

Preparation and evaluation of anti-wrinkle cream containing saffron extract and avocado oil

Atefeh Naeimifar PharmD^{1,2}  | Saman Ahmad Nasrollahi PharmD, PhD² |
Aniseh Samadi MD²  | Roya Talari PharmD, PhD³ | Seyed Sajad Ale-nabi PharmD² |
Amir Massoud Hossini PhD⁴ | Alireza Firooz MD²

¹Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

²Center for Research & Training in Skin Diseases & Leprosy, Tehran University of Medical Sciences, Tehran, Iran

³Food and Drug Administration, Ministry of Health, Tehran, Iran

⁴Departments of Dermatology, Venereology, Allergology and Immunology, Dessau Medical Center, Brandenburg Medical School Theodor Fontane, Dessau, Germany

Correspondence

Roya Talari, Food and Drug Administration, Ministry of Health, Fakhr-Razi St., Enghelab Av., Tehran, Iran.
Email: talari_r@yahoo.com

Funding information

Research and Training in Skin Diseases and Leprosy Center, Tehran University of Medical Sciences, Grant/Award Number: 34332-34-01-96

Abstract

Background: Recently, many compounds or extracts from natural sources are used for reducing facial aging and wrinkles.

Objective: This study investigates the preparation and evaluation of the safety and efficacy of saffron extract and avocado oil used for anti-wrinkle topical cream.

Methods and Materials: Oil in water cream was prepared containing avocado oil and the saffron extract. Accelerated stability tests were carried out due to 6 months accordingly through the International Council for Harmonization (ICH) guideline. For clinical studies, topical cream was applied by twenty healthy volunteers daily, for 12 weeks. Improvement in wrinkle grading by a Global Aesthetic Improvement Scale (GIAS), depth and volume of the nasolabial fold, thickness, and density of dermis, and determination of the skin biophysical parameters were evaluated at the baseline, 6th and 12th week.

Result: During the period of storage, the formulation showed no variation in physico-chemical properties. The clinical evaluation showed at least one-grade improvement in GIAS in 30% and 45% of participants, after 6 and 12 weeks, respectively. Subjects were “moderately satisfied” with the treatment. In addition, the area and volume of nasolabial folds were significantly reduced. Moreover, net elasticity (R5) and gross elasticity (R2) showed a significant increase after 12 weeks of treatment. There was no report of allergic reactions, and no changes in skin hydration occurred during the treatment.

Conclusion: The results of this study showed that saffron extract and avocado oil anti-wrinkle topical cream is an efficient and safe treatment for facial skin rejuvenation.

KEYWORDS

anti-wrinkle, avocado oil, saffron extract, topical cream

1 | INTRODUCTION

Nowadays, the pursuit of youth and beauty has become a vital matter for people. Improvement of observable signs of aging such as wrinkles, lack of firmness, skin pores, and spotted pigmentation has caused anxiety and have affected the people's quality of life.¹

Cosmetic products with different mechanisms are used to protect skin against exogenous and endogenous damaging mediators and to improve their beauty and desirability.²⁻⁴ Recently, customers have focused on products utilizing “natural” ingredients such as herbal extracts, specifically. These ingredients have the appeal of appearing wholesome and “organic” which have potential profits to combat some

signs of aging.⁵ They could offer anti-inflammatory, antioxidant, moisturizing, UV-protective, and other antiaging effects.^{6,7}

Avocado oil (*Persea Americana*) is a rich source of oleic, linoleic, and palmitic acids which are excellent ingredients for moisturizing the skin and enhance skin permeability.⁸ In addition to vitamin E, avocado oil contains potassium, lecithin, and many other ingredients that can nourish and detoxify the skin. Moreover, avocado oil contains compounds known as phytosterols which play a major role in rejuvenating stressed skin.^{9,10}

Saffron is the dried stigma of *Crocus sativus* (Saffron crocus) which belongs to the family of iridaceae.¹¹ Saffron stigmas contain numerous volatile compounds and ingredients including crocin, picrocrocin, and safranal which are accountable for orange color, taste, and odor of saffron respectively.^{12,13} In addition to these compounds, saffron contains little amounts of other pigments like anthocyanin, α -carotene, β -carotene, and zexxanthin.¹⁴ Finally, in the petroleum ether extract from the bulbs of saffron, the essential fatty acids (linoleic and linolenic acid), sterols (campesterol, stigmasterol, and β -sitosterol) as well as ursolic, oleanolic, palmitic, palmitoleic, and oleic acids are found.^{15,16}

The majority of studies related to the therapeutic properties of saffron constituents indicate that it has a potent antioxidant activity, which is mostly due to the presence of unique carotenoids.¹⁷ Crocin with the chemical name of crocetin-di-(β -D-digentiobiosyl)-ester contains carotenoid chain which scavenges free radicals; and thereby possesses the effective properties of preventing aging, fighting wrinkles, eliminating dark spot, and acne when used in the formulation of beauty care products.¹⁷

Skin aging can be examined and calculated objectively and noninvasively by a variety of biophysical techniques in response to treatment with topical preparations. In the present study, the anti-wrinkle effect of the topical cream containing avocado oil and saffron extract on a diversity of skin aging endpoints in a 12-week phase II single group, before-after clinical study was prepared and examined.^{18,19}

2 | MATERIAL AND METHODS

2.1 | Materials

Avocado oil, saffron extract, and the honey fragrance were obtained from Barij Essence Company. Sorbitol, vaseline, lanolin, and cetyl alcohol were procured from Sepidaj. Stearic acid and glycerin were supplied by Pas Addak Company. Methyl paraben, Propyl paraben, and triethanolamine (TEA) were purchased from Merck (Germany). Deionized water was freshly prepared, whenever it was required.

2.2 | Preparation of topical cream

Lipid phase was contained of stearic acid, avocado oil, cetyl alcohol, lanolin, vaseline, and propyl paraben an aqueous phase was composed of glycerin, sorbitol 70, saffron extract, triethanolamine, methyl paraben, and deionized water. At first, the aqueous phase was heated up to 80°C, then methyl paraben was added. After solubilization, other

ingredients were added at 70°C. Lipid phase was heated to 70°C. Then, O/W cream was prepared by adding of the lipid phase to the aqueous phase with nonstop stirring at 1000 rpm (IKA, Germany) for 15 minutes. Afterward, the cream was homogenized about 1 minute and was cooled down to room temperature. In the end, the honey fragrance was added. This formulation is kept at room temperature.

2.3 | Stability studies

The fabricated cream kept in accelerated stability studies ($40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$) for 6 months. A physicochemical analysis including color, odor, homogeneity, pH, viscosity, density, and microbial assessments was done on months 0, 1, 2, 3, and 6.¹⁷

2.4 | Study design

In order to evaluate the antiaging effect of a cream containing avocado oil and saffron extract on facial skin, a 12-weeks, single group, before-after clinical study was conducted. This study was performed at Center for Research and Training of Skin Disease and Leprosy, Tehran University of Medical Sciences, Tehran, Iran from March 2016 to July 2016. The study protocol was approved by the Committee of Research Ethics of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1396.3252). In addition, the study was conducted according to the ethical considerations of the Declaration of Helsinki and Good Clinical Practice (GCP).

The method of the study was explained to all volunteers, and then the written informed consent was obtained. The participants were requested to apply one fingertip unit of the study cream on their face once a day for 12 weeks. All the measurements at baseline, 6th and 12th weeks were noninvasive.

2.5 | Inclusion and exclusion criteria

The following conditions were set as inclusion criteria:

1. Good health condition.
2. Wrinkle grade of 2 (moderate) and 3 (advanced) according to Glogau classification of photoaging.
3. Patients that have signed the written consent form and applied the avocado oil and saffron extract anti-wrinkle cream once a day for 12 weeks and regularly followed up.

The exclusion criteria were as follows:

1. Using any systemic or topical anti-wrinkle treatments or any antiaging procedures and injectable fillers during the last year.
2. History of using isotretinoin over the past three months.
3. Interventions (such as deep chemical peeling or laser therapy) over the past 6 months.

4. Presence of cystic or severe acne on the face.
5. History of smoking during the 2 years before the study.
6. Pregnant or lactating women.

2.6 | Assessments

Objective measurements were the evaluation as follows:

1. Depth, area, and volume of right side nasolabial fold by VisioFace (CK Company, Cologne, Germany and its CSI software)
2. Thickness and echo-density of the dermis by DUB Skin Scanner (TPM Company, Luneburg)
3. Skin biophysical parameters (transepidermal, elasticity and water loss (TEWL) and stratum corneum hydration) by MPA-580 Cutometer (CK Company). All determinations were on right side nasolabial at room temperature with 35%-50% relative humidity.

Subjective assessment methods included:

1. Improvement in wrinkle grading by the independent investigator by using GAIS (global aesthetic improvement scale) which has been explained in Table 1.
2. Each volunteer was asked to rate the level of his/her satisfaction from treatment on a five-grade Likert scale (5 = extremely satisfied, 4 = very satisfied, 3 = moderately satisfied, 2 = slightly satisfied, and 1 = dissatisfied).

All adverse events were reported by the subjects either were observed by the investigators and were documented in all treatments and follow-up visits. The main assessed safety parameters included: erythema, pain, discoloration, and infection.

2.7 | Statistical analysis

Statistical analysis was done by IBM SPSS statistics software version 20 (IBM Corp.). Percentage (%) was used for quantitative data average Standard Deviation (SD) and for qualitative data frequency.

In order to make a comparison between baseline and each treatment value, a paired *t* test or Wilcoxon was used; and all data with a *P*-value <0.05 were acceptable.

3 | RESULTS

In this study, a stable cosmetic product with the appropriate analysis method was designed. This topical anti-wrinkle cream passed accelerated stability tests for a period of six months (Table 2). The freshly prepared cream had a white color and homogenous texture. During the period of storage at $40 \pm 2^\circ\text{C}$ / $75 \pm 5\%$ RH, the formulation showed no change in color, odor, pH, and homogeneity (phase separation). The mean pH at initial, 1st, 2nd, 3rd, and 6th month was 6.2, 6.4, 6.7, 6.6, and 6.7, respectively. Viscosity at initial, 1st, 2nd, 3rd and 6th month was 13 456, 14 236, 15 765, 17 235, and 18 148 cp one to one. Finally, the density of fresh cream was 0.992 and 0.9945, 0.9957, 0.991, and 0.993 at month 1, 2, 3, and 6, respectively. A microbial assessment at initial, 1st, 2nd, 3rd, and 6th month was 2, 0, 0, 2, and 0 bacterial colonies, and fungi counts were 1, 0, 0, 0, and 0 colonies, which indicates that antimicrobial activity has been adequate and efficient. All results were listed in Table 2.

Twenty volunteers aged between 30 and 60 years old (mean age, 40.05 ± 8.76 years) of both genders with moderate to advanced wrinkles were included in the clinical section of our study. No serious adverse effects were seen, and there was no report of skin erythema, burning, itching, and flaking during the treatment. The subjective efficacy evaluation showed at least one-grade improvement in GIAS in 30% and 45% of participants, after 6 and 12 weeks treatment, respectively (Figure 1). Also, subjects were "moderately satisfied" at the end of the treatment (Likert scale, 3.2 out of 5).

In objective assessments, the wrinkle area and volume of nasolabial folds were reduced significantly in both follow-up visits (Figure 2A,B). The wrinkle area dropped from 9.50 ± 1.19 (Baseline) to 9.20 ± 1.05 at 6th week (*P*-value = 0.209) and 9.35 ± 1.42 at 12th week (*P*-value = 0.591). The wrinkle volume reduced from 4.68 ± 1.83 (baseline) to 4.40 ± 1.75 at 6th week (*P*-value = 0.022) and 4.33 ± 1.77 at 12th week (*P*-value = 0.023). There was an

GAIS	Global aesthetic improvement scale
Very much improved	Optimal cosmetic result for the implant in this patient.
Much improved	Marked improvement in appearance, but not completely optimal for this patient. A touch-up would slightly improve the result.
Improved	Obvious improvement in appearance from the initial condition, but a touch-up or re-treatment is indicated.
No change	The appearance is essentially the same as the original condition.
Worse	The appearance is worse than the original condition.

TABLE 1 GAIS scale

TABLE 2 Stability evaluation and antimicrobial test result

Test	Period of storage					Standard
	Initial	1st M	2nd M	3rd M	6th M	
Description	Complies	Complies	Complies	Complies	Complies	White and homogenous cream
Odor	Complies	Complies	Complies	Complies	Complies	Characteristic order
pH	6.2	6.4	6.7	6.6	6.7	NLT 5.00 NMT 7.00
Viscosity (cp)	13 456	14 236	15 765	17 235	18 148	NLT 13 000 NMT 18 000
Density (g/cm ³)	0.992	0.9945	0.9957	0.991	0.993	NLT 0.980 NMT 0.999
Microbial enumeration & tester for specific microorganism						
Total bacterial count (CFU/g)	2	0	0	2	0	NMT 100
Total Fungi & Yeast Count (CFU/g)	1	0	0	0	0	NMT 10
<i>P aeruginosa</i>	Neg.	Neg.	Neg.	Neg.	Neg.	Must be negative
<i>S aureus</i>	Neg.	Neg.	Neg.	Neg.	Neg.	Must be negative
Antimicrobial effectiveness test	Ok	Ok	Ok	Ok	Ok	Bacteria: Not less than 2 log reduction from the initial count at 14 d, and no increase from 14 d count at 28 d. Yeast & Mold: No increase from the initial calculated count at 14 and 28 d.
Remark/ Conclusion	Approved	Approved	Approved	Approved	Approved	-

insignificant decrease in wrinkle depth at 6th and 12th weeks of treatments (Figure 2C).

No significant changes in skin ultrasound parameters neither in follow-up sessions were observed (data not shown).

In Figure 3, the skin elasticity measurements are compared between baseline and 6-week and 12-week treatment. A gross elasticity (R2) had a significant increase after 6 and 12 weeks of treatment (P -value = 0.042 and 0.030, respectively). Moreover, net elasticity (R5) showed a relative increase within 6 weeks of treatment and was significant at 12th weeks (P -value = 0.021). There was also a significant improvement in skin firmness (R0) and tiring effect (skin fatigue, R3) at 12th week (P -value = 0.011 and 0.017, respectively).

As it is seen in Figure 4A, TEWL decreased in follow-up visits; however, this reduction was not significant. Finally, no significant variation occurred in skin hydration (Figure 4B).

4 | DISCUSSION

The dermal layer of the skin comprises of densely packed extracellular matrix (ECM) components, such as collagen and elastin proteins, which provide the structural strength and elasticity to skin. Wrinkles occur when skin loses its elasticity, and it is a visible sign of aging. Increasing the production of these ECM components is one of the major targets for cosmetic active ingredients. Keeping the skin supple by moisturizing and nourishing, as well as protecting it from free radical damage will help to reduce wrinkling, and sign of aging.²⁰ Transparency Market

Research reported the global skin care market stood at \$127.1 billion in 2015 and may exceed \$200 billion by 2024 with antiaging creams leading the charge, particularly for wrinkle reduction. Feasibly the most original and still-trendy antiaging ingredients are antioxidants. Many of these are also naturally derived, so they impact the market from two main viewpoints.²¹ The botanical line presents a prodigious potential in the improvement of new antiaging products considering diverse pathways such as stimulation of collagen synthesis, antioxidant properties, and improvement of the skin elasticities.

In order to increase the efficiency of natural cosmetics, combining several herbal components can be possible to surge the efficacy of plant extracts. One routine combination trick in cosmetic products is comprising oils with antioxidant activity.²² In the present study, it was decided to combine the *avocado oil* with *saffron extract* to get anti-wrinkle and antiaging effects on the skin.

In antiaging creams, saffron extracts are used as an antioxidant for the reason of water-soluble carotenoids (crocin and crocetin) components, polyphenols, and antibacterial properties.²³ Natural phyto-antioxidants (eg, polyphenolic compounds and carotenoids) contribute to inhibit or prevent harmful effects of oxidative stress by acting as free radical scavengers or promoters of enzymatic-based anti-oxidation, subsequently delaying the skin aging process and preventing other pathologies.^{24,25} Saffron main antioxidant crocin and its metabolite crocetin are not absorbed throughout the gastrointestinal tract.²⁶ Thus, a topical preparation of saffron as a target delivery system in skin disorders is a practical usage technique.

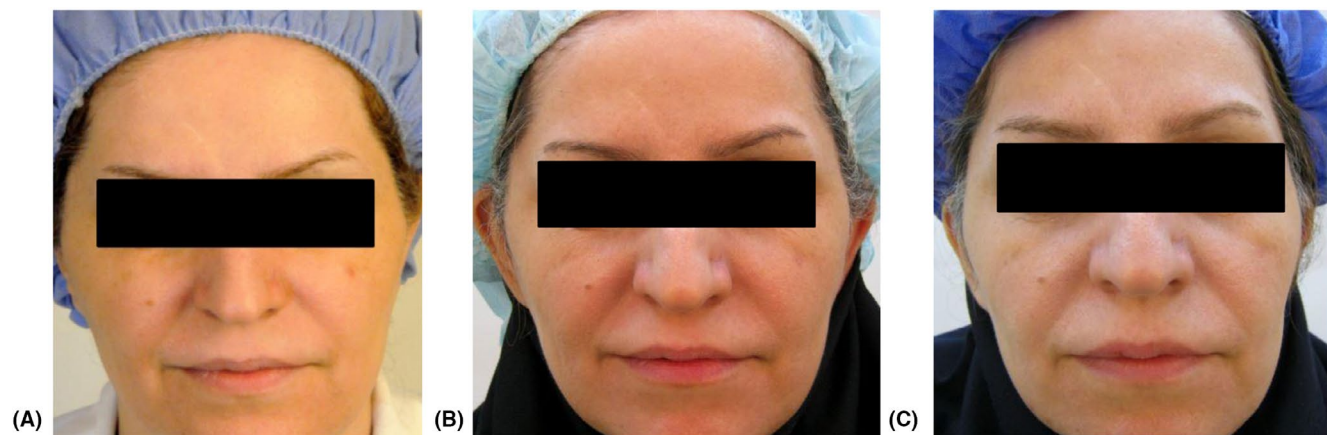


FIGURE 1 Subjective efficacy evaluation of nasolabial fold (A) baseline (B) after 6 wk (C) after 12 wk

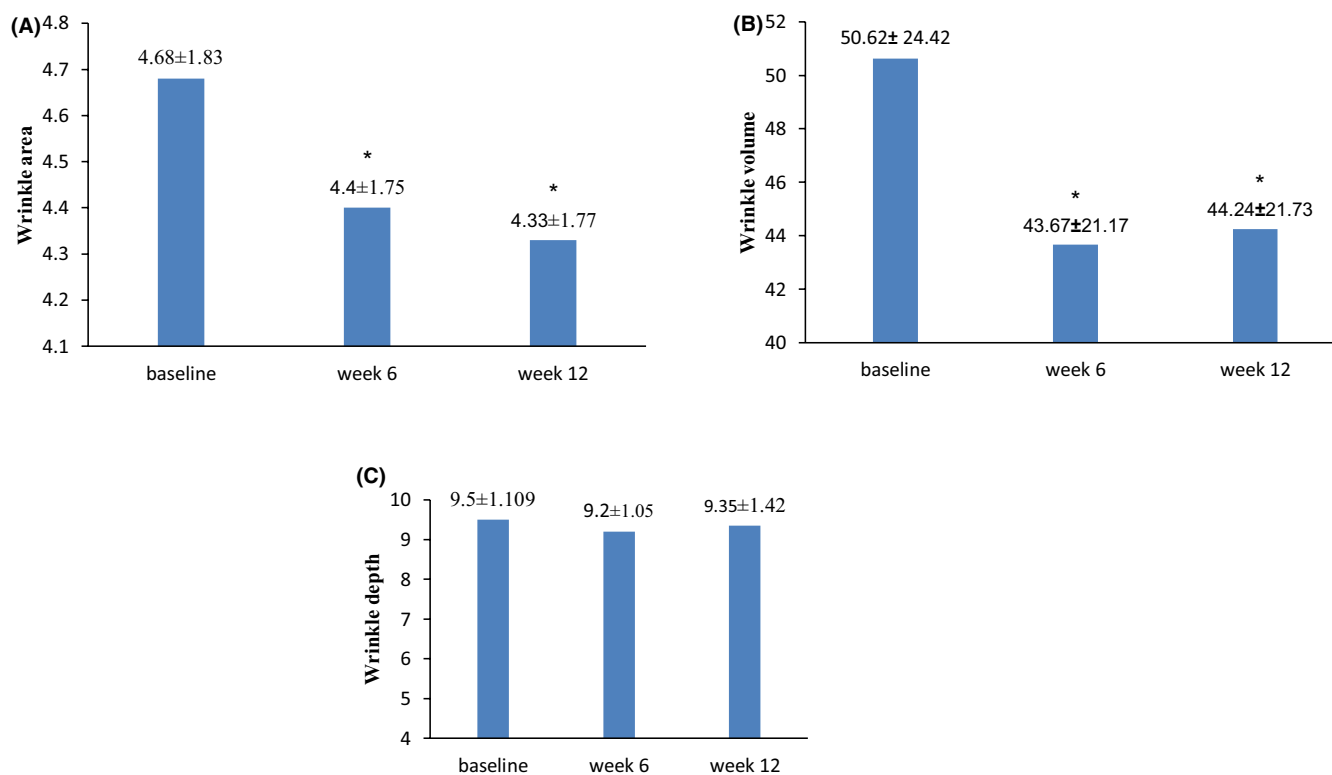


FIGURE 2 (A) The area, (B) volume and (C) depth of nasolabial folds variation in baseline, 6- and 12-week follow-up

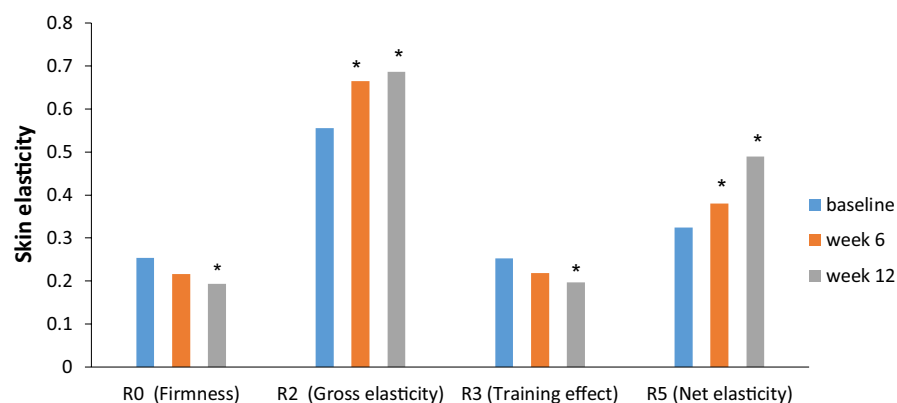


FIGURE 3 Skin elasticity parameters on the nasolabial fold in baseline, 6- and 12-week follow-up

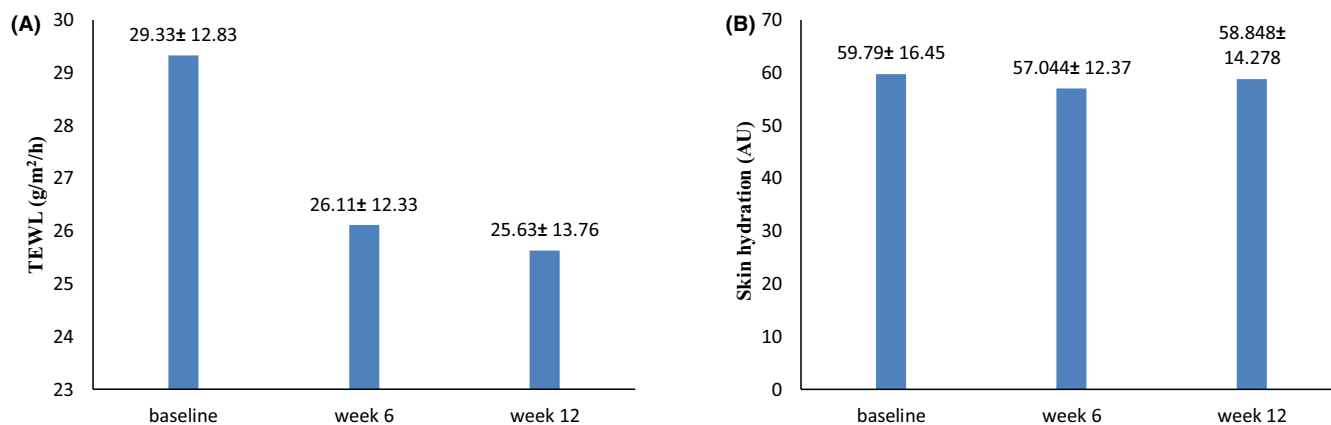


FIGURE 4 (A) TEWL and (B) skin hydration variation in baseline, 6-week, and 12-week follow-up

Natural avocado oil associates a composite of oils and fats that plays a crucial role in restoring the hydro-lipid shielding skin barrier, contributing to vastly softening, nourishing, and moisturizing the skin without leaving any greasy feeling.²⁷ Avocado oil is rich in essential fatty acids (oleic, linoleic acids), phytosterols, polyphenols, triglycerides, proteins, vitamins A, D, and E, having a good geriatric activity on wrinkled skin, therefore reducing fine lines and wrinkle.²⁸

The fabricated topical cream showed proper organoleptic characteristics and stability. Any variation in color, odor or pH, was not seen during accelerated stability tests and it kept the uniformity of its texture. The microbial assessment indicates that the preservative activity of parabens has been adequate and efficient.

All 20 volunteers of both genders, completed the study and informed severe compliance with the given information. Noninvasive skin biophysical measurements were used in the calculation of skin conditions.

After 12 weeks, the ranking image analysis showed that the use of the topical antiaging regimen produced a statistically significant improvement in volume, area of wrinkles when compared with the baseline values. In addition, there was an insignificant reduction in the depth of nasolabial folds at both follow-up visits, which could have been significant if we had chosen larger sample size. This improvement in wrinkles belongs to carotenoids present in saffron extract and polyphenols in both saffron and avocado. By using these ingredients, UV-generated free radicals, repaired laminin, elastic fiber, and collagen network, stimulated collagen synthesis and inhibition of the collagen degradation were significantly reduced.²²

It was observed that the skin elasticity was improved over the study period. Gross elasticity (R2) is stated to as cross-elasticity of skin containing the viscous deformation and is characterized by the proportion of the ability of redeformation of skin to final expansion.^{29,30} Net elasticity (R5) without viscous deformation elastic recovery is represented by the ratio of immediate retraction to immediate distension.^{29,31}

According to the objective results, two major parameters of skin elasticity (R2 and R5) showed significant increase 3 months after treatment. R2 and R5 have been proven to best demonstration of skin mechanical properties at all ages.³² Finally, there was also a

significant improvement in skin firmness (R0) and tiring effect (skin fatigue, R3) at 12th week (Figure 3).

Encouraging obtained results might also be explained by polyphenolic-mediated enzymatic inhibition. Indeed, the enzymes that destroy the skin collagen (eg, collagenases) and induce skin sagging (eg, elastases) can be inhibited by polyphenols present in the saffron extract and avocado oil subsequently improving skin elasticity.³³

With age, impairment of the structural integrity of dermis and fibroblast function and loss of tonus and elasticity lead to increased folding, sagging, and emergence of wrinkles.^{34,35} Those features might partially result from a depletion of vitamin A, a key component of the epidermis, due to chronic exposure to UV, oxidative stress, and nutritional deficiency.³⁵ Avocado oil is rich in vitamin A that can stimulate the production of collagen and elastin fibers and increase the skin's tonicity and elasticity.

Relative improvement of skin appearance was noticeable after 12 weeks of application of the cream as is shown by a decrease in the average TEWL. Avocado oil feeds and rejuvenates the skin due to the fatty acids such as oleic and linoleic acid, omega fatty acids, triglycerides, and phytosterol, which are broadly used in the cosmetic industry as moisturizing agents. Previous studies demonstrated that vegetable oils such as avocado contain unsaturated long chains and are able to contribute improving epidermal barrier repair.²⁸ Also, Akhtar et al in 2014 showed that saffron extract could reduce TEWL and protect the skin hydration.³⁶

In this clinical trial, no change in hydration index was observed. This could be due Tehran's climate which is hot and dry in the summer time. The high UV index (more than 7), raised temperature (up to 40°C) and presence of ozone in the atmosphere in June and July may impact skin hydration.

The physicians and patients assessed the improvement of the skin aging compared with the baseline, as a subjective evaluation. A before-after comparison performed by a dermatologist using the GAIS to judge the wrinkle grading showed improvement in skin aging during the period of treatment. In addition, by the Likert scale subjects were "moderately satisfied" at the end of the treatment. In fact, both subjective assessments confirmed objective and instrumental evaluations.

No serious adverse effects were seen, and there was no report of skin erythema, burning, itching, and flaking during the treatment.

The main limitation of the study was the small number of participants, yet the results showed a significant improvement. Absence of control group and short-term follow-up, are other limitation which would probably affect the reliability of the data.

5 | CONCLUSION

The goal of this study was preparation, evaluation and in vivo evaluation of the skin irritation potential and the efficiency of anti-wrinkle cosmetic cream, with integrated saffron extract and avocado oil. The composition of extracts and base of cream are stable up to 6 months in accelerated stability studies ($40 \pm 2^\circ\text{C}$ / $75 \pm 5\%$ RH). Daily use of this cream was safe, and it is efficient for facial skin rejuvenation by decreasing the signs of skin aging, improvement of skin elasticity and reducing the TEWL.

ACKNOWLEDGMENTS

This study was supported by research grant number 34332-34-01-96 from Research and Training in Skin Diseases and Leprosy Center, Tehran University of Medical Sciences. The technical help of Mrs Fatemeh Amiri and Mr Vahid Mahmoudi in preparation and analytical evaluation of the final cream is appreciated.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ORCID

Atefeh Naeimifar  <https://orcid.org/0000-0002-3958-4918>

Aniseh Samadi  <https://orcid.org/0000-0002-0134-429X>

REFERENCES

- Ainbinder D, Touitou E. H&PC Today - Household and Personal Care Today. 2013;8:2.
- Kaur IP, Kapila M, Agrawal R. Role of novel delivery systems in developing topical antioxidants as therapeutics to combat photoaging. *Ageing Res Rev*. 2007;6:271-288.
- Watson OS, Cotterell LF, Bowden JJ, Bastrilles JY, Long SP, Griffiths CE. A cosmetic 'antiaging' product improves photoaged skin, a double-blind, randomized controlled trial British. *J Dermatol*. 2009;161:419-426.
- Saraf S, Kaur CD. Phytoconstituents as photoprotective novel cosmetic formulations. *Pharmacogn Rev*. 2010;4(7):1-11.
- Nachbar F, Korting HC. The role of vitamin E in normal and damaged skin. *J Mol Med*. 1995;73:7-17.
- Juncan AM, Lung C. Formulation and optimizing of an anti-aging cosmetic. *Studia UBB Physica*. 2016;61(2):101-110.
- Barel A, Paye M, Maibach HI. *Handbook of Cosmetic Science and Technology*. New York: Marcel Dekker; 2001. chapter 46.
- Requejo AM, Ortega RM, Robles F, Navia B, Faci M, Aparicio A. Influence of nutrition on cognitive function in a group of elderly, independently living people. *Eur J Clin Nutr*. 2003;57:54-57.
- Lonseca P, Duarte M, Ahes C, Carolina BD, Madonca RB. Avocado: characteristics, health benefits and uses. *Food Technol Clenca Royal Santa Maria*. 2016;46(4):747-754.
- Schaffer B, Wolestenholme BN, Wiley AW (Eds.). *The Avocado: Botany, Production and Uses* (2nd edn). Croydon, UK: CPI Group (UK) Ltd; 2012.
- Kamalipour M, Akhondzadeh S. Cardiovascular effects of saffron: an evidence-based review. *J Tehran Heart Cent*. 2011;6(2):59-61.
- Melnyk JP, Wang S, Marcone MF. Chemical and biological properties of the world's most expensive spice: Saffron. *Food Res Int*. 2010;43(8):1981-1989.
- Samarghandian S, Borji A. Anticarcinogenic effect of saffron (*Crocus sativus* L.) and its ingredients. *Pharmacog Res*. 2014;6(2):99-107.
- Lage M, Cantrell C. Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. *Sci Hortic*. 2009;121(3):366-373.
- Banker GS, Rhodes CT. Modern Pharmaceuticals. 2001;299-301.
- Frank A. Purpura resulting from artificial abortion. *Deut Med Wochschr*. 1961;86:1618-1620.
- Giaccio M. Components and features of saffron. Proceedings of the International Conference on Saffron, L'Aquila, Italy. 1990;135-148.
- Barel A, Paye M, Maibach HI. *Handbook of Cosmetic Science and Technology*. New York: Marcel Dekker. 2001; chapter 46.
- Berardesca E, Farinelli N, Rabbiosi G, Maibach AND, HI. Skin bio-engineering in the noninvasive assessment of cutaneous aging. *Dermatologica*. 1991;182(1):1-6.
- Menaa F, Badole SL, Menaa B, Menaa A. Promising plant extracts with in vivo anti-melanoma potential. *Bioactive Dietary Factors Plant Extracts Dermatol*. 2013; 283-290, chapter 26. https://doi.org/10.1007/978-1-62703-167-7_26
- <https://www.transparencymarketresearch.com/pressrelease/cosmetic-skin-care-market.htm>
- Boone P. H&PC Today - Household and Personal Care Today. 2011;6:1.
- Asdaq SM, Inamdar MN. *Crocus sativus* (saffron) and its constituent, crocin, as hypolipidemic and antioxidant in rats. *Appl Biochem Biotechnol*. 2010;162(2):358-372.
- Ali Khan B, Akhtar N, Menaa A, Menaa F. A Novel Cassia fistula (L.)-Based Emulsion Elicits Skin Anti-Aging Benefits in Humans. *Cosmetics*. 2015;2(4):368-383. <https://doi.org/10.3390/cosmetics2040368>
- Godevac D, Tesevic V, Velickovic M, Vujisic L, Vajs V, Milosavljevic S. Polyphenolic compounds in seeds from some grape cultivars grown in Serbia. *J Serb Chem Soc*. 2010;75:1641-1652.
- Rezaee Khorasany A, Hosseinzadeh H. Therapeutic effects of saffron (*Crocus sativus* L.) in digestive disorders. A review. *Iran J Basic Med Sci*. 2016;19(5):455-469.
- Lonseca P, Duarte M, Ahes C, Carolina BD, Madonca RB. Avocado: Characteristics, health benefits and uses. *Food Technology Clenca Royal Santa Maria*. 2016;46(4):747-754.
- Soares SE, Mancini Filho J, Della Modesta RC. Sensory detection limits of avocado oil in mixtures with olive oil. *Rev Española Ciencia Tecnol Alimentos*. 1992;32(5):509-516.
- Ryu HS, Joo YH, Kim SO, Park KC, Youn SW. Nutraceuticals and the Skin: Roles in Health and Disease. *Skin Res Technol*. 2008;44:354.
- Murray BC, Wickett RR. Surfactants in Personal Care Products and Decorative Cosmetics. *Skin Res Technol*. 1997;3:101.
- Dobrev H. Use of Cutometer to assess epidermal hydration. *Skin Res Technol*. 2000;6:239.
- Ahn S, Kim S, Lee H, Moon S, Chang I. Correlation between a Cutometer and quantitative evaluation using Moire topography in age-related skin elasticity. *Skin Res Technol*. 2007;13:280.
- Ali A, Akhtar N, Khan H. Enhancement of human cheek skin texture by acacia nilotica bark extract cream. *Trop J Pharm Res*. 2014;12:323-327.
- Fisher GJ, Varani J, Voorhees JJ. Looking older: fibroblast collapse and therapeutic implications. *Arch Dermatol*. 2008;144:666-672.

35. Sorg O, Saurat JH. Topical retinoids in skin aging: a focused update with reference to sun-induced epidermal vitamin A deficiency. *Dermatology*. 2014;228:314-325.
36. Akhtar N, Khan HM, Ashraf S. Moisturizing effect of stable cream containing *Crocus sativus* extracts. *Pak J Pharm Sci*. 2014;27(6):1881-1884.

How to cite this article: Naeimifar A, Ahmad Nasrollahi S, Samadi A, et al. Preparation and evaluation of anti-wrinkle cream containing saffron extract and avocado oil. *J Cosmet Dermatol*. 2020;19:2366–2373. <https://doi.org/10.1111/jocd.13284>