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Antioxidant, UV Protection, and Antiphotoaging Properties of Anthocyanin-Pigmented Lipstick Formulations

ALEXANDRA WESTFALL, GREGORY T. SIGURDSON, and M. MÓNICA GIUSTI, *Department of Food Science and Technology, The Ohio State University, Columbus, OH (A.W., G.T.S., M.M.G.)*

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Synopsis

Consumer demand for foods and cosmetics containing naturally derived ingredients has been increasing. Naturally derived anthocyanins (ACNs), from fruits and vegetables, were previously demonstrated to provide a wide range of hues as lipstick colorants with high stability. Therefore, the objective of this study was to evaluate the use of ACNs as bioactive pigments in lipstick formulations. Commercially available sources of nonacylated and acylated derivatives of the six major ACN aglycones were incorporated into a commercial lipstick base. The ACN-containing lipsticks were evaluated for their ability to act as ultraviolet (UV) absorbers [a source of sun protection factor (SPF)], free radical scavengers against 2,2-diphenyl-1-picrylhydrazyl (DPPH), and preventers of melanin formation through tyrosinase inhibition. All formulas showed increased UV absorption over the lipstick base, and acylated ACNs contributed to the highest *in vitro* SPF (UVB) values (≥15.8) in formulations. All formulas exhibited high inhibition of DPPH free radicals and inhibition of melanin production by tyrosinase at microgram per milligram concentrations similar to or less than kojic acid (2.41 ± 0.06 μg/mg). This is physiologically relevant because lipstick use is on average 24 mg/day. This study suggests the potential for ACNs to be used as biologically active ingredients in lipstick formulations by acting as antioxidants and UV-protection and antiaging compounds.

INTRODUCTION

This is the second article in a series of articles aimed at describing the use of anthocyanins (ACNs) in lipsticks.

Anthocyanins are water-soluble flavonoids responsible for many of the red, purple, and blue colors found in plants. In nature, they occur as glycosylated derivatives of six primary aglycones: cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin (1). The variations in chemical structures of these anthocyanidins (or aglycones) occur at

Address all correspondence to M. Mónica Giusti at giusti.6@osu.edu.
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the 3′ and 5′ positions of the B-ring. Acylation of the sugar moiety with various aromatic and aliphatic acids may also occur (2). The biological activity of ACNs is largely dependent on these variations in their chemical structures.

The intense and attractive colors produced by ACNs have prompted interest in their uses as colorants in the food industry (3). Their potential to act as powerful antioxidants and for use in disease prevention has also been gaining increased attention (4). They have long been considered as strong antioxidants, with the ability to scavenge free radicals and terminate chain reactions demonstrated in many *in vitro* assays (5,6). Their protective effects against oxidative stress–induced damage and regulation of redox signaling pathways have also been demonstrated (7–9).

Unabsorbed ACNs have also been shown to potentially act as chemopreventive agents topically in the gastrointestinal tract by preventing oxidative damage to the mucosal lining (10). This topical activity may also translate to similar benefits when applied to the skin in an appropriate vesicle, such that they are able to react with damaging reactive oxygen species (11). Recent investigations into ACNs' potential to prevent oxidative damage to the skin by ultraviolet (UV)-induced erythema, skin cancer, and photoaging have also demonstrated a protective effect *in vitro* and *in vivo* (12–15). Photoaging is defined as premature aging of the skin caused by repeated exposure to UV radiation, with signs including general skin deterioration and dark spots or abnormal skin pigmentation (16). Anthocyanins have shown a potential to trigger a regenerative effect on the skin (17). Moreover, they have been shown to improve psoriatic lesions *in vitro* and alleviate atopic dermatitis *in vivo* (18,19).

Few studies have investigated the protective effects of ACNs when incorporated into matrices for topical delivery. However, two recent studies positively demonstrated the biological activity of ACNs when concentrated onto protein-rich matrices (20) and also when incorporated into ultradeformable liposomes (11). The results of these studies were promising; however, the technology used does not easily translate into practical or commonly used delivery systems for bioactive ingredients, such as incorporation into pre-existing cosmetic formulas. In cosmetic formulations, the chemical and potential bioactive properties of ACNs could translate into antiphotoaging properties.

When incorporated into some cosmetic formulations, the color imparted by these pigments and their stability must also be considered. We recently reported ACNs to be successful colorants of lipstick formulations with stability comparable with synthetic pigments, determined to exceed 2 years based on the accelerated testing conditions (21). Therefore, the aim of this study was to investigate the potential biological activity of ACNs when incorporated into lipstick formulations as a source of color and as an active ingredient, with ACNs selected based on our previous work.

MATERIALS AND METHODS

MATERIALS

Elderberry, purple carrot, purple sweet potato, and red radish dried extracts were provided by DD Williamson & Co., Inc. (Louisville, KY), and the purple corn and red grape skin dried extracts were provided by Artemis International (Fort Wayne, IN). The base of the lipstick formulations was purchased from MakingCosmetics, Inc. (Snoqualmie, WA);

ingredients for the base were as follows: triglyceride, coconut oil, octyldodecanol, ozokerite wax, polyisobutene, castor oil, isopropyl palmitate, microcrystalline wax, lanolin oil, microcrystalline wax, synthetic wax, glycerin, DL-alpha tocopherol, and butylated hydroxytoluene (BHT). Black lip balm containers were purchased from a local company, Bulk Apothecary (Streetsboro, OH). Compounds used were gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), BHT, mushroom tyrosinase, L-3,4-dihydroxyphenylalanine (L-DOPA), and kojic acid, purchased from Sigma-Aldrich (St. Louis, MO). Reagents used were ethanol and methanol, and were purchased from Fisher Scientific, Inc. (Fair Lawn, NJ).

LIPSTICK SAMPLE FORMULATIONS

Formulations were prepared according to our previous formulation (21) and based on recommendations in the Society of Cosmetic Chemists Monograph Number 8: Lipstick Technology (22). All dried extracts were incorporated as 8% of the final weight (w/w) of each lipstick formulation based on preliminary data (21). Dried extracts were weighed and ground in castor oil at a 1:3 ratio (pigment:oil) by mortar and pestle. Silica was included at 1% of the final weight (w/w) to increase uniformity in the final products. The lipstick base was heated in a water bath at 70°C, and the preground pigment extracts were then added to the hot lipstick base and homogenized. The lipstick formulas were then poured directly into lip balm containers and cooled at 4°C until completely solid.

CHARACTERIZATION OF ACNS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

The ACNs of the extracts used in these lipstick formulations were evaluated by reverse-phase high-performance liquid chromatography. The system (Shimadzu Corporation, Columbia, MD) was composed of an LC-20AD prominence liquid chromatograph and an SPD-M20A prominence diode array detector coupled to an LCMS-2010 mass spectrometer (Shimadzu Corporation). LCMS solution Ver 3.30 software was used to collect and evaluate data. Anthocyanin separation was achieved on a reversed-phase 3.5-µm Symmetry C18 column (4.6 × 150 mm; Waters Corp., MA) fitted with a 4.6 × 150-mm Symmetry 5 microguard column (Waters Corp.) with a binary gradient of 4.5% (v/v) formic acid in water (solvent A) and 100% acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Solvent gradient followed 10–30% B from 0 to 30 min. Spectral information was collected from 260 to 700 nm, and elution was monitored at 280 and 520 nm. For MS analysis, 0.2 mL/min flow was diverted to the MS and ionized under positive ion conditions using an electrospray probe. Data were initially monitored using a total ion scan from m/z 200 to m/z 1,200 and then with selective ion monitoring at m/z 271 (pelargonidin), m/z 287 (cyanidin), m/z 301 (peonidin), m/z 303 (delphinidin), m/z 317 (petunidin), and m/z 331 (malvidin).

SPECTROPHOTOMETRIC ANALYSIS OF TOTAL MONOMERIC ACN CONTENT

The total monomeric ACN content for the extracts and lipstick formulations was measured in 1-cm cuvettes using a spectrophotometer (UV-2450 spectrophotometer; Shimadzu, Kyoto, Japan) using the pH differential method as described by Giusti and

Wrolstad (23). Anthocyanins were extracted from the lipsticks using equal parts: acetone (70%), acidified ethanol (0.01% HCl), and acidified deionized distilled water, followed by phase partition with chloroform as previously described (21). Sample extracts were diluted in pH 1.0 buffer (KCl-HCl) and in pH 4.5 buffer (sodium acetate) and equilibrated for 15 min.

The ACN content, expressed as cyanidin-3-glucoside, was determined using the following equation:

$$\text{Total monomeric anthocyanin content}\left(\frac{\text{mg}}{\text{L}}\right)=\frac{\text{Abs}_{\text{pH1.0}}-\text{Abs}_{\text{pH4.5}}\times\text{DF}\times 1{,}000}{\varepsilon\times d} \quad (1)$$

where DF = dilution factor, ε = molar absorptivity of 26,900, and d = path length (1 cm), and the molecular weight of the monomeric ACN content is 449.2.

TOTAL PHENOLIC CONTENT

The Folin–Ciocalteu method was used to estimate the total phenolic content of the formulas containing ACNs, based on the methods described in the literature (24). Lipstick formulas were dissolved in methanol and briefly sonicated. After sample preparation, they were equilibrated in the dark at room temperature for 2 h before measuring absorbance readings at 765 nm with a spectrophotometer (UV-2450 spectrophotometer; Shimadzu). Analyses were conducted in triplicate. Gallic acid was used as a positive control to determine a standard curve for the test. Linear regression was used to determine a standard curve for the absorbance at 765 nm of the gallic acid solutions (R^2 = 0.99). The results were reported as mg polyphenolic/L of extract solution as gallic acid equivalents (GAEs).

IN VITRO UV ABSORPTION AND SUN PROTECTION FACTOR (SPF) CALCULATION

In vitro SPFs for the lipstick formulas were determined based on the methods described by Sayre et al. (25) and Dutra et al. (26) with modifications based on the Cosmetics Europe-The Personal Care Association revised method (27). Aliquots of each solution were then pipetted into a UV microwell plate, in eight replications. Absorbance readings were measured by using a SpectraMax190 plate reader (Molecular Devices, Sunnyvale, CA) across the 290- to 400-nm UV wavelength range, at 1-nm increments and blanked against ethanol. The absorbance values were then averaged, and the standard deviations (SDs) were calculated for each sample. The *in vitro* SPF values were determined according to the following formula:

$$\text{SPF}_{\text{spectrophotometric}}=\text{CF}\times\sum\text{EE}(\lambda)\times I(A)\text{Abs}(\lambda) \quad (2)$$

where CF = correction factor (10), EE (λ) = erythema action spectrum (CIE 1987), I (λ) = spectral irradiance received from the UV source, and Abs (λ) = spectrophotometric absorbance values at wavelength λ. The EE × I values are constants and were determined by Sayre et al. (25).

DPPH FREE RADICAL SCAVENGING ASSAY

The DPPH free radical scavenging assay was used as a measure of potential antioxidant capacity as described previously (28,29), with modifications described by Montanari et al. (11) for use with cosmetics. Methanolic solutions containing the pigments recovered from the lipsticks, the dried pigment extracts at concentrations equal to their contents in the lipstick formulations, and also BHT were used for the testing of antioxidant capacity. DPPH dissolved in methanol with serial dilutions was used to obtain a standard curve for DPPH based on concentration. To the DPPH solution, methanol solutions containing either BHT, the lipstick base, the dried extract, or the ACN formulas were added in triplicate. Samples were equilibrated for 30 min in the dark at 20°C, and absorbance at 515 nm was measured by using a SpectraMax190 plate reader (Molecular Devices) with 30-min equilibration time. Then the samples were blanked against wells containing only methanol.

The average of the absorbance readings was then used to determine the DPPH inhibitory percentage and 50% inhibitory concentration (IC_{50}) of each sample. The inhibitory percentage of each sample was determined based on the following equation:

$$I\% = \left(\frac{\mathrm{Abs}_{(\mathrm{DPPH})} - \mathrm{Abs}_{(\mathrm{Sample})}}{\mathrm{Abs}_{(\mathrm{DPPH})}} \right) \times 100 \tag{3}$$

IC_{50} values are defined as the concentration necessary to inhibit 50% of the free radical (11). The IC_{50} values of each sample were determined using linear regression of the absorbances at different concentrations. The readings for the ACN formulas were corrected for the absorbance of their respective dried extract in the methanol solution. Samples were tested again after 4 weeks of storage at 4°C, and new IC_{50} values were calculated and compared with the original values.

ANTITYROSINASE ASSAY

Tyrosinase inhibition assay was performed as previously reported (20). Methanolic lipstick extracts and mushroom tyrosinase (5 U) were gently mixed in phosphate buffer (pH 6.5, 50 mM) and incubated for 10 min in a 96-well plate. Absorbance was measured at 475 nm on a SpectraMax190 plate reader (Molecular Devices).

Results were compared with the negative control (phosphate buffer) and positive control (kojic acid). The percentage tyrosinase inhibition was calculated as follows:

$$I\% = \left(\frac{\mathrm{Abs}_{(\mathrm{Control})} - \mathrm{Abs}_{(\mathrm{Sample})}}{\mathrm{Abs}_{(\mathrm{Control})}} \right) \times 100 \tag{4}$$

Results are presented as mean (n = 8) ± SD. IC_{50} values were predicted using linear regression and are expressed in μg/mL.

STATISTICAL ANALYSIS

Results of the Folin–Ciocalteu method, DPPH free radical scavenging assay, and antityrosinase activity were analyzed using two-way ANOVA and regression modeling (α = 0.05)

using Minitab statistical software version 16 (State College, PA) and GraphPad Prism version 6 (La Jolla, CA).

RESULTS AND DISCUSSION

CHARACTERIZATION OF ACNS IN LIPSTICK FORMULATIONS

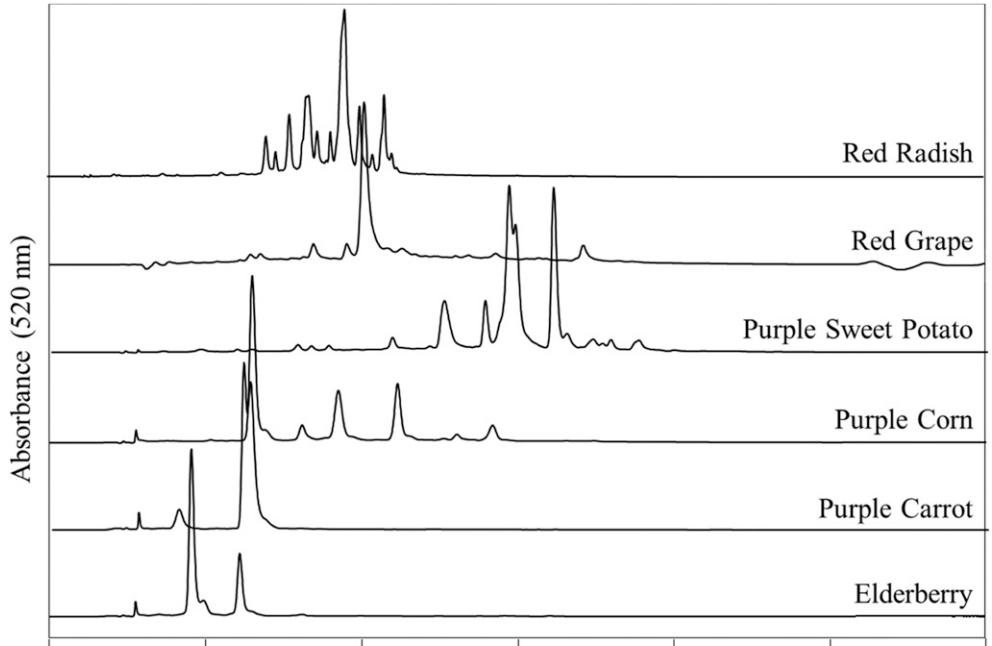
Sources of ACNs used in lipstick formulations were selected to represent a variety of natural structures and commercial availability. The main characteristics of each extract and their concentrations used in these lipstick formulations were characterized by Westfall and Giusti (21) and are summarized in Figure 1. Several sources of cyanidin, such as elderberry (*Sambucus nigra* L.), purple carrot (*Daucus carota* L.), purple corn (*Zea mays* L.), and purple sweet potato (*Ipomoea batatas* L.), were selected because of the high predominance in nature and commercial availability. Sources of acylated ACNs were also included as they have high reported stability (30). Hibiscus (*Hibiscus sabdariffa* L.) is a source of nonacylated delphinidin and was evaluated because of its reported high antioxidant activity (31). Red radish (*Raphanus sativus* L.), a source of acylated pelargonidin, was included as it is reported to be highly stable and as an alternative to synthetic red colorants (32). Red grape (*Vitis vinifera* L.), which contains predominantly malvidin and also all six major aglycones, was also investigated to better understand the effect of chemical structure on color stability. As the lipstick formulations were developed based on inclusion of 8% dried plant extract, ACN concentrations in the lipstick formulations ranged 2.2–16.5 μg/mg of lipstick (Figure 1).

TOTAL PHENOLIC CONTENT

The total phenolic content in the methanolic extracts was expressed as micrograms of GAEs per milligram lipstick (Figure 2). The total phenolic content ranged from 85.8 ± 3.3 μg GAE/mg lipstick for purple sweet potato to 46.1 ± 5.5 μg GAE/mg lipstick for purple carrot. The other lipstick formulations contained 51.6 ± 8.3 (red grape), 56.6 ± 8.1 (red radish), 66.7 ± 3.5 (elderberry), and 76.6 ± 1.8 (purple corn) μg GAE/mg lipstick. The phenolic content was compared with the total ACN content determined in a previous study (21). A high correlation (r = 0.97) was found between the total phenolic and total ACN contents for formulas containing acylated ACNs: purple carrot, purple corn, purple sweet potato, and red radish. These results are expected as the assay also measures the cinnamic and malonic acid acylating groups within the formulas (24). As phenolics are often associated with antioxidant activity, it is an important variable to consider when interpreting the antioxidant activity results of the formulas (33).

UV ABSORPTION AND SPF CALCULATIONS

The UV light absorbance of each formula compared with that of the lipstick base alone, from 290 to 400 nm, is shown in Figure 3. All formulas showed greater absorbance than the lipstick base alone along the entire UV spectrum, although absorption was higher in



Source	Major Aglycone(s)	Acylation Type	Conc. (µg/mg)
Elderberry	Cyanidin	--	16.5
Purple Carrot	Cyanidin	Cinnamic Acids	2.2
Purple Corn	Cyanidin & Peonidin	Malonic Acids	11.1
Purple Sweet Potato	Peonidin & Cyanidin	Cinnamic Acids	9.6
Red Grape	Malvidin	--	14.9
Red Radish	Pelargonidin	Cinnamic & Malonic Acids	12.1

Figure 1. Characterization and quantitation (microgram ACN/milligram lipstick as cyanidin-3-glucoside equivalents) of ACNs in lipstick formulations.

the UVB range than the UVA range for all formulas tested. All formulas showed statistically significant differences when compared with the base. The formula that showed the highest UV absorbance was the red radish formula; the purple sweet potato formula also showed high absorbance across the UV range. Both of these sources contained aromatically acylated ACNs, which is likely related to their increased UV absorbance. Red radish, purple sweet potato, and elderberry showed absorbance values at or above 0.5 for all wavelengths in the UVB (290–320 nm) range (Figure 3). Elderberry ACNs are not typically acylated, and the increased absorbance could be related to the high overall phenolic content of this material (Figure 2).

The calculated *in vitro* UVB SPF values of the ethanolic extracts of the formulas are shown in Table I. Values followed the same pattern as that of the absorbance values in Figure 3; SPF values were highest for red radish, purple sweet potato, and elderberry. Lowest SPF values were calculated for red grape skin and purple carrot; however, all formulations showed an increase in absorbance over that of the lipstick base alone. The increased UV

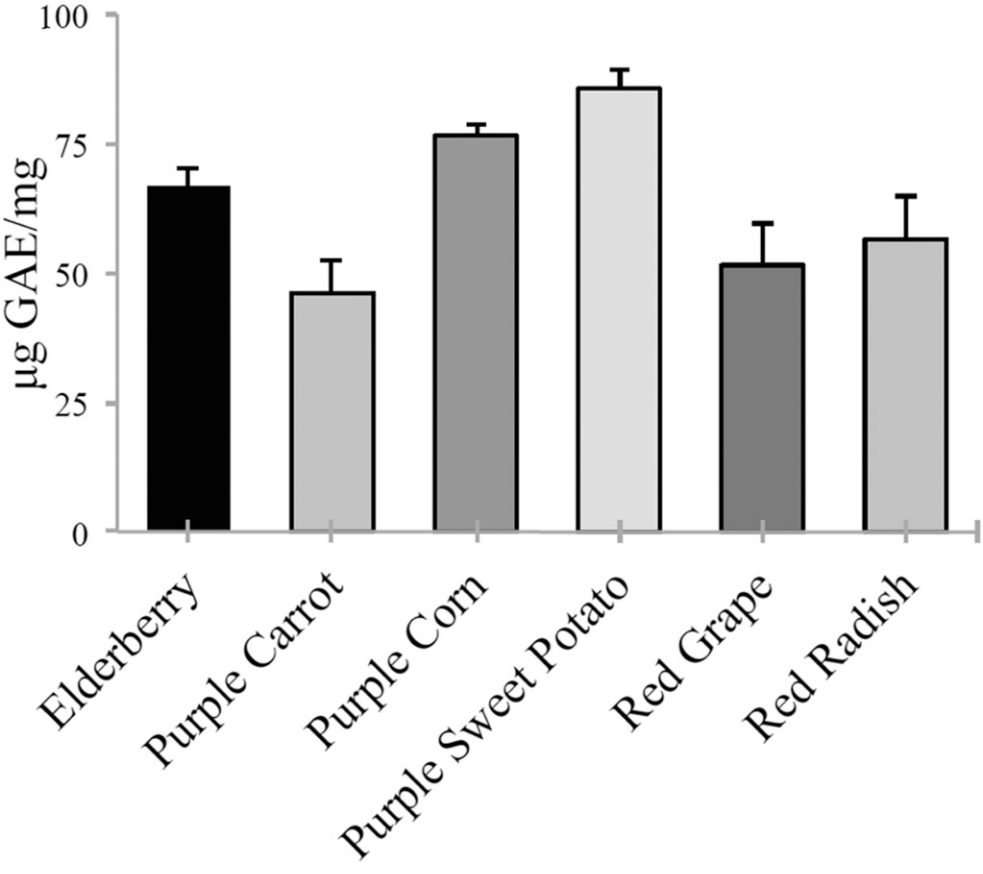


Figure 2. Total phenolic content of ACN–lipstick formulations in methanol, expressed as milligram GAE/milligram lipstick (n = 5 ± SD).

absorption by red radish and purple sweet potato may be due to the addition of acylation by cinnamic acids, which are known to absorb in the UV range (34). These results may suggest a future role for these extracts as photoprotective ingredients in topical formulas.

DPPH FREE RADICAL SCAVENGING ABILITY

The inhibitory percentages of each formula extract against DPPH at three concentrations (40, 60, and 100 µg/mL) were determined to calculate the IC_{50} (µg/mg), or the concentration necessary to inhibit 50% of the DPPH, for each formulation. Inhibitory ability followed the same pattern at all three concentrations, showing dose-dependent effects. Generally, increasing concentration of the lipstick formulation (increasing amounts of ACNs and total phenolics) resulted in greater inhibition of DPPH, consistent with previous reports investigating ACNs as inhibitors of DPPH (35,36). The purple corn and elderberry formulas showed the highest inhibition of DPPH at all three concentrations, whereas the grape skin and purple carrot formulas showed the lowest inhibition of DPPH at all three concentrations. All formula inhibitory values were significantly different from

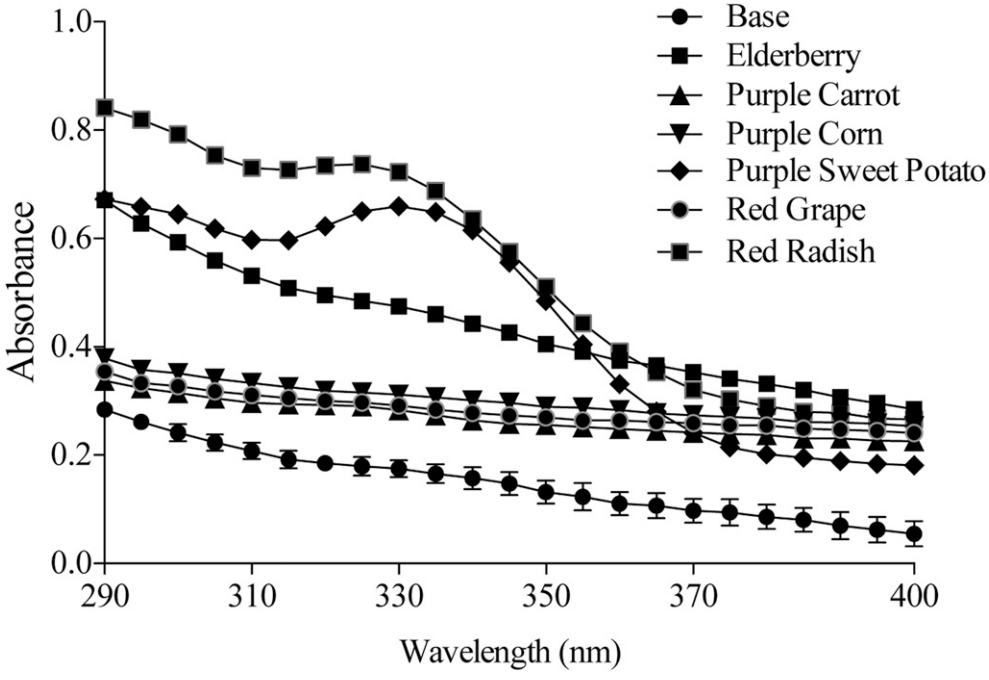


Figure 3. UV absorbance of ACN–lipstick formulations compared with the lipstick base in ethanol. Results are means (n = 8 ± SD).

that of the lipstick base alone when analyzed by one-way ANOVA (p value ≤ 0.05). Interestingly, when the inhibitory percentages of the lipstick formulas were analyzed against that of their respective ACN extract in methanol, no significant differences were found at all three concentrations. These results may be suggesting that the ACNs are behaving in a similar manner to their reducing behavior in solution. The free radical scavenging capacity of each formula extract, expressed as IC_{50} values, is shown in Figure 4.

The antioxidant activity in order from highest activity found was as follows: the purple corn formula (4.87 ± 0.28 μg/mg), elderberry formula (13.35 ± 0.21 μg/mg), purple sweet potato (13.44 ± 0.01 μg/mg), red radish (14.49 ± 1.30 μg/mg), purple carrot (28.59 ± 2.65 μg/mg), red grape skin (31.18 ± 0.68 μg/mg), and, finally, the base lipstick

Table I
Calculated *In Vitro* UVB SPF Values Determined from Ethanolic Extracts of ACN–Lipstick Formulations Compared with the Lipstick Base

Lipstick formulation	Calculated UVB SPF
Base	8.19
Elderberry	13.85
Purple carrot	11.27
Purple corn	11.62
Purple sweet potato	14.44
Red grape	11.39
Red radish	15.84

Calculations based on absorbance values in the UV wavelength range of 290–320 nm.

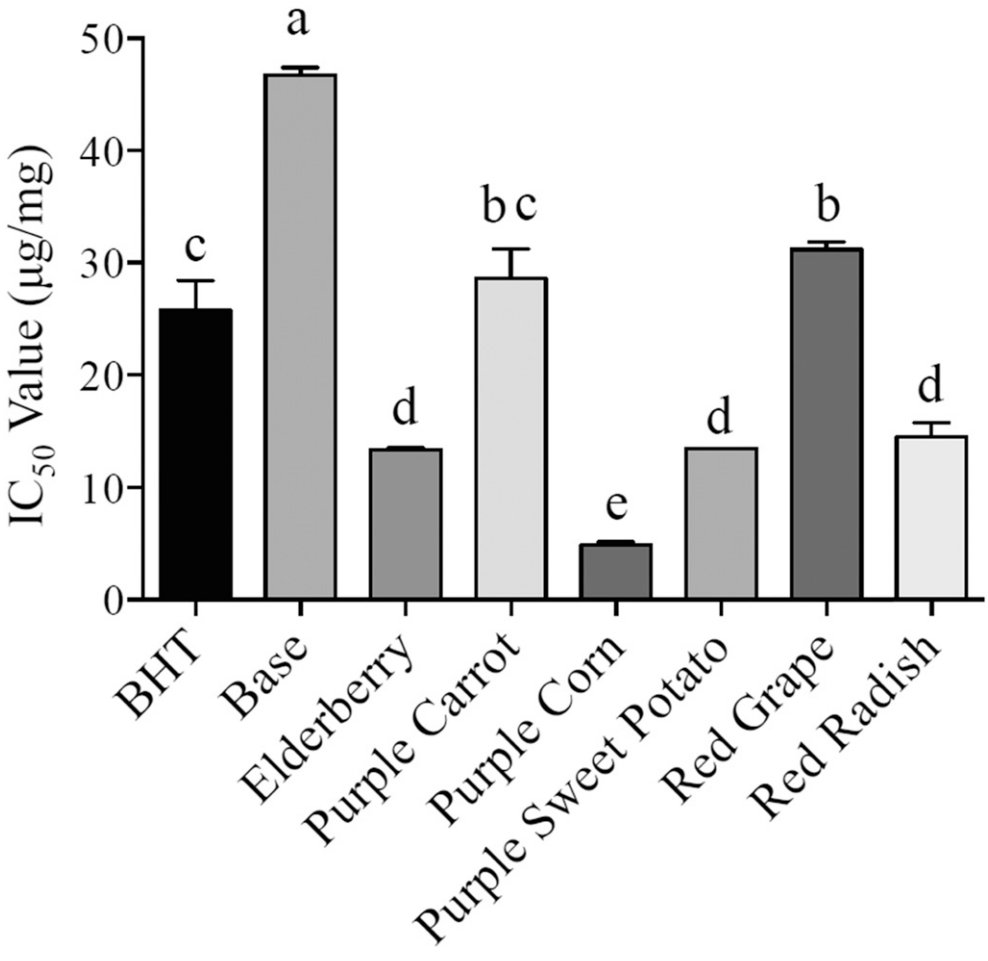


Figure 4. IC_{50} (µg/mg lipstick) of radical scavenging against DPPH determined from methanolic extracts of ACN–lipstick formulations compared with BHT and the lipstick base (n = 5 ± SD). Significant differences are denoted by different letters above bars.

formula (46.72 ± 0.67 µg/mg). All formulations were significantly different when compared with the base formula. All formulas, except purple carrot and red grape, were significantly different when compared with the positive control, BHT (25.78 ± 2.66 µg/mg).

Overall, the antioxidant capacity values found were in agreement with the amount of total phenolic content (r = 0.97). In general terms, those lipstick formulations containing greater amounts of total phenolic compounds demonstrated lower IC_{50} values or greater DPPH inhibition. Purple corn, elderberry, and purple sweet potato were expected to have the highest antioxidant activity because of their higher total phenolic content. However, this trend was not consistent in all cases. The purple sweet potato lipstick formulation had the highest overall total phenolic content; however, the purple corn and elderberry lipstick formulations showed greater DPPH inhibition capacities. This could have been related to the types of ACNs or polyphenols predominant in those extracts. Elderberry

and purple corn ACNs were composed predominantly of cyanidin derivatives, whereas purple sweet potato ACNs were composed of part acylated cyanidin and of majority acylated peonidin derivatives. Peonidin bears a methoxyl and a hydroxyl group on its B-ring, whereas cyanidin bears a catechol moiety on the B-ring, which makes it more reactive. Inversely, the grape skin formula and purple carrot would be expected to have lower relative antioxidant activities, based on their phenolic content. These findings indicate antioxidant capabilities are affected by phenolic composition and concentration.

Samples were tested again after 4 weeks of storage at 4°C, and inhibitory percentages and IC_{50} values were compared with the fresh sample values. There was no statistical significance (p value ≤ 0.05) between the values of the fresh samples and the 4-week old samples. In other studies, ACNs have been demonstrated to have antioxidant properties as investigated in various *in vitro* assays (such as DPPH, FRAP, and ABTS) (36,37); in this study, we demonstrate retention of these capabilities through the DPPH assay when incorporated in lipstick formulations. As these same were previously demonstrated to have high storage stability (≥2 years) (21), it may be suggested that lipsticks could serve as convenient vehicles for delivering ACNs and their antioxidant properties topically. Further investigation will be required to validate this.

ANTITYROSINASE ACTIVITY

Eluates from the base lipstick and the ACN–lipstick formulations were tested for inhibition of L-DOPA oxidation against mushroom tyrosinase as a measure against melanin production. IC_{50} values (μg/mg), or the amount of the extracts necessary to inhibit 50% of the mushroom tyrosinase, were then predicted using linear regression and compared with the positive control, kojic acid (Figure 5). In general, all lipsticks showed the ability to inhibit tyrosinase at similar or lower concentrations than kojic acid (2.41 ± 0.06 μg/mg). The elderberry and purple corn, in particular, exhibited IC_{50} values at, respectively, 11-fold and eightfold lower concentrations than that of the control. Results were analyzed using one-way ANOVA, and all treatments were found to be statistically significant ($p \leq 0.05$) when compared with controls.

IC_{50} values (Figure 5) in order of highest inhibition were as follows: elderberry (0.22 ± 0.06 μg/mg), purple corn (0.31 ± 0.01 μg/mg), purple carrot (0.42 ± 0.03 μg/mg), red grape skin (0.55 ± 0.04 μg/mg), purple sweet potato (0.75 ± 0.06 μg/mg), and red radish (1.78 ± 0.08 μg/mg). These results are in agreement with the expected activity based on the structure of the ACNs present. Anthocyanins with free hydroxyl groups at the 3′ and 4′ positions of their B-ring (cyanidin and delphinidin) would be expected to have the highest inhibition of tyrosinase (38). Conversely, it is believed that the substitution with a methoxyl group at the 3′ position of the B-ring (peonidin, petunidin, and malvidin) causes steric hindrance of the hydroxyl group at the 4′ position, decreasing the inhibition potential. Acylation with cinnamic acids may increase inhibition (39). Acylation with malonic acid, such as that found on purple corn ACNs, may also inhibit tyrosinase through complexation with the copper center of the enzyme. In addition, glucosyl attachments at C3 and C5 of the aglycones are believed to decrease inhibition through steric hindrance. Therefore, the ACN sources with cyanidin with one sugar attachment at the 3′ position (elderberry), and their acylated counterparts (purple carrot and purple corn), would be expected to have the highest inhibition of tyrosinase.

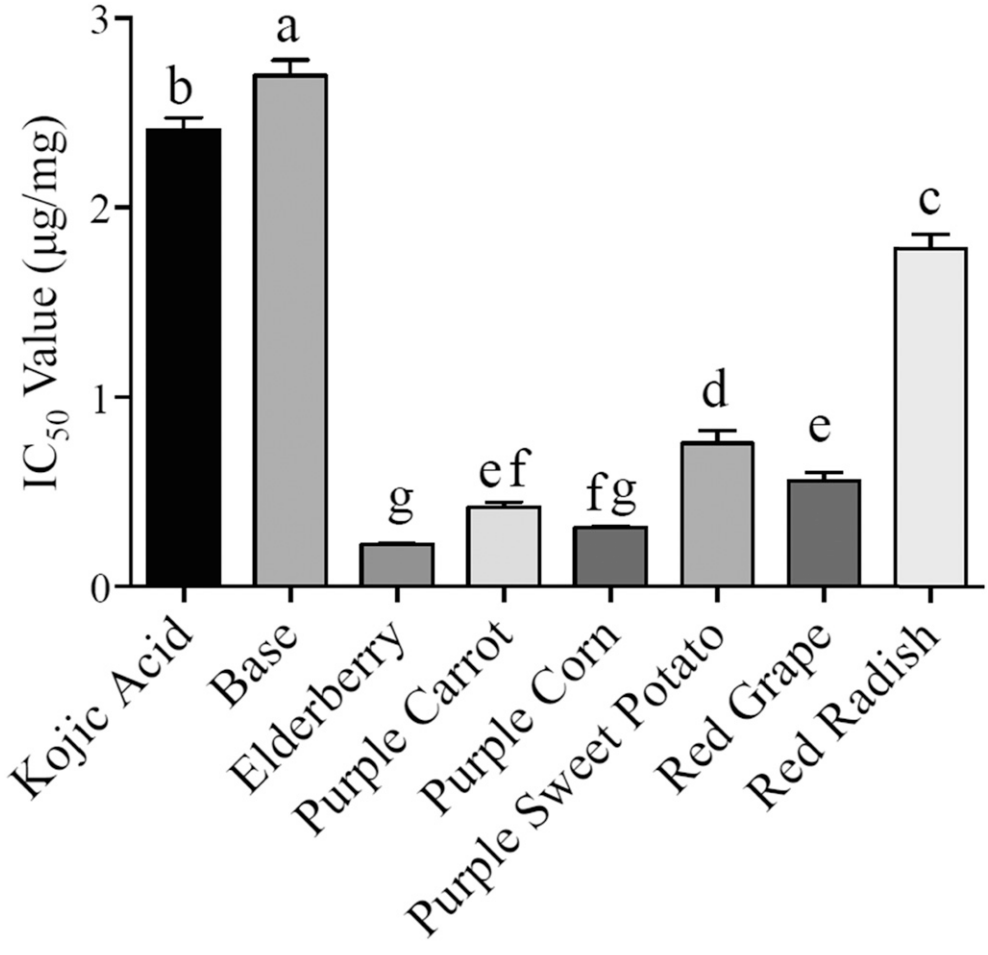


Figure 5. IC_{50} (µg/mg lipstick) against mushroom tyrosinase for ACN–lipstick formulations compared with kojic acid and the lipstick base. Results are presented as means (n = 8) ± SD. Significant differences are denoted by different letters above bars.

It should be noted that the results of IC_{50} values in micrograms per milligram are physiologically relevant to lipstick application. The average woman uses 24 mg lipstick per day (40); therefore, the amounts of ACNs necessary to receive benefits against tyrosinase and other activities in cosmetic formulations could be delivered by these formulations.

CONCLUSIONS

Anthocyanins incorporated into lipstick formulations were shown to retain their tested biological activity *in vitro*. Under the conditions of this study, all formulations showed the ability to act as photoprotective additives through UV absorption, especially those with cinnamic acid acylation. The ACN formulations showed the capability to act as antioxidants, through scavenging of free radicals, in a lipstick matrix. In addition, the formulas showed proficient tyrosinase inhibition, which is a well-known source of melanin formation in the skin. The combination of all these effects may be protective against

photoaging, warranting subsequent *in vivo* investigation. The concentrations necessary to exhibit these activities were all well within physiologically relevant concentrations based on the average uses of lipsticks within the United States. These results suggest ACNs may have uses as potentially bioactive ingredients within cosmetic formulations; further studies are warranted for claim substantiations.

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