THE VALORIZATION OF PLUM SEED OIL FOR THE DEVELOPMENT OF TOPICAL FORMULATION

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The object of this paper was to utilize the plum seed oil for the preparation of the topical formulation for skin care since it can improve elasticity and prevent accelerated skin aging. Soxhlet extraction of plum seed oil was performed using n-heptane. The spectrophotometric methods were applied to estimate the photoprotective effect and antioxidant activity of the samples. The warm-warm emulsification process was used for the preparation of topical formulation based on plum seed oil. The antioxidant activity of the plum seed oil was estimated based on the half-maximal inhibitory concentration (9.39 mg/mL). After the incorporation of the oil in the topical formulation, the IC50 value of 9.33 mg/mL was not significantly changed. The viscosities of the topical formulation and plum seed oil were 1.56×106 mPa·s (at the shear rate of 5 s⁻¹) and 60.48 mPa·s (at the shear rate of 50 s⁻¹), respectively. Rheological analysis showed that the plum seed oil and topical formulation were Newtonian and non-Newtonian pseudoplastic fluids, respectively. The formulation adsorbed the light in the UV-Vis range so that it can be used as a w/o emulsion photoprotective cream. The cream with adequate pH value was stable and microbiologically safe for application to the skin. Due to the use of cheaper ingredients, the formulation is acceptable and suitable for manufacturing.

Keywords: formulation/stability, emulsion, antioxidant activity, quality control

Introduction

The pharmaceutical and cosmetic industries typically use eco-friendly ingredients that are acceptable to consumers. Vegetable oils have a significant place among other ingredients because they are cheaper, ecological, non-toxic, and biodegradable [1]. They can be prepared from different parts of plants, especially from seeds as a by-product of the food industry [2]. The suitable properties of the oil for topical use are fluidity at lower temperatures, thermal and oxidative stability, low viscosity, and small change in the viscosity with changing temperature. In addition to physical properties, the chemical composition (fatty acids, phenolic compounds, carotenoids, waxes, squalene, phospholipids, etc.) is also one of the key factors for the preparation of pharmaceutical and cosmetic products [3]. Vegetable oils have hydrating and emollient properties [4]. They are an integral part of lipid fraction in cosmetic products. The fatty acids can impact the maintenance of the outermost layer of the epidermis (stratum corneum) due to their similarity with human lipids. They act as humectants and have anti-inflammatory, healing, and regenerative effects on the skin [5]. Oleic acid causes the disorder of a protective barrier on the skin and induces dermatitis under continuous topical application [6]. Poly- and mono-unsaturated fatty acids can impact inflammation either as soluble lipid mediators or in the form of phospholipids incorporated in the cell membrane. The topical use linoleic (n-3), linoleic (n-6), and oleic (n-9) fatty acids can regulate the healing of surgically induced skin wounds [7]. The n-9 fatty acids allow the healing of wounds faster than the n-3 and n-6 fatty acids. In addition to fatty acids, vegetable oils also contain phenolic compounds, such as phenolic acids and alcohols, flavonoids, and stilbenes [8]. The most abundant phenolic compounds in vegetable oils are quercetin, rutin, catechins, epicatechin, trans-resveratrol, etc. The phe-nolic compounds have expressed the antioxidant, anti-inflammatory, antimicrobial, photoprotective, anticancer, and other activities [9]. Due to these properties, they are very suitable for application in topical products. The oils of almond [10], peanut [11], sesame seed [12], coconut [13], grape seed [14], olive [15], etc. are the most commonly used vegetable oils for the preparation of topical products.

Plum seed oil is the main source of bioactive compounds with pronounced pharmacological properties [16-18]. It hydrates the skin and improves its elasticity without leaving a greasy film. Due to the presence of
vitamin E, the plum seed oil protects the skin from the effect of external influences and accelerated ageing [16]. It is effective in the treatment of constipation, inflammation, psoriasis, eczema, irritation, dark spots on the skin, and edema, as well as for stimulation of the water circulation in the body. The nail and lip care products based on plum seed oil are commercially available. In this paper, the utilization of plum seed oil has been considered for the preparation of the topical formulation. The antioxidant activity of plum seed oil was determined before and after its incorporation into the topical formulation using the DPPH assay. Also, the oxidative, chemical, physical and functional stabilities of the formulation were investigated.

**Experimental**

**Chemicals and reagents**

In this study, *n*-hexane, *n*-heptane, toluene, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Chemical, Saint Louis, Missouri, USA) were used. The topical formulation was prepared using the following ingredients: liquid paraffin (Swirmd.o.o., Šabac, Republic of Serbia), cetyl palmitate, beeswax white, and sodium tetraborate (Avéna Lab Cosmetics, Vršac, Republic of Serbia). All other chemicals and reagents were of analytical reagent grade.

**Plant material**

The seeds of plum (*Prunus domestica*) were purchased from PD Plemić-komerc (Osečina, Republic of Serbia). The moisture content of the plant material was determined by drying the plant material at 105 °C. The weight was measured after 2 h of drying the plant material and then the procedure was repeated under the same conditions for 30 min to the constant weight. The moisture content was found to be 1.76% (w/w). Before the extraction, the plant material was ground in an electric mill to an average particle size of 0.3 mm.

The extraction of fixed oil from plum seeds

The procedure of oil isolation from plum seeds was performed according to the procedure previously described by Savic et al. [18]. The plant material of 50 g was treated with 500 mL of *n*-heptane as a solvent. The extraction temperature corresponded to the boiling point of the used solvent. After extraction, the oil was separated from the solvent by evaporation under vacuum using a rotary evaporator at 50 °C. The obtained fixed oil was stored in a fridge at 4 °C before further analysis.

**Rheological analysis**

Rheological properties were analyzed on rheometer MCR 302 (Anton Paar, Graz, Austria) supported by RheoCompass™ software (Anton Paar, Graz, Austria) and the system equipped with coaxial cylinder CC27 (ISO3219) at 20 °C. The diameter of the inner cylinder was 13.33 mm, while the other cylinder was 14.46 mm. The sample volume of plum seed oil was 19 mL, while the value of apparent viscosity was interpolated for a shear rate of 50 s⁻¹. The analysis of semi-solid formulation was performed on the system of parallel plates with a distance between them of 1 mm. The plates consisted of PP50/S measure tools, while the lower (P-PTD 200) and upper (H-PTD 200) Peltier element was used for zeroing the temperature gradient. The sample volume was 2.2 mL. The value of apparent viscosity was interpolated for a shear rate of 5 s⁻¹.

**UV-Vis spectrophotometric analysis**

The absorbance of plum seed oil diluted 1:100 (v/v) in *n*-hexane was measured on Varian Cary-100 UV-Vis spectrophotometer (Mulgrave, Victoria, Australia) in a quartz cuvette of 1×1 cm at room temperature (22 °C).

**Preparation of the topical formulation**

The topical formulation (cream with plum seed oil) was prepared by the warm-warm emulsification process. The placebo (cream without plum seed oil) was also prepared to compare its characteristics with the already designed formulation. Instead of plum seed oil, the placebo contained the equivalent amount of liquid paraffin. The percentage composition of the prepared formulations is given in Table 1.

<table>
<thead>
<tr>
<th>Table 1. The composition of the topical formulation and placebo.</th>
</tr>
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<tbody>
<tr>
<td><strong>Ingredients/NCI Nomenclature</strong></td>
</tr>
<tr>
<td><strong>Oily phase</strong></td>
</tr>
<tr>
<td>Cetyl palmitate</td>
</tr>
<tr>
<td>Beeswax</td>
</tr>
<tr>
<td>Paraffinum liquidum</td>
</tr>
<tr>
<td>Prunus domestica (plum) seed oil</td>
</tr>
<tr>
<td><strong>Water phase</strong></td>
</tr>
<tr>
<td>Aqua</td>
</tr>
<tr>
<td>Sodium tetraborate</td>
</tr>
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</table>

Cetyl palmitate and beeswax were melted by heating in an enameled paten using a water bath at 70 ± 1 °C. Liquid paraffin and plum seed oil were added to the melted mixture. Sodium tetraborate was dissolved in the water previously heated to the same temperature. The warm-warm emulsification process implied the gradual addition of sodium tetraborate solution to the oily phase with constant agitation using a laboratory propeller mixer at 800 rpm for 5 min at a constant temperature. After emulsification, the stirring process was continued at 500 rpm until samples were cooled to room temperature (22 °C). The prepared samples of creams were stored in the polypropylene plastic boxes at the controlled room temperature (22 ± 2 °C). The placebo was prepared in the same way.

**Quality assessment of topical formulation**

Investigation of organoleptic parameters

The organoleptic properties (appearance, color, gloss, homogeneity, and phase separation of creams) of the
prepared creams were estimated visually. The applicable and sensory properties (ease of smearing, absorption, stickiness and grease on the skin) were defined after 24 h of the cream application to the skin.

Determining the type of emulsion
The type of emulsion was determined by measuring the conductivity of the cream samples using a conductor-meter (CDM 230, Radiometer, Copenhagen, Denmark). The measurements were performed at room temperature (22 °C) after electrode calibration with 0.01 mol/L potassium chloride solution.

Physicochemical characterization of the topical formulation
The physicochemical characterization of the topical formulation and placebo was done according to the methods described in the official European Pharmacopoeia [19]. The pH value was measured at 22 ± 2 °C on the pH meter (HI 9321, HANNA instruments, Lisbon, Portugal). The acid and saponification values were also determined. The rheological analysis of the topical formulation and placebo was carried out in the same way as the oil except that measurements were made at 20 °C with a parallel plate system, PP25/S.

Stability studies
Oxidative stability of plum seed oil
The oil samples were packed in well-sealed plastic bottles and exposed to daylight for 3 months at room temperature (22 °C). After 0, 7, 14, 21, 28, 60, and 90 days, the peroxide value was determined using a standard procedure [19].

Chemical stability of the topical formulation
The topical formulations were stored at room temperature (22 °C) for 3 months to determine the chemical stability. The peroxide value was carried out after 7, 30, 60, and 90 days.

The physical stability of the topical formulation
Centrifugation assay
Exactly 4 g of the cream was weighed and transferred into a 10 mL cuvette. The samples were centrifuged twice per 15 min at the velocity of 3000 rpm on the laboratory centrifuge (LC 320, Tehtnica, Zelezniki, Slovenia). After centrifugation, the samples were inspected visually to notice possible changes (phase separation).

Accelerated stability studies
The samples were stored at 4 °C and 60% relative humidity (RH), 30 °C, and 60% RH, as well as at 40 °C and 70% RH for 3 months. After 1, 7, 15, and 30 days, the creams were sampled and their pH values were immediately measured [20].

Antioxidant activity of plum seed oil before/after incorporation into the topical formulation was determined using DPPH assay according to the modified procedure [9]. The stock solution was prepared by dissolving 1 g of oil in 10 mL of toluene. A series of solutions were made by dilution of the stock solution. The samples were treated with DPPH toluene solution (3×10⁻⁴ mol/L) and then shaken using the vortex apparatus for 20 s. The negative control sample was prepared by the addition of 1 mL of DPPH solution to 2.5 mL of pure toluene. The topical formulation was dispersed in toluene to obtain the equivalent amount of oil used for analysis. The placebo was also analyzed as a positive control. The functional stability of oil and the topical formulation was estimated based on monitoring their antioxidant activity for 90 days [21]. The samples were stored at 4 °C, room temperature (22 °C), and 40 °C while the RH was 70%. The antioxidant activity of the samples was estimated based on their half-maximal inhibitory concentration (IC₅₀ value) for different time intervals (start, 1, 7, 30, 60, and 90 days). The samples of placebo were stored as positive controls under these conditions.

Determination of microbiological safety
The topical formulation was microbiologically tested to estimate its safety. The method was based on the isolation and identification of bacteria, fungi, and mold present in the topical formulation. The cream samples were seeded on a nutrient medium and incubated under optimal conditions. According to the EN ISO standard [22], the allowed total mesophilic aerobic microorganisms should be lesser than 1×10³ of CFU per 1 gram of the topical formulation. The products should not contain Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans.

Statistical analysis
All data were presented as mean ± standard deviation obtained using OriginPro 2016 software (OriginLab Corporation, Northampton, Massachusetts, USA) at a 95% confidence level.

Results and discussion
n-Heptane was chosen as a solvent of choice for the Soxhlet extraction of plum seed oil due to low density, boiling temperature, toxicity, and price. The high yield of plum seed oil (30%) was probably due to the extraction of non-lipid compounds [23]. The previous results of the moisture content, density, refractive index, pH value, acid, saponification, and peroxide values, as well as the oxidative stability and chemical content have indicated that the oil has a satisfactory quality for further application in topical formulations [18].

Rheological analysis
Rheological analysis of plum seed oil has considered the type of fluid flow and the behavior of shear stress...
and viscosity depending on the shear rate. The linear dependency between shear stress and shear rate (Fig. 1a) indicated that the type of fluid flow can be described using Eq. 1:

$$\tau = \eta \cdot \gamma \tag{1}$$

where \(\tau\) is shear stress, \(\gamma\) is shear rate, and \(\eta\) is viscosity. Since the viscosity remained constant at any value of the shear rate (Fig. 1b), plum seed oil can be considered a Newtonian fluid.

**Figure 1.** The dependency of shear stress (a), and viscosity (b) on the shear rate for plum seed oil.

The dynamic viscosity of 60.48 mPa·s was noticed at a shear rate of 50 s\(^{-1}\) and a temperature of 20 °C. This parameter can be converted in the kinematic viscosity based on the previously reported density of plum seed oil of 0.9 g/mL. The calculated value of kinematic viscosity of 67.2 mm\(^2\)/s was higher compared to the value of 4.29 mm\(^2\)/s for plum seed oil at 40 °C obtained by Górnas et al. [24]. It was expected since the viscosity increases with decreasing the temperature. A similar dynamic viscosity exists in olive (59 mPa·s) and hazelnut oils (63 mPa·s) [25], while the sunflower and grape seed oils have slightly lower viscosity of about 40 mPa·s. This difference in viscosity is due to the different compositions of fatty acids in the oils. The oils with a high content of unsaturated fatty acids (linoleic and linolenic acids) are less viscous because they do not have a solid and fixed structure [3]. The rheological properties of plum seed oil are in agreement with olive oil representing one of the most commonly used commercial oils in cosmetic products. From this point of view, the use of plum seed oil for the preparation of topical formulation is justified.

UV-Vis analysis of the topical formulation

UV-Vis spectrum of plum seed oil in hexane is depicted in Fig. 2. As can be seen, the oil could absorb UVC (100–290 nm), UVB (290–320 nm), and UVA (320–400 nm) irradiations. In the UVC range, the oil had the highest absorbance (about 3 AU) relative to UVA and UVB ranges. Because of that, the oil can be used in the design of formulations with photoprotective activity. In the wavelength range of 290–400 nm, the spectrum can be compared with the spectra of the oils from *Albizia julibrissin* seed [26], *Linum usitatissimum* seed [27], *Nigella sativa* L. [28], *Phoenix dactylifera* L. [29], and *Rubus idaeus* L. [30]. These oils can be used as protective factors for UVB (SPF) and UVA (PFA) irradiations.

**Figure 2.** UV-Vis spectra of plum seed oil dissolved in n-hexane for the wavelength between (a) 200 and 290 nm; (b) 290 and 400 nm, and (c) 400 and 800 nm.
Preparation of the topical formulation

Premature skin ageing characterized by dry and rough skin and the appearance of deep wrinkles could occur after the effect of environmental factors. The corticosteroids, vitamin D, and others that can produce many local and systemic side effects are commonly used in the treatment of these skin conditions. The application of plant extracts and oils with expressed antioxidant, anti-inflammatory, anticancer, and photoprotective properties is one of the new approaches for hydration, protection, prevention, and healing of skin damage. The antioxidants stimulate the generation of collagen and elastin, as well as reduce the degradation of structural components of the skin. Among other eco-friendly substances, plum seed oil is a promising ingredient for the preparation of topical formulations. The oil is readily biodegradable and compatible with skin lipids, so its application in topical formulations could reduce the use of synthetic oil [31].

The topical formulation and placebo were prepared using the technological procedure at the laboratory level. The oily phase was composed of cetaceum (cetyl palmitate), beeswax white (Cera Alba), liquid paraffin (paraffinum liquidum), and plum seed oil. Cetyl palmitate consists of cetyl alcohol and palmitic acid. It increases the emulsifier stability and decreases oily texture as a co-emulsifier. In addition to the emulsifier role, it is also used as a gloss factor in the emulsions. The beeswax white is a mixture of the esters of fatty acids and various long-chain alcohols, free fatty acids, free fatty alcohols, carotenoids, aromatic and mineral matters. It is suitable for dry skin and care in winter due to its regenerative, emollient, and soothing properties. The liquid paraffin is a refined mineral oil without odor, color, and taste. It is a mixture of saturated hydrocarbons that can not penetrate the skin due to their size and can not close the skin pores. The oil does not cause allergic reactions to the skin and is not cancerogenic. It forms the protective layer that inhibits the transepidermal water loss, making it soft and smooth. In addition to the achievement of the occlusion effect, the cetaceum, beeswax white, and liquid paraffin are important for the adjustment of the consistency of the topical formulation [32]. The plum seed oil is quickly absorbed into the skin. It has a light texture and leaves no greasy film. The presence of fatty acids, phenolic compounds, and β-carotenes allows skin protection from external influences and premature ageing. This oil hydrates the skin and improves its elasticity. The water phase consisted of demineralized water and borax (sodium tetraborate) as a preservative. The topical formulation was designed without artificial fragrances and colors. The prepared formulation had an emollient effect and the ability to create a thin protective layer on the skin. In this way, it protects the skin from drying out and the harmful effects of the environment. Due to the presence of plum seed oil, the formulation had a photoprotective effect against UV irradiation.

Topical formulation quality

The pharmaceutical and cosmetic products that use topically are subjected to strict quality control since they can cause some structural and functional changes in the skin. By application of adequate assays for quality control of these products, their reliability and efficiency are checked. If a product works effectively, but is unstable or expresses irritant/allergic effects on the skin, it will not be accepted by the consumer. During the quality control of the developed topical formulation, the organoleptic properties were investigated and the physicochemical parameters (pH value, viscosity, acid, and saponification values) were determined. The physical, chemical, and functional stabilities of topical formulation were also monitored.

Investigation of organoleptic properties

The topical formulation and placebo were white, semi-solid consistency, homogeneous, and with a characteristic odor. They were easily smeared on the skin, leaving a greasy film that was not sticky. Generally, the water from the topical formulation evaporates and creates a cold feeling after application to the skin. The oily phase remains in the skin and inhibits the transepidermal water loss. Because of that, the topical formulation has expressed the emollient effect.

Determination of emulsion type

Based on the values of electrical conductivity for the topical formulation and placebo, the type of emulsion was determined. The low electrical conductivities of about 2 μS/cm indicated that both samples belonged to the w/o emulsions.

Acid and saponification values

The acid values of the topical formulation and placebo were 1.68 mg KOH/g and 1.77 mg KOH/g, respectively. The low acid values for both samples indicated the low content of free fatty acids. The content of free fatty acids in the topical formulation was lower compared to the placebo. The topical formulations with these properties are desirable because they will not cause skin irritation after application. The saponification values for the topical formulation and placebo were 180 mg KOH/g and 184 mg KOH/g, respectively.

Determination of pH value

The pH value of the skin is in the range of 4.5 – 6.0, while the average pH value of 5.5 is desirable [33]. The topical formulations intended for application to the skin should have a pH value in the given range. The topical formulation and placebo had pH values of 5.38 and 5.64, respectively. Based on these results, it can be concluded that the pH value of the topical formulation was decreased due to the addition of the oil.
Rheological analysis of topical formulation
The dependences of shear stress or viscosity on the shear rate for the topical formulation and placebo are depicted in Fig. 3.

Unlike the oil, the viscosities of the topical formulation and placebo were not constant and decreased with increasing the shear rate. This is a characteristic of non-Newtonian pseudoplastic fluid that allows uniform and efficient application on the skin. The viscosities of the topical formulation and placebo at the shear rate of 5 s⁻¹ were 1.56×10⁶ mPa·s and 1.01×10⁵ mPa·s, respectively. Dănilă et al. [34] also found that a topical water emulsion with collagen and a mixture of vegetable oils (coconut, almond, jojoba, and avocado oil), belongs to a non-Newtonian pseudoplastic fluid.

Stability study
Oxidative stability of plum seed oil
Oil stability is very important to define its shelf-life and storage conditions. The plum seed oil was packed in a plastic container and exposed to daylight at room temperature (22 °C) for 3 months. The estimation of oxidative stability was carried out based on peroxide values (Table 2).

Table 2. The peroxide values expressed as mmol O₂/kg of the sample under ambient storage conditions

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plum seed oil</td>
<td>2.47</td>
<td>3.68</td>
<td>4.26</td>
<td>4.80</td>
<td>6.92</td>
<td>8.02</td>
<td>8.86</td>
</tr>
<tr>
<td>Topical formulation</td>
<td>2.21</td>
<td>3.42</td>
<td>3.94</td>
<td>4.71</td>
<td>5.98</td>
<td>7.67</td>
<td>8.11</td>
</tr>
</tbody>
</table>

The sudden increase in the peroxide value of plum seed oil was noticed during the first month of storage, which was most likely the result of primary oxidation due to the presence of air in the packaging itself. During the second and third months of oil storage, the peroxide value was slightly increased due to balancing between the formation and decomposition of peroxides to the secondary products. The plum seed oil was moderately susceptible to oxidation under ambient storage conditions. It had a satisfactory quality after 3 months of storage because the peroxide value was not higher than 10 mmol O₂/kg [35].

Chemical stability of the topical formulation
The chemical stability of topical formulation was estimated based on the change in peroxide value. The topical formulation was packed in a plastic container and stored under ambient conditions for 3 months. A higher increase in peroxide value was noticed in the oil compared with the topical formulation indicating the higher chemical stability of the designed formulation. The plum seed oil is less susceptible to oxidation processes after its incorporation into the topical formulation due to the proper selection of the ingredients.

Physical stability of the topical formulation
Evaluation of emulsion stability by centrifugation
The topical formulation and placebo showed good physical stability because the phase separation has not occurred due to mechanical stress.

Accelerated stability testing
The topical formulation and placebo were exposed to the stress conditions (4 °C and 60% RH, 30 °C and 60% RH, as well as 40 °C and 70% RH) for a month and then the pH values were measured (Table 3). The pH value of the placebo was slightly increased at 4 °C for 15 days, after that it gradually decreased until day 30. Under higher temperatures and relative humidities, the pH value of the placebo was gradually increased for 7 days and then decreased until day 30 with some deviations. At the end of this study, the pH values of the placebo at 4 °C, 30 °C, and 40 °C were 6.01, 5.83, and 5.60, respectively.
Table 3. The pH values of topical formulation and placebo under different storage conditions

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>4 °C</th>
<th>30 °C ± 60% RH</th>
<th>40 °C ± 70% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>TF</td>
<td>P</td>
<td>TF</td>
</tr>
<tr>
<td>0</td>
<td>5.54</td>
<td>5.38</td>
<td>5.38</td>
</tr>
<tr>
<td>1</td>
<td>5.35</td>
<td>5.42</td>
<td>5.79</td>
</tr>
<tr>
<td>7</td>
<td>6.11</td>
<td>5.61</td>
<td>6.01</td>
</tr>
<tr>
<td>15</td>
<td>5.26</td>
<td>5.49</td>
<td>5.92</td>
</tr>
<tr>
<td>30</td>
<td>6.01</td>
<td>5.41</td>
<td>5.83</td>
</tr>
</tbody>
</table>

P - Placebo, TF - Topical Formulation, RH = Relative Humidity

Unlike the placebo, the pH value of the topical formulation was gradually increased for 7 days and then decreased. After 30 days, the pH values of 5.41, 5.46, and 4.99 were noticed for the samples stored at 4 °C, 30 °C, and 40 °C, respectively. The decrease in the pH value of the topical formulation was probably the result of forming acid products under stress conditions. Since the results were in the suggested range, it can be concluded that the samples had adequate stability [36].

Antioxidant activity and functional stability of the topical formulation

The antioxidant activity of the oil was investigated in the concentration range of 0.78 - 50 mg/mL. The calculated IC$_{50}$ value of 9.39 mg/mL indicated the presence of phenolic compounds contained in the oil. Uluta and Özdemir [37] determined the antioxidant activity of 63.3 mg Trolox per 100 g of the plum seed oil from Turkey using a DPPH assay. After incorporation of the oil in the topical formulation, the determined IC$_{50}$ value of 9.33 mg/mL has not been statistically changed. The choice of ingredients was adequate because the antioxidant activity of the oil was saved after incorporation into the topical formulation. The functional stability of the oil and topical formulation at the different temperatures for 3 months was estimated based on the change in their antioxidant activity. Under investigated conditions, a significant change in the antioxidant activity did not occur (Fig. 4a). The decrease in antioxidant activity of the oil for different days and temperatures can be attributed to the experimental errors during the application of the DPPH assay (coefficient of variation of 5%). Also, the small change in the antioxidant activity was the result of the high stability of antioxidants.

The antioxidant activity of the oil in the topical formulation was not statistically changed at 4 °C and room temperature (22 °C) (Fig. 4b). Its activity was decreased by about 7% at the higher temperature of 40 °C. The reduction of antioxidant activity was probably a result of the decomposition of antioxidants.

The obtained results indicated that the polypropylene box with the threaded closure, double bottom, and intermediate cover is recommended for packaging the topical formulation. This packaging has a role to protect the product from the effect of humidity and oxygen. In this case, the packaging was compatible with ingredients, easy to use, lightweight, and economical. The topical formulation should be stored in a cool place or at controlled room temperature (22 °C) and avoid prolonged exposure to temperatures over 30 °C.

Determination of microbiological safety

The topical formulation and placebo were microbiologically safe because the presence of pathogenic bacteria was not confirmed. The total mesophilic aerobic microorganisms in 1 g of the sample were in the suggested range [22].

Conclusion

The rheological analysis showed that the plum seed oil belonged to the Newtonian fluid. The oil has absorbed the UVA, UVB, and UVC irradiations so that it can be used in topical formulations with a photoprotective effect. The topical formulation and placebo were white, with semi-solid consistency, uniform, and with a characteristic odor. They were easy to smear on the skin, leaving a greasy film that was not sticky. The results of the rheological analysis indicated that the uncategorized topical formulation had a pseudoplastic non-Newtonian behavior. The chemical stability of the designed formulation was satisfactory. The incorporated plum seed oil was less exposed to oxidation processes due to the proper selection of ingredients. The oil retained antioxidant ac-
tivity despite its incorporation in the topical formulation. The antioxidant activity was decreased by about 7% at higher temperatures and relative humidities. The storage of topical formulation was desirable in a cool place or at room temperature (22 °C), avoiding prolonged exposure to temperatures above 30 °C. Further studies will be focused on the qualification and quantification of bioactive compounds using the gas chromatographic method to estimate the possibility of application of plum seed oil for other purposes.

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Declarations

The authors report no conflict of interest.

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Advanced technologies


[35] Službeni list SCG br. 23/06, Pravilnik o uslovima u pogledu zdravstvene ispravnosti predmeta opšte upotrebe koji se mogu stavljati u promet, 2019. (in Serbian)

[36] Službeni list SFRJ br. 26/83, 61/86, 50/89, 18/91, 60/19, 78/19, Pravilnik o uslovima u pogledu zdravstvene ispravnosti predmeta opšte upotrebe koji se mogu stavljati u promet, 2019. (in Serbian)

Izvod

VALORIZACIJA ULJA SEMENA ŠLJIVE ZA RAZVOJ TOPIKALNE FORMULACIJE

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Cilj ovog rada bio je da se upotribei ulje semena šljive za pripremu topikalne formulacije za negu kože, s obzirom da ono može da poboljša elastičnost i spreči ubrzano starenje kože. Soxhlet ekstrakcija korišćena je za izolaciju ulja semena šljive pri menom n-heptana. Spektrofotometrijske metode su korišćene za određivanje foto protektivnog efekta i antioksidativne aktivnosti uzoraka. Toplo-toplo emulzifikacioni proces korišćen je za izradu topikalne formulacije na bazi ulja semena šljive. Antioksidativna aktivnost ulja semena šljive procenjena je na osnovu polovine maksimalne inhibitorne koncentracije (IC50 = 9,39 mg/mL). Nakon ugradnje ulja, IC50 vrednost od 9,33 mg/mL nije bila značajno promenjena. Viskozitet topikalne formulacije i ulja semena šljive bio je 1,56×10-6 mPa·s (pri brzini smicanja 5 s-1) i 60,48 mPa·s (pri brzini smicanja 50 s-1), respektivno. Reološka analiza pokazala je da su ulje semena šljive i topikalna formulacija Njutnovski i nenjutnovski pseudoplastični fluidi, respektivno. Formulacija apsorbuje svetlost u UV-Vis oblasti tako da se može koristiti kao v/u emulzioni fotozaštitni krem. Krem sa adekvatnom pH vrednošću bila je stabilna i mikrobiološki bezbedna za primenu na koži. Zbog upotrebe jeftinijih sastojaka, formulacija je prihvatljiva i pogodna za proizvodnju

Ključne reči: formulacija/stabilnost, emulzija, antioksidativna aktivnost, kontrola kvaliteta