In vivo study of safety and efficacy of emulgels with plum seed oil

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Natural plant oils are commonly used in skincare products for their emollient properties. Plum seed oil is a promising cosmetic ingredient since it represents a sustainable material derived from fruit processing waste, recognized in the CosIng Database for its skin-conditioning function. The aim of this study was to incorporate plum seed oil into a topical emulgel and assess its biophysical effects on the skin. An in vivo study on healthy volunteers evaluated the safety (irritation potential) and long-term efficacy (impact on biophysical skin parameters during 28-day application) of emulgel containing 6% (w/w) plum seed oil as a natural emollient and active ingredient. Results showed that plum seed oil exhibited no irritation potential during a 24-hour safety assessment since transepidermal water loss (TEWL) decreased ($p<0.05$), and erythema index didn't change significantly. Results of 28-day efficacy study suggested, potential anti-irritant effects after long-term usage. In addition, pH, skin integrity and skin color were preserved during the study, while skin hydration was increased. To our knowledge, this is the first study to employ non-invasive biophysical methods to assess the influence of plum seed oil in cosmetic formulations. These findings point plum seed oil's potential as a natural, sustainable, safe and effective raw material for cosmetics.

Keywords: plum seed oil; emulgel; in vivo study; efficacy; safety

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1. INTRODUCTION

Natural plant oils are widely used in skin care as raw materials with emollient properties. Since they are composed of different amount of biologically active components such as monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and saturated fatty acids (SFAs), they can contribute to the hydration and soothing of the skin. Additionally, plant oils can integrate with the *stratum corneum*—the skin's outermost layer—and aid in its repair. These oils represent valuable source of essential fatty acids (EFAs), type of PUFAs that unlike their saturated counterparts (SFAs), cannot be synthesized by human body and must be obtained from external sources (Krasodomska and Jungnickel, 2015; Vaughn et al., 2018)
The natural oils should have low potential for irritation or allergy, and should be readily available and economical (Vaughn et al., 2018). Currently, there is a growing interest in applying sustainability approach when choosing raw materials for formulation of cosmetics. Plum seed oil represents perfect example of a recyclable material from fruit processing, a waste product, that can be given "second life". The seeds of plums, found within plum stones, are a rich, yet underexplored source of bioactive compounds. During plum brandy production, a substantial quantity of intact plum stone residues is generated. While the stones enhance the brandy's flavor and aroma, the seeds are often discarded as a by-product (Rodríguez-Blázquez et al., 2024). When the oil is obtained from the seed, this domestic fruit seed oil can be used as potential cosmetic raw material and substitute for popular exotic seed oils (Krasodomska and Jungnickel, 2015).
Plum seed oil is listed in the CosIng Database (INCI: Prunus Domestica Seed Oil, CAS No: 90082-87-4) as "fixed oil expressed from the seeds of the Plum, *Prunus domestica* L., Rosaceae" with skin conditioning function in cosmetic products (European Commission, n.d.). This oil, characterized by light texture, is quickly absorbed by the skin, hydrates it and

improves its elasticity (Savić-Gajić et al., 2022).
In our previous paper, fatty oils from the seeds of four types of plums (Požegača, Čačanska lepotica, Čačanska rodna and Valjevka, cultivated in the territory of Bosnia and Herzegovina) were extracted using supercritical and Soxhlet extraction. Stated oils were characterized in terms of organoleptic inspection, as well as the acid, iodine, saponification and peroxide number. Even though all isolated fatty oils showed desirable characteristics, the best were attributed to oil isolated from Požegača. In addition, Soxhlet extraction proved to be slightly better extraction option compared to supercritical extraction (Kazanović et al., 2024). Therefore, plum seed oil isolated from Požegača plum variete was suggested as potential raw material for cosmetic purpose due to its good stability, as well as high concentration of PUFAs. In this regard, the aim of the current study was to incorporate plum seed oil in topical preparation (emulgel) and to evaluate its influence on biophysical characteristics of the skin. For this purpose, *in vivo* study on healthy human volunteers was conducted in order to evaluate preliminary safety (irritation potential) and long-term effects of emulgel with 6% (w/w) of plum seed oil, added as natural emollient and cosmetic active ingredient.

2. MATERIALS AND METHODS

2.1. Materials

For preparation of emulgels, following raw materials were used: MyrtiolTM318 (INCI: Caprylic/Capric Triglycerides) from Henkel (Germany), Shea Butter (INCI: Butyrospermum Parkii (Shea) Butter) from Sederma (France), Isopropyl Myristate and Glycerin from BASF (Germany), Natrosol™ 250 HR (INCI: Hydroxyethylcellulose) and EUXYL PE9010 (INCI: Phenoxyethanol Ethylhexylglycerin) from Ashland (Oregon, USA). Emulsifier Montanov 202 (INCI: Arachidyl Behenyl Alcohol and Arachidyl Glucoside) was purchased from Seppic (France). Purified water was obtained from Faculty of Medicine, University of Niš. Plum seed oil (INCI: Prunus Domestica Seed Oil) from Požegača plum variete was obtained according to the Soxhlet extraction procedure described in our previous paper (Kazanović et al., 2024).

2.2. Preparation of emulgels

For the purposes of analysis, two samples were prepared – Placebo emulgel (PE) and Active emulgel (AE) (a sample with plum seed oil as a cosmetic active ingredient and natural emollient). The qualitative and quantitative composition of the examined emulgels is presented in the Table 1.
Standard emulgel preparation protocol based on heating and stirring in the closed glass vessel was applied (Berdey and Voyt, 2016; Martinović et al., 2024). The placebo emulgel (PE) was prepared in the following manner. The components of the oil phase (A) were melted on a thermostatic heating plate of magnetic stirrer IKAMAG (IKA, Germany) and heated to 80 °C. After mixing the water phase and gelling the hydroxyethyl cellulose, the water phase was heated to 85 °C and added to the oil phase while stirring with a propeller rotary laboratory stirrer (RW16 basic, IKA Werke, Germany) until cooling.
The active emulgel (AE) was prepared using the same procedure, with plum seed oil added as a component of the oil phase. It was incorporated into the already prepared emulgel at a temperature of 40 °C.

2.3. Participants

The study was performed in accordance with the Helsinki Declaration and with the approval of the Ethics Committee of the Faculty of Medicine, University of Niš, reference number 12-10650/2-6 (03.10.2022), and in compliance with existing guidelines (Nobile, 2016). All participants were informed about the study protocol and signed informed consent to participate in the study. Participants were informed that they could withdraw from the study at any time; however, all participants completed the study as no adverse skin effects were observed in any of them.
The study was conducted in February at the Faculty of Medicine, University of Niš, in a climate-controlled room with a constant temperature of 20–23 °C and consistent airflow. Participants were instructed not to use any skincare products on the day of the study, refrain from showering, and avoid physical exercise prior to the testing.
A total of fourteen healthy volunteers, both male and female, aged 23.42 ± 2.94 years, with no history or clinical signs of dermatological diseases, participated in the *in vivo* study examining the skin irritation potential of the formulated emulgels with a natural-origin emollient. In the long-term *in vivo* study evaluating the impact of the formulated emulgels on the biophysical parameters of the skin, nineteen healthy volunteers, both male and female, with an average age of 23.47 ± 2.63 years and no history or clinical signs of dermatological diseases, participated.

Table 1. Qualitative and quantitative compositions of PE and AE samples

Phase	INCI names	PE (% w/w)	AE (% w/w)	Function in the preparation according to the CosIng Database
A	Caprylic/Capric Triglycerides	12.0	6.0	Emollient, skin conditioning,
	Isopropyl Myristate	4.0	4.0	Emollient
	Butyrospermum Parkii (Shea) Butter	2.0	2.0	Emollient, skin conditioning, viscosity controlling
	Arachidyl Behenyl Alcohol and Arachidyl Glucoside	5.0	5.0	Emulsifier
B	Hydroxyethylcellulose	1.0	1.0	Gelling agent
	Glycerin	10.0	10.0	Humectant
	Phenoxyethanol Ethylhexylglycerin	1.0	1.0	Preservative
	Water	65.0	65.0	Solvent in water phase
C	Prunus Domestica Seed Oil	-	6.0	Skin conditioning, emollient, cosmetic active ingredient

2.4. In vivo biophysical methods

The study was conducted as a double-blind study using non-invasive measurements of skin biophysical parameters. Prior to each parameter measurement, participants were acclimatized for approximately 30 minutes in the room where the measurements were conducted.

The device used for measuring skin biophysical parameters was Multi Probe Adapter System MPA® 9, manufactured by Courage + Khazaka electronic GmbH (Germany) with following probes:

1. Tewameter® TM 300 probe for measuring TEWL (transepidermal water loss),
2. Corneometer® CM 825 probe for measuring EC (epidermal capacitance),
3. Frictiometer® FR 700 probe for measuring friction,
4. Mexameter® MX 18 probe for measuring EI (erythema index) and MI (melanin index),
5. Skin-pH-Meter® PH 905 probe for measuring the pH value of the skin.

Before the study began, the probes were calibrated using appropriate software and according to the manufacturer's guidelines.

2.5. *In vivo* analysis of irritation potential of investigated emulgel with plum seed oil – study protocol

On the volar sides of the participants' forearms, 9 cm² areas were marked using a cardboard template. On the beginning of the study, baseline values of the skin's biophysical parameters were measured at these designated sites. The formulated emulgel preparations were applied to the marked areas in amount of 0.016 g/cm², which were then covered with Parafilm® and Hartmann Omnifix®E elastic adhesive tape. In addition, one square area was marked as non-treated control and one square area was marked as non-treated control under occlusion. After 24 hours, the occlusions were removed, and the biophysical parameters were measured again at the same sites 1 hour after the occlusion removal. The recorded values were subsequently compared and statistically analyzed.

2.6. *In vivo* analysis of efficiency of investigated emulgel with plum seed oil – study protocol

On the initial day of measurements, specific areas on the inner forearms were marked, and baseline parameter values were recorded. Over the following 28 days, participants applied the test preparations twice daily—once in the morning and once in the evening. Additionally, a small area on the lower part of the right forearm was left untreated to serve as a control site. Measurements were conducted at the start of the study (before the application of the preparations), on day 7, day 20, day 28 (before the morning application), and 2 days after stopping the application of the preparations (30 days from the start of the study).

2.7. Statistical analysis

The results of the *in vivo* studies were presented using Microsoft Excel and statistically analyzed with IBM SPSS Statistics software. Descriptive statistics were used to calculate the arithmetic mean and standard deviation. The normality of data distribution was assessed using the Shapiro–Wilk test prior to applying parametric tests. Comparison of variable values between two groups was performed with Student's t-test, while comparisons among more than two groups were performed using analysis of variance (ANOVA) and an appropriate Post Hoc analysis to compare variables individually between each modality of the same categorical variable. A significance level of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

The introduction of dermocosmetic products and cosmeceuticals into the market impose the need for providing evidence of their efficacy and safety through valid and reliable assessment methods. The Regulation (EC) N° 1223 (2009) on cosmetic products, as the main regulatory framework for cosmetics, provides all the requirements that finished cosmetic products must meet to be placed on the EU market (The European Parliament and The Council of the European Union, 2009). *In vivo* testing of biophysical skin parameters on human volunteers (skin bioengineering methods) enables objective evaluation of cosmetic product effects while being ethically acceptable, as it involves non-invasive procedures. Appropriate measuring instruments are used for *in vivo* quantification of specific skin parameters. These biophysical parameters are measured through simple contact between the skin surface and the probe of the instrument, providing insights into the skin's structure and function, such as moisture, elasticity, barrier function, and pH (Ariffin and Hasham, 2020).

3.1. Irritation potential (safety) of emulgels

The results of the *in vivo* study evaluating the irritation potential (preliminary safety) of the tested emulgels—PE and AE with plum seed oil as a natural-origin emollient—were obtained by comparing the measured skin biophysical parameters (TEWL, EC, MI, EI, pH) before and 24 hours after applying the emulgel samples under occlusion, since occlusion amplifies the effects of the applied preparation (Golda et al., 2005).

Transepidermal water loss (TEWL) reflects the skin's condition (hydration) and the integrity of the skin barrier. Measuring TEWL provides insights into the function of the *stratum corneum* (SC) as a diffusion barrier for water. The TEWL level serves as an indicator of the skin's permeability to topically applied substances (Verdier-Sévrain and Bonté, 2007).

Figure 1 shows the mean values and standard deviations of TEWL before and after 24 hours of exposure to the preparations under occlusion. At the non-treated control site without occlusion (NC), there was no statistically significant change in TEWL values, which differed from the sites under occlusion. A statistically significant reduction in TEWL values ($p < 0.05$) was observed at all occluded sites, regardless of whether they were treated with emulgel or not. This finding suggests that the observed change in TEWL values is likely due to the effect of occlusion *per se*. Since elevated TEWL may be a sign of disruption of skin barrier (Ariffin and Hasham, 2020), this result

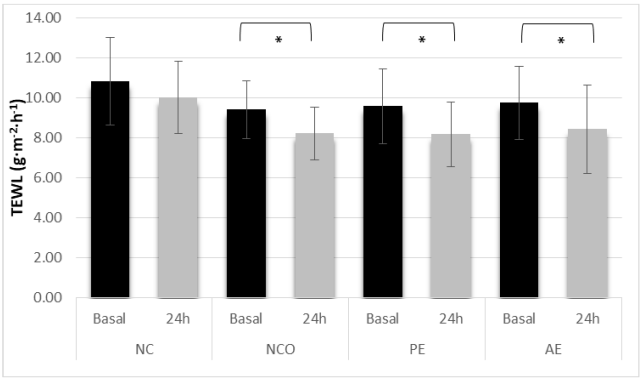

Figure 1. Baseline values and TEWL parameter values measured 24 hours after the application of emulgel samples (PE and AE), on non-treated control site (NC) and non-treated control site under occlusion (NCO), presented as mean values with standard deviations. Differences for the same treatment at different time points (baseline measurement vs. measurement 24 hours after occlusion) were analyzed using Student's t-test, with statistically significant differences ($p<0.05$) marked with (*).

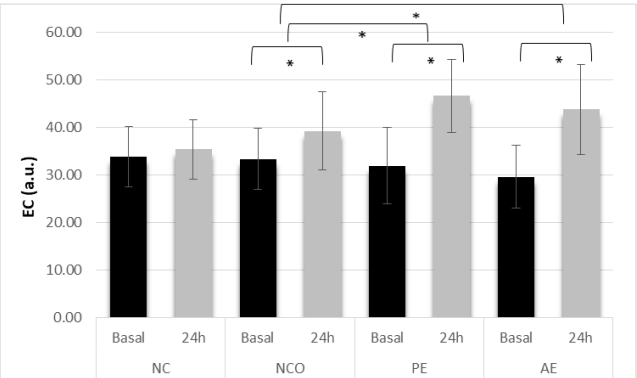

Figure 2. Baseline values and EC parameter values measured 24 hours after the application of emulgel samples (PE and AE), on non-treated control site (NC) and non-treated control site under occlusion (NCO), presented as mean values with standard deviations. Differences for the same treatment at different time points (baseline measurement vs. measurement 24 hours after occlusion) were analyzed using Student's t-test, with statistically significant differences ($p<0.05$) marked with (*).

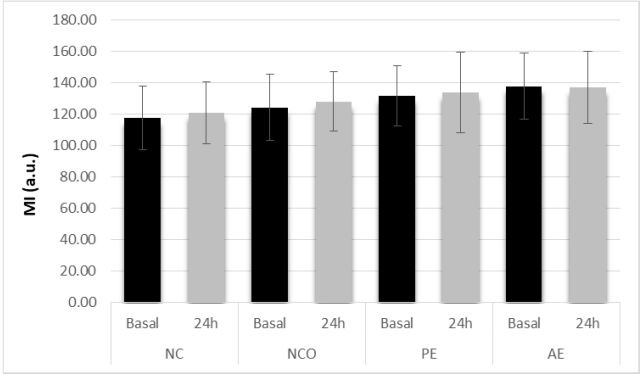

Figure 4. Baseline values and MI parameter values measured 24 hours after the application of emulgel samples (PE and AE), on non-treated control site (NC) and non-treated control site under occlusion (NCO), presented as mean values with standard deviations. Differences for the same treatment at different time points (baseline measurement vs. measurement 24 hours after occlusion) were analyzed using Student's t-test, with statistically significant differences ($p<0.05$) marked with (*).

supports the safety profile of the tested samples, as they indicate the preservation of skin barrier integrity and absence of irritation.

Standard *in vivo* noninvasive methods for evaluating skin hydration are based on the electrical properties of the skin and are dependent on the skin water content (electrical conductance and capacitance) (Ariffin and Hasham, 2020). Figure 2 shows the mean values and standard deviations of EC measurements before and after 24 hours of skin exposure to the preparations under occlusion. The tested emulgel samples, as well as the non-treated control under occlusion (NCO), demonstrated a statistically significant increase in EC values, indicating improved skin hydration compared to baseline values. In contrast, skin areas not exposed to occlusion showed no statistically significant changes in hydration. The results also revealed that areas treated with emulgels exhibited a significantly greater increase in skin hydration compared to non-treated areas under occlusion (NCO). The mean hydration increased from 31.88 ± 8.06 to 46.60 ± 7.68 for PE and from 29.56 ± 6.60 to 43.68 ± 9.48 for AE. This suggests that, while occlusion itself contributed to the increased electrical capacitance of the skin, the application of emulgels further enhanced hydration.

In vivo measurements of skin pH are used in dermatology and cosmetology to evaluate the effectiveness and safety of certain preparations (Ariffin and Hasham, 2020). The normal range of skin pH is between 4.2 and 5.6, which is essential for maintaining the barrier function and lipid production of the skin (Schmid-Wendtner and Korting, 2006).

Figure 3 shows that no significant changes in pH values were observed between the baseline measurements and those taken after one day of occlusion in all areas (non-treated (NCO) and treated with AE or PE). These results suggest that neither the application of preparations nor occlusion disrupted the normal pH values of the skin.

The skin color evaluations include measurements of the melanin index (MI) and erythema index (EI). MI indicates skin tone, while EI primarily refers to the presence of erythema, as a sign of skin irritation. Erythema occurs when the skin is exposed to irritants such as chemical substances, allergens, UV rays, or cleaning agents which cause the dilatation of blood vessels leading to erythema. In various dermatological studies, EI, together with MI, serves as a reliable indicator of skin barrier integrity (Jayabal et al., 2021; Rogiers et al., 1999).

The results of the study on the impact of preparations on skin color (Figure 4) suggest that there were no significant changes in MI values. This was expected, as no redness or irritation was observed in any participant during the study.

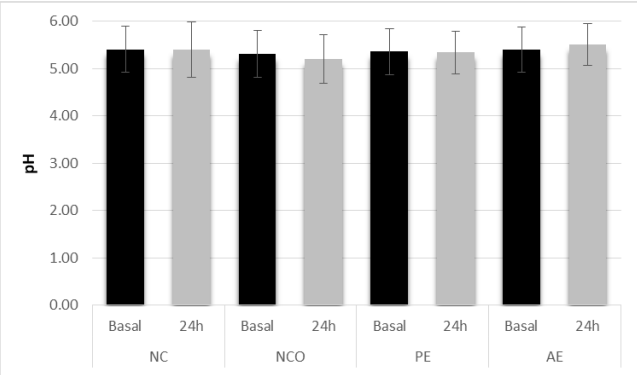

Figure 3. Baseline values and pH parameter values measured 24 hours after the application of emulgel samples (PE and AE), on non-treated control site (NC) and non-treated control site under occlusion (NCO), presented as mean values with standard deviations. Differences for the same treatment at different time points (baseline measurement vs. measurement 24 hours after occlusion) were analyzed using Student's t-test, with statistically significant differences ($p<0.05$) marked with (*).

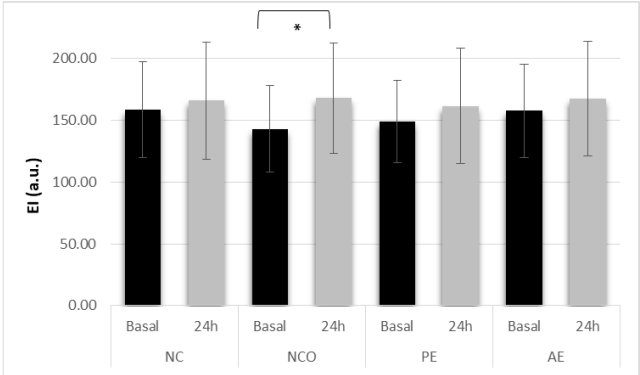

Figure 5. Baseline values and EI parameter values measured 24 hours after the application of emulgel samples (PE and AE), on non-treated control site (NC) and non-treated control site under occlusion (NCO), presented as mean values with standard deviations. Differences for the same treatment at different time points (baseline measurement vs. measurement 24 hours after occlusion) were analyzed using Student's t-test, with statistically significant differences ($p<0.05$) marked with (*).

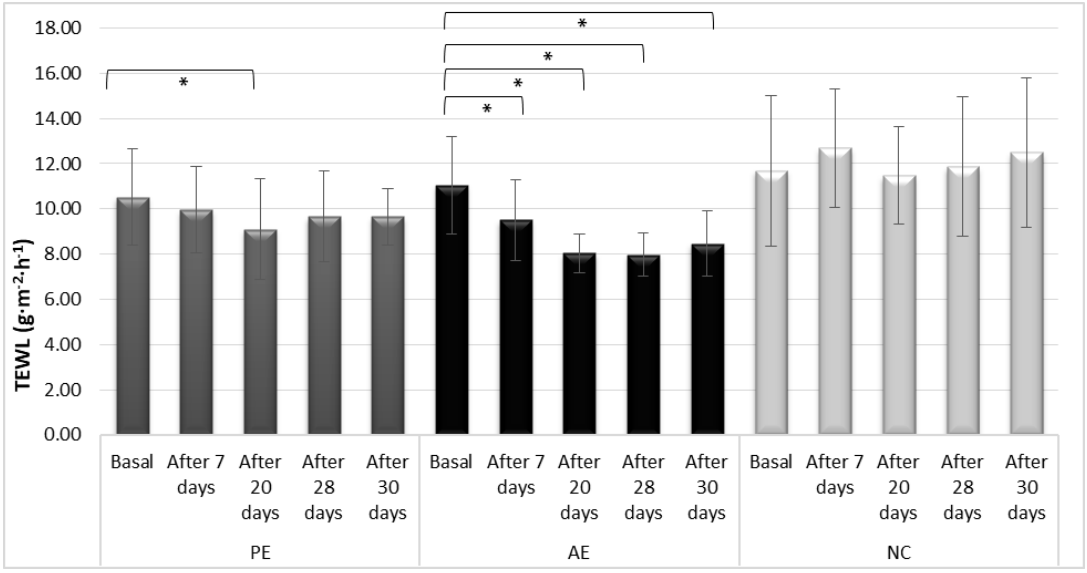

Figure 6. TEWL values presented as mean values with standard deviations, measured across all participants at sites treated with the placebo emulgel (PE), active emulgel (AE), and non-treated site (NC) at the start of the study, as well as after 7, 20, 28, and 30 days from the study's initiation. Statistically significant differences ($p<0.05$) are marked with (*).

The EI values did not show statistically significant differences between measurements, except in the non-treated control area under occlusion (NCO), where a statistically significant increase of average 25.25 units was observed (Figure 5).

The results indicate that no redness or erythema occurred following the application of the emulgel samples. Furthermore, when the statistically significant increase in the erythema index (EI) at the non-treated site under occlusion (NCO) is compared with the change in EI at the sites treated with the tested emulgels, it can be concluded that the presence of the emulgels contributed to the reduction of EI caused by occlusion. Therefore, it might be speculated that the tested emulgels not only do not cause skin irritation but may also have an anti-irritant effect.

3.2. Skin effects of emulgels in a long-term in vivo study

Skin effects of the AE and PE were assessed by comparing the biophysical skin parameters measured at the beginning of the study, after 7 days, after 20 days, after 28 days of application, and 2 days after discontinuing the application (30 days from the study's start).

Figure 6 provides a graphical representation of the baseline TEWL values and those measured on days 7, 20, 28, and 30. At the non-treated site, no statistically significant changes in TEWL were observed throughout the study. However, a reduction in TEWL was observed at the sites treated with both the PE and the AE

For the PE-treated site, a statistically significant reduction in TEWL was observed only after 20 days of application (from 10.52 ± 2.14 to 9.10 ± 2.22). In contrast, at the site treated with the AE, a statistically significant reduction in TEWL compared to baseline values was observed as early as day 7, as well as at all other assessed time-points (on day 20, 28 and 30 i.e. 2 days after discontinuing the emulgel).

Although there was no statistical significance between sites

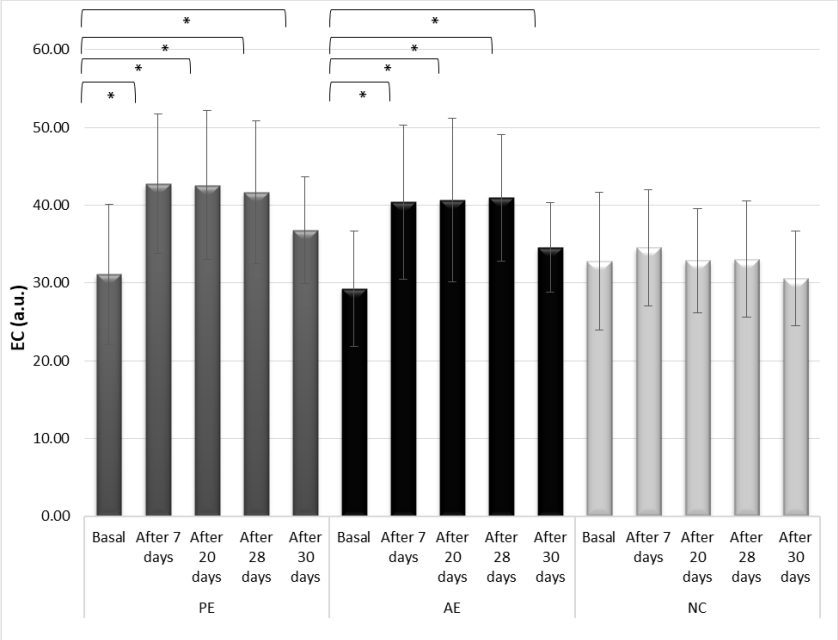

Figure 7. EC values presented as mean values with standard deviations, measured across all participants at sites treated with the placebo emulgel (PE), active emulgel (AE), and non-treated site (NC) at the start of the study, as well as after 7, 20, 28, and 30 days from the study's initiation. Statistically significant differences ($p<0.05$) are marked with (*).

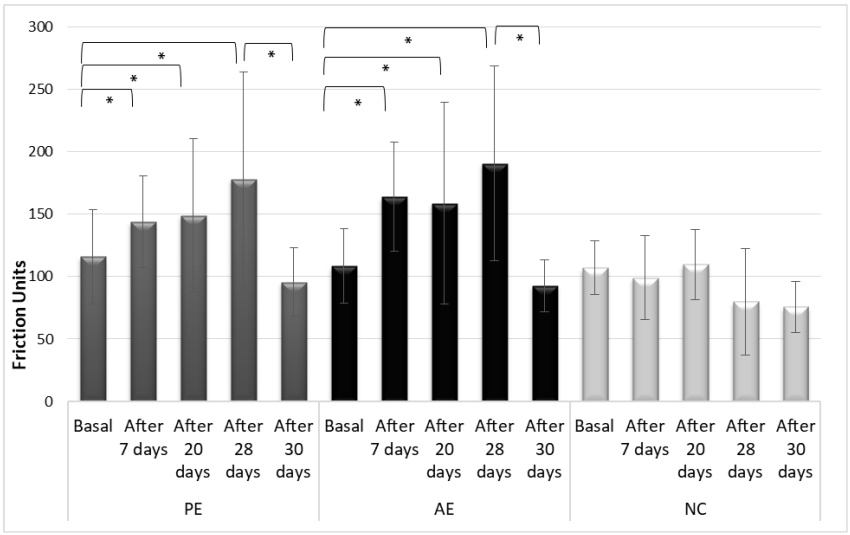

Figure 8. Friction unit values presented as mean values with standard deviations, measured across all participants at sites treated with the placebo emulgel (PE), active emulgel (AE), and non-treated site (NC) at the start of the study, as well as after 7, 20, 28, and 30 days from the study's initiation. Statistically significant differences ($p<0.05$) are marked with (*).

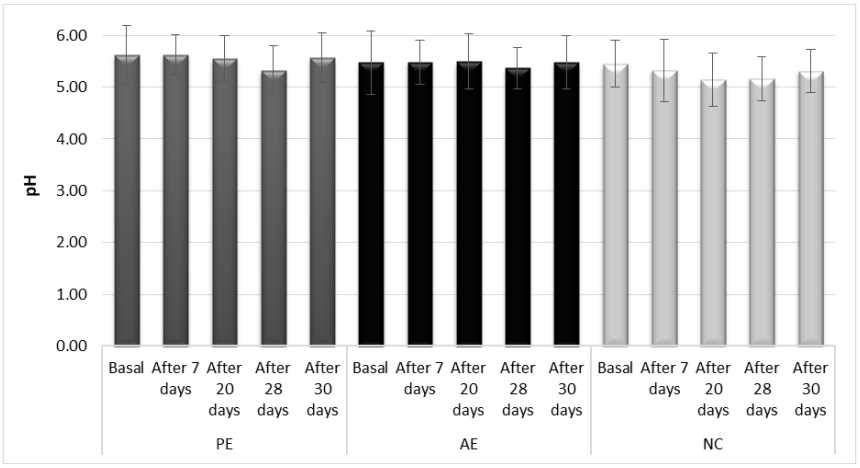

Figure 9. pH values presented as mean values with standard.

treated with AE and PE, these results indicate that plum seed oil at a 6% (w/w) concentration contributed to reducing TEWL, effectively restoring the epidermal lipid layer. The average basal TEWL values were 11.06 ± 2.15, while the lowest average TEWL was measured on the day 28 of AE application (7.97 ± 0.95). Moreover, this effect persisted for 2 days after discontinuing the application of the active emulgel (average TEWL 8.46 ± 1.44).

Preparations that effectively moisturize the skin, commonly referred to as moisturizers, are the most numerous and widely used class of dermocosmetic products. They are employed for prevention, as well as in mono- or adjuvant therapy for treating various skin conditions, particularly ichthyosis, atopic dermatitis, and various dermatoses. These products are formulated to repair damaged SC, act as emollients, and eliminate the subjective and objective symptoms of dry skin, providing a healthier appearance (Stojiljković et al., 2013).

At the sites treated with both the PE and AE, a statistically significant increase in electrical capacitance was observed 7 days after the start of application compared to the baseline values for approximately 37% (Figure 7). This increase persisted throughout the entire application period (28 days). Moreover, 2 days after discontinuing the emulgels (30 days from the start of the study), although a decrease in electrical capacitance was noted compared to the values measured on day 28, a statistically significant increase in hydration compared to the baseline values was still present.

On the other hand, no significant changes in electrical capacitance were observed at the non-treated control site during the study. These results demonstrate that the application of emulgels led to an increase in hydration, which was observed with both the active and placebo emulgels.

However, given the minimal differences in hydration increment between the sites treated with the AE and PE, it can be concluded that the presence of plum seed oil alone did not result in a significantly greater increase in hydration (compared to the PE where it was replaced with caprylic/capric triglycerides).

Skin friction can indicate the state of the skin. There is positive correlation between skin moisture and skin friction coefficient. Dry and oily skin tends to have lower skin friction coefficient (Comaish and Bottoms, 1971; Lodén et al., 1992; Sivamani et al., 2003; Zhu et al., 2011). During the study, a statistically significant increase in skin friction was observed at the sites treated with both the AE and PE after 7, 20, and 28 days of application compared to baseline values, as shown in Figure 8. Upon discontinuation of the emulgels usage, the values of this parameter decreased and returned to their initial levels. At the control site, no significant changes in this parameter were

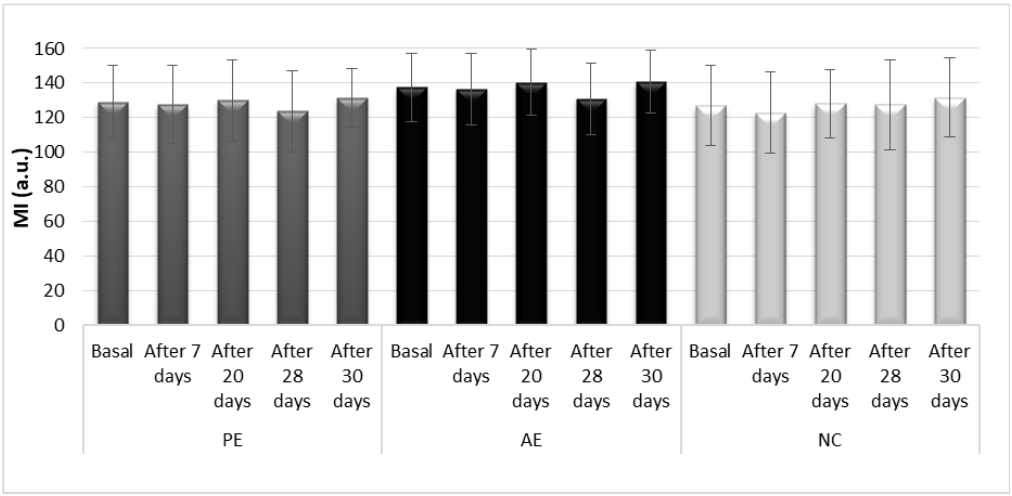

Figure 10. MI values presented as mean values with standard deviations, measured across all participants at sites treated with the placebo emulgel (PE), active emulgel (AE), and non-treated site (NC) at the start of the study, as well as after 7, 20, 28, and 30 days from the study's initiation. Statistically significant differences ($p<0.05$) are marked with (*).

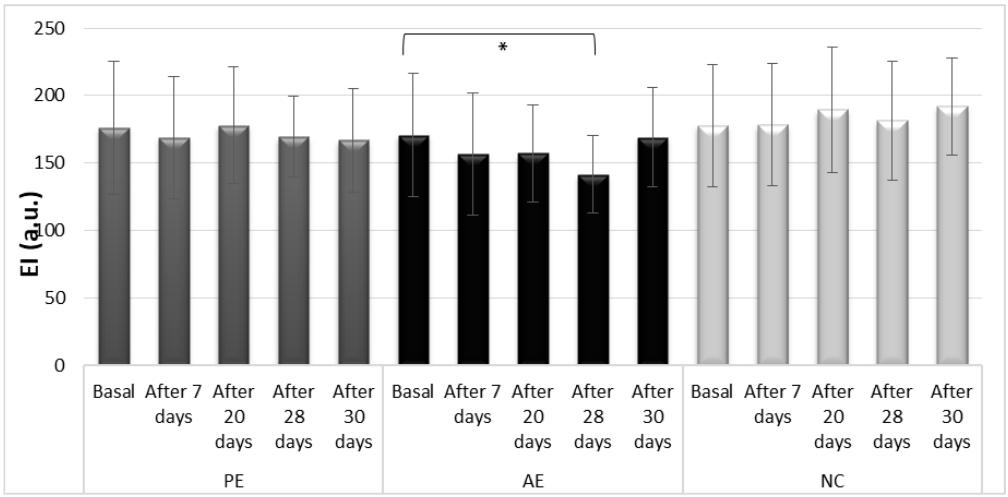

Figure 11. EI values presented as mean values with standard deviations, measured across all participants at sites treated with the placebo emulgel (PE), active emulgel (AE), and non-treated site (NC) at the start of the study, as well as after 7, 20, 28, and 30 days from the study's initiation. Statistically significant differences ($p<0.05$) are marked with (*).

observed, indicating that the increase in skin friction was a result of the emulgel application.

During the study, no statistically significant changes in pH values were observed at any of the tested sites, indicating that the applied emulgels did not disrupt the normal pH of the skin (Figure 9).

Figure 10 shows that no statistically significant changes in the melanin index (MI) were observed at any of the tested sites during the study, indicating that the applied emulgels did not affect skin pigmentation.

During the study, a statistically significant decrease ($p < 0.05$) in the erythema index (EI) was observed only at the site treated with the AE where average EI decreased from 170 ± 45.78 to 142 ± 28.87 (Figure 11). No statistically significant changes in this parameter were noted at the site treated with the PE or at the non-treated site (NC).

At the site treated with the AE, the erythema index (EI) showed a gradual decrease from the beginning of the study throughout the application period. This decrease became statistically significant compared to baseline values after 28 days of emulgel application. However, after discontinuing the AE, the EI was restored to the initial (baseline) value.

These results suggest that plum seed oil may contribute to the potential anti-irritant effect of the emulgel, which was also suggested by the results of the safety study.

4. CONCLUSION

The results of our study demonstrate that plum seed oil is a promising candidate as a natural raw material for use in cosmetics, as it exhibited no irritation potential in a 24-hour safety study. Furthermore, findings from a long-term *in vivo* study suggested its potential anti-irritant effects. To the best of our knowledge, no other studies have applied non-invasive biophysical methods to measure skin parameters for evaluating the influence of cosmetic products containing plum seed oil.

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CONFLICT OF INTEREST

The authors declare that they have no financial and commercial conflicts of interest.

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