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Antioxidant Activity of Plant-Derived Colorants for Potential Cosmetic Application

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Abstract: Application of plant-derived colorants in products, i.e., cosmetics or food, apart from imparting the desired color without harming the environment, may provide other benefits. Valuable ingredients in cosmetic formulations include antioxidants showing an advantageous effect on the skin by neutralizing free radicals that accelerate the aging process and cause skin defects. Antioxidant activity can be determined by chemical-based methods. The aim of this study was to determine the antioxidant activity of plant-derived colorants (purple and red colorant) by two methods: CUPRAC and DPPH free-radical scavenging activity. Antioxidant activity evaluation using both methods for colorants samples was also performed after 5, 15, 30, and 60 min of exposure to UVC irradiation. The results obtained by CUPRAC method were for purple and red colorant unexposed samples as follows: 6.87 ± 0.09 and 4.48 ± 0.14 mg/100 mg colorant expressed as caffeic acid equivalent, respectively. UVC treatment did not affect the results of the antioxidant activity for red colorant and for the purple one only a slight influence was observed. DPPH free-radical scavenging activity for unexposed samples was 70.06 ± 7.74% DPPH/100 mg colorant for the red colorant and 96.11 ± 3.80% DPPH/100 mg colorant for the purple one.

Keywords: antioxidant activity; CUPRAC method; DPPH free radical scavenging activity; plant-derived colorants; UVC irradiation

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1. Introduction

As cosmetic, food, or textile production and consumption are inseparable from the sense of sight, the appropriate color of products is required, which is usually related to the use of colorants. The application of plant-derived ones in various daily-use products such as cosmetics or food, apart from giving the desired color without harming the environment, may provide other benefits and give new properties [1]. For example, in cosmetic products, such essential ingredients include antioxidants, which have an advantageous effect on the skin by neutralizing free radicals that accelerate the aging process and cause damage to the skin. Natural antioxidants applications in skincare cosmetic formulations are the subject of many papers; a review article by Hoang et al. introduces their recent applications, challenges, and perspectives [2], many others concern the characterization of cosmetic formulations containing antioxidants of plant origin such as extracts obtained from *Castanea sativa* shells [3], red dragon fruit [4] or grape pomace, *Pinus pinaster* wood chips, *Acacia dealbata* flowers, and *Lentinus edodes* [5]. Many reports have established that various plant components containing vitamins, flavonoids, or other polyphenolic compounds show antioxidant properties but can also be a source of effective natural

dyes for cosmetic, food, or textile application. Moreover, the antioxidant activity of numerous plant extracts, including those showing coloring potential, have been documented; among others, the following were tested (usually by DPPH free radical scavenging activity or CUPRAC method): dragon fruit [6], black chokeberry, black-thorn and strawberry [7], avocado [8], radish [9], black carrot [10], red currant, black currant, raspberry, blackberry and elderberry [11], purple corn [12], and *Kalanchoe daigremontiana* [13]. Numerous studies prove that plant-derived colorants may comprise promising ingredients in natural cosmetics with both coloring and antioxidant potential. When discussing antioxidant application in cosmetics, it is also worth mentioning that new sources of these compounds are sought not only among plant extracts but also among plant by-products from the industry, for example avocado, grape, opuntia, wine, and brewery by-products, which, aside from their antioxidant properties, sometimes also show dyeing ability [14–19]. Such an approach meets the current requirements of sustainable development and leads to the creation of clean-label products, for instance, cosmetics. Numerous studies concern the application of plant-derived substances in cosmetic formulation. As an example, Hennessey-Ramos et al. in their research applied a colorant extracted from plant waste (avocado seeds) as an ingredient in liquid-soap formulation. Extracts were previously examined for antioxidant properties using the DPPH free-radical scavenging activity, whereby water extract was characterized by the highest antioxidant activity. Furthermore, applied colorant was stable in liquid-soap formulation during one month of storage [8]. Another study indicated that the betacyanin pigment obtained from red dragon fruit can successfully be applied in lipstick formulation, both as an antioxidant, characterized by high antioxidant activity confirmed by DPPH free radical scavenging activity, and as a colorant, leading to the creation of a product with acceptable quality and low lead content [4].

Under the influence of light, plants are able to synthesize and accumulate secondary metabolites, such as phenolic compounds, most of which show antioxidant properties and thus play beneficial roles in human health [20]. Therefore, for various plants, the effect of a sunlight component, i.e., ultraviolet radiation on secondary metabolites production, antioxidant activity, and total phenolic content, was examined [21–24]. Many studies confirmed that UVC irradiation affects the content of antioxidants and other bioactive compounds in fresh fruits and vegetables, but an experiment by Papoutsis et al. investigated the effects of UVC radiation on dried plants. The tested material was dried lemon pomace powder, which was treated with UVC radiation in order to increase the phenolic content, and thus antioxidant activity, before its extraction and application. As expected, the UVC treatment positively affects the quality of the dried lemon pomace powder. The total flavonoid content, total phenolic content, and antioxidant capacity were significantly higher after the application of UVC radiation, which was confirmed using the CUPRAC and FRAP methods. The results indicated that UVC treatment can beneficially influence the content of bioactive substances extracted from dried lemon pomace [25]. As several studies have shown, the treatment of UVC irradiation can contribute to increasing the number of antioxidants in plants and plant extracts and thus enhance the antioxidant activity [26–34].

UV light, ionizing radiation, air pollution, and smoking are just a few examples of factors causing free-radical formation damaging the DNA structure; they contribute to the generation of skin defects and affect the aging process. This is why it is so important to provide the skin and other susceptible natural systems with substances capable of scavenging free radicals. Such antioxidant-rich raw materials are numerous plant components containing various compounds among other vegetable dyes including anthocyanins, carotenoids, betalains, flavonols, and quinones with documented antioxidant properties. All these substances are of increasing interest as they represent a high potential for preventing the aging process and increasing skin health, as confirmed in literature [35].

Due to the high content of various biologically active compounds, plant-derived colorants are also characterized by antioxidant properties, thus the aim of the study was to determine the antioxidant activity by the CUPRAC method and the DPPH free radical

scavenging activity of two plant-derived colorants consisting of a mixture of various plant extracts. Antioxidant activity evaluation by both methods for colorants samples was also performed after 5, 15, 30, and 60 min of exposure to UVC irradiation.

2. Materials and Methods

2.1. Materials

Two commercially available colorants for food applications consisting only of fruit and vegetable concentrates obtained by the physical manufacturing process conducted with water were used in the examination: red and purple. Both colorants were in liquid form and were obtained from EXBERRY by GNT (GNT Group B.V., Mierlo, The Netherlands). Moreover, in determining the antioxidant activity, different reagents were used; neocuproine, caffeic acid, DPPH, and trolox were purchased from Sigma (Poznań, Poland), whereas ethanol, copper chloride, and ammonium acetate from Chemland (Stargard, Poland). All reagents were of analytical grade.

2.2. Sample Preparation

The analyzed samples of red and purple colorants were prepared by dissolving appropriate amounts of colorants in distilled water resulting 0.1% solutions. Next, samples were irradiated at a distance of 5 cm from the UV lamp (ULTRAVIOL NBV 15, Ultra-Viol, Zgierz, Poland) emitting mainly UVC at wavelength 254 nm and intensity of radiation 21.5 W/m^2 during 5, 15, 30, and 60 min.

2.3. Determination of Antioxidant Capacity

2.3.1. Determination of Antioxidant Activity by CUPRAC (Cupric Reducing Antioxidant Capacity) Method

To examine colorants' antioxidant activity by the CUPRAC method, the following reagents were used: neocuproine ethanol solution (0.0075 M), copper chloride solution (0.01 M), ammonium acetate buffer (1.0 M, pH = 7.0), and ethanolic solution of caffeic acid as standard (0.048 mg/mL). Absorbance was measured with the UV-Vis double-beam spectrophotometer Shimadzu UV-1601 (Shimadzu, Kyoto, Japan) in plastic cuvettes [36]. Before measurements, a calibration curve was prepared. The method of making the standard curve and calibration curve is included in the Supplementary Materials (Figure S1).

Sample Analysis

Samples were prepared according to above-described method. Instead of caffeic acid, 0.1 mL of 0.1% colorant solution was added, and the volumetric flask was refilled with distilled water. The measurements were made on unexposed and irradiated samples for 5, 15, 30, and 60 min, respectively. Samples were placed in darkness for 0.5 h, and then the absorbance was performed at a wavelength of λ = 450 nm against the blank (copper (II) chloride, neocuproine solution, ammonium acetate buffer, and distilled water). Three independent measurements were made for each sample.

2.3.2. Determination of Antioxidant Activity by DPPH Assay

To study the colorants' antioxidant activity by the DPPH assay, the following reagents were used: 0.012% 2,2′-diphenyl-1-picrylhydrazyl (DPPH) ethanolic solution and 0.1 mM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) ethanolic solution. Absorbance was measured with the same UV-Vis double-beam spectrophotometer in plastic cuvettes [36]. Before measurements, a calibration curve was prepared. The procedure for making the standard curve and calibration curve is included in the Supplementary Materials (Figure S2).

Sample Analysis

Samples were prepared according to above method, and then, instead of trolox solution, 0.1 mL of colorant solution was pipetted. Measurements were made on unexposed and irradiated samples for 5, 15, 30, and 60 min, respectively. Three measurements for each sample were performed. Before absorbance measurements at a wavelength of $\lambda = 517$ nm, samples were stored in darkness for 15 min. The mixture of ethanol and DPPH solution was prepared as a blank test, and ethanol was used as a reference. The blank test was measured separately for all samples. [36].

3. Results

3.1. Determination of Antioxidant Activity by CUPRAC Method

Using this method, which is based on SET (single-electron transfer) mechanism, a copper (II) complex is reduced to a copper (I) complex under the influence of antioxidants present in the examined sample. Copper (I) forms a yellow-orange complex with neocuproine (2,9-dimethyl-1,10-phenanthroline), with the highest absorbance at wavelength 450 nm. The absorbance of this colored complex is measured spectrophotometrically. The copper-neocuproine complex's redox potential, which equals 0.6 V, is higher than the standard one of −0.16 V, which contributes to the efficiency and rate of polyphenol oxidation. The CUPRAC method is appropriate for examining the antioxidant activity in plant-derived samples, both hydrophobic and hydrophilic antioxidants. The antioxidant activity is expressed as the amount of caffeic acid equivalent in the sample. The linearity of the method was from 0.24 to 2.88 mg/L. The linear regression equation of the calibration curve was obtained as follows: $y = (0.3375 \pm 0.0111)x + (0.2159 \pm 0.0193)$ with $r^2 = 0.9992$. The calculated LOD and LOQ were 0.12 mg/L and 0.28 mg/L, respectively.

The antioxidant activity expressed as a caffeic acid equivalent was calculated in both tested colorants: red and purple after 0, 5, 15, 30, and 60 min of exposure to UVC irradiation. The results of the antioxidant activity from CUPRAC method obtained for red colorant after 0, 5, 15, 30, and 60 min exposure to UVC irradiation were as follows: 4.48 ± 0.14; 4.12 ± 0.29; 4.31 ± 0.25; 4.06 ± 0.21, and 4.16 ± 0.35 mg/100 mg colorant expressed as caffeic acid, respectively. On the other hand, the results obtained for purple colorant after 0, 5, 15, 30, and 60 min exposure to UVC irradiation were as follows: 6.87 ± 0.09; 7.11 ± 0.21; 8.23 ± 0.74; 7.40 ± 0.25; 6.80 ± 0.45 mg/100 mg colorant expressed as caffeic acid, respectively (Figure 1).

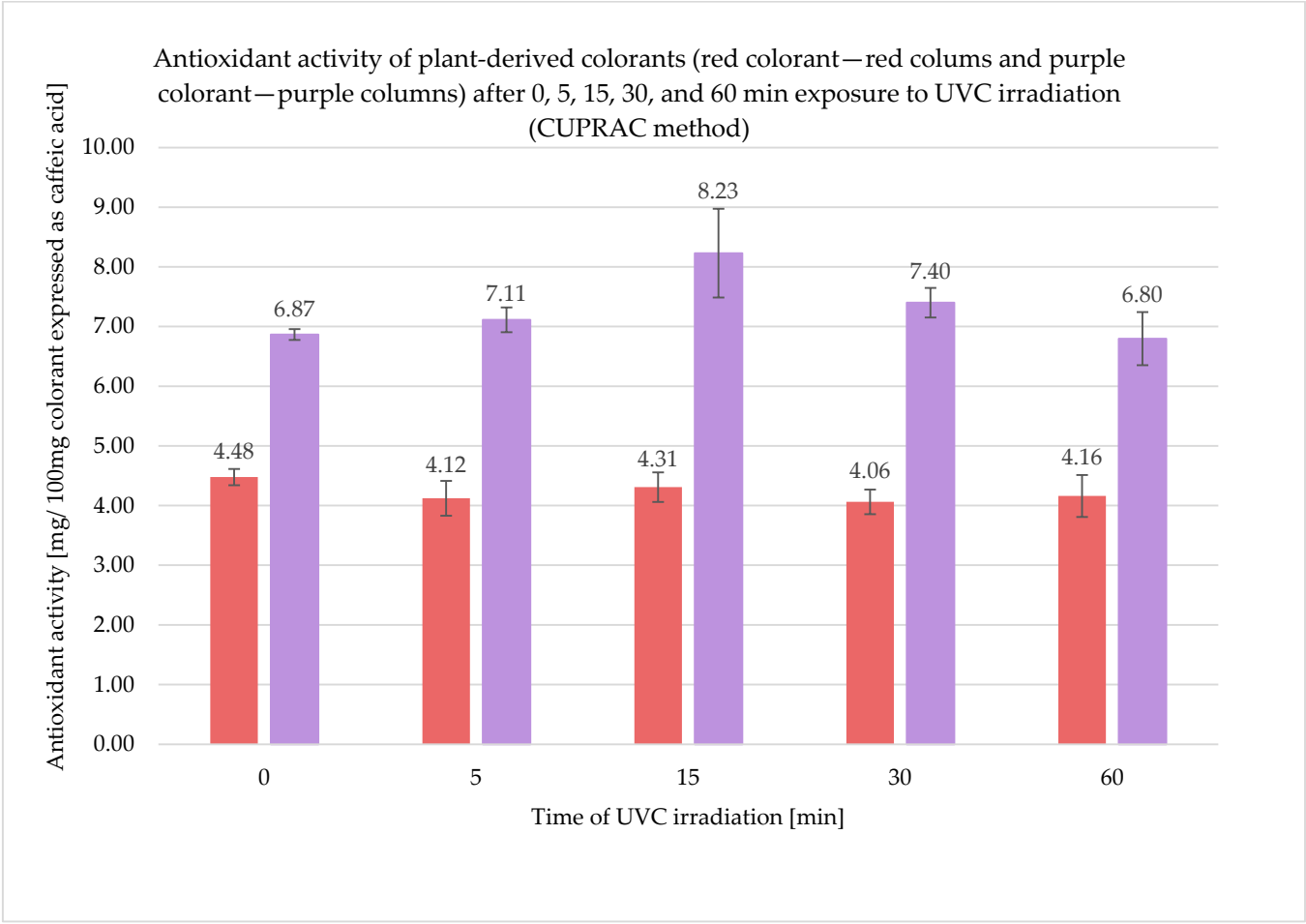

Figure 1. Results of antioxidant activity of plant-derived colorants (red and purple) after 0, 5, 15, 30, and 60 min exposure to UVC irradiation (CUPRAC method).

3.2. Determination of Antioxidant Activity by DPPH Assay

The antioxidant activity of food products or plant-derived raw materials is commonly examined by this method, in which a stable, purple ethanolic solution of DPPH radical is used, indicating the maximum absorption at a wavelength equals 517 nm. This method is based on spectrophotometric measurements: the absorbance decrease of the examined sample containing antioxidants is a consequence of the color change from purple to yellow because of the DPPH reduction. The measured antioxidant activity of the colorant samples was expressed as a percentage of reduction of the DPPH radical. In this method, trolox was used as the standard. The calibration curve was prepared in the range 0.01–0.10 mmol trolox/L. The linear regression equation of the calibration curve was obtained as follows: $y = (730.2 \pm 39.13)x + (0.72 \pm 2.375)$, with r^2 value 0.9985. The calculated LOD and LOQ were 0.0061 mmol trolox/L and 0.0147 mmol trolox/L, respectively.

Antioxidant activity expressed as a percentage of the reduction of the DPPH radical for both tested colorants was calculated, and the results for the unexposed samples were as follows: for the red colorant 70.06 ± 7.74% DPPH/100 mg colorant and for the purple colorant 96.11 ± 3.80% DPPH/100 mg colorant (Figure 2). After 5, 15, 30, and 60 min exposure to UVC irradiation, the results for the red colorant were: 65.48 ± 10.97, 71.51 ± 3.71, 69.58 ± 7.07, and 67.41 ± 2.30% DPPH/100 mg colorant and for purple were 79.59 ± 15.46, 87.67 ± 4.91, 77.30 ± 6.37 and 86.82 ± 4.17% DPPH/100 mg colorant, respectively.

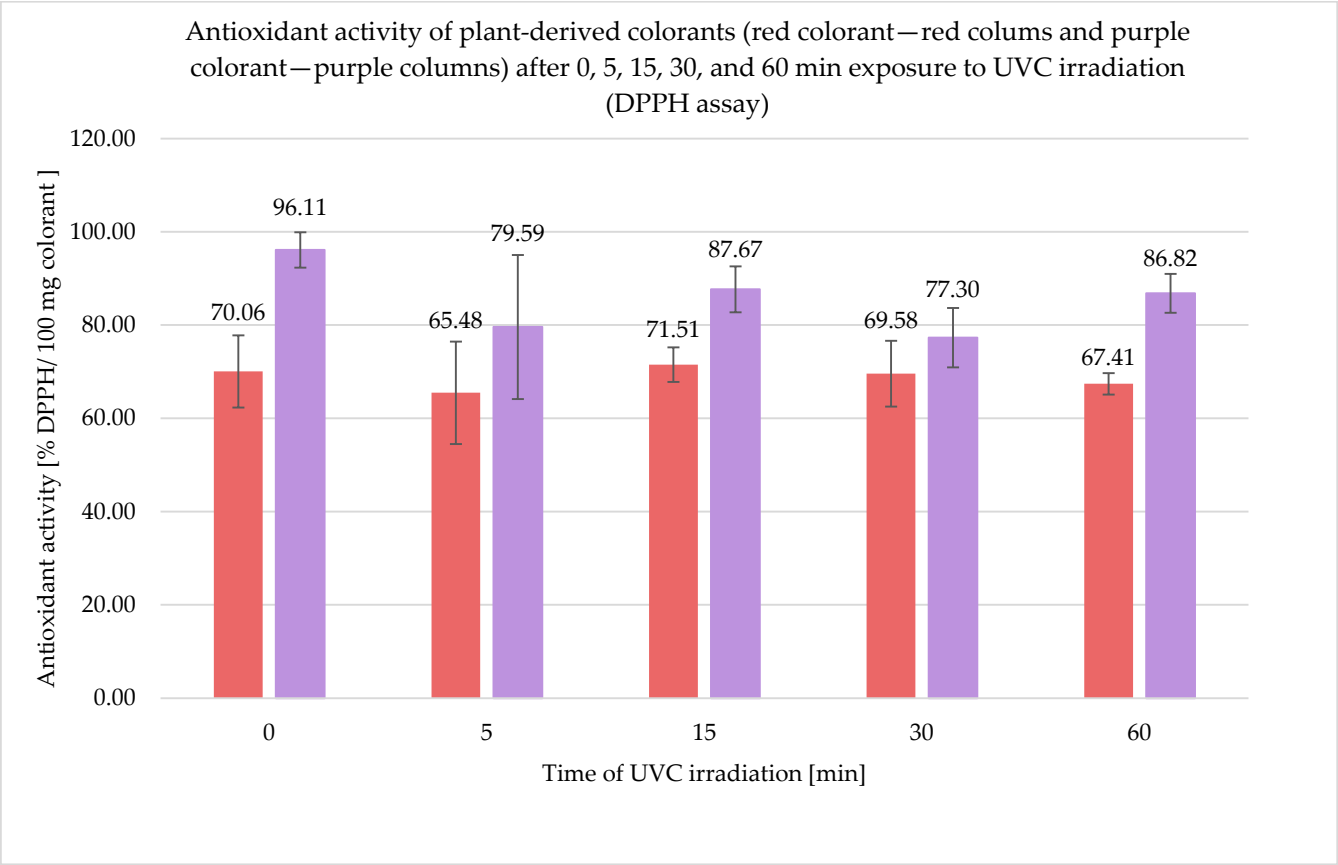

Figure 2. Results of antioxidant activity of plant-derived colorants (red and purple) after 0, 5, 15, 30, and 60 min exposure to UVC irradiation (DPPH assay).

4. Discussion

Examination of the antioxidant activity using the CUPRAC method showed that the purple colorant was characterized by a significantly greater antioxidant activity when compared to the red one. UVC treatment did not affect the antioxidant activity of the red colorant, and the results for the unexposed and irradiated samples were similar; for the purple colorant after 15 min of UVC irradiation, the antioxidant activity slightly increased and subsequently, after 30 and 60 min, decreased, and the results were similar to the initial value. The results of the antioxidant activity determined using the DPPH assay were also greater for the purple colorant than for the red one. UVC irradiation did not significantly affect the antioxidant activity of the red colorant, but, for purple colorant, a decrease in the antioxidant activity was observed.

According to research conducted by Comert et al., there is a relationship between antioxidant activity and the color of fruits and vegetables. Fruits and vegetables can also be classified based on total antioxidant capacity (TAC), which provides information about the general concentration of bioactive ingredients, such as phenolic compounds, carotenoids, and many more, some of which are pigmented [37]. A study indicated that a color's hue can be related to TAC in fruits and vegetables. Red-, blue-, and magenta-colored ones were characterized by the highest antioxidant properties. However, it is worth focusing on researching fruits, vegetables, and other plants as sources of dyes responsible for listed colors to ensure the highest possible content of antioxidants in further product formulation. Nevertheless, there is still an insufficient amount of research focusing on investigating the relationship between TAC and color [37].

The development of cosmetic products should also focus on such a selection of components to ensure skin protection and to prevent damage caused by free radicals. Moreo-

ver, the preparation of cosmetic formulations preceded by an antioxidant activity examination of particular ingredients contributes to its effectiveness. As the intensive search for plant-based cosmetic ingredients with a strong emphasis on natural antioxidants is observed, many plant extracts are evaluated for their valuable properties and beneficial influence on the skin. Simultaneously, new plants that are a source of active compounds such as antioxidants are constantly sought. For instance, in studies conducted by Mota et al., *Araucaria angustifolia* seed extracts were used to prepare various types of cosmetic formulations: W/O emulsion, O/W emulsion, and nonionic gel. Previously, extracts were tested for antioxidant activity using DPPH and ABTS (2,2′-Azino-bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) assay. Due to the fact that different factors, such as storage conditions or type of formulation and its pH values, determine antioxidant activity, this parameter examination was also performed for finished cosmetic formulations. The antioxidant activity of the final products was confirmed [38,39].

It should be also emphasized that one human cell is exposed to more than 100 oxidative hits a day from hydroxyl radicals and other reactive species [40]. Oxidative stress contributes to the initiation of the mechanism causing changes in proteins, lipids, or inflammation and the immunosuppression process. Permanent modification of genetic material resulting from these "oxidative damage" incidents represents the first step of carcinogenesis involved in mutagenesis and aging. DNA alterations caused by radicals are removed by specific and non-specific repair mechanisms. However, a failure to repair DNA damage could result in mutations such as base substitution and deletion, also leading to carcinogenesis. Botanical compounds with anti-carcinogenic and anti-mutagenic properties can use multiple mechanisms to maintain homeostasis and balance interrupted by reactive oxygen species (ROS) [41]. Those compounds are able to stimulate immune and anti-inflammatory responses, changes in gene expression, the detoxification or modulation of antioxidants. Skin-protection mechanisms include the production of antioxidants and other compounds able to absorb UV radiation [42]. Human skin, because of the presence of antioxidant enzymes such as superoxide dismutase, glutathione, or catalase, can protect its cells from free radicals and, as a consequence, from damage they induce. However, antioxidant compounds such as vitamins A, C, and E, and other molecules, which skin possesses, influence the process of its ageing by protecting sensitive biological molecules from oxidizing by free radicals or by reducing the creation of free radicals and quenching formed ones. Nevertheless, different factors, such as environmental ones, for instance, UV irradiation or the natural process of ageing of organisms, can decrease their amount [43,44]. Thus, antioxidants contribute to strengthening the endogenous skin capacity and supporting the neutralization of the ROS-induced process formed under the influence of external factors [41]. To protect the skin, it becomes necessary to apply antioxidants topically onto the skin and provide them with the correct diet. When using plant-derived ingredients in cosmetics with antioxidant properties, one can expect that oxidative damage in human skin can also be reduced [44].

The plant-derived colorants tested in this study may have the potential to impart antioxidant properties or enhance the effect of other antioxidants present in skincare formulations, and, in the same manner, they may have the potential to impart color or enhance the effect of other colorants in cosmetic products. The individual ingredients of the cosmetic formulation and the finished cosmetic product should have confirmed the effectiveness, including the antioxidant properties to be a valuable product for the skin, maintaining it in healthy conditions. This should be taken into account when creating and researching natural skincare cosmetic products based on plant extracts. Confirmation of these properties can be the determination of antioxidant activity, defined as the ability to reduce or inhibit compounds with high oxidation–reduction potential through the use of chemical-based methods such as CUPRAC or DPPH free-radical scavenging activity [45]. However, as various methods provide different results related to the antioxidant activity of plant extracts due to the influence of many factors such as the presence of other components in tested samples, antioxidant distribution or complexity of mechanism, thus

multiple assay methods should be considered simultaneously to evaluate this parameter objectively [46]. Numerous results of various methods of antioxidant capacity evaluation in common fruits are summarized and presented in a review paper by Lu et al., which confirms the complex nature of the study. As vegetable raw materials are rich in many active compounds, chemical methods of antioxidant capacity determination should be still developed [46].

5. Conclusions

The antioxidant activity of plant-derived colorants was confirmed using DPPH and CUPRAC assays. The addition of natural colorants of plant origin may beneficially influence the quality and effectiveness of cosmetic products, thus following the current dominant trend in the cosmetic industry which draws inspiration from the richness of nature and aims to create cosmetic formulations with an application of plant-derived ingredients with minimal impact on the environment. Therefore, they can be valuable cosmetic ingredients and potentially act as effective dyes and antioxidants, but further research is required to create appropriate cosmetic formulations containing plant-derived colorants with antioxidant properties. Conducting extensive studies is necessary, as those colorants are more complicated in application, because they tend to be more variable in the shade, less stable under different conditions, and may introduce undesirable odors to formulations in comparison to synthetic ones [47].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics9040081/s1, Figure S1: Calibration curve for determination of antioxidant activity by CUPRAC method; Figure S2: Calibration curve for determination of antioxidant activity by DPPH method.

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