Original article

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EFFECT OF APPLICATION OF EMULSIONS WITH STANDARDIZED EXTRACT OF WILD APPLE FRUIT (*MALUS SYLVESTRIS* (L.) MILL., ROSACEAE) ON BIOPHYSICAL SKIN PARAMETERS: AN *IN VIVO* STUDY

Ana Kolarević[1], Dragana Stojiljković[2], Sandra Dinić[1], Ivana Nešić[1]

Wild apple fruit (*Malus sylvestris* (L.) Mill., Rosaceae) represents a valuable source of biologically active compounds, such as polyphenols and fruit acids. These compounds have been found to have a positive effect on human health and also have a beneficial effect on the skin by improving its overall condition and appearance. In the present study, the efficacy of the application of oil-in-water emulsions containing 12% and 15% of wild apple fruit extract was examined on healthy volunteers. During a 28-day long-term study, biophysical skin parameters (electrical capacitance (EC), transepidermal water loss (TEWL), pH, erythema index (EI), and melanin index (MI)) were monitored. As a result, a significant increase in EC and decrease in EI and MI parameters were observed after both 14 and 28 days of application, where the emulsion containing 15% of the wild apple fruit extract was more efficient than the emulsion containing 12% of the wild apple fruit extract. On the other hand, no significant changes in TEWL and pH values were observed. Given their beneficial effects on the skin (increased skin hydration, reduced skin irritation, good skin lightening potential), the tested emulsions might have potential application in the formulation of cosmetic products for the treatment of dry and irritated skin, as well as products intended to reduce skin hyperpigmentation.

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Key words: *Malus sylvestris, extract, emulsion, biophysical skin parameters, in vivo study*

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Introduction

Wild apple (*Malus sylvestris* (L.) Mill., Rosaceae) is a shrub up to 4 m high or a deciduous tree that grows to a height of 10 m, creating a branched, wide, irregular and dense canopy. It is widespread in Europe. It grows throughout Serbia along forests, on the border between lower and hilly regions. The healing part of the wild apple is the fruit. The fruit is globose, yellow to red, 6-8 cm in diameter in most wild species. It ripens in late summer and in the fall when harvested (1, 2). Wild apple was mainly used as an decorative plant because of its ornamental features, such as the color of leaves and flowers. The fruit is edible, though extremely acidic and bitter. Due to the significant amount of pectin (~3%), wild apples are used for gelling other fruit products, making jams, fruit juices, wine, brandy or syrups (3). Wild apple fruit is also used to make apple cider vinegar, which, in various combinations, usually with water and honey, is a multifaceted drug and a restorative agent for the human body (1).

Wild apple fruit, primarily red varieties, is a valuable source of biologically active compounds, mostly concentrated in the bark of the fruit. In addition to affecting the nutritional values, appearance, taste and texture of the fruit itself, these compounds have a positive effect on human health (4). Polyphenols are considered to be the main bioactive compounds present in wild apple fruit, including flavan-3-ols (30-90%), flavonols (1-13%), dihydrochalcones (1-10%), anthocyanidins (1-7%) and phenolic acids (Figure 1) (4-6). Flavonoids exhibit a wide range of biological activities, with their antioxidant and anti-inflammatory effects being most pronounced (7-10). Further, wild apple fruit contains α-hydroxy acids, such as malic, citric, succinic, tartaric, pyruvic and lactic acid (Figure 2). In addition to affecting the taste of the apple itself, fruit acids exert

numerous positive effects on the skin (accelerate skin desquamation, hydrate the skin, regulate skin pH, lighten hyperpigmented parts of the skin), thereby improving its overall condition and appearance (11-14).

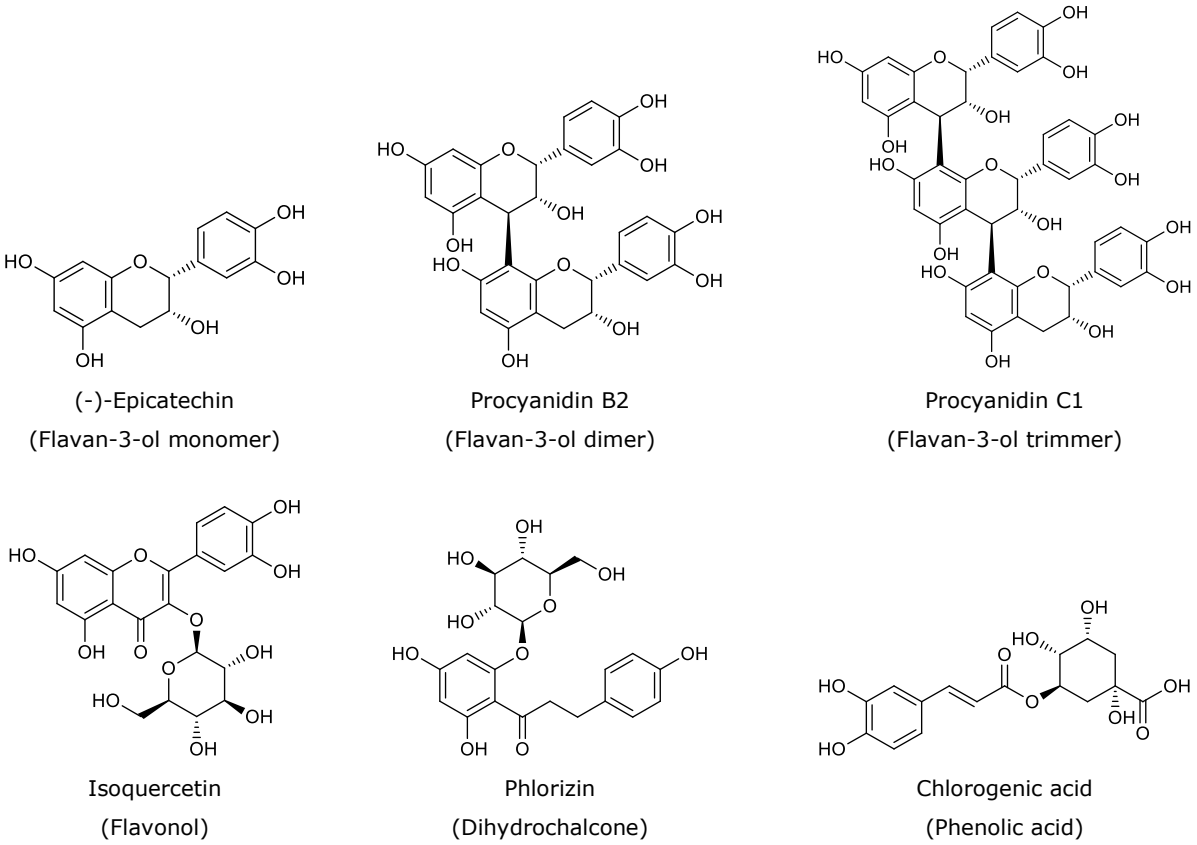


Figure 1. The most common polyphenols in wild apple fruit

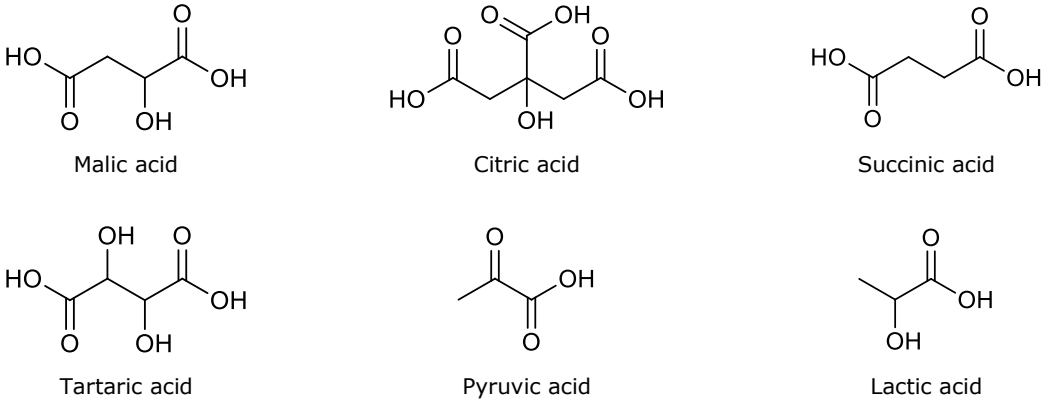


Figure 2. The most common fruit acids in wild apple fruit

Wild apple is also rich in minerals (potassium, calcium, magnesium, iron, phosphorus, zinc), vitamins (A, C and B), plant fibers (cellulose) and sugars (fructose, glucose, galactose and sucrose). Pectin, present in wild apple fruit in large quantities, cleanses the body of heavy metals, prevents the absorption of cholesterol in the small intestine and slows down the absorption of sugar (1).

Extracts rich in bioactive polyphenolic compounds, as well as fruit acids, are often used in the production of phytopreparations for both oral and skin applications. Water as an extraction agent has many advantages over organic extractants, primarily for its safety of application and economy. In addition to polyphenols, aqueous extracts can also contain a large amount of other water-soluble ingredients that can also contribute to better skin appearance (15-17).

Aim of the study

The aim of the study presented in the paper was to evaluate the efficacy of the application of emulsions with aqueous wild apple fruit extract (EWAFE) in an *in vivo* 28-day study on healthy volunteers by monitoring the following biophysical skin parameters:

- ☐ electrical capacitance (EC) - in order to evaluate EWAFE effect on skin humidity;
- ☐ transepidermal water loss (TEWL) - in order to evaluate EWAFE effect on skin barrier function;
- ☐ erythema index (EI) and skin pH - in order to evaluate EWAFE effect on skin irritation and damage;
- ☐ melanin index (MI) - in order to evaluate EWAFE effect on skin color and its lightening.

Materials and methods

Preparation of wild apple fruit extract

The dried and pulverized wild apple fruit was soaked in purified water (as the extractant) in a conical flask and left in an ultrasonic bath for 30 minutes at room temperature. Ultrasonic extraction produced the extract in a drug:extract ratio of 1:5, followed by filtration.

Preparation of O/W emulsions with wild apple fruit extract

The components listed in Table 1 were used to make O/W emulsions containing 12% and 15% of wild apple fruit extract (EWAFE-12 and EWAFE-15, respectively). The fat phase components (coco glucoside and cetearyl alcohol (Montanov™ 82), myristyl alcohol and myristyl glucoside (Montanov™ 14), isopropyl myristate, caprylic-capric triglycerides (Myritol™ 318) and cetearyl alcohol (Lanette 0)) and the aqueous phase components (glycerol, sodium benzoate and purified water) were heated to a temperature of 70 ^{O}C and 72 ^{O}C, respectively. The fat phase was then added to the aqueous phase with stirring (propeller laboratory mixer), first at a speed of 800 rpm for 5 min and then at a speed of 500 rpm for 3 minutes. At the end of the emulsification phase, stirring was continued at a speed of 300 rpm to a temperature of about 40 ^{O}C when wild apple fruit extract was added, followed by cooling to room temperature. The corresponding placebo sample was prepared in the same manner as the test samples, but without wild apple fruit extract (Table 1).

Table 1. Qualitative and quantitative composition of the tested emulsions

Component (INCI name)	Role	EWAFE-12 (g)	EWAFE-15 (g)	Placebo (g)
Coco glucoside and cetearyl alcohol	Emulsifier	7.0	7.0	7.0
Myristyl alcohol and myristyl glucoside	Emulsifier	1.5	1.5	1.5
Isopropyl myristate	Emollient	7.0	7.0	7.0
Caprylic-capric triglycerides	Emollient	7.5	7.5	7.5
Cetearyl alcohol	Emulsifier	1.5	1.5	1.5
Wild apple fruit extract	Cosmetically active compound	12.0	15.0	-
Glycerol	Humectant	2.0	2.0	2.0
Sodium benzoate	Preservative	0.5	0.5	0.5
Purified water	Aqueous phase	61.0	58.0	73.0

In vivo characterization of emulsions tested - biophysical skin parameters determination

In vivo study has been approved by the Ethics Committee of the Faculty of Medicine in Niš, Serbia, No. 12-12123-3, and has been conducted according to previous publications (18-21). The study included 20 healthy volunteers, both sexes, with an average age of 24.1 years, with moderately dry skin, and no history or clinical signs of dermatological disease. The study was conducted in the period January-February 2019. The examinees were instructed not to use any skin care products one week before and throughout the study, but were allowed to wash normally. Volunteers did not take baths, showers or exercise, at least three hours prior to the measurement, and were physically and mentally relaxed.

The measurements of the biophysical skin parameters were carried out in rooms with constant temperature (20-23 ^{O}C) and relative humidity (40-60%), 30 minutes after the acclimatization of the participants. The measuring region (volar parts of the forearmes) was generally hairless or with very little hair among male subjects. The measuring probe was lightly pressed against the skin and placed vertically, and carefully cleaned after each measurement. *In vivo* measurements were performed by only one person using the Multi probe adapter 9 device, Courage & Khazaka Electronic GmbH, Germany, and using the appropriate probes: Corneometer® CM 825 for EC, Tewameter® TM 210 for TEWL, Mexameter® MX 16 for MI and EI, and pH meter® 900 for skin pH measurements.

After the initial measurement of the above mentioned biophysical skin parameters (before the start of the study, basal values), volunteers were instructed to apply samples (EWAFE-12, EWAFE-15 and placebo) at home twice daily, in the morning and evening, after showering, to the skin of the volar side of the forearm for four weeks. Measurements were taken after 14 and 28 days. One part of the volar side of the forearm was left untreated.

Statistical analysis

The results obtained were analyzed by one-factor analysis of variance, followed by Tukey's test, with a statistical significance of $p < 0.05$. Changes in measured parameters at specific time points were checked using the Student t-test.

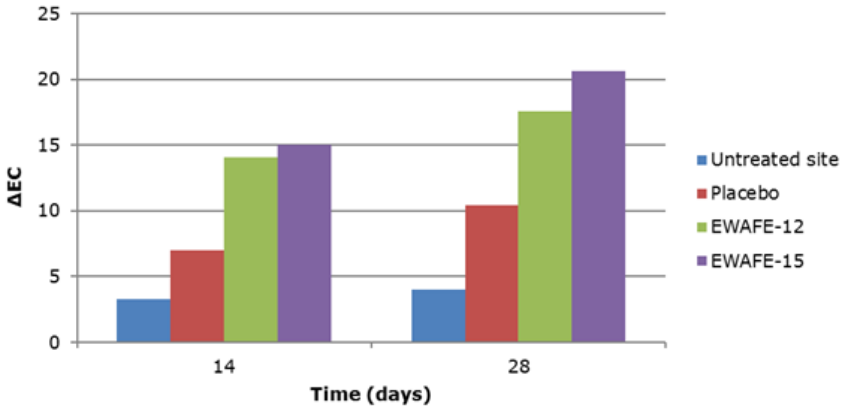
Results and discussion

In vivo efficacy of EWAFE application was examined by measuring the biophysical skin parameters (EC, TEWL, pH, EI, and MI) on healthy volunteers during long-term 28-day study. The results are shown in Graph 1-5.

In vivo testing the effect of EWAFE on EC

According to the results obtained in the present study, the tested emulsions (EWAFE-12 and EWAFE-15) showed, after both 14 and 28 days of application, a statistically significant ($p < 0.05$) increase in EC parameter, that is, skin hydration, compared to the basal values. In addition, both emulsions (EWAFE-12 and EWAFE-15) showed a better moisturizing effect compared to the placebo sample and the untreated site (Figure 3). That is most likely due to the presence of moisturizing compounds in the wild apple fruit extract, such as polyphenolic compounds and fruit acids. The positive effect of emulsions with moisturizing active compounds on the skin humidity has already been shown (18, 22-24). After 14 days of application, both emulsions showed almost the same effect on EC parameter ($\Delta EC_{EWAFE-15}$ = 15.03 ± 7.03, $\Delta EC_{EWAFE-12}$ = 14.1 ± 6.49), while after 28 days EWAFE-15 showed better effect on skin humidity than EWAFE-12 (ΔEC = 20.6 ± 10.51 and 17.6 ± 8.07, respectively) (Graph 1).

The good effect on the skin humidity observed after administration of the placebo sample compared to the untreated site was probably due to the presence of different emollients in the basic formulation (Table 1).



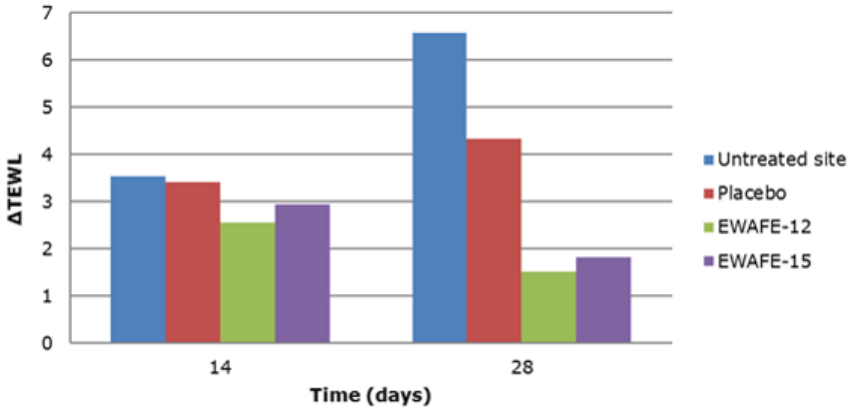
Graph 1. Effect of the tested samples on EC after 14 and 28 days of application

In vivo testing the effect of EWAFE on TEWL

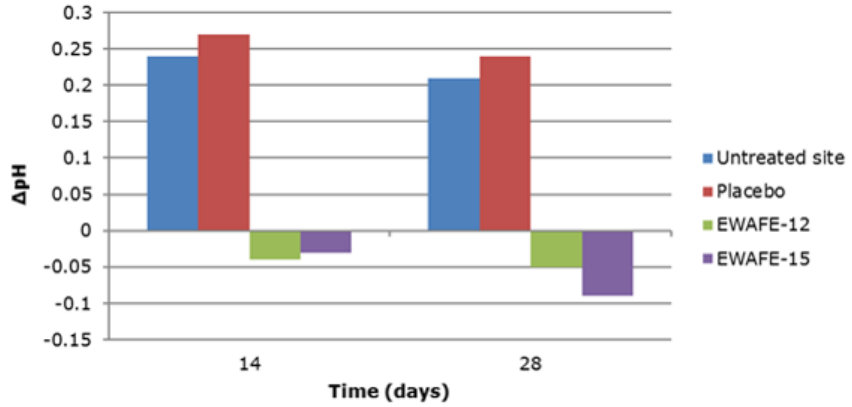
Measurement and monitoring of the TEWL parameter is often used to evaluate changes that occur in the barrier function of the skin after application of the tested samples (19). The obtained results showed that at 14 and 28 days after application of the tested samples, there was no significant increase in TEWL parameter compared to the basal values (Graph 2). Almost unchanged values of the TEWL parameter during the test period indicate that the skin barrier function was not significantly affected by the emulsions tested.

In vivo testing the effect of EWAFE on skin pH

The results obtained by measuring the skin pH of healthy subjects before the application of the tested emulsions, as well as 14 and 28 days after their application, showed that there was no statistically significant change in skin pH (Graph 3). However, a slightly greater decrease in skin pH was observed after EWAFE-15 application than after EWAFE-12 application, which is probably due to the presence of a higher concentration of active ingredients (fruit acids and polyphenolic compounds) from wild apple fruit extract.



Graph 2. Effect of the tested samples on TEWL after 14 and 28 days of application



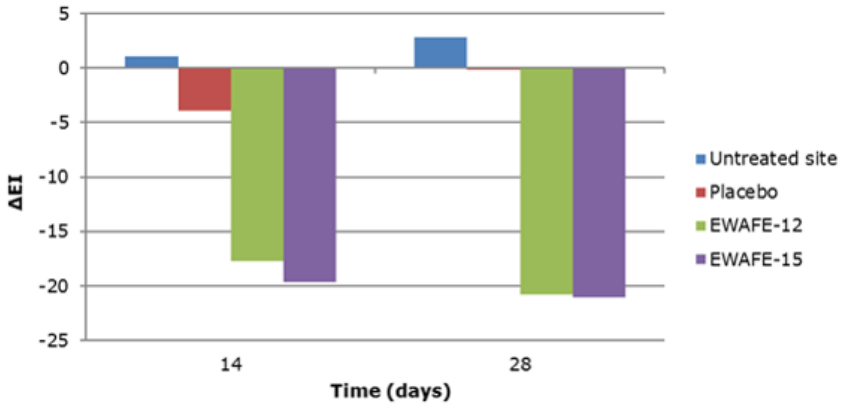
Graph 3. Effect of the tested samples on skin pH after 14 and 28 days of application

In vivo testing the effect of EWAFE on EI

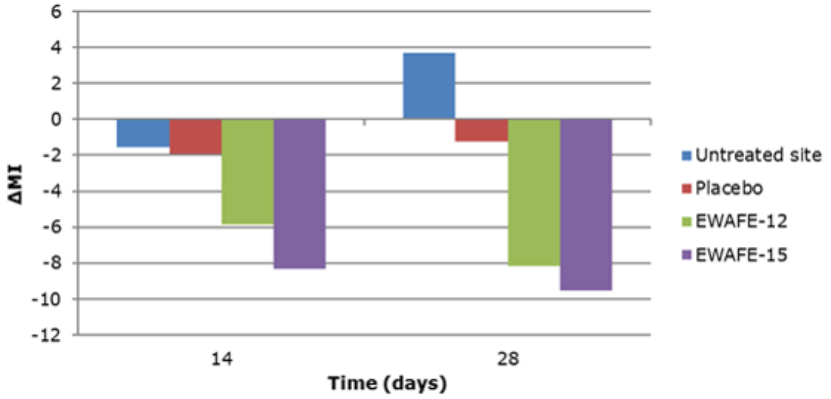
Application of the tested emulsions (EWAFE-12 and EWAFE-15) over a 28-day period showed a statistically significant ($p < 0.05$) decrease in the EI parameter compared to the corresponding controls (untreated site and placebo sample) (Graph 4). Considering that skin irritation is generally accompanied by an increase in the EI parameter, it can be concluded that EWAFEs do not exhibit an irritant effect after prolonged application to the skin. The results showed that slightly better effect was achieved after 28 days than after 14 days of EWAFE application, with EWAFE-15 being slightly more efficient than EWAFE-12 (Graph 4).

In vivo testing the effect of EWAFE on MI

The obtained results indicate the tendency of the tested emulsions to lighten the skin. Namely, at all test sites (untreated site, placebo, and EWAFE sites) there was a decrease in the MI parameter after 14 days of testing. The tested emulsions, including the placebo sample, showed a decrease in the MI parameter also after 28 days of testing, while a slight increase in the MI parameter was observed at the untreated site (Graph 5). During the entire test period, both EWAFE-12 and EWAFE-15 showed a statistically significant decrease in MI parameter ($p < 0.05$), i.e. a slightly better skin lightening effect compared to both controls (untreated site and placebo sample). A slightly better effect was observed after 28 days than after 14 days of the EWAFE application. Additionally, a better effect was observed with the EWAFE-15 than with the EWAFE-12 (Graph 5).



Graph 4. Effect of the tested samples on EI after 14 and 28 days of application



Graph 5. Effect of the tested samples on MI after 14 and 28 days of application

Conclusion

The efficacy of the application of the emulsions containing wild apple fruit extract at concentrations of 12% and 15% was tested *in vivo* on healthy volunteers during 14 and 28 days of application. During the application period, the tested emulsions (EWAFE-12 and EWAFE-15) were shown to lead to increased EC and decreased EI and MI parameters, indicating their positive effect on skin humidity, anti-irritant effect and skin lightening potential, respectively. The best effect on EC, EI and MI parameters was observed after 28 days of EWAFE-15 application. Additionally, the tested emulsions did not significantly affect skin pH and TEWL parameter, i.e. skin barrier function.

The obtained results indicate the potential application of wild apple fruit extract in the formulation of cosmetic products intended for skin hydration, as well as products intended to sanctification of hyperpigmented skin.

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UTICAJ PRIMENE EMULZIJA SA STANDARDIZOVANIM EKSTRAKTOM PLODA DIVLJE JABUKE (*MALUS SYLVESTRIS* (L.) MILL., ROSACEAE) NA BIOFIZIČKE PARAMETRE KOŽE: *IN VIVO* STUDIJA

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Plod divlje jabuke (*Malus sylvestris* (L.) Mill., Rosaceae) predstavlja dragoceni izvor biološki aktivnih jedinjenja, poput polifenola i voćnih kiselina. Utvrđeno je da ova jedinjenja imaju pozitivan uticaj na ljudsko zdravlje, a deluju blagotvorno i na kožu, poboljšavajući njeno opšte stanje i njen izgled. U ovoj studiji, ispitivana je efikasnost primene emulzija „ulje-u-vodi", koje sadrže 12%, odnosno 15% ekstrakta ploda divlje jabuke, na zdravim dobrovoljcima. Tokom dugoročne dvadesetosmodnevne studije, praćeni su biofizički parametri kože (električna kapacitivnost (EK), transepidermalni gubitak vode (TEGV), pH, eritema indeks (EI) i melanin indeks (MI)). Kao rezultat, zabeleženo je značajno povećanje EK i smanjenje EI i MI parametara, kako nakon 14, tako i nakon 28 dana primene, pri čemu je emulzija sa 15% ekstrakta ploda divlje jabuke bila efikasnija od emulzije sa 12% ekstrakta ploda divlje jabuke. Sa druge strane, nisu primećene značajne promene TEGV i pH vrednosti. S obzirom na njihovo blagotvorno dejstvo na kožu (povećana hidratacija kože, smanjena iritacija kože, dobar potencijal posvetljivanja kože), ispitivane emulzije mogu imati potencijalnu primenu u formulaciji kozmetičkih proizvoda namenjenih tretmanu suve i iritirane kože, kao i proizvoda namenjenih posvetljivanju hiperpigmentisane kože.

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Ključne reči: *Malus sylvestris, ekstrakt, emulzija, biofizički parametri kože, in vivo studija*

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