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Research Article

NANOEMULSION LOADED WITH MARIGOLD FLOWER EXTRACT (*TAGETES ERECTA* LINN) IN GEL PREPARATION AS ANTI-WRINKLES COSMECEUTICAL

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ABSTRACT

Objective: Aging process causes the skin problems that can be prevented by using effective cosmeceuticals with antioxidant activity. Our previous study demonstrated that ethyl acetate marigold flower extract (EA) consisting the flavonoids that exhibited potent antioxidant activity with safe. Therefore, the EA loaded nanoemulsion was prepared and incorporated into a gel base for anti-wrinkles purpose.

Methods: The gel containing nanoemulsion loaded with EA (EANG) was investigated physical properties and antioxidant activity (DPPH assay) at before and after various storage conditions for 3 months. The *in vitro* skin permeation study was also carried out. Skin irritation test, clinical evaluation for skin moisturizing effect and skin wrinkles-reducing capability were finally performed in thirty healthy human volunteers for 8 weeks.

Results: The results showed that the EANG was physically stable and exhibited good stability in antioxidant activity after various storage conditions. From the *in vitro* permeation test, a major compound: quercetagetin in the EANG was mostly remained in stratum corneum, viable epidermis and dermis rather than in blood circulation which was related to cosmetic purpose. The results from clinical evaluation revealed that the skin hydration was significantly increased after using the EANG. In addition, it showed significant reduction in all wrinkle parameters (surface, volume, Ra and Rz). The percentages of efficiency between EANG area and placebo area were also significantly different in terms of volume, Ra and Rz.

Conclusion: Our results strongly indicated that the gel containing nanoemulsion loaded with marigold flower extract was a promising anti-wrinkles cosmeceutical.

Keywords: *Tagetes erecta* Linn, marigold flower extract, Quercetagetin, Nanoemulsion, Anti-wrinkles cosmeceutical.

INTRODUCTION

Aging is a process that occurs in all living tissues, but is most visible in the skin. Two main processes are involved in aging of the skin, intrinsic and extrinsic aging. Intrinsic aging occurs from genetic backgrounds and is one of the results from the passage of time. It causes the skin atrophy, flattening, fine lines and wrinkles. Extrinsic aging is affected by environmental factors such as smoking, diet, chemicals, pollutants and also sun exposure [1-4]. As the process of aging occurs, wrinkles and skin pigmentation are the most characteristic morphological changes in the skin. In addition, the skin also shows less moisture and lipids that becomes sagged due to the loss of its elasticity [5-6]. The free radical theory is one of the most widely accepted theories to explain the cause of aging. Reactive oxygen species (ROS) such as superoxide anion ($O2^{-\bullet}$), hydroxyl ($^{\bullet}OH$), peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$) are the group of free radicals that play an important role in the degradation of skin collagen, elastin fiber and cause of DNA damage [3,7]. Antioxidants are called radical scavengers which can protect our body from the harmful of ROS by reducing and neutralizing them. Flavonoids are a group of polyphenolic compounds that occur generally in fruits, vegetables and flowers. Many of the *in vitro* tests and animal studies had shown that flavonoids possessed anti-inflammatory, antiallergenic, hepato-protective, antithrombotic, antiviral and antioxidant activity [8-9]. *Tagetes erecta* Linn or African marigold is a common well-known plant in Family Compositae that consisting of high flavonoids content [10-11]. In traditional medicine, it is used for antimicrobial, antiseptic, wound and ulcer healing, intestine diseases and antioxidant [12]. From our previous study, the ethyl acetate marigold flower extract (EA) which consisted of the high amount of phenolic contents also revealed high potential in antioxidant activity. Besides, quercetagetin as a major flavonoid compound was successfully isolated and also possessed potent antioxidant activity. The marigold flower extracts also showed no cytotoxic effect to human skin fibroblast [13], therefore marigold flower is an interesting source of natural antioxidant. Delivery systems have been found to improve the efficiency and stability of many products. Nowadays, nanotechnology has become an interesting technology, involving to produce various carriers such as microemulsion, nanoemulsion, lipid nanocarriers as well as microparticles. Nanoemulsion is used in various industries such as food technology, pharmaceutical, and also cosmetic [14-18]. It has recently become increasingly important as a potential carrier for the drug or cosmetic delivery and for the optimized dispersion of active ingredients in particular skin layers as well as protects them from environmental degradation. It is also suitable for transporting the lipophilic compounds and increasing the skin permeation of active ingredients. Another advantage of nanoemulsion is to provide the stability against sedimentation, creaming, flocculation and coalescence [19-22]. In this study, we prepared the nanoemulsion loaded with EA and incorporated into gel base for improving the anti-aging effect of the skin. The investigation of skin permeation and clinical efficacy as skin moisturizing and wrinkles reducing capability of the products were also conducted.

MATERIALS AND METHODS

Extract and chemicals

The marigold flowers were grown and collected in Chiang Mai province, Thailand. The specimen was authenticated by a specialist and deposited in the medicinal plant herbarium, Faculty of Pharmacy, Chiang Mai University (No.0212090-0212091). The ethyl acetate marigold flower extract (EA) was received from Northern Research Center, Faculty of Pharmacy, Chiang Mai University. Jojoba oil and PEG 7 glyceryl cocoate were purchased from United Chemical and Trading Co., Ltd (Chiang Mai, Thailand). Span 80 was purchased from NOF Corporation (Tokyo, Japan) and PEG 40 hydrogenated castor oil from O-Basf the Chemical Co., Ltd (Ludwigshafen, Germany). PEG 400 was purchased from INEOS Capital Limited (Rolle, Switzerland). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Inc (St. Louis, USA). Sodium lauryl sulfate was purchased from Kao (Bekasi, Indonesia).

Formulation of gel containing nanoemulsion loaded with EA

Firstly, nanoemulsion was prepared by phase inversion temperature (PIT) technique. PEG 7 glyceryl cocoate and jojoba oil were used as

an oil phase. Span 80 and PEG 40 hydrogenated castor oil were served as surfactants. PEG 400 and EA (1% w/w) were added in deionized water as water phase. Both phases were heated up to above the PIT temperature (>70ºC). The water phase was poured into the oil phase with agitation by a homogenizer (yellow line DI 25 basic, IKA Werke GmbH & Co. KG, Germany) then the emulsion was rapidly cooled with continuous stirring. The nanoemulsion loaded with EA was finally incorporated into a stable gel base at the concentration of 30% w/w with gentle stirring. The final product as gel containing nanoemulsion loaded with EA (EANG) was obtained.

Physical stability of the gel containing nanoemulsion loaded with EA

Gel containing nanoemulsion loaded with EA (EANG) was kept in tight containers and stored at various conditions: room temperature (25±2°C), 4°C, 45°C for 3 months and accelerated condition: heating cooling cycle (alteration of 4°C 48 h and 45°C 48 h) for 6 cycles. The physical characteristics such as texture, color, phase separation, pH (pH meter, EX-20, Horiba Ltd., Japan) and viscosity (Rheometer, R/S-CPS, Brookfield Engineering Laboratories, Inc, USA) were evaluated.

Determination of antioxidant activity

The antioxidant activity of the gel containing nanoemulsion loaded with EA (EANG) was determined by DPPH radical scavenging assay [23]. Briefly, the EA was extracted from each formulation by absolute ethanol (99%) and centrifuged at 10,000 rpm for 30 min. The supernatant of the test samples were added into a 96-well microplate with 20 µl and DPPH in ethanol 180 µl were also added. The reaction mixtures were kept in dark at 25°C for 30 min. The absorbance was then measured at 520 nm with a microplate reader (DTX-880 multimode detector, Beckman Coulter Inc., USA). The experiments were done in triplicates. This was tested to compare between at before and after storage in various conditions for 3 months as mentioned before. The percentage of inhibition was calculated according to the equation:

$$\%inhibition = [(A_{control} - A_{sample}) \div A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the sample.

***In vitro* rat skin permeation using Franz diffusion cells**

The rat skin was isolated from the male Sprague-Dawley rat weighed between 150 to 200 g that was obtained from the National Laboratory Animal Center, Mahidol University in Nakhon Pathom, Thailand. The *in vitro* rat skin permeation study protocol was approved by the Ethical Committee of Faculty of Medicine, Chiang Mai University. Rat skin permeation of the gel containing nanoemulsion loaded with EA (EANG) was performed and compared with gel containing EA (EAG) using Franz diffusion cells [24-26]. Quercetagetin, a main compound in the EA was used as a marker. The abdominal skin of the rat was shaved, cut and removed subcutaneous fat and then mounted on the receiver chamber. The epidermis was faced to the donor compartment while the dermis was in contact with the receiver medium. The contact area between the donor and the receiver chamber was 2.46 cm^2 and the volume of the receiver medium was 13 ml. The receiver chamber contained DI water: absolute ethanol (1:4) that was constantly stirred at 100 rpm, 32±2°C throughout the experiment. The formulations (1 g) were placed into the donor compartment and covered with paraffin film. The medium was then collected at 1, 2, 3, 4, 5 and 6 h [27]. The quercetagetin concentration in receiving solution showed the concentration of samples in blood circulation. After the experiments, each rat skin was swung in 100 ml of distilled water to remove the remaining product. For the determination of quercetagetin concentration in the stratum corneum (SC), the skin was stripped with 20 pieces of an adhesive tape using 3M scotch magic™ tape (1 cm×1 cm) [24-26]. Each tape was charged with a weight of 300 g for 10 s and then removed rapidly. In each skin sample, the first tape strip was discarded. The 2nd to 20th tape strips were used to analyze for quercetagetin contents by extracting with ethanol. Quercetagetin concentration in the viable epidermis and dermis (VED) was determined by cutting the skin into small pieces and extracted with ethanol followed by sonicate for 15 min and filtered. The filtrate was then assayed using UV-VIS spectrophotometer (V-530, Jasco®, Japan) at 360 nm. The experiments were done in triplicate. The percentages cumulative of quercetagetin (%) in SC, VED and receiving solution from each formulation after 6 h was finally calculated using the following equation:

$$\%\,cumulative\ of\ quercetagetin = (A_{sc}, A_{ved}\ or\ A_{so} \div A_{total}) \times 100$$

A_{sc}, A_{ved} and A_{so} are quercetagetin amounts that found in SC, VED and solution, respectively. A_{total} is the total of quercetagetin in 1 g of each formulation.

Skin irritation test and clinical study in human volunteers

Skin irritation test (OECD guideline)

The skin irritation study was carried out by a patch test using Finn chamber® in thirty healthy human volunteers. All the volunteers were lack of skin disease, pharmacological treatment, skin atrophy and tattoo. The back of each volunteer was cleaned with water before the experiment. The test samples were added into the Finn chamber® and stuck on the back. This was removed after 48 h and then erythema and edema formation were observed at 1, 24, 48 and 72 h after patch removal. Sodium lauryl sulfate (SLS, 1% w/v) was used as positive control and untreated skin as negative control. The average score of erythema and edema formation was represented according to Draize scoring system [28-29]. The Primary Dermal Irritation Index (PDII) was calculated following equation:

$$PDII = [(\sum erythema\ grade\ at\ 1/24/48/72h + \sum edema\ grade\ at\ 1/24/48/72h) \div 4] \times number\ of\ volunteers$$

The irritation degree was classified based on the PDII values as non-irritation (PDII= 0-0.4), slight irritation (PDII= 0.5-1.9), moderate irritation (PDII= 2.0-4.9) or severe irritation (PDII= 5.0-8.0).

Skin moisturizing effect evaluation

This protocol was approved by the Ethical Committee of Faculty of Pharmacy, Chiang Mai University. Thirty healthy human volunteers aged between 30-55 years were enrolled and required to sign a consent form to participate in the experiment. All the volunteers had no sign of skin disease and were not using any topical agents on the test area for 4 weeks prior to the study. The volunteers, who were pregnant or lactating or dieting, were excluded from this study. In the experiment, the volunteers were rested in a controlled room at 25±2°C and 40-60% relative humidity for 30 min before the skin measurement. The test samples were applied on both forearms. Left forearm was divided into two areas: untreated skin as negative control and the gel containing nanoemulsion loaded with EA (EANG) area as sample site. Right forearm applied the gel base as a control. All the test areas were treated twice daily with 0.2 g of each formulations for 8 weeks. Moisture capacitance was changed depending on the water content in the stratum corneum. Thus, the skin hydration was investigated using a Corneometer® (CM825, Courage & Khazaka, Cologne, Germany). Three measurements were performed on each tested skin area.

Skin wrinkles reducing evaluation

The same group of volunteers as mentioned above was also used to evaluate skin wrinkles reducing capability. The skin wrinkles parameters: surface, volume, Ra and Rz were determined using the skin Visiometer® (SV 600 FW, CK Electronic GmbH, Germany). The surface parameter showed the size of the wavy surface in comparison to the stretched surface. The volume parameter was the amount of liquid needed to fill the surface. The roughness parameters (Ra and Rz) indicated the number and depth of the furrows, respectively. All the areas were measured at before and after twice daily of application for 8 weeks. The percentage efficiency of all formulations was also calculated to compare between before and after 8 weeks by following equation:

$$\%efficiency = [(V_M - V_0) \div V_0] \times 100$$

V_0 is the value at initial point (day 0), and V_M is the value at measuring point (8 weeks).

Statistical analysis

All data were presented as average ± SD. Paired samples t-test was used to evaluate the significance of differences at the level of P-value<0.05. Statistical analysis was performed using SPSS software version 17.0.

RESULTS AND DISCUSSION

Formulation and stability test of the gel containing nanoemulsion loaded with EA

The prepared nanoemulsion (EAN) by phase inversion temperature (PIT) technique was in nano-size range (approximately 201.80 nm). The EAN were then incorporated into a stable gel base at the concentration of 30% w/w (EANG). The gel containing EA (EAG) in the equally concentration to EANG was also prepared. The results are shown in Table 1. The EANG showed a good appearance with slightly yellow color, smooth texture and homogeneous at both before and after the stability test. The pH of the EANG did not change after storage, except at 45°C and heating-cooling cycles, it showed a slightly decreased. The viscosity of the EANG was not decreased after 3 months of storage except at 45ºC. The EAG also showed a good appearance and after storage at 4°C for 3 months but the sediment of the extract after heating-cooling, room temperature and 45°C were observed. The pH of the EAG showed no change after storage except at 45°C. The viscosity of the EAG showed a slightly decreased after storage. From these results, it revealed that heat was slightly affected the pH and viscosity of the EANG formulation but no cracking, creaming or phase separation occurred. However, the EANG can stabilize the extract better than in the EAG due to a better protection from environmental deterioration as shown in Table 1.

Table 1: The physical appearances of the gel containing nanoemulsion loaded with EA and the gel containing EA at starting time and after storage at various conditions for 3 months

Topics	The gel containing nanoemulsion loaded with EA (EANG)				
	Start	Heating-cooling 6 cycles	3 months		
			Room temperature	4°C	45°C
pH	5.5	5	5.5	5.5	5
Texture	smooth and homogeneous	smooth and homogeneous	smooth and homogeneous	smooth and homogeneous	smooth and homogeneous
Color	yellow	yellow	yellow	yellow	yellow
Viscosity (Pas)	2.33±0.06	2.04±0.03	2.02±0.02	2.06±0.03	1.88±0.10
Topics	**The gel containing EA (EAG)**				
	Start	**Heating-cooling 6 cycles**	**3 months**		
			Room temperature	**4°C**	**45°C**
pH	5.5	5.5	5.5	5.5	5
Texture	smooth and homogeneous	sedimentation of the extract	sedimentation of the extract	smooth and homogeneous	sedimentation of the extract
Color	yellow	yellow and brown spot	yellow and brown spot	yellow	yellow and brown spot
Viscosity (Pas)	2.732±0.05	2.462±0.03	2.564±0.02	2.487±0.05	2.395±0.02

Determination of antioxidant activity

The antioxidant activity of the EANG and the EAG was determined by DPPH assay to compare between before and after storage in various storage conditions for 3 months and was compared between all formulations. The results reported in terms of the percentage of inhibition, as shown in Figure 1.

The differences of decreasing in percentage inhibition of all formulations during various storage conditions were observed. The EANG exhibited a potent activity with 90.29±0.82 percentage of inhibition after preparation. After the stability test, it also showed high activity at room temperature, 4°C and heating-cooling (91.42±0.06, 92.39±0.46 and 91.38±0.29 percentage of inhibition, respectively) which was not significantly different when compared with starting time. But at 45°C, it was significantly decreased to 78.25±2.60% (P<0.05). This can be concluded that the EANG was not stable at more than 45ºC for long storing periods, but it was stable at room temperature, 4ºC and accelerated condition. The EAG also presented potent activity with 90.62±0.26% after preparation which equal to the EANG. However, it showed a significantly decreased in percentage of inhibition at room temperature, 45°C and heating-cooling (50.60±5.41, 55.63±3.66 and 63.81±0.46, respectively) but not significantly decreased at 4°C (82.61±0.10) at P<0.05. These results implied that all formulations were sensitive to the higher temperature that above 45°C, so the products should be kept in a cool place for their high effectiveness. However, it were interestingly found that the EANG showed higher and more stable antioxidant activity than the EAG after long storage for 3 months. This strongly indicated the advantage of loading EA into nanoemulsion that could increase the stability of the active compound.

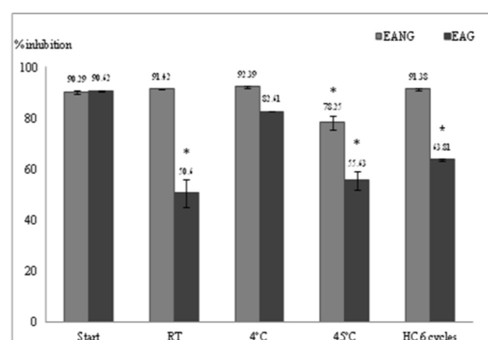

Fig. 1: The Percentage inhibition of gel containing nanoemulsion loaded with EA (EANG) and gel containing EA (EAG) using DPPH assay; before and after stability test–room temperature (RT), 4ºC, 45ºC and heating-cooling cycles (HC), *=significant at P<0.05

***In vitro* rat skin permeation test by Franz diffusion cells**

Quercetagetin, a major compound in the EA was measured as a marker. The amounts of quercetagetin penetration into rat skin were tested with the EANG comparing to the EAG using Franz diffusion cell. The cumulative amounts and percentages of quercetagetin per area in the stratum corneum (SC), viable epidermis and dermis (VED) and receiving solution (blood circulation) from various formulations after 6 h were investigated. The results were shown in Table 2. The cumulative amounts of quercetagetin (μg/cm^2, n=3) in the receiving chamber of all formulations could be detected after 6 h. The EANG exhibited the

cumulative amounts of quercetagetin after 6 h at 88.33±0.01, 8.60±0.00 and 34.20±0.00 μg/cm² which were 10.90±0.91%, 1.06±0.08% and 4.23±0.49% in SC, VED and receiving solution, respectively.

The EAG showed its cumulative amounts after 6 h at 85.90±0.01 μg/cm² (10.60±0.88%), 6.70±0.00 μg/cm² (0.82±0.06%) and 19.00±0.04 μg/cm² (2.34±1.98%) in SC, VED and receiving solution, respectively. The amounts of quercetagetin penetration both in the skin layers (SC and VED) and blood circulation (receiving solution) of the EANG were higher than in the EAG. These results could be explained by two main factors that the small size of nanoemulsion caused a better penetration of the active into the skin, and the incorporation of nanoemulsion into the gel base matrix lead to the expansion of penetration [30]. In addition, nanoemulsion in the gel created larger contact area to help the active compound accessed to skin by surface-to-surface interaction between formulation and the skin [31-32].

The amounts of quercetagetin in receiving solution indicated that both formulations were less detected in blood circulation than in the skin layers which were related to the cosmetic purpose. Although the permeation of the EANG showed a slightly differences to the EAG (Table 2) but the EANG indicated a better stability than the EAG (Table 1 and Figure 1). Therefore, the EANG was further investigated for skin irritation test and clinical evaluations.

Table 2: The accumulative amounts of quercetagetin and the percentage of quercetagetin amounts in stratum corneum, viable epidermis and dermis, and receiving solution after 6 h

Formulations	Calculated at 6 h					
	Stratum corneum (SC)		Viable epidermis and dermis (VED)		Receiving solution (Blood circulation)	
	amounts (μg/cm²)	%	amounts (μg/cm²)	%	amounts (μg/cm²)	%
Gel containing nanoemulsion loaded with EA (EANG)	88.33±0.01	10.90±0.91	8.60±0.00	1.06±0.08	34.20±0.00	4.23±0.49
Gel containing EA (EAG)	85.90±0.01	10.60±0.88	6.70±0.00	0.82±0.06	19.00±0.04	2.34±1.98

Skin irritation test and clinical study in human volunteers

Table 3: The PDII and their classification in thirty healthy human volunteers

Formulations	PDII	Classification
Gel base	0.00	Non-irritation
EANG	0.00	Non-irritation
Positive control (1% w/v SLS)	0.52	Slight irritation
Negative control (DI water)	0.00	Non-irritation

Table 4: The percentage efficiency value of moisture, skin wrinkle parameters (surface, volume, Ra and Rz) after application for 8 weeks

Treatment area	The percentage efficiency (%)				
	Skin moisture	skin wrinkles parameters			
		Surface	Volume	Ra	Rz
Untreated skin	-2.06^{a}	1.31^{a}	5.63^{a}	11.91^{a}	10.29^{a}
Gel	5.63^{a}	-1.45^{a,b}	2.63^{a}	2.99^{a}	2.66^{a}
EANG	24.38^{b}	-4.66^{b}	-5.14^{b}	-3.97^{b}	-4.02^{b}

Note: a, b and c are statistical comparison between groups, same alphabet indicate not statistically significant

Skin irritation test

The skin irritation test is used to determine the safety of all formulations. Accordingly, the erythema and the edema scores were recorded and calculated in terms of PDII. The data were shown in Table 3. It was found that all the formulations caused no skin irritation whereas SLS (positive control) showed a slight irritation.

Skin moisturizing effect evaluation

To evaluate the moisturizing effect, the Corneometer® was used to measure the skin moisture content after 8 weeks of twice daily application in thirty healthy human volunteers. The moisture values of treated areas (EANG), placebo area (gel base) and untreated area (intact skin) were compared between at before and after application, and also calculated the percentage of efficiency as shown in Figure 2 and Table 4.

The results demonstrated that the skin moisture of the volunteers was significantly increased after using the EANG for 8 weeks (37.72±2.07 to 46.42±2.45) whereas no change occurred in the untreated skin and the gel base. The differences between untreated, placebo treatment and active treatment, in terms of the percentage efficiency values, were analyzed by paired t-test using SPSS (P<0.05). The application of the EANG indicated significantly increased in percentage of moisture efficiency against untreated and placebo area. This can be concluded that the gel containing nanoemulsion loaded with EA possessed a good skin moisturizing effect.

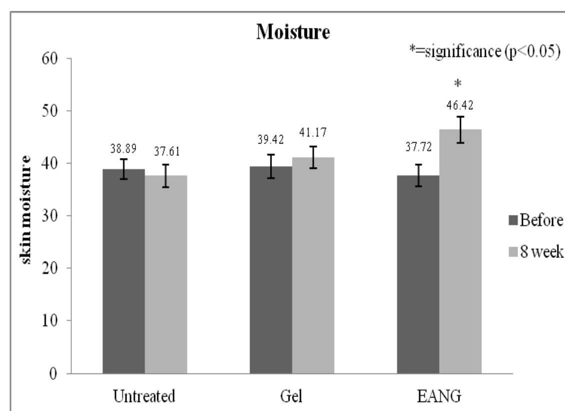

Fig. 2: The skin moisture after 8 weeks in thirty healthy human volunteers (Data show average±SD, *=significantly different at P<0.05)

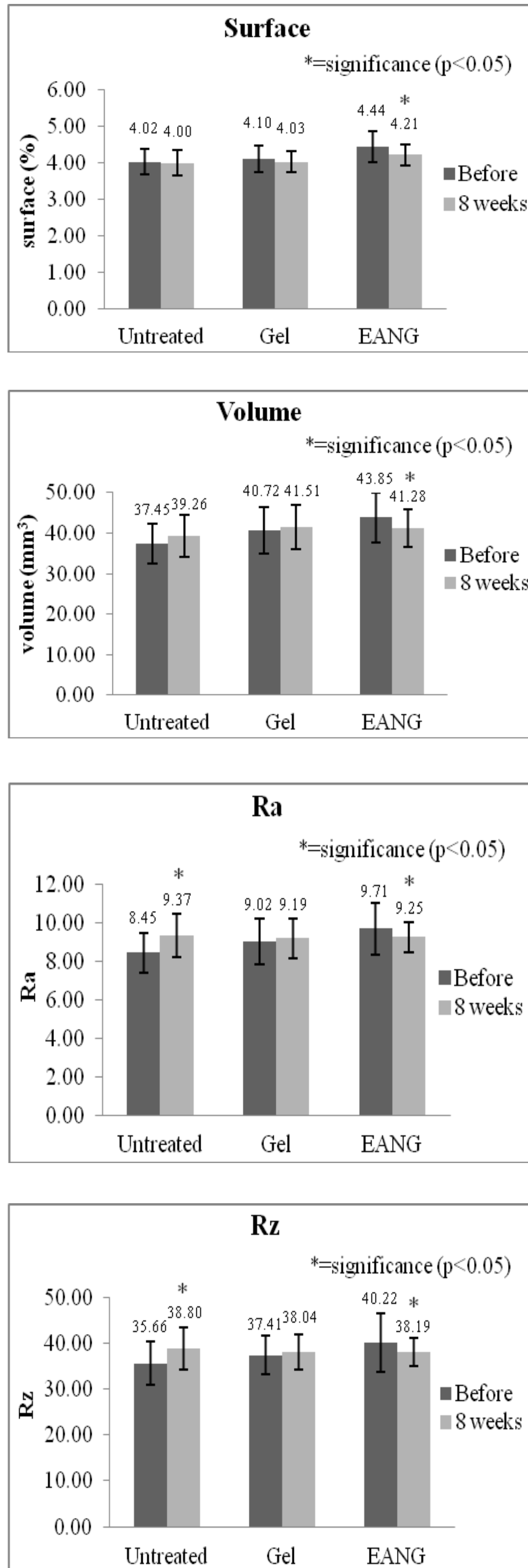

Fig. 3: Mean values of the skin wrinkles parameters (surface, volume, Ra, Rz) before and after 8 weeks of application (*=significantly different at P<0.05)

Skin wrinkles reducing evaluation

Thirty healthy human volunteers applied the gel base and the EANG on their lower forearms twice daily for 8 weeks. The assessment of wrinkles reducing capacity by Visiometer® was presented in four parameters (surface, volume, Ra and Rz). The values of treated area (EANG), placebo area (gel base) and untreated area (intact skin) were compared at before and after treatment, and also computed into the percentage of efficiency (P<0.05). The results are presented in Figure 3 and 4. The EANG area showed the changes of all parameters with significantly reduced in surface, volumn, Ra and Rz (4.44±0.43 to 4.21±0.28, 43.85±6.06 to 41.28±4.67, 9.71±1.34 to 9.25±0.76 and 40.22±6.35 to 38.19±3.04, respectively) while the placebo area showed no significant change after 8 weeks of application (P<0.05). For untreated area, the tendency of wrinkles increasing was observed in terms of roughness (Ra and Rz) as shown in Figure 3. The differences between untreated, placebo treatment and active treatment, in terms of percentage efficiency values, were analyzed and presented in Table 4. The results were obviously showed that the application of the EANG produced significant improvement in all parameters against untreated area. Between the EANG area and placebo area, they were statistically different in volume, Ra and Rz parameters while surface parameter exhibited non-significantly difference (P<0.05). Our studies demonstrated that the antioxidant capacity of the EA plays an important role in skin wrinkles reducing efficacy. Additionally, the nanoemulsion can help the penetration of the extract into the skin layers due to its small particles size and high surface area and it can also stabilize the extract in the formulation. Therefore, the gel containing nanoemulsion loaded with EA presented better effects than placebo.

CONCLUSION

This study demonstrated that the gel containing nanoemulsion loaded with EA was stable at various storage conditions and more stable than EA in gel. It exhibited a high antioxidant activity (DPPH assay) and good stability. From the *in vitro* test in rat skin, the penetration of quercetagetin from EANG was mostly detected in the stratum corneum, viable epidermis and dermis rather than in blood circulation. Therefore the EANG could help to deliver the active compound into the skin which was related to the cosmetic purpose. The clinical evaluation strongly indicated that the gel containing nanoemulsion loaded with EA could increase skin hydration after treatment. It could also reduce skin wrinkles compared with untreated and placebo area. In addition, it showed no skin irritation throughout the study. The results also revealed that the antioxidant capacity of the EA plays an important role in skin wrinkles reducing efficacy. Moreover, the loading of EA into nanoemulsion also showed the better stability and efficiency that can be used as a promising anti-aging cosmeceutical product.

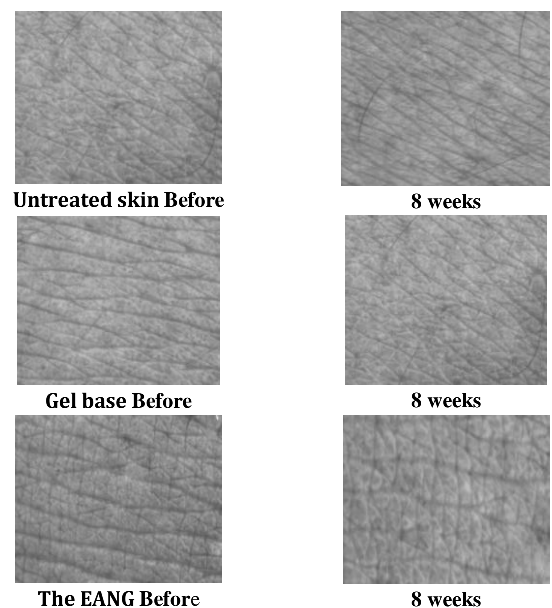

Fig. 4: Skin surfaces of lower forearms using skin visiometer® before and after 8 weeks of treatment

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