

DEVELOPMENT AND EVALUATION OF A COSMETIC W/O EMULSION SYSTEM CONTAINING A MODEL PLANT EXTRACT

Gülay Büyükköroğlu*, Yasemin Yazan

Anadolu University, Faculty of Pharmacy, Department of Cosmetology
26470, Eskişehir, TURKEY

Abstract

In this study, a model plant material, Centaurium erythraea, in a w/o type emulsion system was investigated regarding its physicochemical and cosmetic evaluation. W/O emulsion system without the incorporation of active ingredient was formulated prior to formulations containing 2% and 4% extracts obtained from the leaves of Centaurium erythraea. The emulsions were investigated under the light microscope, Droplet distributions were determined using laser diffraction equipment. Centrifugation, microbiological and thermal stress tests and rheological analyses were also performed on the formulations. The most stable formulations selected according to the tests mentioned above were used for dermatological evaluation on 10 volunteers. Statistical test (SPSS) was used to evaluate any variation between the cosmetic efficacy of the formulations and weeks. The three emulsion systems formulated showed appropriate consistency, homogeneity and physicochemical properties for application to the skin. New w/o emulsion systems were developed for a plant extract of cosmetic value. The systems were found to be stable and physicochemically acceptable. All three preparations formulated in this study have shown variation regarding skin moisture, skin pH and sebum content. Variation was not found for the skin elasticity between the formulations.

Key Words: W/O emulsion, Formulation, Physicochemical evaluation, Cosmetic efficacy

Model Bitki Ekstresi İçeren Kozmetik S/Y Emülsiyon Sisteminin Geliştirilmesi ve Değerlendirilmesi

Bu çalışmada, bitkisel bir madde olan Centaurium erythraea'nin s/y tipi emülsiyon sistemi içindeki fizikokimyasal ve kozmetik değerlendirmesi yapılmıştır. Centaurium erythraea'nin yapraklarından elde edilen %2 ve %4'lük ekstraktları içeren formülasyonlardan önce etkin madde içermeyen s/y emülsiyon sistemi formüle edilmiştir. Emülsiyonlar ışık mikroskopunda incelenmiştir. Damlacık dağılımları lazer kırınım cihazı ile belirlenmiştir. Formülasyonlarda, santrifüjleme, mikrobiyolojik ve hızlandırılmış termal testler ve reolojik analiz de gerçekleştirilmiştir. Bahsedilen testlere göre en kararlı olduğu saptanan formülasyonlar dermatolojik değerlendirme için 10 gönüllüde kullanılmıştır. Formülasyonlar ve haftalar arasında kozmetik etkinlik açısından farklılık olup olmadığı istatistiksel (SPSS) olarak değerlendirilmiştir. Formülasyonu gerçekleştirilen her üç emülsiyon sistemi de cilde uygulanacak uygun kıvam, tekdüzelik ve fizikofarmasötik özelliklere sahiptir. Kozmetik değeri olan bir bitki için yeni s/y emülsiyon sistemi geliştirilmiştir. Sistemlerin kararlı ve fizikofarmasötik olarak kabul edilebilir oldukları bulunmuştur. Bu çalışma kapsamında formülasyonu yapılan preparatların tümü, cilt nemi, pH'sı ve sebum içeriğine etkisi yönünden birbirlerinden farklılık göstermiştir. Tüm formülasyonların uygulanması sonucu elde edilen cilt esnekliği değerleri arasında farklılık saptanmamıştır.

Anahtar Kelimeler: S/Y emülsiyonu, Formülasyon, Fizikofarmasötik değerlendirme, Kozmetik etkinlik

* Corresponding author:

☎ +90.222.335 05 80 (10 lines) ext. 3631, 3656

☎ +90.222.335 07 50

✉ gbuyukko@anadolu.edu.tr

Introduction

Plant materials are being widely used in cosmetic products during the last 30 years. *Erythraea centaurium* Rafin. (Gentianaceae) contains considerable amounts of polyphenolic compounds, namely xanthones and phenolic acids as the main components (1, 2). Phenolic groups exhibit activity as radical scavengers and/or metal chelators. By virtue of effectively scavenging deleterious radicals and suppressing radiation-induced oxidative reactions, the phenolic compounds may serve an important antioxidant function in preserving the physiological integrity of cells, such as skin cells, exposed to both air and UV radiation. An *Erythraea centaurium* lotion prepared for external use was found to remove skin blemishes like freckles and spots (3). Some antioxidants in cosmetics are known to suppress pigmentation, stimulate collagen production and refine keratinization (4).

Preparation and cosmetic evaluation of a novel emulsion system with *Centaurium erythraea* extract, which is known to contain active groups with expected free radical scavenging effect and thus delaying skin aging, was aimed in this study. The study also has a novelty considering the cosmetic evaluation of the *Centaurium erythraea* extract. Almond oil was used as the oily external phase of the emulsions owing to its skin moisturizing, softening and non-irritant properties. Three different emulsion systems with 2% and 4% plant extracts and without the extract were prepared and their characteristics and cosmetic values were compared. The main challenge was whether the potential cosmetic value of *Centaurium erythraea* extract could be obtained at selected concentrations in a w/o emulsion system.

Experimental

Materials

Almond oil (Detsan, Turkey); *Centaurium erythraea* plant (Sivrihisar, Turkey); cetyl dimethicone copolyol (Abil EM 90[®], France) and silicone oil (Abil 350[®], France) (Goldschmidt A.G., Germany); Vitamin E and methyl paraben (Nipagin M, Johnson&Johnson, Turkey); sodium chloride (E.Merck, Germany) were used as received.

Preparation of plant extract

Centaurium erythraea extract was obtained by the decoction of leaves dried in the shadow. The decoction prepared was 5%. The liquid extract was freeze-dried (Edwards, U.K.) and diluted with water just prior to addition to the formulations.

Preparation of emulsions

For maintaining the stability of the emulsion systems, preparation method, equipments used, oil and surfactant selected and their concentration and temperature are of importance. Contents of the emulsion formulations were kept constant in order to follow the cosmetic value of the plant extract. Following the preformulation studies, aqueous phase which contained plant extract, methyl paraben (preservative) and sodium chloride (stabilizer) was added (Heidoph RZR 2051, Germany) to the oily phase containing the lipophilic surfactant (Abil EM 90[®]), Vitamin E (antioxidant) and silicone oil (Abil 350[®]) in almond oil. When the temperature was around 40°C, 10 drops of perfume was added to the formulation to mask the odor of the almond oil.

Emulsion without the plant extract was defined as F₀, while F₁ and F₂ contained 2% and 4% plant extract in the aqueous phase, respectively. Formulations of all the emulsions contained 22 % almond oil, 2 % Abil EM90[®], 0.5 % Abil 350[®], 0.2 % Vitamin E, 0.6 % sodium chloride, 0.25 % methyl paraben and sufficient quantity distilled water for 100 %.

Characterization of emulsions

Macroscopic analysis

Organoleptic characteristics (color, consistency, appearance), homogeneity (creaming, phase separation) were investigated visually.

Determination of the emulsion type

External phase of the emulsion was tested by dilution with water and oil.

Globule size distribution

The freshly prepared emulsion systems were dispersed in almond oil and their globule sizes were examined using particle size analyzer (Malvern Mastersizer 2000, U.K.).

Stability tests on emulsions

Since emulsions are thermodynamically unstable, they are open to physical instability. Accelerated stability tests were performed in this study to ensure the physical, chemical and biological characteristics of the formulations developed (5).

Centrifugation test

Centrifugation tests were performed to determine the physical stability of the emulsions under accelerated gravitational conditions. Freshly prepared samples and samples kept at room temperature for 24 hr were centrifugated at 5 000 rpm for 10 minutes. Any change in the macroscopic appearance was recorded.

Thermal stability

Samples of the emulsions were kept at $4^{\circ}\text{C}\pm 1^{\circ}\text{C}$ (refrigerator), $25^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$ (room temperature), $40^{\circ}\text{C}\pm 0.1^{\circ}\text{C}$ (oven) and in a cabin of $40^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ temperature and 60% relative humidity (Aymes, Turkey). Phase separation, creaming and any change in color were examined periodically.

Microbiological test

For the microbiological analyses, emulsions were first emulsified with TLP (5% polysorbate 80, 0.1% lecithin, 0.1% peptone) solution. Samples were inoculated in blood EMB (Eosin Methylene Blue) agar, SDA (Sabraud Dextrose Agar) and buillon containing thioglycolate. Samples inoculated in Blood EMB agar were evaluated following 24 hr incubation at 37°C . Inoculations in buillon containing thioglycolate were incubated at 37°C for 7 days. Two samples from each emulsion were inoculated in SDA. Those inoculations were incubated at 37°C for 24-28 hr and for 3 weeks at room temperature. All incubations were performed at normal atmospheric conditions.

Rheological analysis

For the rheological studies, approximately 0.2 g samples of the emulsions prepared freshly, samples kept at oven ($40^{\circ}\text{C}\pm 0.1^{\circ}\text{C}$) and room temperature ($25^{\circ}\text{C}\pm 0.1^{\circ}\text{C}$) for 15 days were used. Shear rate was increased at constant temperature ($25\pm 0.1^{\circ}\text{C}$) and the changes in shear stress and viscosity were examined using a cone-plate rheometer (Brookfield Model DV-III, U.S.A.).

Dermatological tests

Volunteers

10 female volunteers within an age range of 24 and 45 were used. To ensure that all subjects were at a baseline value, they were instructed not to use any other cosmetic products 1 month before the study. Skin uniformity of the foreheads and cheeks of all volunteers were examined visually prior to the study.

Each volunteer was asked to read and sign a written consent before the dermatological study. The volunteers were not informed about the content of the emulsions.

Test methods

Basic values of skin pH, moisture, sebum and elasticity were determined objectively (Courage&Khazaka, Germany) before the facial application of the formulations. Volunteers were asked to apply the preparations as instructed, twice daily for a period of 4 weeks; and the cheek and forehead measurements were repeated every week. All values were measured using skin pH meter, sebumeter, corneometer and cutometer in a conditioned room of 25°C and 40 % RH. Percentages of change in skin pH, moisture, sebum and net elasticity were calculated.

Analysis of data

Results of the skin measurements were evaluated statistically using SPSS (two-way ANOVA and paired samples t-test) on the computer.

Results and Discussion

Natural materials provide many cosmetic benefits, but since they are also potent chemicals, thorough investigation of their formulation, safety and efficacy has to be done. Therefore, physicopharmaceutical tests, stability tests and cosmetic evaluation tests have to be conducted consecutively in developing a new cosmetic product.

Characterization of the emulsions

Macroscopic analyses of the cosmetic emulsions include examination of the color, opacity, homogeneity, phase separation or creaming and consistency. The three emulsion

systems formulated showed the appropriate consistency and homogeneity for application to the skin. All the colors were homogeneous and darkened depending on the percentage of the extract in the formulation.

External phases of F₀, F₁ and F₂ emulsions were determined to be oily upon dilution with almond oil.

Microscopic analysis (Olympus Bx-50, China) in this study was carried out just for the confirmation of the formation of an emulsion system and to follow the homogeneity and shapes of the globules. Emulsion systems with homogeneously distributed water droplets were achieved.

As a result of the globule size analysis, mean globule sizes of F₁ and F₂ were found to be 3.1 µm and 5.4 µm and their specific surface areas were 1.4960 m²/mL and 1.2675 m²/mL, respectively. The globule sizes obtained in this study seem to be appropriate to maintain the stabilities of the systems (6).

Stability tests on the emulsions

Color change, phase inversion and separation, chemical decomposition, pH change, drying, oxidation, loss of perfume and unwanted scent are among the many changes that may happen in cosmetic products. Stability tests are performed to ensure the stability of the products under normal and accelerated conditions in the countries of production and consumption (6).

Centrifugation tests may be used in the prediction of long-term stabilities of emulsions. No phase separation and creaming was observed for the formulations prepared.

Cosmetic preparations exposed to various climate changes are significantly affected by the temperature. Time determined for phase separation of F₀, F₁ ve F₂ emulsions kept under conditions of 4°C ± 0.1°C, 25°C ± 0.5°C, 40 ± 0.1°C and 40 ± 0.1°C and 60 % RH are summarized in Table 1.

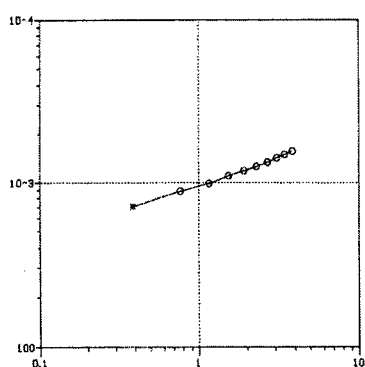
TABLE 1. Time determined for phase separation in the emulsions prepared

Formulation	40°C	40°C and 60% relative humidity	25°C	4°C
F ₀	9 months	4 months	> 12 months	> 12 months
F ₁	8 months	3 months	> 12 months	> 12 months
F ₂	8 months	2 months	> 12 months	> 12 months

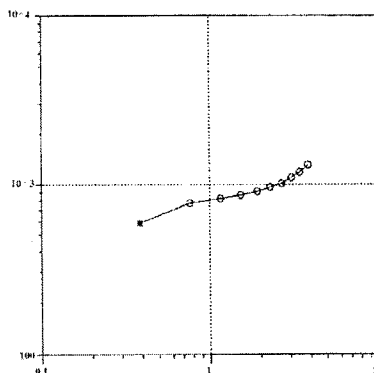
Cosmetic emulsions have to be free of pathogenic microorganisms since they are in direct contact with the human tissues. However, they are open to contamination during consumption. No microbiological growth (bacteria-aerob and anaerob-, fungi) was determined for F₀, F₁ and F₂ emulsions during 4 weeks. This finding shows the efficiency of the preservative material incorporated. Bacterial contamination is usually seen in O/W emulsions. It is rarely seen in W/O emulsions since the aqueous globules are surrounded by oil (7).

The cone selected for rheological analysis had a radius of 1.2 cm, an angle of 1.565° and a shear rate of 3.84 N (N=RPM) sec⁻¹. Rheograms of the samples prepared freshly, kept at room temperature and oven for 15 days are given in Figure 1. Rheological data was found to fit the Bingham model with a correlation coefficient of greater than % 99 following calculations of several mathematical models, and Bingham curves are demonstrated in Figure 2.

Freshly prepared emulsions

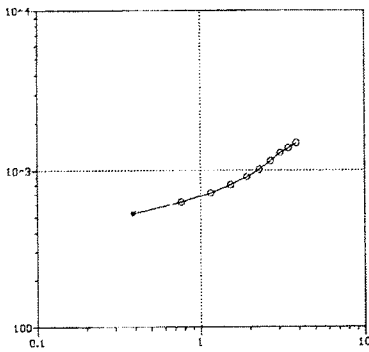


F₁

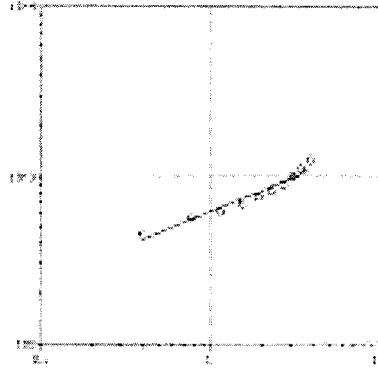


F₂

Emulsions kept at room temperature for 15 days

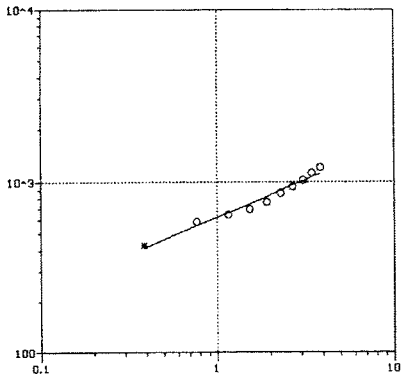


F₁

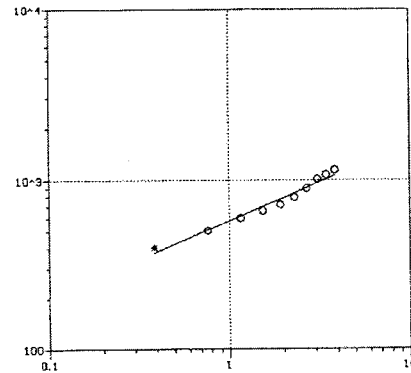


F₂

Emulsions kept at 40°C for 15 days



F₁



F₂

Figure 1. Rheograms (shear stress versus shear rate) of F₁ and F₂ prepared freshly, kept at room temperature (25°C) and at 40°C for 15 days (n=3).

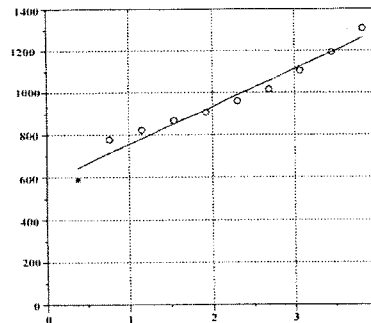
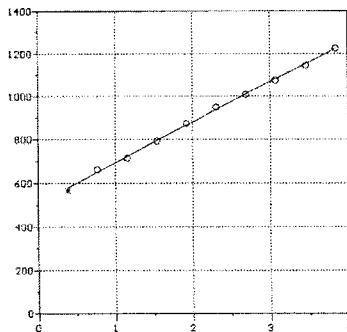


Figure 2. Profiles of the freshly prepared F1 and F2 emulsions according to the Bingham model (shear stress versus shear rate)

The first stage in ensuring the various functions of a specific formulation is to determine its physicochemical characteristics and follow any changes in long term. In our study, direct and microscopic observation of the products formulated was done on freshly prepared samples and throughout the storage period. In order to test the stability of the emulsion systems prepared, accelerated temperature tests, rheological and microbiological analyses were performed. According to the data obtained, all the emulsions formulated were found to be within the technological limits of acceptance.

Dermatological tests

Objective measurements on the skin provides data for the efficacy and acceptability of a cosmetic finished product. Therefore, objective measurements of skin pH, moisture, sebum and elasticity were done.

Skin pH

Comparison of skin pH values following application of F₀, F₁ and F₂ formulations is given in Figure 3. Average skin pH value of 5.5 for females seems to be decreased slightly on the foreheads following application of F₀, F₁ and F₂. For the cheeks, skin pH values obtained were increased with F₁ and F₂ while slightly decreased with F₀. There was no significant difference between F₀ and F₁ as a result of statistical analysis ($p>0.05$); there was difference between F₁ and F₂ and F₀ and F₂ ($p<0.05$). For the cheek pH values, difference was determined between F₁ and F₂ ($p<0.05$) and F₀ and F₂ ($p<0.01$). There was also significant difference between the weeks for every emulsion ($p<0.05$).

Skin pH has to be determined to define the existing condition and quality of the skin. Aqueous solution characteristic of the skin surface with its secretions and moisture content makes pH measurements possible (8). Exposure of the skin to cosmetic products which have a pH value out of the weak acidic range leads to damage and premature aging of the skin. There were no dramatic changes in the skin pH values following the application of all the formulations prepared and therefore no volunteer complained about irritation.

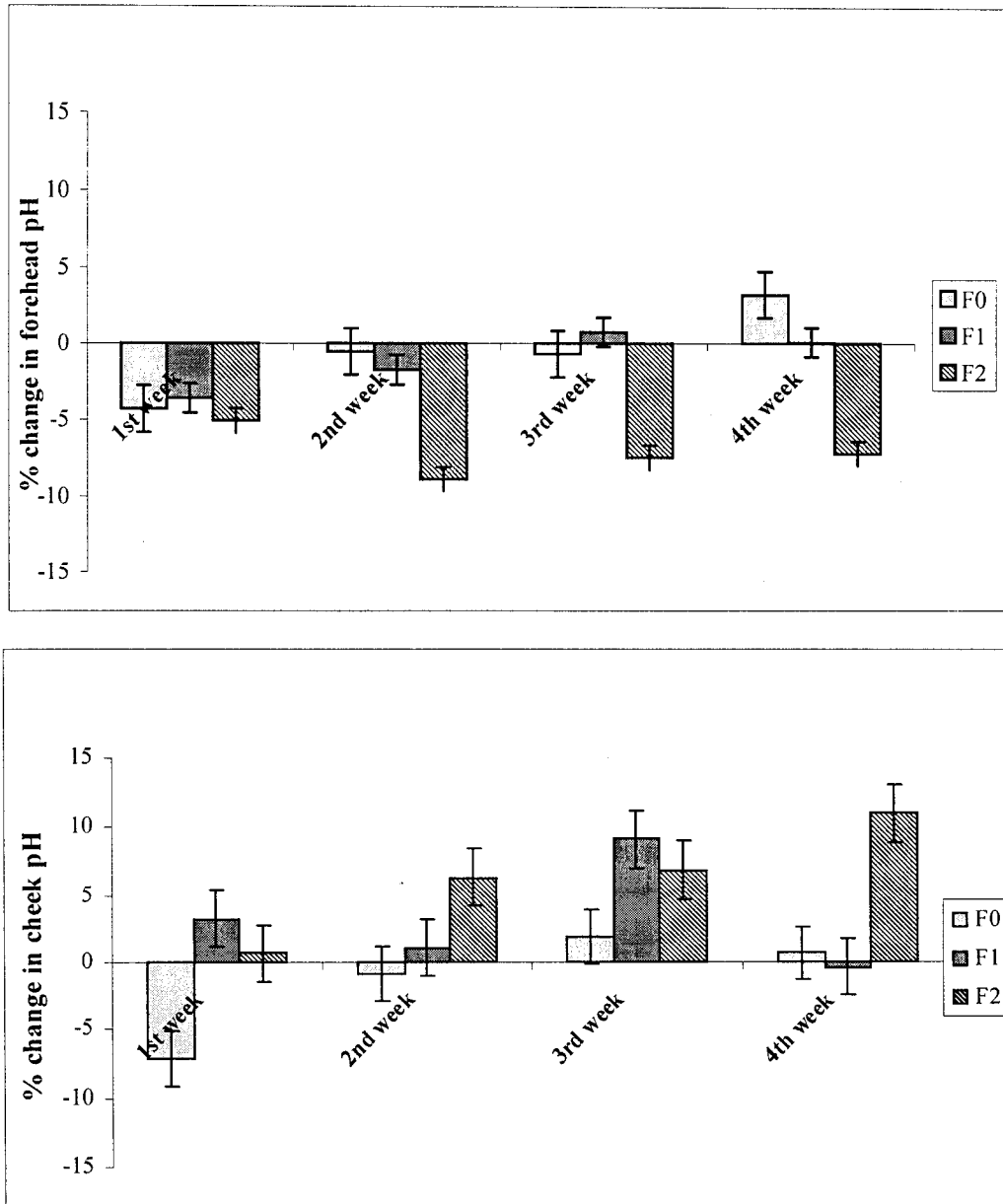


Figure 3. Comparison of skin pH values following application of F₀, F₁ and F₂ formulations (n=10).

Skin sebum

Comparison of percentages of change in skin sebum following the application of the three formulations is given in Figure 4. All the formulations have lead to an increase on the sebum content of the forehead and cheek; the highest increase was obtained with the formulation containing 4% extract (F₂).

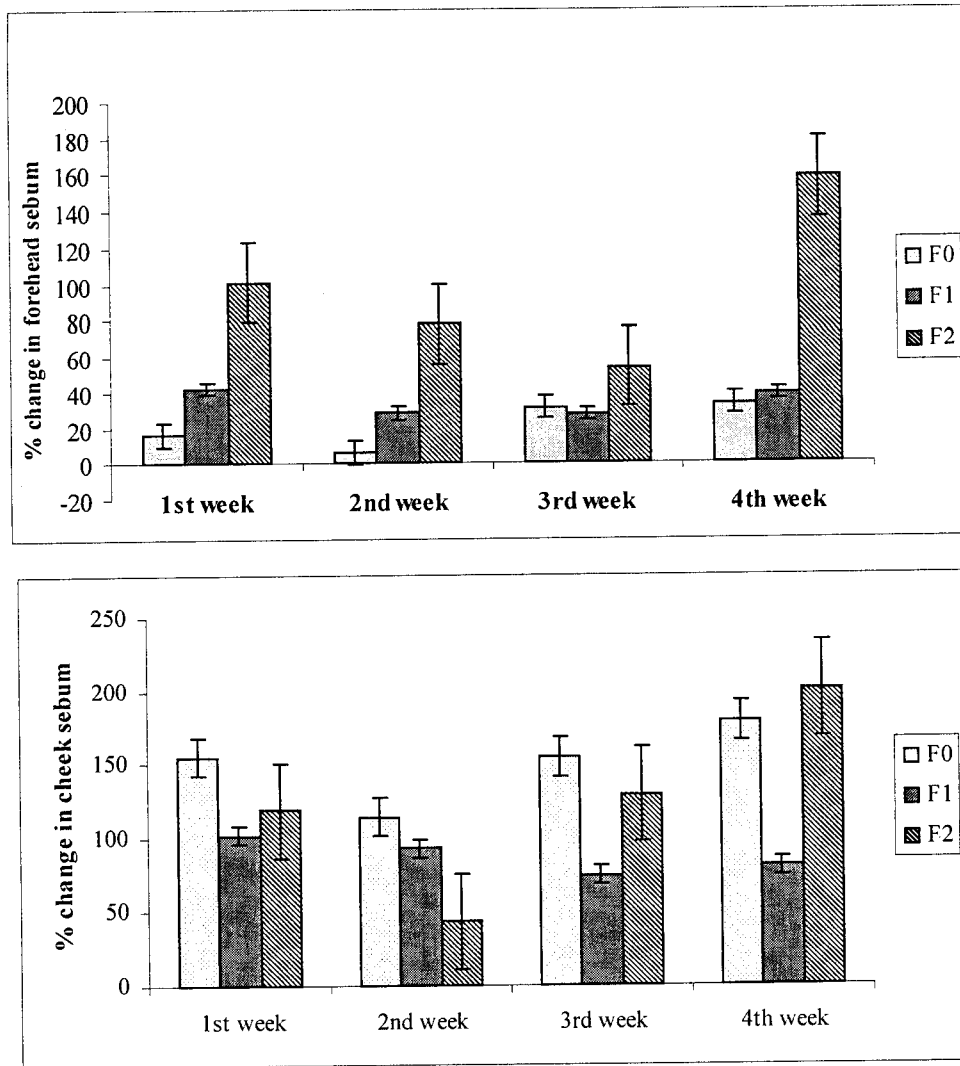


Figure 4. Comparison of skin sebum values following application of F₀, F₁ and F₂ formulations (n=10).

There was significant difference between the weeks upon statistical analysis both for the forehead ($p < 0.01$) and cheeks ($p < 0.001$). There was no statistical difference between the three formulations for the cheeks ($p > 0.05$); but for the forehead, there was significant difference between F₀ and F₁ ($p < 0.01$) and between F₁ and F₂ ($p = 0.001$) regarding the skin sebum.

Sebum, lipid on the skin surface, is the secretion of glands in the horny layer. There is no unique content of sebum due to the changes in physiology of glands, temperature, humidity and UV light (8). To decide on the right skin type (dry, normal,

oily) for the cosmetic products, effect of the specific product on skin sebum has to be determined. The objective sebum measurements obtained for the preparations demonstrated contribution of all the formulations to the skin sebum content. This indicates that the use of the creams on dry skin is the most appropriate.

Skin moisture

Comparison of percentages of change in skin moisture following the application of the three formulations is given in Figure 5.

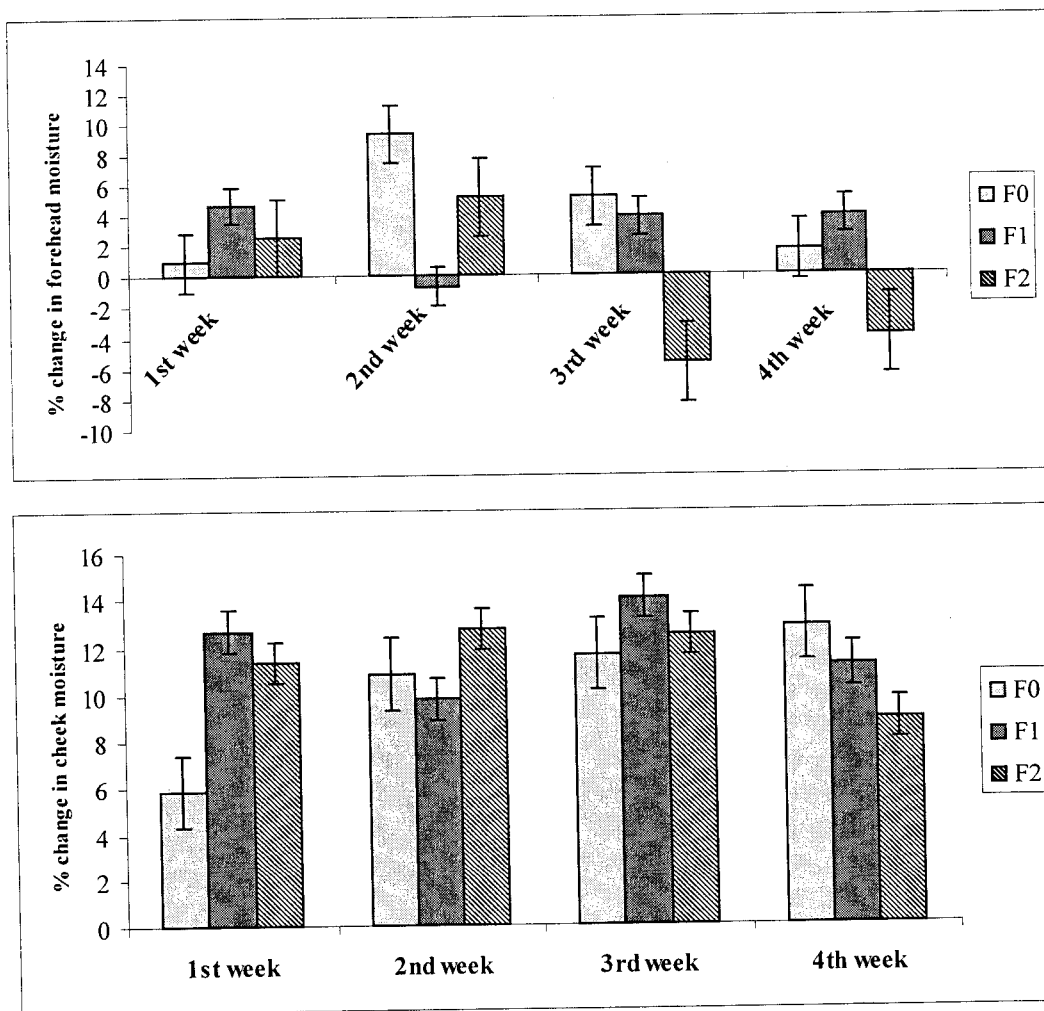


Figure 5. Comparison of skin moisture values following application of F₀, F₁ and F₂ formulations (n=10).

Formulation containing no extract (F_0), formulation containing 2% extract (F_1) and formulation containing 4% extract (F_2) lead to an increase in forehead and cheek moisture almost equally. The highest increase was seen with F_0 . There was significant difference between the weeks for the cheek ($p < 0.05$) but no difference for the forehead ($p > 0.05$). Skin moisture values of the forehead and cheeks after application of the three formulations did not lead to any statistical difference between the formulations F_0 and F_2 ($p > 0.05$). There was significant difference between F_0 and F_1 for the forehead and cheek moisture ($p < 0.001$) and between F_1 and F_2 for the forehead ($p < 0.05$) and cheek ($p = 0.001$) moisture.

Moisture content of the skin depends on many endogenous and exogenous factors (9). There is no one value for skin moisture since it depends on age, sex and region of the skin (10). Measurement of skin moisture has gained popularity because moisture content affects the physical characteristics of the skin. Loss in skin moisture results in aging of skin. For the formulations prepared in this study, the plant extract which was supposed to be the active agent, have not lead to any contribution in the skin moisture at the end of the 4-week period.

Skin elasticity

Net elasticity (R_5) values measured after application of F_0 , F_1 and F_2 are given in Figure 6. F_1 and F_2 seem to increase the skin elasticity at the end of 4 weeks more than F_0 . However, no difference was found between the R_5 values of all formulations for a period of 4 weeks ($p > 0.05$). Significant variation was found between the weeks regarding the net elasticity of the forehead skin ($p = 0.01$). There was no variation between weeks for the cheek values ($p > 0.05$).

Skin has a viscoelastic property which has to be examined for the efficacy of an antiaging product. Various regions of the body have different degrees of elasticity and plasticity; for example, the elasticity of the forehead is less than the elasticity of the cheek. Cutometer, existing with a suction and relaxation phase, displays curves which show the viscoelasticity of the skin. The curves consist of two parts, suction phase created by a negative pressure (400 mB) for 3 seconds and then a relaxation phase.

During the relaxation phase, skin returns back to its original status. R_5 value (net elasticity) calculated is the ratio of the skin plasticity when the pressure is applied to the plasticity when the skin is relaxed. This value approximates 1 for the maximum elasticity (11). The preparations containing the plant extract was expected to increase the

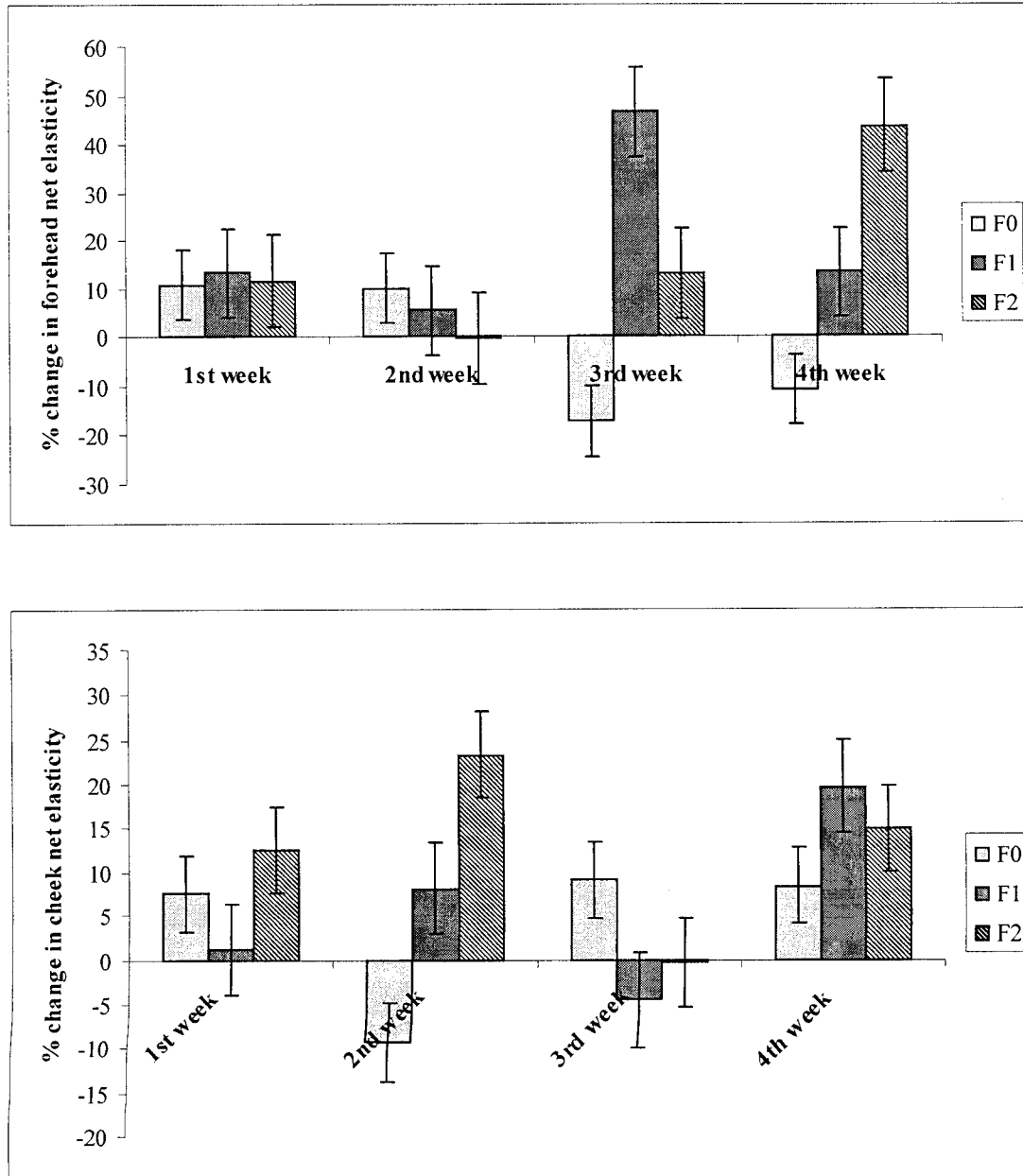


Figure 6. Comparison of skin net elasticity values following application of F_0 , F_1 and F_2 formulations (n=10).

skin elasticity due to its predicted antioxidant effect. However, no significant increase could be found. This may be due to the partial loss of active ingredients in the plant extract during the extraction procedure even though the extract was prepared in the light of the literature. Further attempt has to be made to improve the extraction process since the formulation parameters have been obtained successfully.

Conclusion

The emulsions prepared in this study are stable systems and dermatologically acceptable. No one of the formulations is superior to the other in the cosmetic sense. The idea of cosmetic value of the plant extract did not give promising results upon objective skin evaluation. This result may be due to the partial loss of the active ingredients of *Centaureum erythraea* during the extraction procedure. The cosmetic value of *Centaureum erythraea* has to be further investigated using a different plant extraction procedure and different formulation types.

References

1. **Valentao, P., Fernandes, E., Carvalho, F., Andrade, P.B., Seabra, R.M. and Bastos, M.L.**, "Antioxidant activity of *Centaureum erythraea* infusion evidenced by its superoxide radical scavenging and xanthine oxidase inhibitory activity" *J.Agric.Food Chem.*, 49 (7), 3476-3479, **2001**.
2. **Tapiero, H., Tew, K.D., Nguyen Ba, G. and Mathé, G.**, "Polyphenols: do they play a role in the prevention of human pathologies?" *Biomed.Pharmacother.*, 56, 1-8, **2002**.
3. www.naturedirect2u.com/medicinal_herbs/eucentauryherb.htm
4. **Lupo, M.P.**, "Antioxidants and vitamins in cosmetics" *Clin.Dermatol.*, 19 (4), 467-473, **2001**.
5. **Kaş, H.S.**, Kozmetik ürünlerde stabilite çalışmaları, deri yaşlanması ve kozmetiklere bir bakış. In: Kozmetoloji Günleri-1, pp. 41-57, Şafak Matbaacılık, Ankara, 1997.

6. **Öner, F.**, Methods for testing the physical stability of emulsion type cosmetic formulation. In: Proceedings of the First International Cosmetics Symposium, Eds.: K.H.C. Başer, Y. Yazan, pp. 134-146, Anadolu Üniversitesi Basımevi, Eskişehir, 1993.
7. **Patel, N.K. and Romanowski, J.M.**, "Heterogeneous systems II: influence of partitioning and molecular interactions on in vitro biologic activity of preservatives in emulsions" *J.Pharm.Sci.*, 59 (3), 372, 1970.
8. **Elsner, P.**, Sebum. In: Bioengineering of the Skin: Methods and Instrumentation, Eds.: E. Berardesca, P. Elsner, K.P. Wilhelm, H.I. Maibach, pp. 81-85, CRC Press, Inc., Florida, 1995.
9. **Berardesca, E., Farinelli, N. and Maibach, I.H.**, Stratum corneum water content and TEWL. In: Cosmetic Dermatology, Eds.: R. Baran, H.I. Maibach, pp. 383-389, Martin Dunitz Ltd., London, 1994.
10. **Warner, R.R. and Lilly, N.A.**, Correlation of water content with ultrastructure in the stratum corneum. In: Bioengineering of the Skin: Water and the Stratum Corneum, Eds.: E. Berardesca, P. Elsner, K.P. Wilhelm, H.I. Maibach, CRC Press, Inc., Florida, 1995.
11. **Elsner, P.**, Skin Elasticity. In: Bioengineering of the Skin: Methods and Instrumentation, Eds.: E. Berardesca, P. Elsner, K.P. Wilhelm, H.I. Maibach, pp. 53-64, CRC Press, Inc., Florida, 1995.

received : 25.03.2004

accepted : 29.04.2004