cosmetics

MDPI

Article

Coreopsis tinctoria Nutt. As a New Raw Material for Natural Hair Coloration: Discovering the Dyeing Potential of Chalcones

Jule Marie Sauler [1,2], Volkmar Vill [2] and Fabian Straske [1,2,*]

[1] Henkel AG & Co. KGaA, Ruhrstraße 19, 22761 Hamburg, Germany; jule.sauler@henkel.com
[2] Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany; volkmar.vill@uni-hamburg.de
* Correspondence: fabian.straske@henkel.com

Abstract

This study presents the development of a natural hair dye based on *Coreopsis tinctoria* Nutt. plant extract with and without subsequent mordanting. The dye molecules behind the color development have been investigated to gain better understanding of the relationship between flavonoid structure and color on hair. Yak hair was dyed under different conditions and wash fastness tests were carried out to evaluate the performance of the new hair dye. Analysis of *Coreopsis tinctoria* Nutt. plant extract was performed to assess the chemical constitution of hair dye. *Coreopsis tinctoria* Nutt. extract solely led to yellow colors (as represented within $L^*a^*b^*$ color space: $L^* = 65.5$; $a^* = 0.7$; $b^* = 46.6$), whereas the treatment combined with ferrous lactate led to dark brown colors ($L^* = 26.4$; $a^* = 2.3$; $b^* = 10.0$). Wash fastness demonstrated a very good color stability with a maximum loss in color intensity of $\Delta E = 12.4$ after 24 hair washes. Dyeing experiments using the most abundant flavonoids marein, flavanomarein, okanin, and isookanin gave insight into the responsible flavonoids for color outcome. In combination with ferrous lactate, chalcones led to brown colors and flavanones to gray colors. The chalcone okanin presented itself as the most powerful dye, leading to intense colors in combination with ferrous lactate ($\Delta E = 56.6$), at low dye concentrations of 0.1 mg mL^{-1}.

Keywords: *Coreopsis tinctoria* Nutt.; flavonoid; chalcone; flavanone; mordant; metal flavonoid complex; natural hair coloration; ferrous lactate; plant-derived dye

1. Introduction

Oxidative permanent hair dyes are popular with consumers, resulting in a multibillion dollar industry worldwide [1]. Long-lasting and reliable color results, paired with an easy application done by the consumers themselves or by hairdressers, are the main factors contributing to their popularity. The synthetic dyes contained in these hair colorations are well-known since their discovery by Hofmann 150 years ago. Hofmann found that *p*-phenylenediamine (PPD) turns into brown hair color under certain conditions [1]. Since then, much research has been done in the field of oxidative hair dyes, leading to a huge product palette nowadays which comprise cheap and easy-to-handle synthetic dyes [2]. Nevertheless, there are concerns about the safety of the dye precursors being shown due to the development of contact allergies [1]. Resorcinol, a dye precursor used in oxidative hair colorants, is suspected of possessing endocrine-disrupting properties and potential skin-sensitizing attributes. However, its use is considered safe by the Scientific Committee on Consumer Safety (SCCS) [3]. Despite possible safety issues, environmental issues have

Check for updates

Academic Editor: Enzo Berardesca

Received: 6 March 2026
Revised: 29 April 2026
Accepted: 30 April 2026
Published: 20 May 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

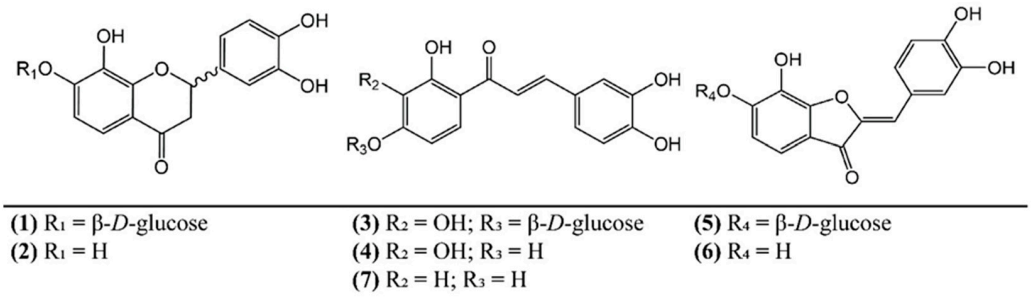
been reported in this context. Up to 84% of PPD remains unused during the application of permanent hair dyes, leading to wastewater pollution and possible ecotoxic effects [4]. Moreover, consumer demands concerning cosmetics are changing. Majorly influenced by the COVID-19 pandemic, consumers have become more aware of their own wellbeing and, thus, the use of cosmetic products. Natural, eco-friendly ingredients and manufacturing processes have been associated with this trend [5].

The possibility to meet consumer demands and combine environmentalism, the use of safe and sustainable raw materials focuses on natural raw materials such as plants or their extracts for hair colorations. Plants being used as (hair) dyes have been widely explored in the past. A common dye used to achieve orange to brown colors on hair and skin is henna (*Lawsonia inermis* L.). Known for almost 5000 years, it is still the topic of current research [6–9]. This suggests that the exploration of (forgotten) dyes from antiquity offers a great possibility to bring new color to our present-day lives. Ancient dyes comprise a wide variety of molecules, ranging from tannins and flavonoids to carotenoids or quinones, as exemplified by the naphthoquinone lawson from the henna plant [6]. The analysis of (ancient) textile samples can help bring back dyes from natural sources. Analytical methods, in particular liquid chromatography coupled with spectrophotometric and spectrometric techniques, are being used to reveal the dye molecules and their origin. Zhang et al. analyzed ancient Peruvian textile samples originating from 1050 to 1200 AD using high performance liquid chromatography coupled with diode array detector and mass spectrometry (HPLC-DAD-MS). Chalcones, e.g., okanin glycosides or butein glycosides, as well as flavanols, e.g., luteolin-7-glycosides, were identified as being responsible for yellow-colored textiles. Although the specific plant origin of these dyes could not be found, it was assumed that it may be plants belonging to *Coreopsis* species [10]. In a similar context, León et al. investigated traditional dyes used in Peru. Among other things, the plant family of *Asteraceae* was found to color textiles in yellow shades, with the *Coreopsis senaria* variety as an example [11].

The *Coreopsis* species comprises a huge variety of subspecies, one of which is *Coreopsis tinctoria* Nutt. (*C. tinctoria*). Originating from Northern America, the annual plant is now distributed worldwide, making it an easy and fast to grow crop [12,13]. Primarily, the flower is cultivated in China and already commercially available as an herbal beverage [14]. During the last few years, it has been mainly investigated due to its medicinal properties, showing, e.g., antioxidant, anti-inflammatory or anti-diabetic properties [12,15–19]. Analysis has shown that the phytochemicals present include phenylpropanoids, polyacetylenes, essential oil, polysaccharides, and around 10% of flavonoids [15]. Among the flavonoids, chalcones, flavanones, flavones, and aurones represent the main subclasses, whereas the chalcone marein (**3**) and the flavanone flavanomarein (**1**) have been reported to be most abundant (see Figure 1 for all molecular structures of mentioned flavonoids) [13,15,20,21]. These flavonoids can also be present as their aglycones, namely okanin (**4**) and isookanin (**2**) [17,21,22]. Despite the chalcones marein and okanin, other chalcones such as butein (**7**) have been mentioned [22]. A notable example for aurones is the glycoside–aglycone pair maritimein (**5**) and maritimetin (**6**) due to their close connection to the previously mentioned chalcones and flavanones [12,21,23,24]. Oxidation processes and hydrolysis within the plant's biosynthesis, whether catalyzed enzymatically or occurring as an artifact during extraction and storage, need to be considered [23–25].

The dyeing properties of *C. tinctoria* have recently been explored by Velmurugan et al. [26]. They investigated the dyeing outcome of different colored parts of the *C. tinctoria* flower petals on leather. It was shown that upon varying the dyeing conditions with regard to dyeing time, pH, and dyeing method, uniform yellow or brownish colors were

achieved [26]. Nevertheless, there has been no research so far on the specific molecules responsible for the developed colors nor on their applicability as hair dyes.



(1) R_1 = β-*D*-glucose
(2) R_1 = H
(3) R_2 = OH; R_3 = β-*D*-glucose
(4) R_2 = OH; R_3 = H
(7) R_2 = H; R_3 = H
(5) R_4 = β-*D*-glucose
(6) R_4 = H

Figure 1. Molecular structures of selected flavanones, chalcones and aurones found in the flower petals of *C. tinctoria*: (**1**) flavanomarein, (**2**) isookanin, (**3**) marein, (**4**) okanin, (**5**) maritimein, (**6**) maritimetin, (**7**) butein.

Besides using plants and their extracts directly to color textiles, hair, or leather, as in the case of Velmurugan et al. [26], additives in the form of metal salts can be used. This technique known as mordanting helps to attach the dye molecules to the fiber, leading to increased color strength, light, and wash fastness. In addition, the phenomenon behind metal mordanting, a complex formation between metal ions and plant polyphenols, can lead to color changes due to the formation of charge transfer (CT) complexes. Common mordants used include $FeSO_4$ or $AlK(SO_4)_2$ [2,27]. This principle has already been explored in the field of hair dyes [28–36]. Nevertheless, in the case of using metal ions in hair coloration, the safety of consumers and aquatic life must be considered. The metal salts should be non-irritant to human skin and safe to release in the environment. At this point in time, the European Union allows the use of metal ions in cosmetic products under certain circumstances. Silver nitrate is commonly used as a dye for eyelashes and eyebrows, whereas iron-containing compounds, such as iron oxides, are used as a colorant in cosmetic products [37]. Other examples show that metal salts, such as ferrous lactate (E585) and ferrous gluconate (E579), are allowed as food additives in the European Union [38]. These facts and the use of minimal concentrations of metal salts in hair color formulations could pave the way for the use of said metal salts in future hair dyes.

This study presents a novel hair dye comprising *C. tinctoria* as a source for flavonoids with and without the combination of metal salts as mordants. It highlights the versatility and wash fastness of color shades produced on hair. A selection of the most abundant flavonoids present in *C. tinctoria* extract are evaluated in terms of their dyeing performance. These experiments give insight into the influence of flavonoid structure on color outcome.

2. Materials and Methods

2.1. Materials

The dried flower heads of *C. tinctoria* were purchased from Livadenn Plantes Tinctoriales (Kerbors, France). The flowers were harvested manually from July to October in 2022 and cultivated under organic farming certified by Ecocert®.

Ferrous(II)–lactate hydrate (≥98%, CAS-RN: 5905-52-2), potassium titanium oxide oxalate dihydrate (CAS-RN: 14402-67-6), Folin & Ciocalteu's phenol reagent (2 M acid), quercetin (≥95%, CAS-RN: 117-39-5), and formic acid (98–100%, CAS-RN: 64-18-6) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The sodium molybdenum oxide anhydrous (46.2% Mo; CAS-RN: 7631-95-0) was acquired from Thermo Fisher Scientific (Waltham, MA, USA). Gallic acid monohydrate (≥99%, CAS-RN: 5995-86-8) was purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Sodium carbonate decahydrate

(CAS-RN: 6132-02-1) and HCl (37%, CAS-RN: 7647-01-0) were acquired from VWR Chemicals (VWR International GmbH, Darmstadt, Germany). Ethanol absolute (99.8%, CAS-RN: 64-17-5) and acetonitrile (≥99.9%, CAS-RN:75-05-8) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Marein (98%, CAS-RN: 535-96-6) was purchased from Apollo Scientific (Manchester, UK). Flavanomarein (99%, CAS-RN: 577-38-8), isookanin (97%, CAS-RN: 1036-49-3), maritimein (98%, CAS-RN: 490-54-0), and maritimetin (99.09%, CAS-RN: 576-02-3) were purchased from Synchem UG & Co. KG (Felsberg, Germany). Okanin (99%, CAS-RN: 484-76-4) was purchased from Biosynth AG (Staad, Switzerland). Butein (≥98%, CAS-RN: 487-52-5) was acquired from TCI Deutschland GmbH (Eschborn, Germany). A 25% (*w*/*w*) sodium laureth sulfate (SLES) was obtained from Henkel AG & Co. KGaA internally. Cellulase-mix containing ß-*D*-glycosidase was obtained from Novonesis (Bagsværd, Denmark).

For all pH measurements, the FiveEasy F20 pH-meter from Mettler-Toledo (Columbus, OH, USA) was utilized. Unless stated otherwise, ultrapure water was used for all experiments, purified using the Arium Pro Ultra-Pure Water System (Sartorius AG, Göttingen, Germany).

For hair coloring experiments, hair tresses of white, unbleached yak belly hair (Kerling International Haarfabrik GmbH, Backnang, Germany) were used. They exhibited a total length of 7 cm, and the weight of hair fibers was 0.70 g. Yak belly hair was chosen as a dye substrate as it naturally does not contain melanin and therefore no pretreatment such as bleaching is necessary. Moreover, it could be shown that there was a high resemblance to human hair in terms of molecular composition [39]. For comparison, untreated white human hair (Kerling International Haarfabrik GmbH, Backnang, Germany) was used.

2.2. Extraction of C. tinctoria Raw Material

Dried *C. tinctoria* flower heads were pulverized for 30 s using a grinder (EGK 200, Rommelsbacher ElektroHausgeräte GmbH, Dinkelsbühl, Germany). A total of 25 g pulverized plant material was weighed and mixed with 400 mL of 55% (*v*/*v*) ethanol. This solvent ratio has already been proven to display a suitable solvent for extracting *C. tinctoria* [13,20,40]. The suspension was extracted for 5 h under reflux (60 °C, 370 mbar). Afterwards, the extract was cooled down to room temperature and filtered through a polyvinylidene fluoride (PVDF) filter (pore size: 0.22 µm; VWR international GmbH, Radnor, USA) under vacuum. The solvents were evaporated under reduced pressure (50 °C) to an amount of approximately 200 mL. Subsequently, the remaining extract solution was frozen at −20 °C for 12 h and subjected to freeze-drying using the Alpha 1-4 LSCbasic (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) to produce the final extract. The procedure described above was repeated three times in total. The resulting extracts were mixed and stored at 4 °C until further use. To calculate the yield of the extract, the total amount of extract was divided by the amount of raw material used for extraction.

2.3. HPLC Method Setup, Procedure, and External Calibration

To determine the amounts of individual flavonoids present in *C. tinctoria*, the AZURA® analytical HPLC (Knauer GmbH, Berlin, Germany), equipped with the autosampler AS 6.1L, column oven AZURA® CT 2.1, diode array detector DAD 2.1 L, and reversed-phase column Eurosphere II 100-3 C18 (3 µm; 150 mm × 4,6 mm) was used. A gradient elution was chosen, adapted from the method of Lam et al. [20], using acetonitrile (A) and 0.1% formic acid in ultrapure water (B) as eluents with the following gradient profile: 0–5 min, 10–15% A; 5–15 min, 15–22% A; 15–25 min, 22–48% A; 25–27 min, 48–80% A; and 29–30 min, 10% A. The separation was carried out at 1 mL/min flow rate, and the temperature of the column oven was set to 30 °C. For analysis, a volume of 100 µL was injected into the device.

The following wavelengths were used for the detection of the flavonoids: 285 nm, 375 nm, and 410 nm.

For external calibration, standards of marein, okanin, flavanomarein, isookanin, maritimein, maritimetin, and butein were used. The general procedure was as follows. Ca. 5 mg (m_{SL}) of each compound were accurately weighted and dissolved in 10 mL of 55% (*v*/*v*) ethanol in water. From this stock solution, defined amounts were diluted to 10 mL of water shortly before measurement. Hereby, at least six different concentrations were prepared and measured. To establish the calibration curve, the area under each peak was calculated using the ClarityChrom (Version 9.0.0.85, Knauer GmbH, Berlin, Germany) software considering the maximum wavelength of the respective compound (λ_C). In Table 1, the parameters for each compound are summarized. For the calculation of the parameters for the linear regression, Excel (Version 2503, Microsoft, Redmond, WA, USA) was used.

Table 1. Summarized parameters for calibration curves of marein, okanin, flavanomarein, isookanin, maritimein, maritimetin, and butein. t_R: retention time, λ_C: wavelength used for calibration, R^2: coefficient of determination, y: area below peak, b: slope of linear regression, x: concentration of the respective compound, a: y-intercept of linear regression, *Nc*: number of calibration points, *LOD*: limit of detection, *LOI*: limit of inclusion, *LOQ*: limit of quantification, m_{SL}: amount of respective compound in stock solution.

Compound	t_R (min)	λ_C (nm)	R^2	Linear Regression Equation $y=bx+a$	Concentration Range (µg mL^{-1})	*Nc*	*LOD* (µg mL^{-1})	*LOI* (µg mL^{-1})	*LOQ* (µg mL^{-1})	m_{SL} (mg)
Marein	14.7	375	0.999	$y = 405x + 132$	0.243–24.3	11	0.477	0.954	1.430	4.86
Okanin	19.7	375	0.999	$y = 785x + 20.4$	0.107–4.28	11	0.088	0.177	0.263	4.28
Flavanomarein	8.5	285	0.998	$y = 194x + 20.4$	0.502–10.0	8	0.403	0.819	1.209	5.02
Isookanin	12.2	285	0.999	$y = 238x - 7.24$	0.247–7.90	11	0.089	0.180	0.268	4.94
Maritimein	14.2	410	0.999	$y = 367x - 24.6$	0.227–10.9	9	0.212	0.430	0.636	3.63
Maritimetin	18.2	410	0.998	$y = 547x - 14.0$	0.121–2.42	6	0.120	0.248	0.361	4.84
Butein	23.3	375	0.991	$y = 732x - 5.35$	0.126–2.51	6	0.309	0.618	0.927	5.02

For method validation, the limit of detection (*LOD*) was calculated using the slope b and y-intercept of linear regression a as shown in Equation (1), whereas y_c is defined by Equation (2) [41].

$$LOD = \frac{y_c - a}{b} \quad (1)$$

$$y_c = a + t_{f,\,5\%}\sqrt{\frac{\sum(y-\hat{y})^2}{N_C - 2}}\sqrt{1 + \frac{1}{N_C} + \frac{\overline{x}^2}{\sum\left(x_i - \overline{x}\right)^2}} \quad (2)$$

With $f = Nc - 2$,

$\hat{y} = a + bx_i$, with x_i as the concentration of flavonoid used for calibration,

and $\overline{x} = \frac{1}{N_C}\sum x_i$.

The limit of inclusion (*LOI*) and the limit of quantification (*LOQ*) were calculated using Equations (3) and (4), with $k = 3$ representing a result uncertainty of 33% [41,42].

$$LOI = 2\ LOD \quad (3)$$

$$LOQ \approx k\ LOD \quad (4)$$

The slightly lower coefficient of determination obtained for butein (R^2 = 0.991) can be attributed to the narrower calibration range and the lower number of calibration points compared to the other flavonoids. In addition, the chalcone aglycone butein shows increased sensitivity toward minor baseline variations under DAD detection. Nevertheless, the achieved linearity was considered sufficient for its qualitative and comparative use in subsequent dyeing experiments.

To prepare the *C. tinctoria* extract (as described in Section 2.2.) for HPLC analysis, ca. 10 mg of the extract (m_E) was weighed in accurately and dissolved in 10 mL of 55% (v/v) ethanol in water. From this stock solution, 1 mL was diluted to 10 mL using water to receive a final concentration of ca. 100 µg mL^{-1}. This procedure was repeated three times in total. The flavonoids present inside the extract were identified based on their retention time as well as their UV–Vis absorbance (see Table 1 and Figure S3 and Table S11. The amount of the respective flavonoid in µg mL^{-1} (x_{F1}) was calculated using the parameters of slope b and y-intercept a from the linear regression equations (see Table 1), and then converted according to Equation (5) as follows.

$$x_{F1} = \frac{y - a}{b} \quad (5)$$

To receive the amount of each flavonoid in mg per g extract (x_{F2}), the following equation was used (see Equation (6)).

$$x_{F2} = \frac{x_{F1}}{m_E} \quad (6)$$

Mean values of x_{F2} and standard deviation (SD) were calculated using Excel. For the flavonoids marein, okanin, flavanomarein, isookanin, and butein, the determined concentrations were above the *LOD*, *LOI*, and *LOQ* and thereby accepted. For maritimein and maritimetin, the calculated concentrations within the extract were determined as above *LOD*, but below *LOI* and *LOQ*, and marked as those.

2.4. Determination of Total Polyphenolic Content (TPC)

The method determining the total polyphenolic content (TPC) using Folin–Ciocalteu reagent was performed according to the International Organization for Standardization (ISO) 14502-1 [43] with minor modifications. The total polyphenol content and total flavonoid content (TFC) were calculated using gallic acid and quercetin as standard compounds, respectively [44].

Stock solutions of 10% (v/v) Folin–Ciocalteu solution and 7.5% (w/v) Na_2CO_3 in water were prepared. Ca. 25 mg of *C. tinctoria* extract were dissolved in 10 mL of 10% (v/v) ethanol and filtered through 0.22 µm PVDF syringe filters. From this stock solution, 1 mL was mixed with 5 mL of Folin–Ciocalteu stock solution. The mixture was left for 6 min at room temperature for the reaction to proceed. Then, 4 mL of Na_2CO_3 stock solution was added. After 1 h of reaction time, the solution was diluted from 1 to 39 using 10% (v/v) ethanol prior to measuring the absorption at 765 nm using an UV–Vis spectrophotometer (PerkinElmer LAS GmbH, Rodgau, Germany) equipped with UV WinLab 7.3.0.340 software and a quartz glass cuvette (1.00 cm light path, Carl Roth GmbH + Co. KG, Karlsruhe, Germany). A solution of 10% (v/v) ethanol served as the blank. The reaction was performed in triplicate.

For external calibration, stock solutions of gallic acid or quercetin were prepared at 0.1 mg mL^{-1} using 10% (v/v) ethanol. From these stock solutions, different amounts were withdrawn and diluted to a volume of 1 mL (see Table 2) to receive five different concentrations per standard compound. These samples were prepared and measured using the above-described reaction procedure in triplicate.

The total polyphenol (x_{TPC}) and total flavonoid contents (x_{TFC}) were calculated in mg per g extract using Equation (7), whereas mean values of x_{TPC} and standard deviation (SD) were calculated using Excel.

$$x = 1000\,\frac{y - a}{b\,m_E} \quad (7)$$

Table 2. Summarized parameters for calibration curves of gallic acid and quercetin for TPC determination. R^2: coefficient of determination, y: absorption at 765 nm, b: slope of linear regression, x: concentration of the respective compound, a: y-intercept of linear regression, Nc: number of calibration points, m_{SL}: amount of compound in stock solution.

Compound	R^2	Linear Regression Equation $y=bx+a$	Concentration Range (µg mL^{-1})	Nc	m_{SL} (mg)
Gallic Acid	0.9990	$y = 0.1108x + 0.01651$	1.000–10.00	5	10.00
Quercetin	0.9942	$y = 0.1417x - 0.003936$	1.000–10.00	5	10.00

2.5. Hair-Dyeing Procedure

2.5.1. Pre-Cleansing the Hair Tresses

All yak hair tresses were pre-cleansed before dyeing using the following procedure. A solution of 12.5% (w/w) SLES at pH 4.5 was prepared using 25% (w/w) SLES, deionized water, and freshly diluted 10% (w/w) HCl. Each hair tress was moistened using deionized water and placed into the SLES solution, using 20 g per hair tress. After 30 min, the strands were rinsed using tap water with a total flow rate of 25 mL s^{-1}, then combed and air-dried.

2.5.2. General Hair-Dyeing Procedure

The general method for hair dyeing is adapted from the method previously described by Sargsyan et al. [32] and comprises a two-step dyeing protocol. In detail, the first step included dyeing the hair using either *C. tinctoria* raw material or *C. tinctoria* extract. Here, the respective amount was suspended or dissolved in 50 mL deionized water. The hair was then incorporated into the suspension/solution and stirred at 150 rpm for 30 min at room temperature. Afterwards, the hair strand was rinsed under running tap water, combed 20 times, and then blow-dried. Next, the hair tress was mordanted in a solution containing the metal salt. For this second step, the metal salt was dissolved in 50 mL deionized water. After that, the hair strand was added to the solution and stirred at 150 rpm for 30 min at room temperature. The hair strand was rinsed under running tap water, combed 20 times, and then blow-dried.

In general, a concentration of 1% (w/v) raw material or mordant was set as the standard concentration for the following experiments. It presented a compromise between raw material consumption and color intensity. Furthermore, by using higher concentrations, color saturation should be achieved. From experience, this is the case for extract/raw materials or metal salt solutions of 1% (w/v).

For hair strands dyed using *C. tinctoria* raw material, hair strands were dyed using the shredded *C. tinctoria* flower heads in a concentration of 1% (w/v) for 30 min. Following this, the hair tresses were mordanted using 1% (w/v) ferrous lactate for 30 min.

To determine the influence of different metal salts, hair strands were dyed using 1% (w/v) *C. tinctoria* extract for 30 min. The dyed hair strands were mordanted using each 1% (w/v) of ferrous lactate, sodium molybdate, and potassium titanium oxide oxalate for 30 min. For combinatory treatment of ferrous lactate and sodium molybdate, each metal salt was dissolved separately in 25 mL deionized water. Both solutions containing either 0.45 g (0.459 g) sodium molybdate or 0.05 g (0.005 g) ferrous lactate were combined shortly before adding the hair strand to receive a final concentration of 0.9% (w/v) (0.99% (w/v)) for sodium molybdate and 0.1% (w/v) (0.01% (w/v)) for ferrous lactate; this corresponded to a ratio of 9 to 1 or 99 to 1, respectively.

For evaluation of dyeing time, all hair strands were dyed using 1% (w/v) *C. tinctoria* extract for 5, 10, and 15 min. Afterwards, the hair strands were mordanted using 1% (w/v) ferrous lactate for 30 min. For evaluating the mordanting time, first, all hair strands

were dyed using 1% (*w*/*v*) *C. tinctoria* extract for 30 min. Second, the hair strands were mordanted using 1% (*w*/*v*) ferrous lactate for 5, 10, or 15 min.

To determine the influence of *C. tinctoria* extract concentration, hair strands were dyed using 0.01, 0.1, or 0.5% (*w*/*v*) of *C. tinctoria* extract for 30 min in the first step. The mordanting step comprised treating the hair strands in 1% (*w*/*v*) ferrous lactate for 30 min. The influence of mordant concentration was evaluated by dyeing hair strands with 1% (*w*/*v*) *C. tinctoria* extract for 30 min. Then, the hair strands were mordanted using 0.01, 0.1, and 0.5% (*w*/*v*) ferrous lactate for 30 min.

For evaluation of the comparability of yak and human hair, white human hair was first dyed with 1% (*w*/*v*) *C. tinctoria* extract for 30 min. Following this, the hair was treated with 1% (*w*/*v*) ferrous lactate solution for 30 min.

To evaluate the comparability between *C. tinctoria* based mordant dye and oxidative permanent dyes, yak hair strands were dyed with Igora Royal permanent color crème 5–13 combined with Igora Royal oil developer 6% (Henkel AG & Co. KGaA, Hamburg, Germany). A total of 5 g of permanent color crème and oil developer were combined in a ratio of 1:1 and applied to the hair strand. The dye was left in for 30 min at room temperature. The hair strand was rinsed under running tap water, combed 20 times, and then blow-dried.

All described experiments were repeated in triplicate.

2.5.3. Hair Coloration Using Individual Compounds and Enzymatic Hydrolysis

To assess the influence of the individual compounds present inside *C. tinctoria* extract, flavanomarein and marein were used directly to color hair, as well as their aglycones isookanin and okanin, which were released after enzymatic hydrolysis. Butein was used to directly color hair as well. Here, yak hair strands, which had been cut in half and re-bound, were used for dyeing, utilizing half of the dye bath concentration, i.e., 25 mL of dye solution.

For the evaluation of dyeing strength of the individual flavonoids in direct comparison, 12 mg (0.048% (*w*/*v*)) of each flavonoid were used. To calculate the theoretical amount of aglycones after completed enzymatic release, Equation (8) was used, where m_A (m_G) corresponds to the amount of aglycone (glycoside) in mg and M_A (M_G) are the molar masses of aglycon (glycoside).

$$m_A = m_G \frac{M_G}{M_A} \tag{8}$$

To evaluate the dyeing strength of the individual flavonoids that can be found in 1% (*w*/*v*) of *C. tinctoria* extract, Equation (4) was also used to assess the amount of aglycones released from theoretically completed glycoside hydrolysis. The given amounts of each flavonoid are summarized in Table 3.

For experiments using the glycosides flavanomarein, marein, and butein, the given amount (see Table 3) of the respective flavonoid was dissolved in 25 mL of 4% (*v*/*v*) ethanol in deionized water. The halved hair strands were dyed for 30 min, rinsed, and blow-dried as described in the standard protocol of hair coloration in Section 2.5.2. Afterwards, the halved hair strands were mordanted using 1% (*w*/*v*) ferrous lactate in 25 mL deionized water for 30 min, rinsed, and blow-dried.

To color hair using the corresponding aglycones, isookanin and okanin, enzymatic hydrolysis using ß-glucosidase containing cellulase-mix was performed. An amount of the glycoside (see Table 3) was dissolved in 25 mL of 4% (*v*/*v*) ethanol in deionized water. A total of 250 µL cellulase-mix was added to the solution and stirred at 150 rpm at room temperature for 30 min. Consecutively, the halved hair strands were incorporated into the solution and dyed as described above for 30 min, rinsed, and blow-dried. Afterwards, the

halved hair strands were mordanted using 1% (*w*/*v*) ferrous lactate in 25 mL deionized water for 30 min, rinsed, and blow-dried.

Table 3. Summarized parameters for dyeing halved hair strands with individual flavonoids present in *C. tinctoria* extract. Target compounds represent the actual flavonoid used for dyeing, where starting compounds display the glycoside used to receive the corresponding aglycone via enzymatic hydrolysis.

	Target Compound	Starting Compound
Amount that can be found in 1% (*w*/*v*) *C. tinctoria* extract	8.51 mg marein	-
	2.70 mg okanin	4.20 mg marein [1]
	5.00 mg flavanomarein	-
	3.30 mg isookanin	5.00 mg flavanomarein [1]
Identical amount of flavonoids	12.0 mg marein	-
	12.0 mg okanin	18.8 mg marein [1]
	12.0 mg flavanomarein	-
	12.0 mg isookanin	18.8 mg marein [1]
	12.0 mg butein	-

[1] Glycosides, for which enzymatic hydrolysis using ß-glucosidase containing cellulase-mix was performed to receive the targeted aglycone.

To assess enzymatic hydrolysis and monitor the completed transformation of the glycosides into their aglycones, HPLC analysis was performed using similar enzyme-to-flavonoid ratios as described above for hair coloration. Therefore, stock solutions of ca. 5 mg mL^{-1} of marein or flavanomarein in 10 mL of 55% (*v*/*v*) ethanol and ultrapure water were prepared. From these solutions, 100 µL were combined with 1 µL of β-glucosidase-containing cellulase mix, and ultrapure water was added to receive a final volume of 10 mL. After 30 min of reaction time at room temperature, the solution was injected directly into the HPLC using the conditions described in Section 2.3. Reference solutions without the addition of cellulase mix were measured and recorded as well.

2.6. L, a* and b* Measurement*

To objectify the color of (dyed) hair strands, the *L*a*b** color space, implemented by the Commission Internationale de l'Èclairge (CIE), was used. Each *L*a*b** value represents a coordinate setting up a 3D color space to describe all colors visible to the human eye. The brightness is indicated by the *L** value, reaching from 0 (black) to 100 (white). The *a** coordinate represents the green–red value (−*a**–+*a**) and the *b** coordinate includes all colors from blue (−*b**) to yellow (+*b**). From these coordinates, the distance between two colors can be expressed by the Δ*E* value, calculated using Equation (9) according to ISO-CIE-11664-4-2029.

$$\Delta E = \sqrt{(L^*_S - L^*_R)^2 + (a^*_S - a^*_R)^2 + (b^*_S - b^*_R)^2} \quad (9)$$

Here, *S* refers to the *L**, *a**, *b** values of the sample and *R* to the reference hair strand—an untreated hair strand [45]. Since not only color plays a role in the perception of hair dyes but also shine, light, and color undertone, this study reported the perception by the human eye as well. Several studies indicated that a difference of around Δ*E* = 2 marked the threshold of observable color difference [46–48].

2.7. Wash Fastness

The method to determine the wash fastness of hair dyes was previously described by Hachmann et al. [49] in detail and is briefly summarized here. It comprises four wash cycles

consisting of six hair washes to receive a total of 24 hair washes. One wash cycle included shampooing one hair strand using 0.5 g shampoo (Schauma 7 Kräuter, Schwarzkopf, Düsseldorf, Germany) from bond to tip for ten times. Then, the hair strand was rinsed and combed. This procedure was repeated five times and completed by a blow-drying step. After each wash cycle, hair strands were photographed and $L^*a^*b^*$ values were measured.

3. Results and Discussion

3.1. Dyeing Outcomes Using C. tinctoria and Mordants

Dyeing outcomes using *C. tinctoria* raw materials were evaluated by treating hair with the plant material solely and in combination with the metal salt ferrous lactate, as described in Section 2.5.2. Figure 2 shows that the hair using the raw material gave an intense yellow color, with a color difference in ΔE = 38.7 compared to untreated hair. Subsequent treatment with ferrous lactate resulted in the formation of a dark brown color with high intensity, as shown by a decrease in L^* value (L^* = 22.6) and an increased ΔE value of 54.8. Despite the intense color formation, the usage of shredded plant raw material was not suitable for product development and incorporation into a consumer-friendly hair-dye formulation in a later stage. The presence of plant tissue not only led to decreased formulation stability but also influenced the sensory properties of the formulation. Extraction, however, provided enrichment of desired flavonoids and separation from unwanted plant compounds [50]. Therefore, *C. tinctoria* extract represents a more compatible ingredient that needs to be evaluated.

The application of *C. tinctoria* extract led to the formation of a yellow color, similar to the one that emerged when using *C. tinctoria* raw material. A slight but visible difference in ΔE = 2.6 was observed originating from the marginally darker and less reddish color of the hair strand dyed with the extract. By mordanting using 1% (w/v) aqueous ferrous lactate solution and *C. tinctoria* extract, a dark brown shade of hair was developed, similar to the treatment using the *C. tinctoria* raw material. Minor differences in L^* value (3.8 units) and b^* value (3.3 units) appeared, leading to slightly lighter and more yellowish-colored hair compared to dyeing using *C. tinctoria* raw material. Overall, a visible color difference in ΔE = 5.0 occurred when comparing these hair strands.

To assess the influence of different metal ions on color formation in combination with *C. tinctoria* extract, potassium titanium oxide oxalate, sodium molybdate, as well as the combination of sodium molybdate and ferrous lactate have been tested. The application of molybdenum and titanium ions on hair treated with *C. tinctoria* extract led to the formation of orange colors with ΔE = 55.9 and 43.4, respectively, compared to untreated hair. The combination of molybdenum and iron ions can additionally be used to create more color shades. A ratio of 9:1 using Mo^{6+} and Fe^{2+} ions as mordants led to a color shift toward warmer brown shades compared to using ferrous lactate alone. This is represented by higher a^* (1.1 units) and lower b^* values (1.2 units). This effect became even more apparent when using a ratio of 99:1 Mo^{6+} to Fe^{2+}. The a^* value compared to using ferrous lactate alone increased by 5.3 units. Nevertheless, the b^* value showed a higher value as well (b^* = 16.2), suggesting an overall color shift toward an orange color. In both cases, the dark brown shade resulting from ferrous lactate was dominant within the mixture, even though very little ferrous lactate was used. Comparing color of hair and concentration of the respective metal ions, the interaction between flavonoids and ions needs to be examined. Malešev and Kuntić investigated the complex stability between different metal ions and flavonoids. They showed a correlation between metal ions and complex stability constants. However, there were no experiments directly comparing the behavior of Mo^{6+} and Fe^{2+} [51]. The complex stability has already been studied as part of the Hard and Soft Acids and Bases (HSAB) concept. Accordingly, the interaction between Fe^{2+} and the functional groups

of flavonoids could be preferred due to their similarity in hardness/softness [52]. Despite this, the interaction of metal ions and hair could influence the actual concentration of metal ions adsorbed on hair. Experiments performed by Bate revealed differences in their affinity to hair [53]. Nevertheless, there has been no evidence of the different complexation or adsorption behavior of Fe^{2+} and Mo^{6+} so far.

	***C. tinctoria* raw material**		***C. tinctoria* extract**					
Mordant	w/o	Fe^{2+}	w/o	Fe^{2+}	Mo^{6+} : Fe^{2+} 9 : 1	Mo^{6+} : Fe^{2+} 99 : 1	Mo^{6+}	Ti^{4+}
Hair strand								
L^*	68.0 ± 0.6	22.6 ± 0.3	65.5 ± 0.3	26.4 ± 0.1	23.4 ± 1.3	28.3 ± 1.8	48.7 ± 0.2	54.6 ± 0.3
a^*	1.4 ± 0.9	2.5 ± 0.1	0.7 ± 0.4	2.3 ± 0.1	3.4 ± 0.3	7.6 ± 0.6	28.1 ± 0.9	17.4 ± 0.1
b^*	46.3 ± 1.2	6.7 ± 0.3	46.6 ± 0.4	10.0 ± 0.7	8.8 ± 1.0	16.2 ± 1.7	47.9 ± 0.7	41.7 ± 0.4
ΔE	38.7 ± 1.1	54.8 ± 0.3	39.3 ± 0.4	51.0 ± 0.1	54.1 ± 1.3	50.2 ± 1.5	55.9 ± 0.7	43.5 ± 0.4
After 24 hair washes	Hair strand							
	L^*		70.4 ± 0.9	34.2 ± 2.3	30.9 ± 0.9	38.4 ± 1.2	56.9 ± 2.9	59.5 ± 1.1
	a^*		−3.2 ± 0.3	1.7 ± 0.3	2.7 ± 0.5	7.4 ± 0.4	22.8 ± 2.6	14.7 ± 2.2
	b^*		42.1 ± 0.7	14.0 ± 0.8	13.6 ± 0.3	23.3 ± 1.4	48.6 ± 2.2	42.0 ± 0.7
	ΔE		7.8 ± 1.0	8.8 ± 2.5	8.9 ± 2.4	12.4 ± 1.4	9.9 ± 3.8	5.8 ± 2.1

Figure 2. Dyeing outcomes of 1% (*w*/*v*) *C. tinctoria* raw material and 1% (*w*/*v*) *C. tinctoria* extract with and without subsequent treatment using different mordants: 1% (*w*/*v*) ferrous lactate; combinatory treatment using 0.9% (*w*/*v*) sodium molybdate and 0.1% (*w*/*v*) ferrous lactate; combinatory treatment using 0.99% (*w*/*v*) sodium molybdate and 0.01% (*w*/*v*) ferrous lactate; 1% (*w*/*v*) sodium molybdate; and 1% (*w*/*v*) potassium titanium oxide oxalate. For strands dyed using *C. tinctoria* extract with or without mordants, color outcomes after 24 hair washes are shown. Results are represented by $L^*a^*b^*$ and ΔE values; mean value ± standard deviation; $n = 3$) and photos taken of the dyed hair strands. For dyed and unwashed hair, ΔE values were calculated to untreated hair. For dyed and washed hair, ΔE values were calculated to dyed and unwashed hair.

Since all experiments were carried out using white yak hair, white human hair was used to validate the described findings and prove their transferability. As shown in Table S3, human hair dyed with *C. tinctoria* extract showed the bespoken yellow color ($L^* = 67.2$; $a^* = 2.6$; $b^* = 46.2$). The values were similar to yak hair dyed with the extract; however, human hair revealed a minor decrease in L^* and a^* value, resulting in slightly darker and more reddish colors. The mordanted human hair using ferrous lactate displayed a dark brown color ($L^* = 26.4$; $a^* = 1.9$; $b^* = 8.2$). Again, this color was highly similar to yak hair, dyed under identical conditions. Only a slight increase in the b^* value (1.8 units) for human hair was observable. Overall it can be concluded, that the colors achieved by dyeing human hair were in accordance with the results obtained using yak hair. In this case, yak hair seemed to be an appropriate substitute to human hair.

To prove that the color was a result of plant polyphenols interacting with metal salts and not an the interaction of metal salts and hair, strands treated with metal salts only under identical conditions were examined (see Table S1). The maximum difference compared to untreated hair (ΔE = 4.7) was produced for the treatment with 1% (w/v) ferrous lactate and the combinatory treatment with 0.9% (w/v) sodium molybdate and 0.1% (w/v) ferrous lactate. For the other tested metal salts, ΔE values between 0.7 and 3.4 compared to untreated hair were shown. This suggests that the treatment of the metal salt itself does not lead to a decisive color formation.

All shown hair strands, dyed with *C. tinctoria* extract with and without subsequent treatment with metal ions, underwent wash fastness tests. The results, depending on the number of washes, mordant and ΔE values, are summarized in Figures 2 and 3. Further underlying data are included in Supplementary Materials Tables S4–S9.

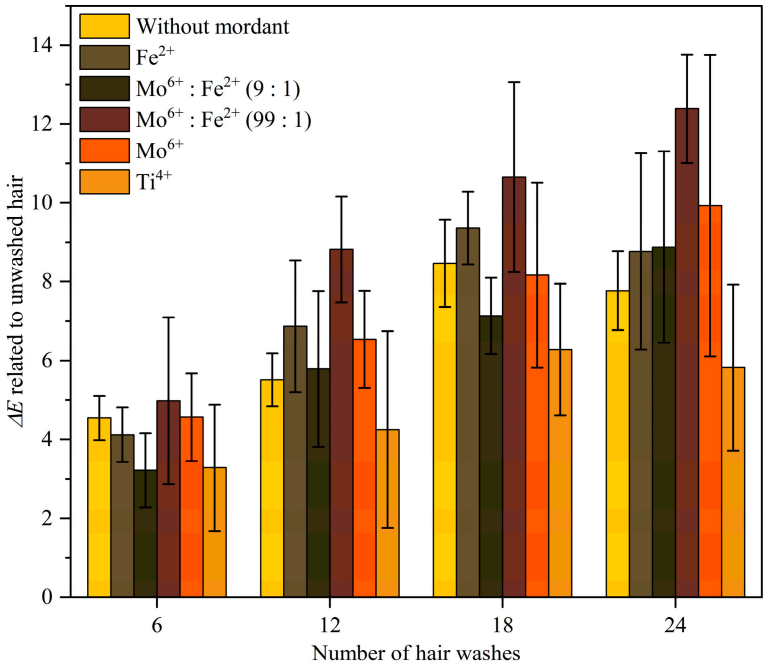


Figure 3. Comparison of wash fastness of hair strands dyed with 1% (w/v) *C. tinctoria* extract with or without subsequent metal ion treatment using different mordants: 1% (w/v) ferrous lactate; combinatory treatment using 0.9% (w/v) sodium molybdate and 0.1% (w/v) ferrous lactate; combinatory treatment using 0.99% (w/v) sodium molybdate and 0.01% (w/v) ferrous lactate; 1% (w/v) sodium molybdate; and 1% (w/v) potassium titanium oxide oxalate. Wash fastness are reflected as ΔE values related to unwashed hair and measured after the given number of hair washes (6, 12, 18, and 24), with error bars indicating standard deviation (n = 3).

The results showed that the presented hair colorations had good wash stability over 24 hair washes. A continuous loss of color intensity was visible and represented by increasing ΔE values related to unwashed hair. In detail, the hair strand treated with potassium titanium oxide oxalate showed the lowest overall increase in ΔE value (5.8), whereas the combinatory treatment using sodium molybdate and ferrous lactate in a ratio of 99 to 1 led to the highest value of 12.4 after 24 hair washes. All washed hair strands showed a steady increase in ΔE, with maximum increases of 4.6 (sodium molybdate), 5.0 (combinatory treatment of sodium molybdate and ferrous lactate in a ratio of 99 to 1), and 4.5 (without mordant) after six hair washes. Subsequent differences in color appeared to have ΔE values below 4.5, indicating a smooth and barely visible color removal. By comparing the initial $L^*a^*b^*$ values (unwashed hair strands) to the color coordinates after 24 hair washes (Supplementary Materials Tables S4–S9), an increasing L^* value was identified, indicating

that color change during washing was mainly based on shift toward lighter colors. Nevertheless, small changes in a^* and b^* values were detected, showing that minimal changes in color nuance appeared during washing.

The wash fastness of human hair dyed with 1% (*w*/*v*) *C. tinctoria* extract and 1% (*w*/*v*) ferrous lactate was evaluated to examine the comparability to yak hair. As presented in Table S10, an increase in L^* value was observable after 24 hair washes (4.2 units) compared to unwashed hair. Similarly, an increase in b^* value appeared from $b^* = 8.2$ for unwashed hair to $b^* = 10.1$ for hair that was washed 24 times. Overall, the color difference to unwashed hair amounted to $\Delta E = 4.6$, which confirmed very good wash stability. Comparing these results to yak hair that was dyed and washed under identical conditions, it can be concluded that, in this case, human hair showed better wash fastness compared to yak hair. Nevertheless, the results were comparable and yak hair could used as a substitute for human hair.

To compare these results with a commercially available permanent oxidative dye, a wash fastness test was conducted under identical conditions (see Table S2). The oxidative dye showed an intense color directly after dyeing ($L^* = 24.5$; $a^* = 1.5$; $b^* = 3.0$; $\Delta E = 52.1$). After performing the hair washes, a loss in lightness was observed after 18 hair washes ($L^* = 26.5$), as well as an increase in the b^* value ($b^* = 3.8$). After 24 hair washes, similar results to an unwashed hair strand were observed ($L^* = 25.8$; $a^* = 1.4$; $b^* = 3.5$) with a ΔE value to unwashed hair of 1.9. These results showed that the hair dye presented in this study exhibited less wash stability compared to the benchmark product of an oxidative permanent dye. Nevertheless, individual results proved that the hair dye comprising *C. tinctoria* extracts with and without mordants approximated the wash fastness of permanent dyes. Examples of this are hair treated with *C. tinctoria* extract and potassium titanium oxide oxalate with a ΔE value compared to unwashed hair of 5.8, or hair dyed with *C. tinctoria* extract only ($\Delta E = 7.8$). Other studies investigating the wash fastness of oxidative permanent dyes evaluated differences in ΔE to unwashed hair reaching from 3.2 to 11.18 after 10 hair washes, from 1.5 to 4.3 after one hair wash, and up to 16.2 after 48 hair washes [54,55]. Sargsyan et al. described a color loss of up to $\Delta E = 11.01$ after 24 hair washes [32]. In these cases, a direct comparison to the *C. tinctoria*-based hair dye was not given; however, they have shown that wash fastness is not only dependent on the type of dye but also surfactant, user, application of conditioning products, and oxidative dye composition. It can be assumed that a difference in color of 1.9 to 11 after 24 hair washes fit within the frame that is expected from permanent dyes. Thereby, the *C. tinctoria*-based dye could be classified as a permanent dye.

To summarize, the results proved that the presented dye showed very good color stability in terms of wash fastness. Nevertheless, further parameters, e.g., light fastness, hair damage, or resistance to mechanical stress, need to be considered to entirely characterize the hair color and compare it to oxidative benchmark products. Although no formal statistical comparison was conducted, many of the observed differences between dyeing conditions exceeded the commonly accepted perceptibility threshold of $\Delta E \approx 2$, indicating visually relevant and reproducible color differences rather than random variation.

3.2. Influence of Dyeing Time and Dye Concentration

The influence of dyeing time was evaluated by treating hair strands with 1% (*w*/*v*) *C. tinctoria* extract for 30 min while varying the ferrous lactate treatment time from 5 to 30 min. Results indicated by ΔE values (see Figure 4A, Table 4) showed an increase in color intensity from 49.6 units after 5 min of treatment time to 53.7 units after a treatment time of 15 min. After a treatment time of 30 min, ΔE values decreased to a value of 51.0. A detailed look at the $L^*a^*b^*$ values revealed slight decreases in L^* and b^* values, while the a^* value remained constant. It can be assumed that a longer treatment time of ferrous lactate led to

a darker and less yellow color on hair. Nevertheless, intense and dark brown colors can already be achieved after 5 min of treatment time.

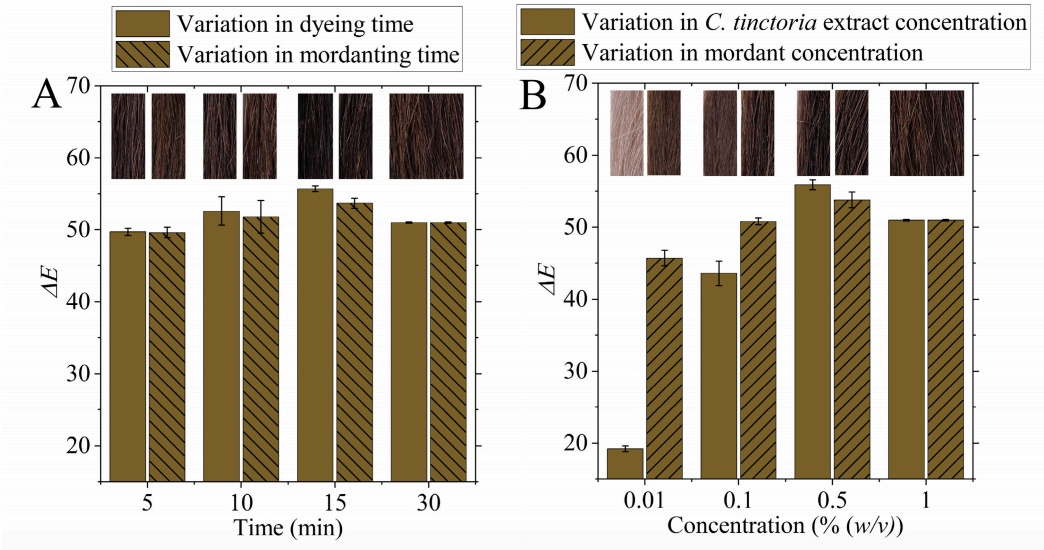


Figure 4. (**A**): Influence of dyeing time on color outcomes of mordanted hair. Brown bars indicate variation in *C. tinctoria* extract treatment time while maintaining the ferrous lactate treatment time at 30 min. Brown striped bars indicate variation in ferrous lactate treatment time while maintaining the *C. tinctoria* extract treatment time at 30 min. (**B**): Influence of mordant and dye concentration on color outcome of mordanted hair. Brown bars indicate variation in *C. tinctoria* extract concentration while maintaining the ferrous lactate concentration at 1% (*w*/*v*). Brown striped bars indicate variation in ferrous lactate concentration while maintaining the *C. tinctoria* extract concentration at 1% (*w*/*v*). Results are presented as Δ*E* values (mean value ± SD, *n* = 3) and pictures taken of hair strands.

Table 4. Influence of dyeing time on color outcomes of mordanted hair evaluated by varying the *C. tinctoria* treatment time for 5, 10, and 15 min while maintaining the ferrous lactate treatment time at 30 min and by varying the ferrous lactate treatment time for 5, 10, and 15 min while maintaining *C. tinctoria* treatment time at 30 min. Results are presented as *L***a***b** and Δ*E* values (mean value ± SD, *n* = 3).

	Variation in *C. tinctoria* Treatment Time			**Variation in Ferrous Lactate Treatment Time**		
Time (min)	5	10	15	5	10	15
*L**	28.2 ± 0.5	25.3 ± 2.0	22.2 ± 0.4	28.4 ± 0.7	26.1 ± 2.4	24.1 ± 0.7
*a**	3.0 ± 0.1	2.6 ± 0.2	2.3 ± 0.0	2.2 ± 0.2	2.3 ± 0.0	2.2 ± 0.0
*b**	7.6 ± 0.4	7.5 ± 0.6	6.3 ± 0.3	13.0 ± 0.5	10.5 ± 1.7	9.3 ± 0.6
Δ*E*	49.7 ± 0.5	52.6 ± 2.0	55.7 ± 0.4	49.6 ± 0.7	51.8 ± 2.3	53.7 ± 0.7

Similar results for Δ*E* values could be obtained while varying the *C. tinctoria* dyeing time from 5 to 30 min with a constant ferrous lactate treatment time of 30 min. However, the influence represented in Δ*E* values became even more apparent. After 15 min, an increase in Δ*E* from 49.7 to 55.7 revealed a visible difference in color, which decreased after 30 min of treatment time to Δ*E* = 51.0. *L***a***b** values showed a similar trend, demonstrating that the most intense results can be achieved after 15 min of treatment time, while results, comparable to treatment time of 30 min, can already be achieved after 5 min.

Investigating the influence of mordant concentration (see Figure 4B, Table 5) while treating the hair strands with a constant concentration of 1% (*w*/*v*) *C. tinctoria* extract revealed a trend toward higher color intensities when using higher ferrous lactate concentrations. The Δ*E* increased from values of 45.7 for 0.01% (*w*/*v*) to 53.8 for 0.5% (*w*/*v*) of ferrous lactate. However, treating hair with a concentration of 1% (*w*/*v*) ferrous lactate

led to a decrease in ΔE (51.0) as well as the individual $L^*a^*b^*$ values. The use of 0.5% (w/v) ferrous lactate caused the darkest (L^* = 23.8) and least yellow (b^* = 8.8) colors on hair. A further increase in ferrous lactate concentration did not increase color intensity and darkness of hair.

Table 5. Influence of dye concentration on color outcomes of mordanted hair evaluated by varying the *C. tinctoria* concentration to 0.01, 0.1, and 0.5% (w/v) while maintaining the ferrous lactate concentration at 1% (w/v) and by varying the ferrous lactate concentration to 0.01, 0.1, and 0.5% (w/v) while maintaining *C. tinctoria* concentration at 1% (w/v). Results are presented as $L^*a^*b^*$ and ΔE values (mean value ± SD, n = 3).

	Variation in *C. tinctoria* Concentration			**Variation in Ferrous Lactate Concentration**		
Concentration (% (w/v))	0.01	0.1	0.5	0.01	0.1	0.5
L^*	58.6 ± 0.3	34.0 ± 1.7	21.7 ± 0.6	32.2 ± 1.2	26.8 ± 0.6	23.8 ± 1.1
a^*	2.8 ± 0.1	3.4 ± 0.2	2.2 ± 0.1	1.8 ± 0.1	2.0 ± 0.2	2.1 ± 0.3
b^*	11.6 ± 0.4	10.0 ± 0.2	6.6 ± 0.5	15.5 ± 0.9	11.3 ± 0.9	8.8 ± 1.3
ΔE	19.2 ± 0.4	43.6 ± 1.7	55.9 ± 0.7	45.7 ± 1.1	50.8 ± 0.5	53.8 ± 1.1

The treatment of hair with different concentrations of *C. tinctoria* extract while maintaining the ferrous lactate concentration at 1% (w/v) led to the largest differences in color outcomes observed so far. A *C. tinctoria* extract concentration of 0.01% (w/v) led to light colors with a ΔE value of 19.2 compared to the hair strand treated with 1% (w/v) and a ΔE of 51.0. The highest ΔE value of 55.9 was achieved using a *C. tinctoria* extract concentration of 0.5% (w/v). The main influence on ΔE values was ascribed to the L^* coordinate, which decreased from 58.6 units to 21.7 units when dyeing hair with *C. tinctoria* extract concentrations of 0.01% (w/v) and 0.5% (w/v), respectively. Despite this, there was a decrease in b^* values visible for the concentrations between 0.01% (w/v) and 0.5% (w/v). The use of higher concentrations of *C. tinctoria* extract did not lead to further decreases in L^* and b^* values. These results are in accordance with pictures taken of the dyed hair strands.

The results showed that the parameters dyeing time and dye concentration can influence the color outcome on hair. Time is an important factor when it comes to the application of the hair color by hairdressers or consumers. The color formation should ideally take place within a certain time frame, allowing the consumer or hairdresser to cover the hair completely with the dye formula without significant color changes happening during that time. Despite this, long application times are not desired as well. The hair color developed in this study consisted of a two-step procedure. It was proven that one of the application steps could independently be reduced to an application time of five minutes while still achieving intense color results on hair. An application time of 15 min for *C. tinctoria* extract or ferrous lactate represented an optimum time to achieve the highest color intensity and darkness. Longer exposure times of 30 min did not cause significant color changes visible to the human eye. This effect could be attributed to the saturation of flavonoids and/or Fe^{2+} ions adsorbed on hair. Furthermore, longer treatment times included a higher chance of interactions between flavonoids on the one hand and Fe^{2+} on the other hand with water and oxygen. This interaction could lead to the degradation of flavonoids and the oxidation of Fe^{2+} to Fe^{3+}. The reduced amount of flavonoids and/or Fe^{2+} could cause the slight decrease in color intensity after dyeing and mordanting for 30 min. The rapid color formation within five minutes of application time and the consistent color results after 15 min are promising regarding future development and application of the presented hair dye.

Variations in dye concentration were shown to have an impact on the color result the most. In general, a saturation effect could be observed using a concentration of 0.5% (w/v) or higher of ferrous lactate or *C. tinctoria*. The color intensity did not increase

proportionally when increasing concentrations from 0.5% (*w*/*v*) to 1% (*w*/*v*). Several studies observed a similar phenomenon for plant extract and mordant concentrations. They found that an increase in plant dye or mordant concentration did, in some cases, only lead to a negligible change in color strength on hair [9,28,56,57]. This effect was observed by Sargsyan et al. as well, where concentrations of more than 10% of plant raw material did not lead to a visible nor significant color change on hair [32]. The slight decrease in ΔE for 1% (*w*/*v*) of *C. tinctoria* extract or ferrous lactate could be based on a saturation regarding the possible adsorption places on hair that are already occupied and/or due to a saturated dye solution. In addition, for mordant concentration, an increase in concentration may only lead to increasing color strength if there were still flavonoids with free binding sites on hair available to form coordination bonds. This could be the case for the use of 1% (*w*/*v*) *C. tinctoria* extract and ferrous lactate. In general, the ferrous lactate concentration could be reduced to 0.01% (*w*/*v*) without losing much color intensity on hair. However, a ferrous lactate concentration of 0.5% (*w*/*v*) gives the darkest and most intense colored hair strands. Lower ferrous lactate concentrations are favored in terms of reducing health risks for consumers and aquatic life, as well as targeting the category of sustainable hair dyes. *C. tinctoria* extract concentration was shown to have the highest impact on color nuance of hair. The variation in *C. tinctoria* extract concentration influenced the darkness of color outcomes, indicated by higher L^* values for lower concentrations and vice versa for higher concentrations. A future product comprising the *C. tinctoria* extract could benefit from these properties, as multiple color nuances can be achieved without having to add different dyes. Color nuances can be controlled via concentrations.

3.3. Identification of Polyphenols Responsible for Dyeing Outcome

The extraction procedure for *C. tinctoria* flower heads as described in Section 2.2 showed a yield of 41.3% (Table 6). The use of 55% (*v*/*v*) ethanol as extraction solvent has already been shown to represent an efficient solvent to reach high extract yields and polyphenolic contents [13,20,40]. A total of 130.45 mg gallic acid equivalents per g extract (TPC) and 110.03 mg quercetin equivalents per g extract (TFC) were determined. Lam et al. investigated 13 different compounds present in *C. tinctoria* flowers, of which 10 compounds were defined as flavonoids. Lam et al. determined their total content to be 57.38–98.46 mg per g extract, depending on the regional origin of the plants. Taking the different origins (France) of the *C. tinctoria* flower used within this work into account, a TFC of 110.03 mg quercetin per g extract was comparable [20]. The slightly higher value of TPC can be explained by the presence of further compounds that do not belong to the flavonoid subclass, such as phenolic acids, but have formed complexes with the Folin–Ciocalteu reagent and thus were detected erroneously [15].

Table 6. Quantitative analysis of the *C. tinctoria* extract represented by the amounts of the seven identified flavonoids (x_{F2}), extract yield related to initial weight of the plant raw material, total polyphenol content (x_{TPC}), and total flavonoid content (x_{TFC}). Mean value ± standard deviation; $n = 3$.

x_{F2} (mg per g Extract)							Extract Yield (%)	x_{TPC} (mg per g Extract)	x_{TFC} (mg per g Extract)
(1) Flavano-Marein	(2) Isookanin	(3) Marein	(4) Okanin	(5) Mariti-Mein	(6) Mariti-Metin	(7) Butein			
19.93 ± 1.28	12.76 ± 0.98	34.03 ± 4.20	10.61 ± 1.42	2.89 ± 0.93 [1]	1.88 ± 0.13 [1]	0.93 ± 0.13	41.3 ± 9.7	130.45 ± 3.40	110.03 ± 2.86

[1] Concentrations were determined as above *LOD* and below *LOI* and *LOQ*.

The assessment of individual flavonoids present in *C. tinctoria* extract done by analytical HPLC is visualized in Figure 5. Four major peaks were identified as flavanomarein (**1**), isookanin (**2**), marein (**3**), and okanin (**4**). Additionally, three minor peaks were assigned to

maritimein (**5**), maritimetin (**6**), and butein (**7**). All mentioned flavonoids were identified based on their retention time as well as their UV–Vis absorbance spectra, which were in good accordance with the reference substances used for HPLC (see Table 1, Figure S3, and Table S11). The quantitative evaluation (see Table 6) specified flavanomarein (19.93 mg per g extract) and marein (34.03 mg per g extract) as major compounds present inside the *C. tinctoria* extract, which was in accordance with the previously published literature [20]. Guo et al. found the content of flavanomarein to be 6.20% and of marein to be 3.92% [58]. Maritimein and maritimetin could be detected within the chromatogram; however, a statistically reliable quantitative analysis could not be conducted as the amounts were below the limit of quantification.

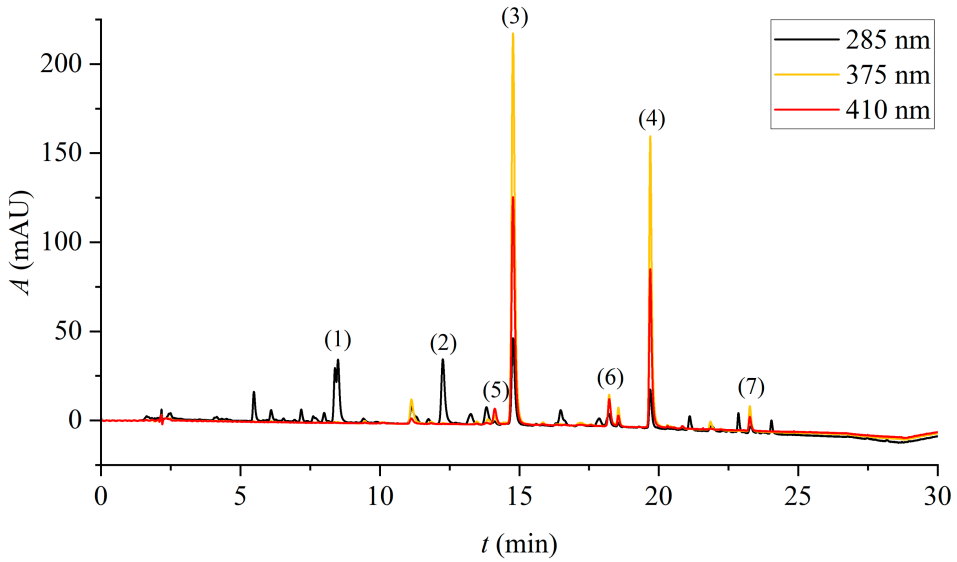


Figure 5. HPLC chromatogram of *C. tinctoria* extract blend (n = 3) showing the seven identified flavonoids—(**1**) flavanomarein, (**2**) isookanin, (**3**) marein, (**4**) okanin, (**5**) maritimein, (**6**) maritimetin, and (**7**) butein—detected at 285 nm (black line), 375 nm (yellow line), and 410 nm (red line).

To identify the influence of the major flavonoids on hair color outcomes, dyeing experiments using flavanomarein and marein were performed (see Figure 6). To investigate the individual dyeing properties of each flavonoid, 12 mg of each flavonoid were used. In a second experiment, the actual amounts of each compound found in *C. tinctoria* extracts were used to mimic the influence and color result when the extract was applied. Additionally, enzymatic hydrolysis was performed to examine the influence of the flavonoid aglycones isookanin and okanin. As shown in Supplementary Materials Figures S1 and S2, a complete conversion of the glycosides flavanomarein and marein into isookanin and okanin after the addition of a β-glucosidase-containing cellulase mix was achieved. Hence, enzymatic hydrolysis was proven to be an appropriate procedure that made use of dyeing hair with the aglycones only.

For color results without mordants, light colors similar to untreated hair (see Supplementary Materials Table S1) were achieved using the flavanones flavanomarein and isookanin. Regardless of the concentration used for hair dyeing, no meaningful color development was visible. However, the chalcones marein and okanin showed a yellow color on hair. Comparing the identical amounts of flavonoid used for dyeing, the glycoside marein showed a pale yellow color with a b^* value of 32.7, whereas the aglycone okanin revealed an intense yellow color with a b^* value of 55.6. This demonstrated the high color strength of okanin compared to its glycoside marein. When comparing the color results of hair dyed with 12 mg to 2.7 mg okanin, there was a slight decrease in lightness (1.1 units) and in b^*

value (3.1 units) observable. By calculating the Δ*E* between these two strands, a difference of 3.4 units was observable, displaying a slight visible difference between the colors.

		Identical amount of flavonoids used for dyeing					**Amount as found in extract used for dyeing**			
Amount of flavonoid used for dyeing (mg)		Flavano-marein	Isookanin	Marein	Okanin	Butein	Flavano-marein	Isookanin	Marein	Okanin
		12.0	12.0	12.0	12.0	12.0	5.0	3.2	8.5	2.7
Without mordant	Hair strand									
	*L**	76.8	74.2	69.7	67.5	74.5	77.8	76.3	70.3	66.4
	*a**	−0.7	−0.6	4.4	0.5	−1.9	−0.7	−0.7	2.1	1.3
	*b**	7.9	9.7	32.7	55.6	28.0	8.2	8.0	33.7	52.5
	ΔE	1.9	3.2	24.6	47.9	19.1	1.7	2.1	25.7	45.2
Fe^{2+} as mordant	Hair strand									
	*L**	68.1	32.3	40.8	21.6	56.9	69.8	47.4	39.6	20.8
	*a**	−0.2	0.2	4.3	2.2	2.3	−0.1	−0.3	4.5	2.4
	*b**	9.6	−0.6	10.1	7.6	25.2	10.8	0.8	10.2	6.7
	ΔE	8.3	45.2	36.5	55.8	25.8	6.7	30.3	35.7	56.6

Figure 6. Dyeing outcomes using the flavonoids flavanomarein and marein and their glycosides isookanin and okanin derived through enzymatical hydrolysis, as well as the chalcone butein, with and without subsequent treatment using 1% (*w*/*v*) ferrous lactate. For comparison, an identical flavonoid amount (12 mg, left) and a flavonoid amount as found in the C. tinctoria extract (right) were used for dyeing. Results are represented by *L*a*b** and ΔE values and photos were taken of the dyed hair strands.

For hair treated with flavanones and ferrous lactate, there is no meaningful color development visible when using flavanomarein. This shows, that regardless of its high amount present inside the *C. tinctoria* extract, flavanomarein seems not to be involved in the overall color outcome when dyeing with the extract. The hair strand dyed with 12 mg isookanin, and ferrous lactate shows an intense gray color, with a *L** value of 32.3 and a Δ*E* value of 45.2. When using the amount of isookanin as found in the *C. tinctoria* extract (3.2 mg), the hair strand appears slightly lighter in color with an increase in *L** value to 47.4 and decrease in Δ*E* value to 30.3. Both chalcones show a color development in combination ferrous lactate. For marein, a medium intense warm brown color is developed, with a Δ*E* value of 36.5 for dyeing with 12 mg marein. This color is similar to the one formed by dyeing with 8.5 mg of marein and ferrous lactate. Okanin shows an intense dark brown color on hair. The color of the chalcone aglycone has a higher color strength, compared to the glycoside marein, when the identical amount of 12 mg is used for dyeing. This is indicated by an increase in Δ*E* value to 55.8. When using the same amount of okanin (2.7 mg) as found in the *C. tinctoria* extract, color intensity and lightness do not change significantly compared to the dyeing result using 12 mg okanin.

Comparing the *L*a*b** coordinates of the individual flavonoids with the color of *C. tinctoria* extract on hair, the occurring yellow color can be assigned to the direct dyeing properties of okanin. Still, marein showed a yellow color on hair as well. But due to the decreased color strength compared to okanin, this color might be covered by the intense yellow of okanin. The flavanones presented did not have direct dyeing properties. The subsequent ferrous lactate treatment revealed brown colors for the chalcones and a gray color for isookanin. It is most likely that a combination of isookanin, marein and okanin provides the resulting color as can be seen by applying *C. tinctoria* extract and ferrous lactate. However, the high color strength of okanin might be the dominant dye within the extract.

To highlight the importance of okanin as a dye molecule, butein, a similar chalcone present in *C. tinctoria*, was used for dyeing hair. For the treatment without ferrous lactate, pale yellow colors with ΔE = 19.1, similar to marein, were found. A subsequent treatment with ferrous lactate led to a light yellowish-brown color with a ΔE value of 25.8 and a high b^* value of 25.2. These color results differed highly from those of the chalcone okanin in color strength and darkness. It can be assumed that okanin indeed possesses the highest influence on color outcomes inside *C. tinctoria* extracts compared to other possible chalcones—not only due to its high color strength but also its high amount. However, to fully confirm this hypothesis, all polyphenols present in *C. tinctoria* flowers would need to be evaluated within dyeing experiments.

A closer look at the color outcome of glycosides compared to aglycones revealed a decrease in color strength for all examined glycosides. This phenomenon was most distinct for flavanomarein, which did not show a meaningful color result when applied on hair at all. However, isookanin displayed an intense gray color when combined with a subsequent treatment using ferrous lactate. The same trend can be applied to marein, which revealed medium intense colors, and its aglycone okanin, exhibiting the highest color strength observed. It can be deduced that aglycones show darker and more intense colors on hair than their corresponding glycoside.

It is already known that glycosylated polyphenols can act as precursor molecules for hair and textile coloration [8]. Enzymes, e.g., ß-*D*-glucosidase, can be used to promote enzymatic hydrolysis into the polyphenolic aglycones shortly before application on hair. Thus, higher stability of dye molecules during storage, e.g., within a formula, is ensured [8]. Prominent examples of this approach are the henna and indigo plant, containing the precursor molecules hennoside and indican. Before applying these plant dyes on hair, enzymatic hydrolysis provokes the formation of lawson and the indigo molecule [2,8,59–61]. Relating to the application of *C. tinctoria* as a hair dye, the use of glycosides in formulations could also be applied to flavanomarein and marein. Though the application in combination with metal salts such as ferrous lactate would be preferred, marein could work as a precursor molecule together with hennoside and indican to open up new color nuances for direct dyes. Since the commercial cultivation of *C. tinctoria* has already been performed, e.g., in China, the development and availability of *C. tinctoria* extracts for future hair colorations are within future reach [14].

To summarize, naturally sourced nuances with high coverage can preferably be achieved by applying the combination of marein and okanin—warm color nuances derived from the glycoside and intense colors from the aglycone. Future research regarding *C. tinctoria* extract modification and enrichment in certain flavonoids could lead to the development of customized extracts. These could comprise the desired dyeing properties and flavonoid amounts to receive the best possible dyeing outcome.

4. Conclusions

This study presents a new hair coloration based on *C. tinctoria* extract with or without subsequent mordanting. *C. tinctoria* raw material and *C. tinctoria* extract applied to hair lead to yellow colors. Colors arising from the combinatory treatment of *C. tinctoria* and metal ions lead to natural and dark brown colors on hair, making it appealing to future consumers. It was demonstrated that the presented hair dyes show very good wash fastness. In particular, for the combination of *C. tinctoria* extract and ferrous lactate applied to human hair, small differences in color compared to unwashed hair were observed. By comparing the results to oxidative permanent dyes, these differences were located within the range that can be expected from permanent dyes. Investigation of dyeing times and dye concentrations highlighted the possibility to develop further color shades, as well as lowering application time and metal salt concentration. Marein and flavanomarein were found to be the most abundant flavonoids present in *C. tinctoria* extract, followed by their aglycones, isookanin and okanin. Dyeing experiments with these compounds proved the importance of chalcones for brown color development in combination with ferrous lactate. However, while focus was laid on these flavonoids, to fully characterize *C. tinctoria* as a hair dye, the dye performance of all polyphenols present in *C. tinctoria* extract must be kept in mind for prospective studies. For the future development of colorations, the combination of different flavonoids could be used to develop browns with different hues. Together with other polyphenolic extracts and different metal salts, the extension of the color portfolio seems realistic.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics13030127/s1, Table S1: Reference hair strands comprising untreated hair; hair treated with 1% (*w*/*v*) ferrous lactate; combinatory treatment using 0.9% (*w*/*v*) (0.99% (*w*/*v*)) sodium molybdate and 0.1% (*w*/*v*) (0.01% (*w*/*v*)) ferrous lactate; and 1% (*w*/*v*) potassium titanium oxide oxalate, represented by *L*a*b** and ΔE values and photos taken of the hair strands, as well as the natural pH value of the metal salts in solution. Table S2: Reference hair dyed with oxidative permanent hair color (Igora Royal permanent color crème 5–13 combined with Igora Royal oil developer 6% in a ratio of 1 to 1), directly after dyeing (0 hair washes), and after 6, 12, 18, and 24 hair washes. Results represented by *L*a*b** and Δ*E* values calculated to untreated or unwashed hair (mean value ± standard deviation; *n* = 3) and photos taken of hair strands. Table S3: Dyeing outcomes on white human hair using 1% (*w*/*v*) *C. tinctoria* extract with and without treatment using 1% (*w*/*v*) ferrous lactate, as well as untreated hair as a reference. Results represented as *L*a*b** and Δ*E* values calculated to untreated human hair (mean value ± standard deviation; *n* = 3) and photos taken of the hair strands. Table S4: Comparison of wash fastness of hair strands dyed with 1% (*w*/*v*) *C. tinctoria* extract, reflected as *L*a*b** and Δ*E* values related to unwashed hair measured after the given number of hair washes (mean value ± SD; *n* = 3). Table S5: Comparison of wash fastness of hair strands dyed with 1% (*w*/*v*) *C. tinctoria* extract and 1% (*w*/*v*) ferrous lactate, reflected as *L*a*b** and Δ*E* values related to unwashed hair measured after the given number of hair washes (mean value ± SD; *n* = 3). Table S6: Comparison of wash fastness of hair strands dyed with 1% (*w*/*v*) *C. tinctoria* extract and combinatory treatment using 0.9% (*w*/*v*) sodium molybdate and 0.1% (*w*/*v*) ferrous lactate, reflected as *L*a*b** and Δ*E* values related to unwashed hair measured after the given number of hair washes (mean value ± SD; *n* = 3). Table S7: Comparison of wash fastness of hair strands dyed with 1% (*w*/*v*) C. tinctoria extract and combinatory treatment using 0.99% (*w*/*v*) sodium molybdate and 0.01% (*w*/*v*) ferrous lactate, reflected as *L*a*b** and Δ*E* values related to unwashed hair measured after the given number of hair washes (mean value ± SD; *n* = 3). Table S8: Comparison of wash fastness of hair strands dyed with 1% (*w*/*v*) *C. tinctoria* extract and 1% (*w*/*v*) sodium molybdate, reflected as *L*a*b** and Δ*E* values related to unwashed hair measured after the given number of hair washes (mean value ± SD; *n* = 3). Table S9: Comparison of wash fastness of hair strands dyed with 1% (*w*/*v*) *C. tinctoria* extract and 1% (*w*/*v*) potassium titanium oxide oxalate,

reflected as $L^*a^*b^*$ and ΔE values related to unwashed hair measured after the given number of hair washes (mean value ± SD; $n = 3$). Table S10: Comparison of wash fastness of human hair strands dyed with 1% (w/v) *C. tinctoria* extract and 1% (w/v) ferrous lactate, reflected as $L^*a^*b^*$ and ΔE values related to unwashed human hair measured after the given number of hair washes (mean value ± SD; $n = 3$). Table S11: UV–Vis absorbance maxima of reference compounds (flavanomarein, isookanin, marein, okanin, maritimein, maritimetin, and butein) compared to UV–Vis absorbance of compounds identified within the *C. tinctoria* extract. Figure S1: HPLC chromatogram of flavanomarein before (black line) and after incubation with β-glucosidase-containing cellulase mix for 30 min (red line), detected at 285 nm. Figure S2: HPLC chromatogram of marein before (black line) and after incubation with β-glucosidase-containing cellulase mix for 30 min (red line), detected at 375 nm. Figure S3: UV–Vis absorbance of (A) flavanones flavanomarein and isookanin, (B) chalcones marein and okanin, (C) aurones maritimein and maritimetin, and (D) butein, recorded for HPLC reference compound (continuous line) and for compound identified within the *C. tinctoria* extract (dashed line).

Author Contributions: Conceptualization, J.M.S. and F.S.; methodology, J.M.S.; software, J.M.S.; validation, J.M.S., F.S. and V.V.; formal analysis, J.M.S.; investigation, J.M.S.; resources, F.S. and V.V.; data curation, J.M.S.; writing—original draft preparation, J.M.S.; writing—review and editing, J.M.S. and F.S.; visualization, J.M.S.; supervision, F.S. and V.V.; project administration, F.S. and V.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in this article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank Novonesis for providing the β-glucosidase-containing cellulase blend.

Conflicts of Interest: J.M.S. and F.S. are employees of Henkel AG, Düsseldorf, Germany. The research was conducted within an established scientific collaboration between Henkel AG and the University of Hamburg. All aspects of the study, including its design, data collection, analysis, and the decision to publish, were carried out by the authors. The company did not exert any additional influence on the interpretation of the results or the publication of the study.

Abbreviations

The following abbreviations are used in this manuscript:

a	y-intercept of linear regression
a^*	Green–red coordinate in CIE-Lab color space
b	Slope of linear regression
b^*	Blue–yellow coordinate in CIE-Lab color space
C. tinctoria	*Coreopsis tinctoria* Nutt.
CAS-RN	Chemical Abstracts Service Registry Number
CIE	Commission Internationale de l'Èclairge
CT	Charge transfer
DAD	Diode array detector
ΔE	Distance between two colors in CIE-Lab color space
f	Degrees of freedom
HPLC	High performance liquid chromatography
HSAB	Hard and Soft Acids and Bases
ISO	International Organization for Standardization
L^*	Lightness, black–white coordinate in CIE-Lab color space
λ_C	Wavelength used for calibration
LOD	Limit of detection

LOI	Limit of inclusion
LOQ	Limit of quantification
m_A	Amount of aglycon
M_A	Molar mass of aglycon
m_E	Amount of extract
m_G	Amount of glycoside
M_G	Molar mass of glycoside
m_{SL}	Amount of compound in stock solution
MS	Mass spectrometry
n	Number of samples
N_C	Number of calibration points
PPD	*p*-phenylenediamine
PVDF	Polyvinylidene fluoride
R^2	Coefficient of determination
SD	Standard deviation
SLES	Sodium laureth sulfate
TFC	Total flavonoid content
TPC	Total polyphenolic content
t_R	Retention time
UV–Vis	Ultraviolet–visible
x	Concentration
x_{F1}	Concentration of flavonoid in µg mL^{-1}
x_{F2}	Concentration of flavonoid in mg per g extract
x_i	Concentration of flavonoid in µg mL^{-1} used for linear calibration
x_{TFC}	Total flavonoid content in mg per g extract
x_{TPC}	Total polyphenolic content in mg per g extract
y	Area below peak

References

1. Morel, O.J.X.; Christie, R.M. Current Trends in the Chemistry of Permanent Hair Dyeing. *Chem. Rev.* **2011**, *111*, 2537–2561. [CrossRef]
2. Stevens, C.; Bechthold, T.; Manian, A.; Pham, T. (Eds.) *Handbook of Natural Colorants*; John Wiley & Sons: Chichester, UK, 2023.
3. European Commission; Directorate General for Health and Food Safety. *Opinion on Resorcinol: SCCS/1619/20*; European Comission: Brussels, Belgium, 2021.
4. Mishra, V.; Sharma, U.; Rawat, D.; Benson, D.; Singh, M.; Sharma, R.S. Fast-Changing Life-Styles and Ecotoxicity of Hair Dyes Drive the Emergence of Hidden Toxicants Threatening Environmental Sustainability in Asia. *Environ. Res.* **2020**, *184*, 109253. [CrossRef]
5. Shim, J.; Woo, J.; Yeo, H.; Kang, S.; Kwon, B.; Jung Lee, E.; Oh, J.; Jeong, E.; Lim, J.; Gyoo Park, S. The Clean Beauty Trend Among Millennial and Generation Z Consumers: Assessing the Safety, Ethicality, and Sustainability Attributes of Cosmetic Products. *Sage Open* **2024**, *14*, 2. [CrossRef]
6. Cui, H.; Xie, W.; Hua, Z.; Cao, L.; Xiong, Z.; Tang, Y.; Yuan, Z. Recent Advancements in Natural Plant Colorants Used for Hair Dye Applications: A Review. *Molecules* **2022**, *27*, 8062. [CrossRef] [PubMed]
7. Dallmann, N.; Vill, V.; Straske, F. A Novel and Reliable Analysis Method Utilizing Hennosides to Improve the Quality Assessment of *Lawsonia inermis* L. Material Used in Cosmetic Formulations. *Cosmetics* **2025**, *12*, 99. [CrossRef]
8. Dallmann, N.; Vill, V.; Straske, F. Reinventing Henna: Enzyme-catalysed Colour Release from Stabilized *Lawsonia inermis* L. Extracts. *Int. J. Cosmet. Sci.* **2025**, *48*, 310–326. [CrossRef]
9. Lueadnakrob, K.; Juntrapirom, S.; Rongthong, T.; Kanjanakawinkul, W.; Chaiyana, W. Functional Performance and Safety Evaluation of Optimized Plant-Based Dye Mixtures for Intense Hair Coloration. *Cosmetics* **2025**, *12*, 78. [CrossRef]
10. Zhang, X.; Boytner, R.; Cabrera, J.L.; Laursen, R. Identification of Yellow Dye Types in Pre-Columbian Andean Textiles. *Anal. Chem.* **2007**, *79*, 1575–1582. [CrossRef]
11. Mostacero León, J.; López Medina, S.E.; Yabar, H.; De La Cruz Castillo, J. Preserving Traditional Botanical Knowledge: The Importance of Phytogeographic and Ethnobotanical Inventory of Peruvian Dye Plants. *Plants* **2017**, *6*, 63. [CrossRef] [PubMed]
12. Begmatov, N.; Li, J.; Bobakulov, K.; Numonov, S.; Aisa, H.A. The Chemical Components of *Coreopsis tinctoria* Nutt. and Their Antioxidant, Antidiabetic and Antibacterial Activities. *Nat. Prod. Res.* **2020**, *34*, 1772–1776. [CrossRef]

13. Zălaru, C.; Crişan, C.; Călinescu, I.; Moldovan, Z.; Ţârcomnicu, I.; Litescu, S.; Tatia, R.; Moldovan, L.; Boda, D.; Iovu, M. Polyphenols in *Coreopsis tinctoria* Nutt. Fruits and the Plant Extracts Antioxidant Capacity Evaluation. *Cent. Eur. J. Chem.* **2014**, *12*, 858–867. [CrossRef]
14. Yu, Q.; Chen, W.; Zhang, J.; Huang, D.; Shi, W.; Chen, H.; Yan, C. Purification, Structural Characterization, and Bioactivities of a Polysaccharide from *Coreopsis tinctoria*. *Food Front.* **2022**, *3*, 736–748. [CrossRef]
15. Shen, J.; Hu, M.; Tan, W.; Ding, J.; Jiang, B.; Xu, L.; Hamulati, H.; He, C.; Sun, Y.; Xiao, P. Traditional Uses, Phytochemistry, Pharmacology, and Toxicology of *Coreopsis tinctoria* Nutt.: A Review. *J. Ethnopharmacol.* **2021**, *269*, 113690. [CrossRef]
16. Zhi, Q.; Li, Y.; Li, F.; Tian, Y.; Li, F.; Tang, Y.; Yang, Y.; Yin, R.; Ming, J. Polyphenols Extracted from *Coreopsis tinctoria* Buds Exhibited a Protective Effect against Acute Liver Damage. *J. Funct. Foods* **2018**, *44*, 201–208. [CrossRef]
17. Ma, Z.; Zheng, S.; Han, H.; Meng, J.; Yang, X.; Zeng, S.; Zhou, H.; Jiang, H. The Bioactive Components of *Coreopsis tinctoria* (Asteraceae) Capitula: Antioxidant Activity in Vitro and Profile in Rat Plasma. *J. Funct. Foods* **2016**, *20*, 575–586. [CrossRef]
18. Li, N.; Meng, D.; Pan, Y.; Cui, Q.; Li, G.; Ni, H.; Sun, Y.; Qing, D.; Jia, X.; Pan, Y.; et al. Anti-Neuroinflammatory and NQO1 Inducing Activity of Natural Phytochemicals from *Coreopsis tinctoria*. *J. Funct. Foods* **2015**, *17*, 837–846. [CrossRef]
19. Li, Y.; Huang, C.; Fu, W.; Zhang, H.; Lao, Y.; Zhou, H.; Tan, H.; Xu, H. Screening of the Active Fractions from the *Coreopsis tinctoria* Nutt. Flower on Diabetic Endothelial Protection and Determination of the Underlying Mechanism. *J. Ethnopharmacol.* **2020**, *253*, 112645. [CrossRef] [PubMed]
20. Lam, S.; Liu, X.; Chen, X.; Hu, D.; Zhao, J.; Long, Z.; Fan, B.; Li, S. Chemical Characteristics of Different Parts of *Coreopsis Tinctoria* in China Using Microwave-assisted Extraction and High-performance Liquid Chromatography Followed by Chemometric Analysis. *J. Sep. Sci.* **2016**, *39*, 2919–2927. [CrossRef]
21. Shimokoriyama, M. Anthochlor Pigments of *Coreopsis tinctoria*. *J. Am. Chem. Soc.* **1957**, *79*, 214–220. [CrossRef]
22. Yang, Y.; Sun, X.; Liu, J.; Kang, L.; Chen, S.; Ma, B.; Guo, B. Quantitative and Qualitative Analysis of Flavonoids and Phenolic Acids in Snow Chrysanthemum (*Coreopsis tinctoria* Nutt.) by HPLC-DAD and UPLC-ESI-QTOF-MS. *Molecules* **2016**, *21*, 1307. [CrossRef]
23. Crawford, D.J.; Smith, E.B. Leaf Flavonoid Chemistry and Taxonomy of Coreopsis Sect. Coreopsis. *Biochem. Syst. Ecol.* **1985**, *13*, 115–118. [CrossRef]
24. Geissman, T.A.; Harborne, J.B.; Seikel, M.K. Anthochlor Pigments. XI. The Constituents of Coreopsis Maritima. Reinvestigation of Coreopsis Gigantea. *J. Am. Chem. Soc.* **1956**, *78*, 825–829. [CrossRef]
25. Nakayama, T. Biochemistry and Regulation of Aurone Biosynthesis. *Biosci. Biotechnol. Biochem.* **2022**, *86*, 557–573. [CrossRef] [PubMed]
26. Velmurugan, P.; Shim, J.; Seo, S.-K.; Oh, B.-T. Extraction of Natural Dye from *Coreopsis tinctoria* Flower Petals for Leather Dyeing—An Eco-Friendly Approach. *Fibers Polym.* **2016**, *17*, 1875–1883. [CrossRef]
27. Su, X.; Liu, G.; Zheng, M.; Li, X.; Song, K.; Shi, D. Integrating the Dual Functionality of Plant Tannins for Natural Hair Dyeing: A Critical Review on Mechanisms, Sustainability and Formulation Innovation. *Color. Technol.* **2026**, *142*, 177–195. [CrossRef]
28. Cui, H.; Cai, R.; Hua, Z.; Tang, Y. Plant Colorants for Natural Hair Coloration: Dyeing Optimization and Photostability Assessment. *Sustain. Chem. Pharm.* **2023**, *36*, 101285. [CrossRef]
29. Fang, C.; Ma, L.; Chen, T.; Chen, Y.; Li, Z.; Yan, X.; Wang, J. Multifunctional Amino Acid-Assisted Hair Dyeing with Metal-Polyphenol Networks. *Fibers Polym.* **2025**, *26*, 4933–4942. [CrossRef]
30. Jia, D.; Shen, Y.; Zhang, X.; Wang, Y.; Su, R.; Qi, W. Colorful Pigments Based on Multicomponent Metal-Phenol Network Nanoparticles for Hair Dyeing. *ChemistrySelect* **2022**, *7*, e202203886. [CrossRef]
31. Geng, H.; Zhuang, L.; Li, M.; Liu, H.; Caruso, F.; Hao, J.; Cui, J. Interfacial Assembly of Metal–Phenolic Networks for Hair Dyeing. *ACS Appl. Mater. Interfaces* **2020**, *12*, 29826–29834. [CrossRef]
32. Sargsyan, L.; Hippe, T.; Manneck, H.; Vill, V. Tannin-Mordant Coloration with Matcha (Camelia Sinensis) and Iron(II)-Lactate on Human Hair Tresses. *Molecules* **2021**, *26*, 829. [CrossRef] [PubMed]
33. Sargsyan, L.; Vill, V.; Hippe, T. Investigations of Vegetable Tannins as Hair Dyes and Their Interactions with Pre-bleached Hair Fibres. *Int. J. Cosmet. Sci.* **2020**, *42*, 320–327. [CrossRef]
34. Han, S.Y.; Kang, E.K.; Choi, I.S. Iron Gall Ink Revisited: A Surfactant-Free Emulsion Technology for Black Hair-Dyeing Formulation. *Cosmetics* **2021**, *8*, 9. [CrossRef]
35. Han, S.; Hong, S.-P.; Kang, E.; Kim, B.; Lee, H.; Kim, W.; Choi, I. Iron Gall Ink Revisited: Natural Formulation for Black Hair-Dyeing. *Cosmetics* **2019**, *6*, 23. [CrossRef]
36. Prasetyo, S.; Bertha, S.; Sugih, A.K.; Tan, D.; Kristianto, H. Valorization of Mangosteen (*Garcinia mangostana* L.) Waste as Green Hair Dyeing Pigment: Effect of Mordant and Formula Optimization. *Sustain. Chem. One World* **2026**, *9*, 100176. [CrossRef]
37. European Union. Regulation (EC) No 1223/2009 of the European Parliament and of the Council on Cosmetic Products. *Off. J. Eur. Union* **2009**, *L 342*, 59–209.
38. European Union. Regulation (EC) No 1333/2008 of the European Parliament and of the Council on Food Additives. *Off. J. Eur. Union* **2008**, *L 354*, 16–33.

39. Müllner, A.R.M.; Pahl, R.; Brandhuber, D.; Peterlik, H. Porosity at Different Structural Levels in Human and Yak Belly Hair and Its Effect on Hair Dyeing. *Molecules* **2020**, *25*, 2143. [CrossRef] [PubMed]
40. Chen, L.X.; Hu, D.J.; Lam, S.C.; Ge, L.; Wu, D.; Zhao, J.; Long, Z.R.; Yang, W.J.; Fan, B.; Li, S.P. Comparison of Antioxidant Activities of Different Parts from Snow Chrysanthemum (*Coreopsis tinctoria* Nutt.) and Identification of Their Natural Antioxidants Using High Performance Liquid Chromatography Coupled with Diode Array Detection and Mass Spectrometry and 2,2′-Azinobis(3-Ethylbenzthiazoline-Sulfonic Acid)Diammonium Salt-Based Assay. *J. Chromatogr. A* **2016**, *1428*, 134–142. [CrossRef]
41. Funk, W.; Dammann, V.; Donnevert, G. *Qualitätssicherung in Der Analytischen Chemie*, 2nd ed.; Wiley-VHC: Weinheim, Germany, 2005.
42. Kromidas, S.; Kuss, H.-J. (Eds.) *Chromatogramme Richtig Integrieren Und Bewerten: Ein Praxishandbuch Für Die HPLC Und GC*; Wiley-VHC: Weinheim, Germany, 2008.
43. *ISO 14502-1:2005*; Determination of Substances Characteristic of Green and Black Tea Part 1: Content of Total Polyphenols in Tea—Colorimetric Method Using Folin-Ciocalteu Reagent. ISO: Geneva, Switzerland, 2005.
44. Yao, X.; Zhang, T.; Wang, B.; Chin, W. Determination Method of Total Flavonoids in *Coreopsis tinctoria* Nutt. from Xinjiang. *J. Shihezi Univ. Nat. Sci.* **2015**, *33*, 183–187.
45. *ISO/CIE 11664-4:2019*; Colorimetry-Part 4: CIE 1976 L*a*b* Colour Space. ISO: Geneva, Switzerland, 2019.
46. Machado, A.V.; Menezes, L.R.O.; Santana, A.A.M.; Marques, M.N.; De Lima, A.K.; Cano, A.; Severino, P.; Souto, E.B.; Cardoso, J.C. *Genipa americana* L.: A New Phytochemical for White Hair Coloring. *Chem. Biodivers.* **2023**, *20*, e202300083. [CrossRef]
47. Karma, I.G.M. Determination and Measurement of Color Dissimilarity. *IJEET* **2020**, *5*, 67–71. [CrossRef]
48. Simon, K. *Farbe Im Digitalen Publizieren: Konzepte Der Digitalen Farbwiedergabe Für Office, Design Und Software*, 6th ed.; Springer: Berlin/Heidelberg, Germany; Thalwil, Switzerland, 2008.
49. Hachmann, J.K.; Sauler, J.M.; Ruhmlieb, C.; Vill, V.; Straske, F. Red Era: Dyeing Yak Hair Fibres Red with In Situ Generated Silver Nanoparticles Using Silver Nitrate and a Flavonoid-containing Plant Extract. *Color. Technol.* **2026**, *142*, 439–455. [CrossRef]
50. Lefebvre, T.; Destandau, E.; Lesellier, E. Selective Extraction of Bioactive Compounds from Plants Using Recent Extraction Techniques: A Review. *J. Chromatogr. A* **2021**, *1635*, 461770. [CrossRef] [PubMed]
51. Malesev, D.; Kuntic, V. Investigation of Metal-Flavonoid Chelates and the Determination of Flavonoids via Metal-Flavonoid Complexing Reactions. *J. Serbian Chem. Soc.* **2007**, *72*, 921–939. [CrossRef]
52. Ho, T.-L. Hard Soft Acids Bases (HSAB) Principle and Organic Chemistry. *Chem. Rev.* **1975**, *75*, 1–20. [CrossRef]
53. Bate, L.C. Adsorption and Elution of Trace Elements on Human Hair. *Appl. Radiat. Isot.* **1966**, *17*, 417–423. [CrossRef]
54. Hetherington, K.; Tidder, A.; Tack, B.J.; Benohoud, M.; Nowlan, D.; Zahar, A.; Prater, D.; Zguris, J.C.; Rayner, C.M.; Blackburn, R.S. Analysis and Quantification of the Propensity of Hair Dyes to Desorb from Human Hair Fibre Using an Accelerated 48-Wash Method. *Microchem. J.* **2026**, *220*, 116613. [CrossRef]
55. Zhou, Y.; Foltis, L.; Moore, D.J.; Rigoletto, R. Protection of Oxidative Hair Color Fading from Shampoo Washing by Hydrophobically Modified Cationic Polymers. *J. Cosmet. Sci.* **2009**, *60*, 217–238. [CrossRef]
56. Mallya, R.; Ravikumar, P. Formulation and Evaluation of Natural Hair Colorants. *Int. J. Pharm. Pharm. Sci.* **2015**, *7*, 347–349.
57. Boga, C.; Delpivo, C.; Ballarin, B.; Morigi, M.; Galli, S.; Micheletti, G.; Tozzi, S. Investigation on the Dyeing Power of Some Organic Natural Compounds for a Green Approach to Hair Dyeing. *Dye. Pigment.* **2013**, *97*, 9–18. [CrossRef]
58. Guo, L.; Zhang, W.; Li, S.; Ho, C.-T. Chemical and Nutraceutical Properties of *Coreopsis tinctoria*. *J. Funct. Foods* **2015**, *13*, 11–20. [CrossRef]
59. Klaas, S.; Vill, V.; Straske, F. Indirubin as a Red Hair Colourant from *Indigofera tinctoria* L. *Int. J. Cosmet. Sci.* **2025**, *47*, 877–886. [CrossRef]
60. Blackburn, R.S.; Bechtold, T.; John, P. The Development of Indigo Reduction Methods and Pre-reduced Indigo Products. *Color. Technol.* **2009**, *125*, 193–207. [CrossRef]
61. Minami, Y.; Nishimura, O.; Hara-Nishimura, I.; Nishimura, M.; Matsubara, H. Tissue and Intracellular Localization of Indican and the Purification and Characterization of Indican Synthase from Indigo Plants. *Plant Cell Physiol.* **2000**, *41*, 218–225. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.