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Organic Compounds of Natural Origin as Hypopigmentation Dermocosmetic Active Substances—In Vitro and In Vivo Study †

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Abstract: Organic compounds of natural origin (alpha-hydroxy acids (AHAs) and polyphenols (PPs)) are frequently used as a good and safe hypopigmentation substances in dermocosmetic products for lightening of skin hyperpigmentation. The aim of study was in vitro analysis (*HPLC analysis*) of content of AHAs and PPs and in vivo investigation (by biophysical methods) of hypopigmentation potential of dermocosmetic creamwith 6% of extract of wild apple fruit (*Mali sylvestris fructus*, (L.) Mill., Rosaceae), as a source of these organic compounds. Investigation revealed good content of AHAs and PPs, as well as, good lightening and anti-irritating effects on the skin after cream application, probably due to the synergistic effects between identified AHAs and PPs. Cream with organic compounds of natural origin, as active hypopigmentation substances, might be suitable for possible usage as dermocosmetic product for lightening of skin hyperpigmentation.

Keywords: bioactive organic compounds; wild apple fruit extract; dermocosmetic cream; hypopigmentation effect

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1. Introduction

There is a growing interest in the investigation of plants rich in bioactive compounds which can be used in dermocosmetic industry [1–5]. The use of plant extracts rich in natural compounds, primarily in polyphenolic compounds and alpha-hydroxy acids are very important in the prevention and/or treatment of skin changes and diseases caused by oxidative stress and photodamages. The use of cosmeceuticals in dermocosmetic industry, rich in these organic compounds of natural origin, represents a good basis for health improving and prevention of age-related chronic diseases [1,2,5–8].

Polyphenols, as most common natural organic compounds,possess antioxidant and anti-inflammatory properties and might participate in the prevention and treatment of many diseases, caused by oxidative stress and cell aging [9–11]. AHAs, as organic acids of natural origin, due to their antioxidant, moisturizing and lightening activity, have been used for skin moisturizing, reduction of wrinkles and deep chemical peeling of the skin [1,3,4,8,12]. Therefore, alpha-hydroxy acidsand polyphenols, as organic compounds of natural origin, could be used as good and safe hypopigmentation substances in dermocosmetic products for lightening of dark spots appearing on the skin due to oxidative stress.Because of all of these activity, AHAs and PPs are widely used and popular, and

dermocosmetic products containing these organic compounds are usually designed for home based application [8,12].

Wild apple fruit (*Mali sylvestrisfructus, Malussylvestris* (L.) Mill., Rosaceae), as a biomarker of territory of Serbia, is a potential source [1–4] of these organic compounds. Therefore, the aim of this study was in vitro analysis of content of PPs and AHAs and in vivo investigation of hypopigmentation potential of dermocosmetic cream with extract of investigated wild apple fruit.

2. Materials and Methods

2.1. Plant Materials

Wild apple fruit (*Malussylvestrisfructus* (L.) Mill., Rosaceae) was collected in September on Kopaonik mountain, Serbia and dried for three weeks at 22 ± 2 °C. The voucher specimen is kept at the Department of Botany, Faculty of Pharmacy, University of Belgrade, Serbia, under the number 3709HFF.

2.2. Methods

2.2.1. Preparation of Wild Apple Fruit Extract and Dermocosmetic Cream with Extract

A liquid extract of wild apple fruit (EWAF) was made in drug:extract ratio of 1:5 (w:V—weight:Volume) using 96% (*v*/*v*) ethanol as solvent and ultrasonic extraction as the extraction method [2]. Dermocosmetic cream was made with 6% of EWAF, as a source of AHAs and PPs, and stabilized by alkyl-polyglucoside emulsifier. Placebo sample (PL), as a control, was prepared in the same way, but without the extract. Investigated creams were prepared using ingredients and procedure used in our previous study (preparation of cream of o/w type) [1,3].

2.2.2. HPLC Analysis of Bioactive Organic Compounds

HPLC analysis of PPs and AHAs in investigated dermocosmetic cream was achieved by "fingerprinting" applying the method described by Pereira et al. [13] with necessary modification and preliminary preparation of the cream [2], 7 days after preparation.

2.2.3. In Vivo Investigation of Hypopigmentation Potential of Dermocosmetic Cream

In vivo hypopigmentation potential was investigated employing the biophysical methods on the skin of healthy volunteerswithout a history or clinical signs of dermatological diseases, after 7 days of cream application, after artificially induced skin hyperpigmentation using dihydroxyacetone. Investigation was carried out in a long double-blinded study by measuring melanin index (MI) and erythema index (EI) using MexameterMX (Multi Probe Adapter System MPA®9, Courage&Khazaka, Germany), according to the guidelines, earlier publications and our previous study [2,14,15]. Measurements were carried out baseline and after 7 days of cream application. The obtained results were analyzed using the software package SPSS 16.0.

3. Results and Discussion

3.1. HPLC Analysis of Bioactive Organic Compounds

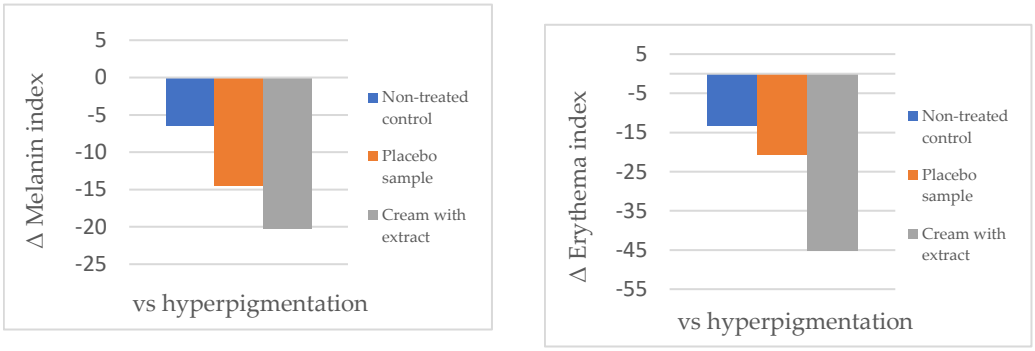
HPLC analysis has shown good content of organic compounds (AHAs and PPs) in investigated cream containing wild apple fruit extract, as a source of these organic compounds. Total content of identified PPs was 1.07 mg/100 g of cream. The most common PPs were phloridzin ((0.47 mg/100 g of cream) and chlorogenic acid (0.41 mg/100 g of cream).The presence of polyphenolic compounds(especially identified flavonoid and phenolic acid) is very important for the quality of the wild apple fruit extract, as well as for the quality of the obtained cream, containing extract. Polyphenols with their antioxidant and antibacterialactivitiescould show a beneficial effect on the human skin after local application of the products containing plant extract as bioactive substance [2,16,17]. Total

content of identified AHAs was 252 mg/100 g of cream. The most common AHAs were malic acid (19.75 mg/100 g of cream) and lactic acid (21.07 mg/100 g). Malic acid is one of the most important acids in wild apple fruit [2,3,18]. These AHAs, as a good hydration and exfoliant substances, potentially, could show positive effects on the skin after application [8]. Therefore, investigated dermocosmetic cream might be considered for potential use for care and treatementof skin with hyperpigmentation.

3.2. In Vivo Investigation of Hypopigmentation Potential

The research and promotion of organic compounds of natural origin exhibiting a cosmetic lightening effect on the skin are popular and current recently [1–4,8]. In vivo investigation in our study revealed good hypopigmentation potential of dermocosmeticcream, containing organic compounds of natural origin.

Application of investigated cream with PPs and AHAs, as active antioxidant and exfoliant agents, induced significant decrease of MI (−20,25 ± 41,61) and decrease of EI (−45,25 ± 23,54).The results of potential lightening effects of investigated creams, that is values of measured MI and EI, were shown in Figure 1.



(**a**) (**b**)

Figure 1. In vivo determined absolute changes of investigated parametersmeasured after 7 days of application of cream with extract and both controls compared to same parameters measured after hyperpigmentation: (**a**) absolute changes of melanin index; (**b**) absolute changes of erythema index.

The changes in the measured values of MI parameter after application of cream (Figure 1) indicated a colour change in treated skin (i.e., lighteningof the skin). Cream after application, generally, showed a whitening and anti-irritating effects compared to the non-treated control and placebo sample. After 7 days of application of cream with 6% of extract, containing PPs and AHAs, as a good antioxidant, moisturize and exfoliant substances [1–4,8,12], showed a decrease in MI (ΔMI was −20.25 ± 41.61) compared to MI after hyperpigmentation. Value of MI after 7 days of application of cream was almost similar to basal value, which might indicate a good lightening effect of cream after long-term application. In vivo investigation revealed decrease in EI (−45.25 ± 23.54) after cream application, which might indicate a good anti-irritating effect of the cream. Decrease in MI and EI after artificial induced skin hyperpigmentation was probably due to presence of AHAs and PPs in the cream. The obtained results might indicate a favourable potential of cream containing organic compounds of natural origin for skin hyperpigmentation lightening.

4. Conclusions

Formulated dermocosmetic cream, containing 6% of extract of wild apple fruit, as a source of organic compounds of natural origin, and stabilized by alkyl-polyglucoside emulsifier, demonstrated good lightening and anti-irritating effects on the skin, probably due to the synergistic effects between identified organic compounds (polyphenolic compounds and alpha-hydroxy acids). Cream with organic compounds of natural origin, as

active hypopigmentation substances, might be suitable for possible usage as dermocosmetic product for lightening of skin hyperpigmentation.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement:

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Conflicts of Interest: The authors declare no conflicts of interest.

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