DOI: 10.15386/cjmed-668

FORMULATION AND EVALUATION OF A WATER-IN-OIL CREAM CONTAINING HERBAL ACTIVE INGREDIENTS AND FERULIC ACID

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Abstract

Background and aims. *The main aims of the present study were to formulate an anti-age cream based on vegetal ingredients and ferulic acid and to evaluate the physical characteristics and the efficacy of the cream.*

Methods. *The active ingredients were Centella asiatica oil, Spilanthes acmella oil, Zingiber officinale extract and ferulic acid. Formulation 1 (F1) was prepared using glyceryl stearate and Ceteareth-25® as emulsifiers and Formulation 2 (F2) using glyceryl stearate and potassium cetyl phosphate, all other ingredients remaining the same. The physical characterization of the creams was performed and the following parameters were analyzed: viscosity, oil droplet size, polydispersity index; also, texture analysis was performed. The anti-aging effect of the F2 was evaluated by assessing the cutaneous density before and after cream application using DUB-cutis® scanner.*

Results. *The mean diameter of oil drops was 10.26±4.72 mm (F1) and 22.72±7.93 mm (F2) and the polydispersity index was 35.4% and 45.7%, respectively. The mean values for consistency were 594.7±10.3 g (F1) and 300.5±14.5 g (F2), the average values for adhesion were 15.61±0.8 mJ (F1) and 22.25±4.3 mJ (F2), for firmness were 51.2±0.8 g (F1) and 30.3±4.3 g (F2) and the spreadability had values between 72.63 mm2 (F1) and 73.3 mm2 (F2). In vivo study revealed that the mean values of the cutaneous density increased from 9.21±1.39 % to 12.50±1.44 % after 8 weeks of cream application. The herbal ingredients incorporated in the O/W cream base for the antioxidant activity and anti-wrinkle effect, induced changes of the cutaneous density, an important parameter which quantifies the regeneration process of the skin.*

Conclusions. *An anti-age cream containing herbal active ingredients and ferulic acid with appropriate physical characteristics was obtained. In vivo study of clinical efficacy revealed a positive effect on skin density, which increased after 8 weeks of cream application.*

Keywords: anti-age, O/W cream, herbal extracts, texture analysis, ultrasound skin imaging

Background and aims

Ageing is a biological process that involves a multitude of external and internal factors as genetic and environmental influences and induces characteristic molecular, cellular, tissular and clinical changes. Age-related changes in skin appearance, including wrinkles, irregularities of pigmentation, xerosis and laxity are related to the thinning of the epidermis. A progressive and significant thinning (with 6.4% in each decade), flattening of the

Manuscript received: 29.03.2016
Received in revised form: 23.05.2016
Accepted: 25.05.2016
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dermo-epidermal junction and reduction of extracellular matrix components were also observed [1,2,3]. General atrophy of the extracellular matrix is influenced by the decrease of the number of fibroblasts, while the decrease of the protein synthesis influences types I and III collagen in the dermis and determines an increased breakdown of extracellular matrix proteins [4]. These changes lead to a more vulnerable skin especially to mechanical stress, loss of elasticity, fragility of blood vessels [5].

Among all factors, oxidative stress is considered to have a major importance in the ageing process. The original free radical theory postulated by Harman in 1956 purported that the reactive oxygen species interfere with the cellular and subcellular systems, inducing molecular degradations [1,4]. Even if skin antioxidant activity is very efficient, during ageing reactive oxygen species levels increase and intrinsic antioxidant activity decreases [4]. Reactive oxygen species such as superoxide anion radical, hydrogen peroxide, hydroxyl radical and oxygen singlet cause oxidative damage to cellular macromolecules including lipids, proteins and DNA and their oxidized forms [4,6,7,8]. Ageing is associated with changes in the molecular structure of these compounds and with other pathways such as spontaneous errors and other protein alterations. At the same time, the production of reactive oxygen species plays an important role in signaling processes and in cellular homeostasis [4].

The identification of the mechanisms of the skin aging and the research of new anti-age cream formulation represent a continuous challenge, which is especially important because skin aging reflects aging of the entire organism [9]. Herbal extracts present a great potential in the development of new anti-age products considering antioxidant effects of phytochemical compounds.

Phytochemical analysis of *Centella asiatica* (fam. Apiaceae) indicates the presence of asiaticoside, centelloside, madecassosidasiatic acid, centellin, asiaticin and centellicin. *Centella asiatica* possess a strong antioxidant activity in three different pathways including superoxide free radical scavenging activity, inhibition of linoleic acid peroxidation and DPPH free radicals scavenging activity. Due to the complex composition, *Centella asiatica* oil has been used as anti-photoaging agent because it increases the synthesis of type I collagen in fibroblasts and regulates the activity of matrix metalloproteinase enzymes responsible for the degradation of collagen. Also, the mechanism of action involves the increasing of mitotic activity in the germ layer and the increasing of the level of intracellular fibronectin, as well an improved tensile strength of newly formed skin [10,11,12,13].

Spilanthes acmella is a plant belonging to the Asteraceae family that is used in cosmetology for an anti-wrinkle effect by inhibiting contractions in subcutaneous muscles and by reorganizing and strengthening the collagen network. The main constituent, spilanthol (N-isobutyl-2, 6, 8-decatrienamide) is responsible for the anti-age effect while trans-ferulic acid possess antioxidant activity [14,15,16,17].

Zingiber officinale extract (Family Zingiberaceae) presents a high antioxidant capacity due to the large number of antioxidants such as betacarotene, ascorbic acid, terpenoids, alkaloids and polyphenols such as flavonoids, flavones, glycosides and rutin. In addition, *Zingiber officinale* extract contains other compounds like gingerols, shogaols and paradols, also responsible for the activity of this plant. In a previous study, Tsukahara et al. found that the topical application of *Zingiber officinale* extract inhibited the wrinkle formation induced by chronic UVB irradiation and prevented the decreasing of skin elasticity [18,19,20,21].

Ferulic acid is one of the most abundant phenolic acids in plants with an important structural role in the cell wall. Due to its radical scavenging activity, ferulic acid offers protection against UV radiation and possesses strong antioxidant properties. Additionally, ferulic acid has been proven effective for alleviate cutaneous pigmentation, sun-induced darkening, inflammatory reactions and skin aging process [22,23,24].

Among the methods used to evaluate skin structure, ultrasonography is a sensitive and noninvasive tool, which is able to quantify the ageing process and also the efficacy of anti-ageing therapies. This technique is able to measure the small but clinically important changes in thickness of various skin layers [1,25]. Using a 20 MHz transducer skin thickness may be accurately measured by transmitting ultrasounds into skin tissues and a visual image of structures up to 6-7 mm in depth can be obtained (Figure 1).

The incident ultrasonic energy is partially transmitted, partially reflected, which generates echoes. The amplitudes of these echoes are characteristic to the nature of the media. Tissues have the property to reflect sound waves distinctly based on their intrinsic variation in their structure, such as density and vascularity, reflecting differences in collagen, keratin and water content. An increased dermal density appears as a more increased echogenity of this area [3,26,27].

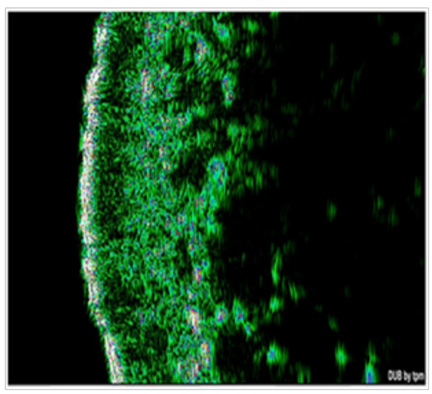


Figure 1. Ultrasonografic aspect of skin (DUB® cutis, Lüneburg, Germany).

The main aims of the present study were to formulate an anti-age cream based on vegetal ingredients- *Centella asiatica* oil, *Spilanthes acmella* oil, *Zingiber officinale* extract and ferulic acid and to evaluate the physical characteristics and the efficacy of the cream.

Methods

Cream preparation

Centella asiatica oil (Elemental, Oradea, Romania), *Spilanthes acmella* oil (Elemental, Oradea, Romania) and *Zingiber officinale* extract (Elemental, Oradea, Romania) were selected as active ingredients of the O/W emulsion. The surfactants chosen were: glyceryl stearate (Elemental, Oradea, Romania), potassium cetyl phosphate (DSM Nutritional Products, Basel, Switzerland), Ceteareth -25® (BASF, Ludwigshafen, Germany). The other materials used were the following: caprylic/capric triglycerides (Croda, Snaith, United Kingdom), isopropyl miristate (Sigma-Aldrich, St. Louis, Missouri, United States), silicone oil (Elemental, Oradea, Romania), Ferulan® (GfN, Wald-Michelbach, Germany), Sepigel 305® (polyacrylamide & C13-14 Isoparaffin & laureth-7) (Seppic, Paris, France), Euxyl PE 9010® (phenoxyethanol & ethylhexylglycerin) (Schülke & Mayr, Norderstedt, Germany). Cetyl stearyl alcohol, cetaceum and glycerol were supplied by Vitamar, Bucharest, Romania and Persea gratissima oil was purchased from Elemental, Oradea, Romania.

Formulation 1 (F1) was prepared using glycerol stearate and Ceteareth-25® as emulsifiers, while Formulation 2 (F2) was prepared using glyceryl stearate and potassium cetyl phosphate, all other ingredients remaining the same. The cream was prepared as an oil-in-water disperse system by heating separately the aqueous phase and the oily phase containing the lipophylic surfactants. The hydroalcoholic extract of *Zingiber officinalis* was added in the aqueous phase, while *Spilanthes acmella* oil, *Centella asiatica* oil and Ferulan® were added to the oily phase. The aqueous phase was added in the oily phase at 50°C±2°C under continuous stirring (DLS Stirrer, Velp Scientifica Germany, 500 rpm). The composition of the two formulations is presented in Table I.

Rheological analysis

In order to find the viscosity values, rheological measurements were performed using a rotational rheometer DV-III Ultra (Brookfield Engineering Laboratories, Middleboro, Massachusetts), at rotation speed of the spindle of 2 rpm (spindle 64). The measurements were carried out in triplicate at 22±2°C, the average values are presented.

Microscopic analysis

Microscopic analysis was performed with an optical microscope (Optika, Ponteranica, Italy) equipped with 40x and 100x objectives and Optikam 3 digital camera. The images were analyzed using Image Tool®, version 3.0 software to determinate the size of the droplets.

Texture analysis

Texture profile analysis was performed using CT3 Texture Analyzer (Brookfield Engineering Laboratories,

Table I. Qualitative and quantitative ingredients of the creams.

Formulation 1		**Formulation 2**	
Ingredients	Amount (%)	Ingredients	Amount (%)
Caprylic/ Capric Triglycerides	5.0	Caprylic/ Capric Triglycerides	5.0
Isopropyl Miristate	3.0	Isopropyl Miristate	3.0
Persea gratissima oil	2.0	Persea gratissima oil	2.0
Silicone oil	2.0	Silicone oil	2.0
Cetylstearyl alcohol	4.0	Cetylstearyl alcohol	4.0
Cetaceum	2.0	Cetaceum	2.0
Gliceryl Stearate	2.7	Gliceryl Stearate	0.4
Ceteareth-25	2.3	Potassium cetyl phosphate	4.6
Centella asiatica oil	1.0	Centella asiatica oil	1.0
Spilanthes acmella oil	1.0	Spilanthes acmella oil	1.0
Zingiber officinale extract	1.0	Zingiber officinale extract	1.0
Ferulic acid	5.0	Ferulic acid	5.0
Glycerol	5.0	Glycerol	5.0
Sepigel 305	1.5	Sepigel 305	1.5
Euxyl PE 9010	1.0	Euxyl PE 9010	1.0
Water	to 100.0	Water	to 100.0

Middleboro, Massachusetts), following parameters being calculated: firmness, consistency, adhesiveness, stringiness and spreadability.

The assessment of firmness, consistency, adhesiveness and stringiness of the creams was performed using fixture back extrusion cell (TA-DEC), while the probe has been set to penetrate into the sample containers to a depth of 25 mm at a rate of 2.0 mm/s. The force exerted to the probe was recorded using Texture Pro Software. The spreadability test was performed using fixture base table spreadability accessory (TA-SF), using a conical shape sample holder. The measurements were performed while the cone analytical probe was forced down into each sample at 2 mm/s test speed to 15 mm depth. The samples were analyzed in triplicate, the average values and the standard deviation were calculated.

Ultrasonographic evaluation

The ultrasonographic evaluation was performed using DUB® cutis device (Taberna pro medicum, Lüneburg, Germany), a high resolution ultrasound systems equipped with 22 MHz transducer that allows acquirement of cross-sectional images of the dermis and skin density measurements.

Subjects

F2 was chosen for the evaluation of the anti-aging effect efficacy, because in the case of F1 an oily layer could be noticed at the cream surface, even if it was not a true separation, the two phases being still emulsified. For F2 we couldn't observe this phenomenon during the surveillance time of six months after preparation. Also, in case of F2, textural parameters such as firmness and consistency presented a lower value, which is a desirable factor for the simple pick-up of the cream from the container and the facility of spreading on the skin. The adhesiveness had a higher value than F1, but without providing a sticky feeling after cream application. *In vivo* efficacy study was performed in the Dermatology Clinic, Cluj-Napoca, Romania on ten female volunteers aged between 30-57 years who used the tested cream. The study was carried out according to Helsinki declaration regarding Ethical Principles for Medical Research Involving Human Subjects. Every subject was informed about the nature and the purpose of the study and signed an informed consent before enrolling into the study. The subjects included in the study belonged to Fitzpatrick phototype class II and class III. The study excluded patients without static wrinkles, with known allergies to one of the cream components, with cutaneous facial lesions and those who used other anti-aging therapies in the last two months, phototherapy or oral contraception.

During the study each volunteer followed the same skin care routine, using the same cleansing product and the instructions regarding its application. No other cosmetic product was used on the tested areas. The cosmetic product was applied on the zygomatic area, once daily, in the evening, by lightly massaging the area for two minutes.

All measurements were made in the same area in controlled temperature and humidity conditions (T=22°C, R.H=50±5%). The volunteers had been preconditioned in the test room for at least fifteen minutes before the measurements.

Ultrasonographic images were taken for each volunteer at the beginning of the study, before product application (T0), after four weeks (T1) and after eight weeks of cream application (T2). For every subject the density of dermis (%) has been assessed.

Statistical analysis

The analysis was performed by relating the data of the cosmetic product treated sites to the corresponding starting value. The obtained data were analyzed by calculating the mean value and standard error; the difference of means before and after eight weeks was tested using a *t*-test for paired samples. A p value <0.05 was considered significant.

Results

1. Physical characterization of the creams

The physical measurements were performed to characterize the cream and to study the effect of the emulsifying agent on the quality of the final product. Table II presents the results for the physical characterization of the two formulations.

Table II.Characterization of the creams: viscosity, oil droplet size, polydispersity index, consistency, adhesiveness, firmness, spreadability.

Parameter	F 1	F 2
Viscosity (cP, mean±SD, 2 rpm)	159000±5656.8	112500±3535.5
Oil droplets (mm, mean diameter±SD)	10.26±4.72	22.72±7.93
Polydispersity index (%)	45.7	35.4
Consistency (g, mean±SD)	594.7±10.3	300.5±14.5
Adhesiveness (mJ, mean±SD)	15.61±0.8	22.25±4.3
Firmness (g, mean±SD)	51.2±0.8	30.3±4.3
Spreadability (mm2)	72.63	73.3

The rheological behavior of the investigated samples is shown in Figure 2. As confirmed by the appearance of the plot of viscosity vs. shear rate, the two creams exhibited thixotropic behavior, which is a desirable characteristic for topical applied products.

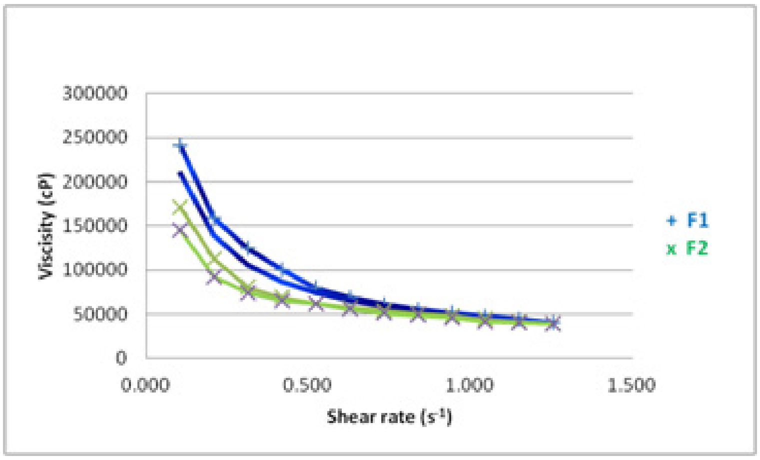


Figure 2. Viscosity of the two creams (cP) vs. shear rate (s-1).

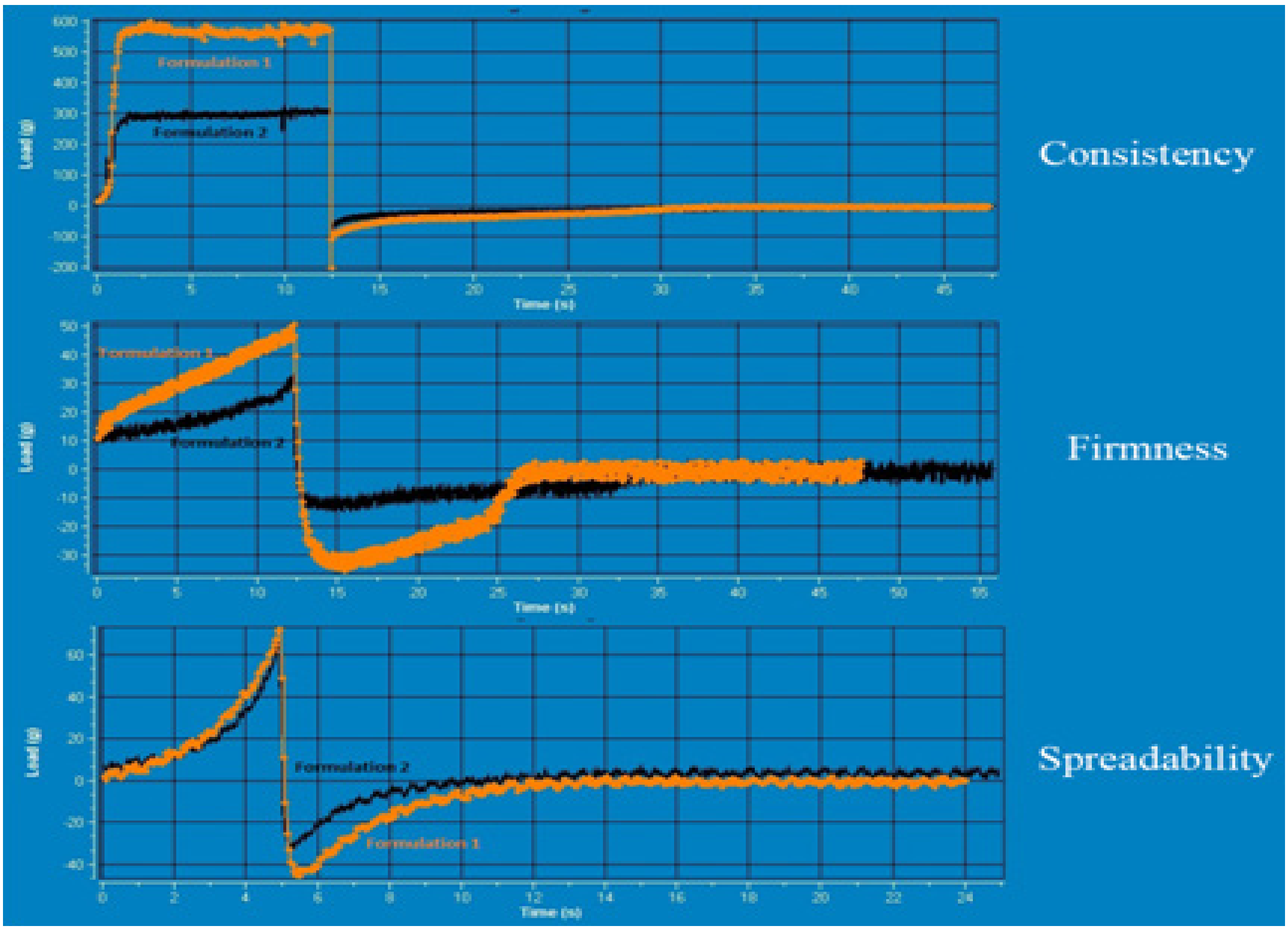
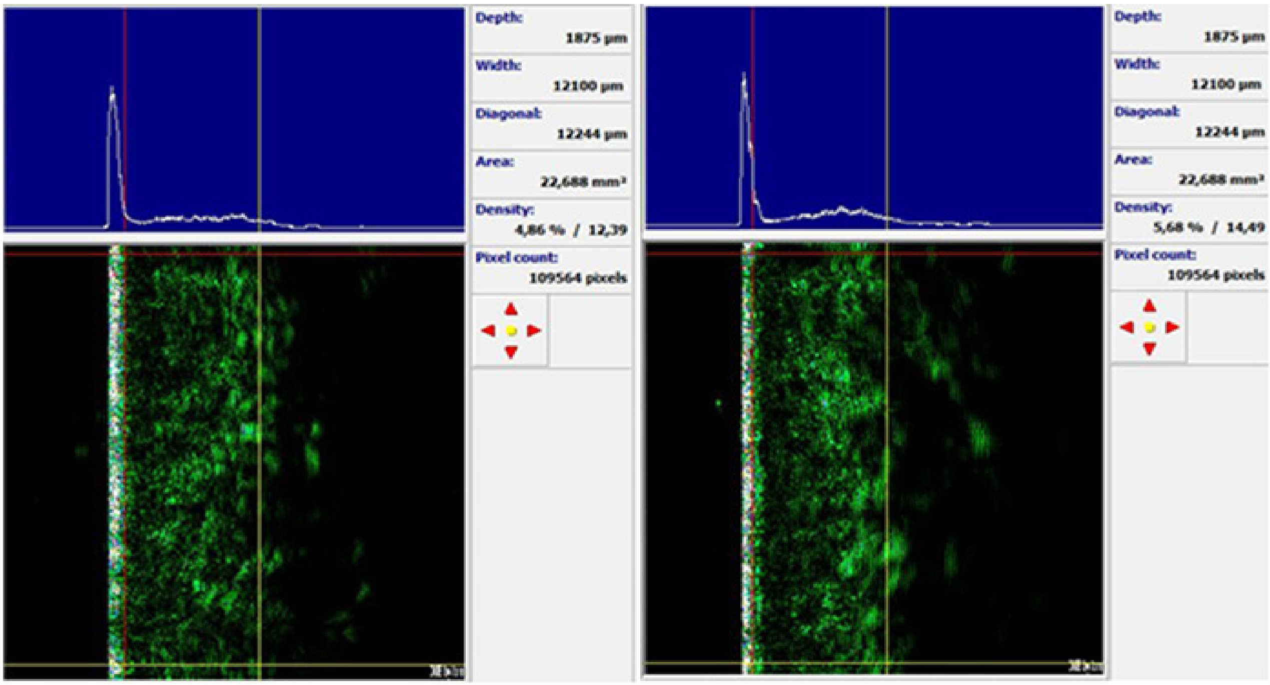


Figure 3. Texture profile plot of the creams: F1 (orange) compared with F2 (black); (a) - consistency, (b) - firmness, (c) - spreadability.

2. In vivo results

Our data shows significant ultrasonographic changes at cutaneous level after 8 weeks of cream application, as visible in Figure 4.



Before After

Figure 4. Ultrasonographic aspect of the skin before and after 8 weeks of cream application.

The obtained results were statistically significant ($p=0.000022$) and revealed an increase of skin density from 9.21% (±1.39) at the beginning of the study to 12.50% (±1.44) at the end of the study. The results were presented as mean±standard error.

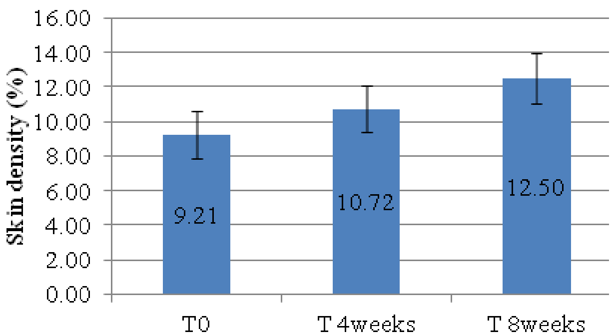


Figure 5. Relative change of the skin density (%) after 4 and 8 weeks(zygomatic area, mean values±SE, N=10).

Discussion

Despite the multitude of alternatives available at present, the interest for anti-age natural products is increasing. The medicinal plants present a great potential in the development of new anti-age products considering different pathways such as antioxidant effects, stimulation of collagen synthesis and improvement of the skin elastic properties. Besides, natural ingredients are well tolerated and the incidence of side effects to herbal extracts is extremely rare [28].

In these formulations, the vegetal extracts were incorporated in an O/W cream base for the strong antioxidant activity and anti-wrinkle effect. Moreover, ferulic acid presents antioxidant and anti-inflammatory properties. Persea gratissima oil was chosen for the emollient effect due to the high content of fatty acids, in order to improve skin barrier function [29]. The other ingredients of the oily phase were chosen for their appropriate characteristics in anti-age formulations: caprylic/capric triglycerides and silicone oil were used for texturizing properties, isopropyl myristate, cetylstearylic alcohol and cetaceum for emollient effect. Glycerol was chosen in the cosmetic formulations as moisturizer to increase hydration level of stratum corneum and Sepigel 305® as thickening and stabilizing agent for its capacity to form gel creams with an excellent sensory profile.

Generally, creams with lower droplet size have higher viscosity values. In our case, F1 which has smaller particle size has higher viscosity values than F2. F1 had also higher consistency and firmness values (Figure 3). These differences in texture parameters between the two formulations are obtained due to the different emulsification system, which confirms the importance of their choice in a cosmetic formulation. Thus, the mixture of glyceryl stearate and Ceteareth-25® from F1 determines higher consistency and firmness values, smaller adhesiveness values, while the spreadability was less influenced, both creams having similar values for this parameter.

F2 presents higher values for particles size, but a narrower particles size distribution, which contribute to the stability of the cream system. The adhesiveness value for F2 is greater than F1, but still being cosmetically acceptable, as appreciated by the volunteers who participated to the study. Based on these measurements we selected F2 for the clinical study.

In these formulations, vegetal extracts were incorporated in the cream base as redensifying agents. Reports in literature have evidenced that local application of *Centella asiatica* oil stimulates the synthesis of type I collagen in fibroblasts and decrease the degradation of collagen, *Spilanthes acmella* oil accelerates the collagen synthesis and also, *Zingiber officinale* extract exerts potent inhibitory activity against fibroblast elastases [12,15,19].

Cutaneous density is an ultrasonographic marker which can quantify the efficacy of anti-age cosmetic formulations. Previous studies revealed that dermal density is an important parameter that evaluates the regeneration process of the skin as a result of the neosynthesis of protein structures which induces an increase of the dermal echogenicity and density [1,30].

According to our results, an increase of the dermal density was observed, therefore the present study suggests that these herbal active ingredients may reduce cutaneous aging process by improving the dermal density. Other similar studies also reported the modification of dermal density during the anti-ageing therapy [30,31].

Conclusions

Two anti-age creams containing *Centella asiatica* oil, *Spilanthes acmella* oil, *Zingiber officinale* extract and ferulic acid were formulated. Formulation with glyceryl stearate and potassium cetyl phosphate is a homogeneous cream, with an appropriate texture. Ultrasonographic evaluation evidenced a positive effect skin density, which increased after 8 weeks of cream application, suggesting that the active ingredients used are efficient as anti-ageing therapy.

Acknowledgement

The authors gratefully acknowledge Sanorama Company, Cluj-Napoca, Romania for allowing the use of the ultrasound equipment and Farmec Company, Cluj-Napoca, Romania for the substances supplied.

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