Instrumental evaluation of anti-aging effects of cosmetic formulations containing palmitoyl peptides, *Silybum marianum* seed oil, vitamin E and other functional ingredients on aged human skin

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Abstract. Anti-aging cosmetics are widely used for improving signs of aged skin such as skin wrinkles, decreased elasticity, low dermal density and yellow skin tone. The present study evaluated the effects of cosmetic formulations, eye cream and facial cream, containing palmitoyl peptides, *Silybum marianum* (*S. marianum*) seed oil, vitamin E and other functional ingredients on the improvement of facial wrinkles, elasticity, dermal density and skin tone after 4 weeks period of application on aged human skin. Healthy volunteers (n=20) with aged skin were recruited to apply the test materials facially twice per day for 4 weeks. Skin wrinkles, elasticity, dermal density and skin tone were measured instrumentally for assessing the improvement of skin aging. All the measurements were conducted prior to the application of test materials and at 2 and 4 weeks of treatment. Crow's feet wrinkles were decreased 5.97% after 2 weeks of test material application and 14.07% after 4 weeks of application in comparison of pre-application. Skin elasticity was increased 6.81% after 2 weeks and 8.79% after 4 weeks. Dermal density was increased 16.74% after 2 weeks and 27.63% after 4 weeks. With the L^* value indicating skin brightness and the a^* value indicating erythema (redness), the results showed that brightness was increased 1.70% after 2 weeks and 2.14% after 4 weeks, and erythema was decreased 10.45% after 2 weeks and 22.39% after 4 weeks. Hence, the test materials appear to exert some degree of anti-aging effects on aged human skin. There were no abnormal skin responses from the participants during the trial period. We conclude that the facial and eye cream containing palmitoyl peptides and *S. marianum* seed oil, vitamin E and other ingredients have effects on the improvement of facial wrinkles, elasticity, dermal density and skin tone.

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Key words: palmitoyl peptides, *Silybum marianum* seed oil, facial wrinkles, elasticity, dermal density, skin tone

Introduction

Skin aging is a complex phenomenon that induces numerous changes to the skin components (1). Aging is accompanied by various symptoms such as wrinkles, dryness, darkening, pigmentation, decreased dermal thickness and a loss of elasticity (2,3). Due to the gradual aging of various populations worldwide, and the advance of science associated with aging, a variety of anti-aging procedures have been developed, such as photoprotection, cosmetics, cosmeceuticals and antioxidants for prevention and treatment of skin aging (4). The business of anti-aging cosmetics, including cosmeceuticals, is growing rapidly in the skin care market (5), and may potentially benefit the care of wrinkles, elasticity, dermal density and skin tone. However, although clinical experience suggests an important role for topical anti-aging formulations, such as eye cream and anti-wrinkle cream, further empirical studies are required to investigate the underlying mechanisms and confirm their effects.

Palmitoyl peptides, *Silybum marianum* (*S. marianum*) seed oil and vitamin E are generally used antioxidants for protecting and restoring the skin from damage that may result in wrinkles and low elasticity (6-8), in anti-aging cosmetics such as alpha and beta hydroxyl acids (9) and kinetin (10). Palmitoyl peptides are the agents by the action of which matrikines are transformed in palmitoyl derivatives to increase transepidermal penetration (11,12). Although matrikines are

short chains of amino acids contributing to tissues repair activity by facilitating collagen synthesis (13), these are hydrophilic and very weak to be absorbed across epidermis (11,12). However, palmitoyl peptides, the modified formation of these small peptides, have potential anti-aging functions (14). *S. marianum* seed oil contains silymarin flavonoids including silibinin, silidianin and silichrystin, which may exert antioxidative activity (15), and show potential anti-aging effects as a cosmetic cream formulation by decreasing transepidermal water loss and surface wrinkles (16). Vitamin E has been demonstrated to be an antioxidant in numerous studies (17-19). Additionally, cosmetic application of vitamin E protects skin from ultraviolet (UV) damage, which may exacerbate wrinkles, loss of elasticity and dehydration (20-22). Creams, lotions or emulsions generally serve as vehicles for the penetration and cutaneous absorption of vitamin E (21).

Although various formulations of anti-aging cosmetics containing functional components are used and developed extensively for relieving skin aging such as wrinkles, low elasticity, low dermal density and photo damage (23), further clinical studies are required to validate their effects on aged skin. The evaluation of anti-aging products is considered appropriate to prove the effects of the substances that improve skin wrinkles, elasticity, dermal density and skin tone.

The aim of the present study was to evaluate the anti-aging effects of cosmetic formulations, eye cream and facial cream, containing palmitoyl peptides, *S. marianum* seed oil, vitamin E and other functional ingredients on aged human skin after 4 weeks period of application, using skin bioengineering techniques. The effects of the substances on wrinkles, elasticity, dermal density and skin tone were determined.

Materials and methods

Subjects. This study complied with the principles of the Declaration of Helsinki and Korean and was reviewed and approved by the Institutional Review Board of Korea Institute for Skin and Clinical Sciences (Seoul, Republic of Korea).

A total of 20 female volunteers (age, 30-65) were selected on the basis of predetermined inclusion and exclusion criteria. Inclusion criteria were as follows: Volunteers were female and >30 years old; subjects voluntarily signed the informed consent form; subjects were healthy without acute or chronic physical diseases, including any skin diseases; and subjects were available for follow-up during the testing period. A person with any of the following factors was excluded from the study: Pregnant, breast feeding or potentially pregnant; person who had been treated with any external application containing steroids for a skin disease treatment for >1 month; had participated in the a similar test within the last 6 months; person with sensitive or hypersensitive skin; person with skin abnormality on the test site, including moles, acne, erythema, and dilated capillaries; person who received any treatment on the test area within the last 6 months. Participants were withdrawn for the following reasons and these were reported: Adverse events, such as itching or erythema at the test area; hindrance of the evaluation due to a medical treatment, application of another product, excessive sun exposure, or excessive drinking or smoking during the test period; inability to participate in a follow-up appointment during the test period due to personal reasons; and person who did not comply with the study directions without specific reason.

Adverse events, including erythema, edema, scaling, itching, stinging, burning, tightness, prickling and other abnormalities, were visually examined and described on the case report form at every visit. The records included the degree of symptoms and whether these were mild, moderate or severe. Each subject's attendance was also recorded. Whether the participant was excluded from the study due to withdrawal was also noted. If a subject was unable to continue in the study, she signed an abandonment consent form.

Preparation and application of test materials. The facial cream and eye cream were freshly prepared for this study. The facial cream contains 1% palmitoyl oligopeptide and palmitoyl tetrapeptide-7 (BulkActives, Keelung City, Taiwan), 1% *S. marianum* seed oil (Botanic Innovations LLC, Spooner, WI, USA), 1% vitamin E (BulkActives), 1% xylitylglucoside, xylitol, and anhydroxylitol (Seppic S.A., Puteaux, France), 1% *Rosmarinus officinalis* leaf extracts (Flavex Naturextrakte GmbH, Rehlingen-Siersburg, Germany), 3% jojoba oil (Biocosmethic, Bonnelles, France), 3% avocado oil (Biocosmethic) and 1% squalane (BulkActives). The eye cream contains 1% palmitoyl oligopeptide and palmitoyl tetrapeptide-7 (BulkActives), 1% hesperidin methyl chalcon, dipeptide-2 (Sederma, Le Perray-en-Yvelines, France), 1% *S. marianum* seed oil (Botanic Innovations LLC), 1% *Hordeum vulgare* extracts (Presperse Corporation, Somerset, NJ, USA), 1% sodium hyaluronate (Jinwoo Bio, Inc., Seoul, Republic of Korea), 1% glycosphingolipid (Wha Costech Inc., Gyeonggi-Do, Seoul, Republic of Korea), 1% vitamin E (BulkActives), 3% jojoba oil (Biocosmethic), 1% jojoba esters (International Flora Technologies, Ltd., Chandler, AZ, USA), 1% squalane (BulkActives) and 1% acacia wax (Hangzhou Reb Technology Co., Ltd., Hangzhou, China).

Following facial washing, subjects applied the eye cream around eyes and face cream on the facial area twice per day, morning and night. Except for the test materials supplied by the institution, subjects were prohibited from using other products that may affect the results during the trial period. These other products included eye cream, functional cosmetics against aging and treatments such as masks or massages.

Evaluation of wrinkle improvement. All clinical analyses were conducted after cleansing face with same cleanser (Cleansing Foam; Anna Holtz Skin Care, Incheon, Republic of Korea) and resting in a controlled temperature and humidity room (temperature, 22±1˚C; humidity, 45±5%) for 30 min. All measurements were conducted prior to any test material application and subsequently after 2 and 4 weeks of application.

For evaluation of wrinkle improvement, a PRIMOS Lite 3D Face and Skin Scanner Analyzing System (GFMesstechnik GmbH, Berlin, Germany) was utilized. The outer corner of the right eye was measured three consecutive times with the PRIMOS Lite after placing subjects' face onto a special PRIMOS face-held-equipment (GFMesstechnik GmbH) and focusing the outer corner of eye on a same pattern of the PRIMOS Lite to prevent the test area from moving. The images adjusted to the same position each time by applying 3D matching and were analyzed with the PRIMOS Lite software (version 5.6E; GFMesstechnik GmbH). The measurement

Table I. Changes in skin wrinkle values (Ra) after applying test materials.

Application period	Wrinkle values	P-value
Pre-application	42.58±12.09	
2 weeks	40.04±11.32	0.043[a]
4 weeks	36.59±8.15	0.001[b]

Results presented as the mean ± standard deviation. [a]P<0.05, [b]P<0.001.

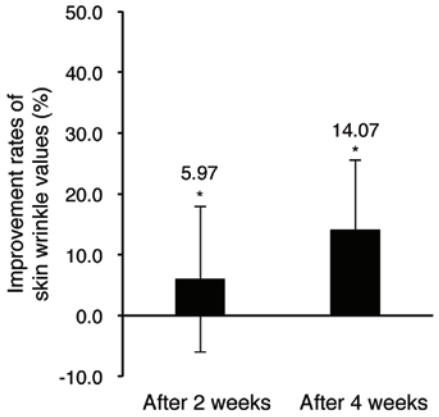

Figure 1. Improvement rates of skin wrinkle values after applying test materials. Data were presented as means ± standard deviation. A paired t-test was performed to determine statistical significant results. *P<0.05, vs. the control.

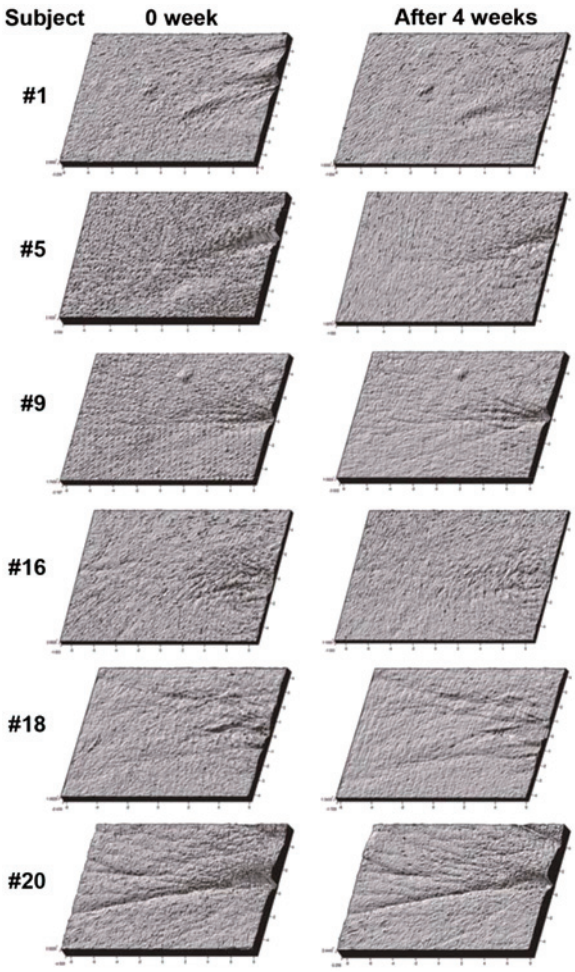

Figure 2. Representative 3D images of an area around the right eye. The outer corner of the right eye was measured three consecutive times using the PRIMOS Lite equipment. 3D images before and after 4 weeks of test material application are shown.

variable Ra (average of all heights and depths to the reference plane) was used for wrinkle analysis as the most common surface roughness index worldwide, which represents the maximal mathematical average of the profile within the entire measurement range. The Ra value decreases with a lower depth of wrinkles, indicating that skin wrinkles were improved.

Evaluation of skin elasticity improvement. For evaluation of skin elasticity improvement, the DermaLab USB elasticity probe (Cortex Technology ApS, Hadsund, Denmark) was applied. After attaching the probe to the skin with tape, the left cheek under the eye was measured only once for prevention of skin fatigue caused by repeated measurement. The DermaLab USB elasticity probe quantifies skin changes and restoring forces in accordance with inhalation of skin and the duration of the inhalation, and the results were analyzed using DermaLab USB analysis software, version 1.09 (Cortex Technology ApS). Young's modulus (E) was used for elasticity analysis, which is the value representing the difference in forces to raise surface skin as much as 1.5 mm, the distance between two infrared sensing wires within the probe. Its unit of measure is the mega pascal (MPa). Young's modulus (E) increases with a higher elasticity, indicating that skin elasticity was improved.

Evaluation of dermal density improvement. For evaluation of dermal density improvement, an ultrasonographic DUB® SkinScanner (Tpm Taberna Pro Medicum GmbH, Lüneberg, Germany) was applied. After applying ultrasonography gel, 3 cm from the outer corner of left eye was measured by using the probe at a right angle with skin and pressing skin with same pressure. The range of analysis was set in limits from epidermis to upper subcutaneous fat layer. The value increases with a higher density of dermis, indicating that dermal density was improved.

Evaluation of skin tone improvement. For evaluation of skin tone improvement, a CR-2600D spectrophotometer (Konica Minolta, Inc., Tokyo, Japan) was used. The right cheek was measured three consecutive times, and the average value was determined. The L^* and a^* of three measurement values were determined as a measure of skin tone. L^* indicates brightness, a^* indicates red and b^* indicates yellow.

Evaluation of abnormal skin response. During the trial period, medical doctors (Dr Kyu Joong Ahn and Dr Hyung Jin Hahn) determined using a visual inspection whether subjects had any visual dermatological side-effects (including erythema, edema and scaling) or not. We indicated the degree of symptoms and reported the results. A survey was conducted to ask subjects about abnormal skin responses.

Table II. Changes in skin elasticity after applying test materials.

Application period	Skin elasticity (MPa)	P-value
Pre-application	12.85±2.55	
2 weeks	13.73±2.36	0.045[a]
4 weeks	13.98±1.67	0.013[a]

Results presented as the mean ± standard deviation. [a]P<0.05.

Table III. Changes in dermal density after applying test materials.

Application period	Dermal density	P-value
Pre-application	42.87±7.35	
2 weeks	50.05±9.81	0.001[a]
4 weeks	54.72±14.67	0.000[b]

Results presented as the mean ± standard deviation. [a]P<0.01, [b]P<0.001.

Table IV. Changes in L^* value after applying test materials.

Application period	L^* value	P-value
Pre-application	64.34±2.97	
2 weeks	65.44±2.27	0.007[a]
4 weeks	65.72±2.71	0.001[a]

Results presented as the mean ± standard deviation. [a]P<0.01.

Statistical analysis. The data were analyzed using paired t-tests with SPSS 17.0 software for Windows (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant result.

Results and Discussion

General characteristics and abnormal skin responses of subjects. No subjects discontinued their participation due to lack of effectiveness or adverse events. The average age of the 20 female subjects was 45.60±8.18 years (data not shown). On average, the subjects used 80-98% of the quantity of test materials expected (data not shown).

There were no abnormal responses, including allergic contact dermatitis or irritant contact dermatitis, following application of the test material on the subjects, and the survey answered by subjects resulted in no special abnormal skin response for this trial period (data not shown).

Evaluation of wrinkle improvement. The results of the evaluation of the facial wrinkles using the wrinkle value (Ra) obtained using the PRIMOS Lite are shown in Table I and Fig. 1.

Table V. Changes in a^* value after applying test materials.

Application period	a^* value	P-value
Pre-application	10.61±2.11	
2 weeks	9.50±1.76	0.001[a]
4 weeks	8.23±2.16	0.000[b]

Results presented as the mean ± standard deviation. [a]P<0.01, [b]P<0.001.

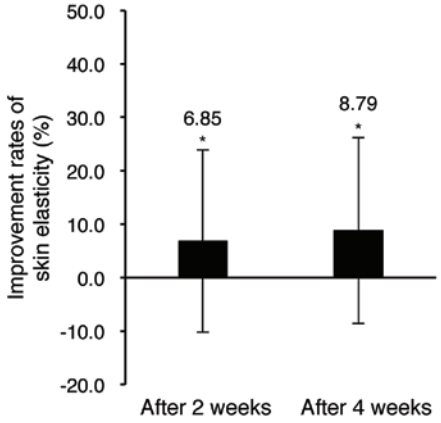

Figure 3. Improvement rates of skin elasticity after applying test materials. Data were presented as means ± standard deviation. A paired t-test was performed to determine statistical significant results. *P<0.05, vs. the control.

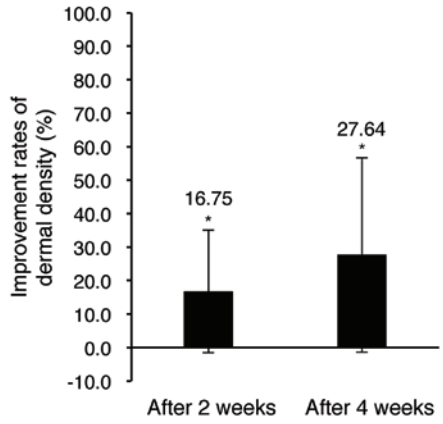

Figure 4. Improvement rates of dermal density after applying test materials. Data were presented as means ± standard deviation. A paired t-test was performed to determine statistical significant results. *P<0.05, vs. the control.

The evaluation results of the wrinkle value (Ra) showed a 5.97% decrease after 2 weeks of the test material application and a 14.07% reduction after 4 weeks of application in comparison of pre-application. The results were statistically significant (P<0.05).

The representative 3D images before application and after 4 weeks of the test material application were shown in Fig. 2. The outer corner of the right eye was measured three consecutive times with the PRIMOS Lite. Collectively, these

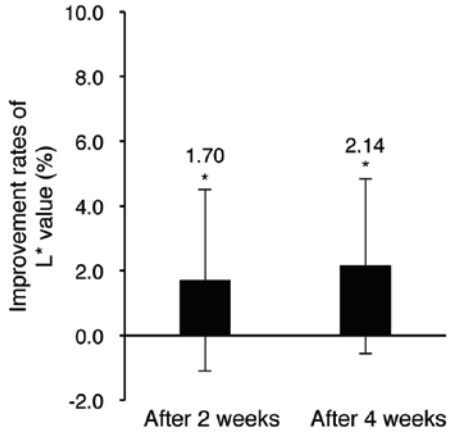

Figure 5. Improvement rates of L^* value after applying test materials. Data were presented as means ± standard deviation. A paired t-test was performed to determine statistical significant results. $^*P<0.05$, vs. the control.

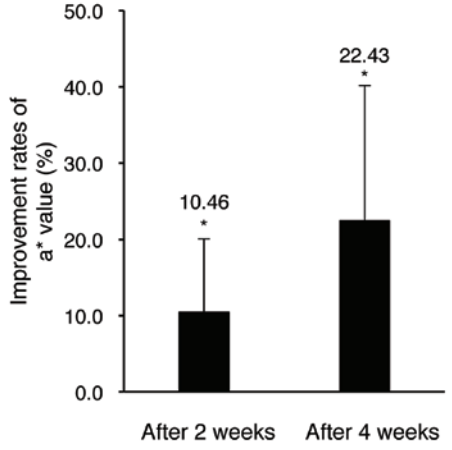

Figure 6. Improvement rates of a^* value after applying test materials. Data were presented as means ± standard deviation. A paired t-test was performed to determine statistical significant results. $^*P<0.05$, vs. the control.

suggest that the facial cream and eye cream tested in this study contribute to the improvement of skin wrinkles.

Evaluation of skin elasticity improvement. Next, we have determined the skin elasticity improvements with the DermaLab USB elasticity probe (Table II and Fig. 3).

The evaluation results of skin elasticity showed 6.85 and 8.79% increase after 2 and 4 weeks of test material application, respectively, in comparison with pre-application skin. The results were statistically significant (P<0.05) and suggest that the facial cream and eye cream contribute to improve skin elasticity.

Evaluation of dermal density improvement. The dermal density improvements were evaluated using the DUB® SkinScanner (Table III and Fig. 4).

The dermal density evaluation results showed 16.75 and 27.64% increase after 2 and 4 weeks of test material application, respectively, in comparison of pre-application. The results were statistically significant (P<0.05) and indicate that the facial cream and eye cream tested here improves dermal density.

Evaluation of skin tone improvement. The evaluation results of skin tone (L^* and a^* values) improvement obtained with the spectrometer are shown in Tables IV and V and Figs. 5 and 6.

As shown in Table IV and Fig. 5, the evaluation results of the L^* value indicating skin brightness showed a 1.70% increase after 2 weeks of the test material application and a 2.14% increase after 4 weeks of application in comparison of pre-application. The results were statistically significant (P<0.05). As shown in Table V and Fig. 6, the evaluation results of a^* value indicating erythema (redness) showed 10.46 and 22.43% reduction after 2 and 4 weeks of application, respectively, in comparison with pre-application. The results were statistically significant (P<0.05). These suggest that the facial cream and eye cream examined here help improve skin tone.

Cosmetic formulations containing palmitoyl peptides, *S. marianum* seed oil, vitamin E and other functional ingredients showed anti-aging effect in the improvement of facial wrinkles, elasticity, dermal density and skin tone on human aged skin. Following a 4-week period of application, skin wrinkles were decreased by 14.07% and elasticity was increased by 8.79% in comparison with pre-application. Dermal density showed a 27.63% increase and skin tone indicated a 2.14% increase in L^* value indicating skin brightness and a 22.39% reduction in the a^* value indicating erythema (redness) after 4 weeks of application.

Thus, formulations containing palmitoyl peptides, *S. marianum* seed oil, vitamin E and other functional ingredients may have some anti-aging effects on aging skin over a 4-week application period.

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