijap.2022v14i1.43481. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

UV radiation in the skin induces oxidative free radicals, such as superoxide, hydrogen peroxide, and highly reactive hydroxyl radicals that strongly attack macromolecules such as proteins, lipids, RNA, and DNA, changing their structure and interfering with their function, which can cause oxidative stress [1]. Oxidative stress can cause cell damage and death in the skin and cause skin aging. The visible signs of aging on the skin, including dry skin appearance, scalping, wrinkling, pigmentation, and photoaging correlate with cancer risk [2, 3]. The use of sunscreen is necessary for the precautions recommended by dermatologists and public health campaigns aimed at reducing sunburn, premature skin aging, and skin cancer [4].

Some chemical sunscreen ingredients show undesirable effects from sunscreen products such as irritation, allergy, phototoxicity, and photoallergy [4]. Therefore, the use of natural sunscreen ingredients such as carrot seed oil can reduce this effect. Carrot seed oil is an essential oil obtained by extraction from carrot seeds (Daucus carota L.). The most essential oil content is carotol, fatty acids, β-carotene in large amounts and α and γ carotene in small amounts, α-carotene, and β-carotene are partially metabolized to vitamin A [5, 6]. Carrot seed oil is an antioxidant, antiseptic, antifungal, and fragrant with a high vitamin A content and can be used as a sunscreen, anti-aging, skin revitalizing, and rejuvenating ingredient [7, 8]. Topical administration of preparation containing active ingredients from natural ingredients provides sunscreen and skin anti-aging effects with a broad spectrum, namely the effect of sunscreen on UV radiation and as antioxidant might help to reduce the reactive oxygen species causes of aging [9]. Nowadays, the use of cosmetics with active ingredients that have dual anti-aging and sun activity protection (sunscreen) is very useful and practical. Nanotechnology has become a technology that can improve the stability and efficiency of cosmetic preparation [10].

The purpose of this study was to investigate sunscreen effectiveness on SPF value of nanoemulgel containing carrot seed oil by *in vitro* spectrophotometry method and anti-aging effectiveness by using skin analyzer. Nanoemulgels were made by incorporating nanoemulsions into a gel matrix. The nanoemulgel has the advantage that it can increase the stability of the nanoemulsion by reducing surface tension, improve the patient's acceptability because it had good dispersibility, was not greasy, easy to apply, and increase the penetration of lipophilic active ingredients like vegetable oil (carrot seed oil) into the skin thereby increasing the effectiveness of the active ingredients [11, 12].

MATERIALS AND METHODS

The active ingredient used in this study was Carrot Seed Oil (Happy green store, Jakarta Indonesia), Carbopol 940, sodium carboxymethyl cellulose, glycerol, propylene glycol, sorbitol, methylparaben, propylparaben, Tween 80, Triethanolamine, Span 80, buffer solution pH 7.01, buffer solution pH 4.01 were purchased from CV Rudang Jaya, Medan, Indonesia. All ingredients were analytical grade.

Nanoemulsions containing carrot seed oil were prepared through a high-energy emulsification method [13, 14] by using a magnetic stirrer and ultrasonicator. Tween 80 and sorbitol are used as surfactants and co-surfactans. The nanoemulsion system consists of the oil and the water phase. The oil phase consists of carrot seed oil and sorbitol. In the water phase, methylparaben and propylparaben as preservatives were dissolved in distilled water, then heated by using the hot plate (Fisons); this solution was added Tween 80 and stirred at 3000 rpm by magnetic stirrer (Boeco, Germany) for 8 h. The oil phase was mixed into the water phase and stirred at 3000 rpm with a magnetic stirrer (Boeco, Germany) for 8 h, then sonicated for 30 s by ultrasonicator (Ultrasonic Cleaner Branson 1510 E-MT, USA) until a transparent nanoemulsion was formed. Carbopol 940 was dispersed in pure water and added with TEA to pH-6.5 to obtain a gel base of 1% Carbopol 940 solution [15]. Nanoemulgels were prepared by mixing the obtained nanoemulsion containing carrot seed oil with a gel base of 1% Carbopol 940 (ratio of nanoemulsion and gel base 4:1), then stirred using a magnetic stirrer at 3000 rpm for 10 h and ultrasonicated for 2 h until a transparent nanoemulgels were produced [16].

The preparation of emulgel containing 4% carrot seed oil was prepared by mixing carrot seed oi and Span 80, then this oil phase was heated to 70 °C. The preservatives (methyl and propyl parabens) were dissolved in propylene glycol and glycerol and added CMC Na solution and this water phase was heated to 70 °C. After that, the oil phase was added to the water phase and stir with a magnetic stirrer for 45 min to produce a homogenous emulsion [17]. Emulgel was obtained by mixing the obtained emulsion with a gel base of 2% of Carbopol 940 (ratio of emulsion and gel 4:1) with gentle stirring for 10 min.

The mean droplet size for nanoemulgel and emulgel formulations was measured by Particle Size Analyzer Fritsch Analysette 22 NanoTec. The pH measurement is done using 1% nanoemulgel solution in pure water with a digital pH meter (Hanna instrument). Viscosity measurement was carried out using the NDJ-8S Viscometer and viscosity value was measured every 2 w for 12 w of storage at room temperature physical stability evaluation was done by storing it is at 25±2 °C (room temperature), 40±2 °C (high temperature), 75±5% RH and 4±2 °C (low temperature) for 12 w, then observed the nanoemulgels and gel preparation through visual inspection for their color, odor, and phase separation with observation every w [18].

Cycling test of the nanoemulgels and emulgel containing 4% carrot seed oil was done by putting it is in the freezer at 4±2 °C for 24 h and then put in Climatic Chamber (Memmert, Germany) at 40±2 °C for 24 h and repeated in 6 cycles. Centrifugation test was done using centrifuges (Hitachi CF 16 R X II, Japan) with a rotation of 3750 rpm for 5 h at 25±2 °C. The physical stability of the nanoemulgels and emulgel was observed [19].

The morphology and droplet size of nanoemulgel and emulgel containing 4% carrot seed oil was analyzed using a transmission electron microscope (JEOL JEM 1400, Japan). Sunscreen activity was evaluated by diluting the preparation using 96% ethanol to a concentration of 200 µg per ml. Absorption spectra of nanoemulgels and emulgel were measured by using UV–visible (Shimadzu UV 1800, Japan). After that, the sunscreen activity (SPF) was calculated by the Mansur equation. Six determinations were made for each sample [20].

Anti-aging efficacy measurement was performed on 6 volunteers by using a skin analyzer (Aram, Huvis, Co., Ltd, Korea). The volunteers were accommodated in an air-conditioned room at 25±1 °C and 45±5% relative humidity for 15 min before the measurements. The selected nanoemulgel preparation containing 4% carrot seed oil was applied 2 times every day for 28 d. The moisture content, pore, evenness, spot, and wrinkles of skin were measured before the application of nanoemulgel on day 0 and after application at days 7, 14, 21, and 28. Approval to conduct the *in vivo* studies was obtained from the Research Ethics Committee of Universitas Sumatera Utara (No. 166/KEP/USU/2020).

RESULTS

Carrot seed oil nanoemulsion was prepared by using a variation of the ratio between Tween 80 as surfactant and sorbitol as co-surfactant. The resulting nanoemulsion was added with a gel solution of carbopol 940 to produce nanoemulgel. The composition of nanoemulgel and emulgel can be seen in tables 1 and 2.

Table 1: Composition of carrot seed oil nanoemulgels

	Qantity of 100 ml (%w/v)			Ratio of nanoemulsion with gel base
	F1	F2	F3	
Ingredients of nanoemulsion				
carrot seed oil	5	5	5	
Tween 80	40	38	36	
Sorbitol	20	22	24	80 ml
Methylparaben	0.10	0.10	0.10	
Propylparaben	0.02	0.02	0.02	
Distilled water to	100	100	100	
Ingredients of gel base				
Carbopol 940	1.0	1.0	1.0	20 ml
TEA	1.0	1.0	1.0	
Distilled water to	100	100	100	

Table 2: Composition of carrot seed oil emulgel

	Quantity of 100 ml (%w/v)	Ratio of nanoemulsion with gel base
Ingredients of emulsion carrot seed oil	5.00	
Tween 80	1.26	
Span 80	3.73	
CMC Na	1.00	80 ml
Propylen glycol	10.00	
Methylparaben	0.10	
Propylparaben	0.02	
Distilled water to	100	
Ingredients of gel base		
Carbopol 940	1.00	
TEA	1.00	20 ml
Distilled water to	100	

All preparations of carrot seed oil nanoemulgel were yellow and translucent and this appearance did not change during 12 w of storage at room temperature but the consistency of carrot seed oil emulgel becomes liquid as shown in fig. 1.

The nanoemulgel containing carrot seed oil obtained in this study had a smaller droplet size than the emulgel formulation. The emulgel preparation has a droplet size of 5028.37 nm. The droplet size of the nanoemulgel containing carrot seed oil increased during 12 w of storage at room temperature, but the droplet was still in the nanosize as presented in table 3.

Table 4 shows that the decrease in pH and viscosity value of the emulgel containing carrot seed oil was greater than that of the nanoemulgel preparations during 12 w of storage at room temperature.

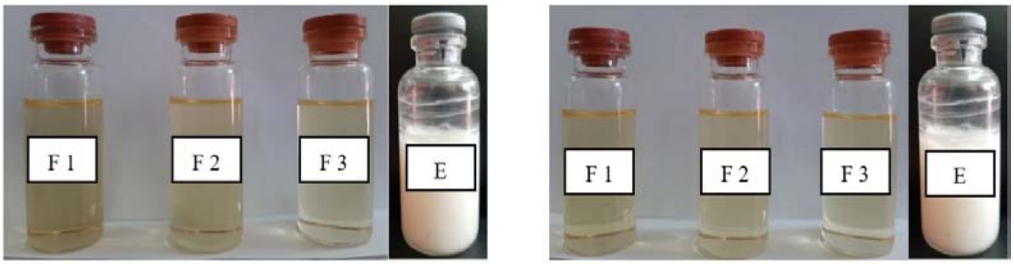

Fig. 1: Appearance of carrot seed oil nanoemulgels (F1, F2, and F3) and emulgel (E)

Table 3: The mean droplet size for nanoemulgel preparations

Formula code	Time (w)	Mean droplet size (nm)
	0	338.34
F1	4	411.86
	8	512.27
	12	607.23
	0	401.29
F2	4	416.27
	8	532.15
	12	645.20
	0	401.29
F3	4	470.31
	8	583.63
	12	688.15

Table 4: Viscosity and pH value of nanoemulgel and emulgel containing carrot seed oil

Formula code	Time (w)	pH (n=3)	Viscosity (mPas) (n=3)
F1	0	6.90±0.00	499,00±0.00
	4	6.80±0.00	454.33±1.15
	8	6.53±0.06	439.00±1.73
	12	6.13±0.06	422.33±2.52
F2	0	6.87±0.06	486.33±1.15
	4	6.70±0.00	455.00±0.00
	8	6,47±0,06	441.33±1.15
	12	6.13±0.06	417.33±2.31
F3	0	6.87±0.06	475.33±3.51
	4	6.77±0.06	454.00±1.73
	8	6.43±0.06	437.67±1.15
	12	6.07±0.06	411.67±2.89
Emulgel	0	6.97±0.06	1998.00±0,00
	4	6.67±0.06	1530.00±4.62
	8	6.37±0.06	1118.33±2.89
	12	5.87±0.06	946.67±2.89

The color and odor of nanoemulgel remain unchanged; there is no phase separation after storage for 12 w at low and high temperatures as can be seen in fig. 2.

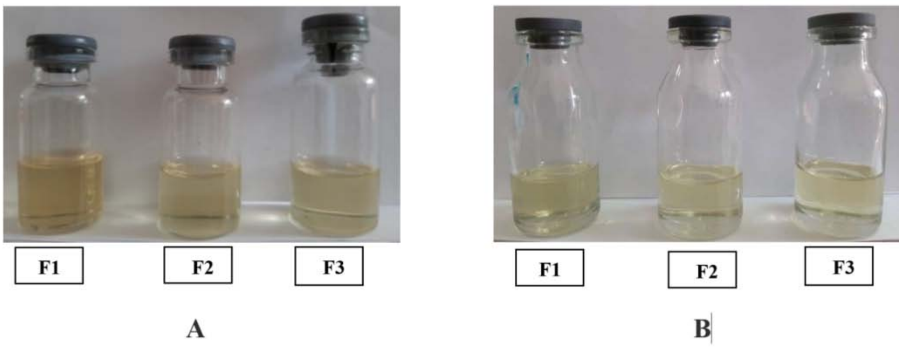

Fig. 2: Appearance of carrot seed oil nanoemulgels after storage for 12 w at low temperature (A) and high temperature (B)

The result of the cycling test of nanoemulgel preparation did not find any phase separation (fig 3). There was phase separation in the emulgel preparation after the centrifugation test, but the physical form of nanoemulgel preparations did not change as shown in fig. 4.

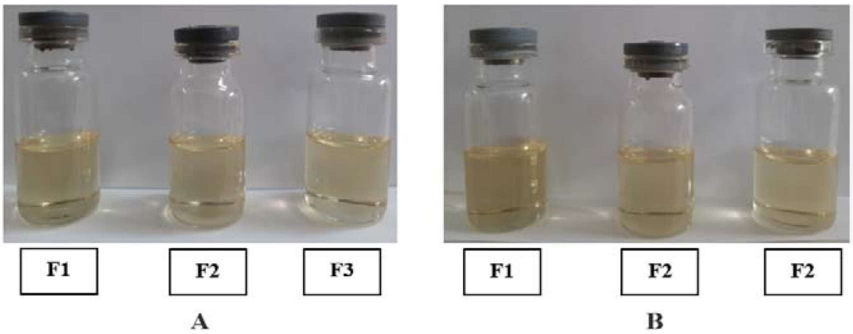

Fig. 3: Cycling test result of carrot seed oil nanoemulgel preparations (A: Before Test, B: After Test)

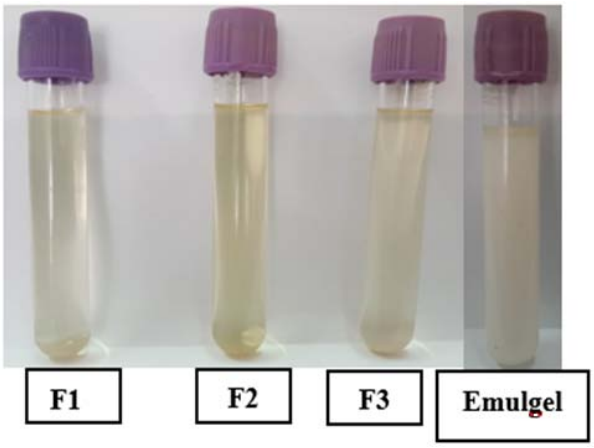

Fig. 4: Centrifugation test result of carrot seed oil nanoemulgels and emulgel

To study the morphology and distribution of nanoemulgel and emulgel, transmission electron microscopy photomicrographs were taken, as shown in fig. 5. It was observed that nanoemulgel has a uniform dispersion, spherical without any aggregation and the droplet size was smaller than emulgel preparation.

The nanoemulgel containing carrot seed oil showed a higher SPF value than emulgel preparation (table 5). The results of SPF determination revealed that carrot seed oil in the form nanoemulgel exhibits greater UVR protection.

Table 5: SPF value of nanoemulgel and emulgel containing carrot seed oil

Formulation code	Sun protection factor (SPF) value
F1	20.28±0.218
F2	19.33±0.280
F3	16.59±0.190
Emulgel	13.94±0.266

Notes: Data is presented as mean±SD, n=6

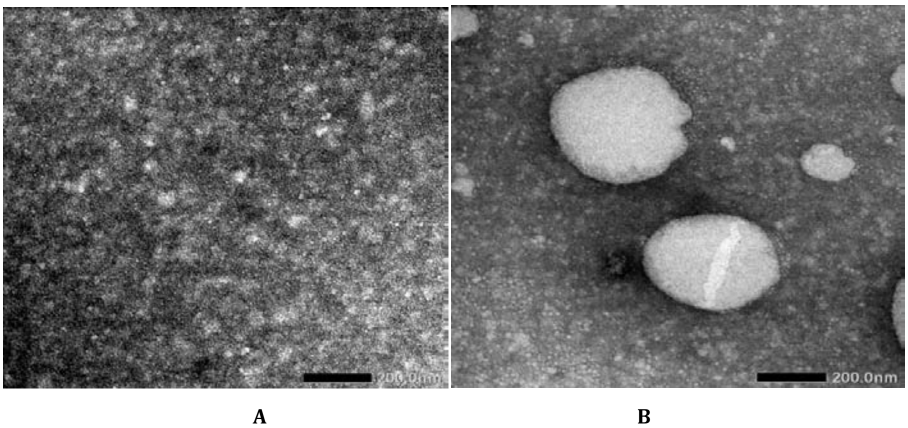

Fig. 5: TEM images of nanoemulgel (A) and emulgel (B) containing carrot seed oil 4%

Nanoemulgel F1 which has the highest SPF values were chosen to evaluate the effectiveness of anti-aging on skin volunteers. The parameters of anti-aging activity evaluation include measurement of moisture content, pore size, evenness, spot, and wrinkles. The skin anti-aging measurement results can be seen in table 6 and table 7. The moisture content of volunteers increases after the application of nanoemulgel and emulgel from dehydrated skin to normal skin. The pore size of the volunteer skin was reduced from some large (41.33±2.8) to excellence (18.00±1.4), the spot from many spots (40.50±2.7) to little spot (18.66±3.0), and wrinkles from wrinkles (43.33±3.5) to approach fine line (20.50±2.1) after using the nanoemulgel containing 4% carrot seed (F1) for 4 w. The volunteer's skin becomes smooth (27.16±1.4) after using nanoemulgel for 2 w. The condition of pore size, spot, evenness, and wrinkles of volunteer skin did not change after using emulgel for 4 w.

Table 6: Moisture content (%) results of nanoemulgel (F1) and emulgel containing carrot seed oil 4% on skin volunteers

Formulation code	Time (Week)				
	0	1	2	3	4
F1	27.83±1.4	31.5±1.3	35.33±1.2	38.66±1.2	42.16±0.7
Emulgel	28.10±1.4	29,83±1.4	32.16±1.1	34.00±1.2	35.83±1.1

Notes: Data is presented as mean±SD, n=6, Dehidrated 0-29%, Normal 30-50, Hidrated 51-100

Table 7: Pore, evenness, spot, wrinkles measurement results on skin volunteers

Formulation code	Time (week)	Average of score			
		Pore	Evenness	Spot	Wrinkles
Nanoemulgel (F1)	0	41.33±2.8	36.33±1.6	40.50±2.7	43.33±3.5
	1	35.50±2.1	33.50±3.3	34.83±2.8	37.83±3.0
	2	28.83±1.4	31.10±2.2	29.00±3.0	30.66±3.0
	3	24.00±1.4	29.33±1.6	23.83±3.3	23.83±2.5
	4	18.00±1.4	27.16±1.4	18.66±3.0	20.50±2.1
Emulgel	0	40.00±2.0	38.00±1.5	41.00±2,3	41.66±2.1
	1	37.66±2.0	36.30±1.7	36.33±2.3	38.50±1.8
	2	35.66±1.9	34.30±0.8	34.16±1.7	33.66±3.3
	3	33.83±1.7	32.80±0.7	32.16±1.7	30.66±3.3
	4	31.66±1.8	31.10±0.7	29.83±1.7	27.16±2.2

Notes: Data is presented as mean±SD, n=6

DISCUSSION

There are two methods for nanoemulsion preparation: the high-energy method in which a mechanical device is used and the low-energy method in which the chemical potential of the component is used [21]. In this study nanoemulgel containing carrot seed oil preparations have been successfully formulated using Tween 80 as a surfactant, Sorbitol as a co-surfactant, and Carbopol 940 as a gelling agent used in addition to the formulation to facilitate the stabilization process. Carbopol 940 has advantages such as being non-toxic, biocompatible, bioadhesive (long residence time on the skin) [22]. The amount of Carbopol 940 used is 0.5%, which was sufficient to produce nanoemulgels translucent, non-greasy, and easy to spread. The addition of Trietanolamin in the formulation can form a good and stable gel matrix at a pH around 6-7. The pH value of nanoemulgels obtained still meets the pH standard for topical preparations suitable with skin pH balance (4.5-6.5) [23] and the viscosity only slightly decreases after storage for 12 w at room temperature. The nanoemulgels with small droplets around 200-300 nm were achieved by using the high-energy method (combining high shear stirring and ultrasonic emulsification) and the nanoemulgels have remained stable, no phase separation, creaming, sedimentation, or color change occurred after storage for 12 w under three different temperature (25±2 °C, 40±2 °C, and 4±2 °C). The nanoemulgel formulation F1 with the optimum amount of surfactant and co-surfactant shows the smallest droplet size.

The nanoemulgels were stable after the centrifugation test. The small droplet size of the nanoemulgels can resist the physical destabilization caused by gravitational separation, flocculation, and/or coalescence. It also avoids the creaming process because the droplet's Brownian motion is enough to overcome the gravitational separation force. Due to very small globule size and less surface tension between the oil and water molecules in nanoemulgel, it almost has not the tendency to agglomerate or precipitate, which reduces the possibility of creaming or sedimentation. As a result, nanoemulgel is much more stable than emulgel system, and the nanoemulgels would be stable for at least one y [24].

The SPF value of nanemulgel preparations is higher than that of emulgel, this is due to the small size of the globules, which will increase the solubility of carrot seed oil, so it will absorb more ultraviolet light, which results in higher SPF values.

Oxidative stress is considered to be one of the main mechanisms involved in skin aging [25] Carrot seed oil contains beta carotene that has antioxidant activity can be useful for preventing and treating skin aging. However, beta carotene is very lipophilic, so that penetration into deep layers of the skin is only slightly; therefore in this study, the carrot seed oil was formulated in nanoemulgel to increase the penetration of antioxidants for increasing anti-aging activity.

The anti-aging activity of nanoemulgel containing 4% carrot seed oil (F1) was higher than that of emulgel preparation with the same concentration of carrot seed oil. This is because the smaller globule size of nanoemulgel can pass through the pores on the skin easily, thereby increasing the penetration of the active ingredients into the skin [26, 27].

CONCLUSION

Carrot seed oil can be formulated into nanoemulgels using Tween 80 as surfactant and Sorbitol. as co-surfactant and physically stable on stability test. Nanoemulgel containing 4% carrot seed oil with a ratio of Tween 80 and Sorbitol 40 and 20 (formulation code F1) has the smallest droplet size of 338.34 nm and showed the highest SPF value of 20.28±0.22 and most effective of activity skin anti-aging. The SPF value and activity skin anti-aging of nanoemulgels are more effective compare with emulgel preparation. The emulgel preparation showed phase separation after the centrifugation test.

ACKNOWLEDGMENT

The Authors are extremely grateful to the Head of the research institute, Universitas Sumatera Utara, Prof. Dr. Erman Munir, M. Sc. who has facilitated this research.

FUNDING

This work was financially supported by Universitas Sumatera Utara, Medan, Indonesia, through the TALENTA Research, scheme of Penelitian Dasar 2020 (research contract number: 44/UN5.2.3.1/PPM/SPP-TALENTA USU/2020).

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors report no conflicts of interest in this work.

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