Journal of Applied Pharmaceutical Science Vol. 15(01), pp 243-255, January, 2025
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2024.198274
ISSN 2231-3354

Formulation and evaluation of hair dye shampoo using *Genipa americana* L. (Huito) fruit extract

Dany Gutierrez-Ninachoque[1], Jimmy Oscar Ortiz de Orue-Meza[1], Carla del Carpio-Jiménez[2*]

[1]School of Pharmacy and Biochemistry, National University of San Antonio Abad del Cusco, Cusco, Peru.
[2]Institutional Laboratory of Pharmaceutical Technology, National University of San Antonio Abad del Cusco, Cusco, Peru.

ARTICLE HISTORY

Received on: 18/04/2024
Accepted on: 30/08/2024
Available Online: 25/11/2024

Key words:
Natural dye, hydroalcoholic extraction, tristimulus colorimeter, hair dye formulation, *Genipa americana*.

ABSTRACT

Natural dyes are in demand due to their inherent goodness and minor side effects. However, color fastness and poor reproducibility after application in human hair still need to be improved for natural hair dyeing. This study investigates the efficacy of *Genipa americana* fruit coloring extracts for hair dyeing. The dye was extracted from green fruit using a hydroalcoholic solution of 70% ethanol and 30% water. The study analyzed the physicochemical properties of the shampoo dye and evaluated its microbiological quality. The study also assessed the ability of the coloring shampoo to dye bleached hair by measuring the total color difference using a tristimulus colorimeter. The shampoo was applied for one month, with different numbers of applications (30, 15, and 10) and concentrations of 7.5% and 10% of the extract. A tress of bleached hair was also used as a control, but it did not receive any treatment. The results showed that the physicochemical characteristics were within the parameters established for this type of formulation. Likewise, the microbiological quality was satisfactory. Regarding the coloring capacity, it was shown that the 10% formulation presented the most significant color difference concerning the hair of the bleached tresses after 30 applications of 45 minutes each compared to the control. It was also shown that the concentration of *G. americana* extract directly affected the coloring.

INTRODUCTION

Hair coloring products comprise a significant beauty and personal care market segment [1]. The hair industry is moving towards using less harmful hair dyes that do not contain parabens (the most commonly used preservatives), persulfates (used in bleaching powders), or ammonia, which is an alkaline solution that opens up the hair cuticles to allow the color to be deposited on the hair cortex [2]. Synthetic hair dyes that contain heavy metals such as Pb, Cd, and Ni have toxic properties that can harm the environment and human health [3]. Specific hair dye formulations may cause skin sensitivity in the consumers and the professionals who apply them [4]. Contact dermatitis is a common condition that can be caused by hair dye, and its typical clinical presentation is redness, scaling, and itching of the face, along with dermatitis on the neck, hairline, and ears. Severe reactions can include facial swelling and ulceration of the scalp and ears after using hair dye [5,6]. In some cases, hair loss, scalp irritation, and changes in skin pigmentation, such as facial-pigmented contact dermatitis and lichen planus pigmentosus, may be present [7,8,9]. Additionally, certain oxidative hair dyes can cause painful sensory irritation of the scalp [10].

The use of natural dyes for hair coloration has a long history, dating back to ancient times when they were used medicinally, decoratively, and in religious ceremonies [11,12]. Before synthetic dyes were invented, metallic compounds, plant extracts, and dried plants were used for hair dyeing [13]. Various herbal preparations and plant extracts, such as saffron, birch bark, mullein, and turmeric, were utilized for hair staining. Ancient Egyptians used henna to maintain their hair color,

*Corresponding Author
Carla Del Carpio Jiménez, Institutional Laboratory of Pharmaceutical Technology, Universidad Nacional de San Antonio Abad del Cusco, Cusco, Perú.
E-mail: delcarpiojc_daqf @ unsaac.edu.pe

while ancient Greeks lightened their hair using a potassium lye solution and a yellow flower petal ointment [14]. Romans darkened their hair using lead combs and walnut stains [15].

Natural dyes are non-oxidative hair dyes that can be either temporary or semi-permanent. They can be absorbed into the hair's cuticle and certain parts of the fiber's cortex to create color. Derived from plants, such as flowers, fruits, seeds, leaves, and roots, these dyes are low-irritating, less allergenic, and eco-friendly. They also have health properties, such as being anti-inflammatory, antioxidant, and antimicrobial [16].

There are two main methods of natural plant dyes to color hair: direct dyeing and mordant dyeing. This process involves two distinct steps: first, dye molecules diffuse into the hair fibers; second, chemical bonds form between the dye molecules (which contain hydroxyl or carboxyl groups) and the amino/sulfhydryl groups in the hair keratin. This bond formation can occur with or without a mordanting product [17]. Diffusion is a three-stage process: (i) Dye molecules are transported to the fiber/water interface through aqueous diffusion and agitation. (ii) Dyes are absorbed by the outer layer of the hair cuticle. (iii) Dye molecules with low molecular weight penetrate the inner structures of the hair, mainly through the cell membrane complex (CMC), which connects the cortical cells and cuticle [18]. The research indicates that dye substances primarily enter the hair cortex through CMC penetration [19,20]. Meanwhile, less ionized small molecules can pass through and spread over the lipid bilayer of CMC [21]. Additionally, the condition of hair fibers impacts the absorption and diffusion of external dye substances. For instance, hydrogen peroxide in hair dye formulations can break down the disulfide bonds in hair keratin, damaging the hair fibers. As a result, this allows for deeper penetration and stronger dye bonding to the hair [12,22].

Direct dyeing is a process of coloring hair without using an oxidizing agent. During direct dyeing, the dye forms a bond with the hair fiber. The intensity of the color depends on how well the dye molecules bond with the hair fiber's surface. Smaller dye molecules, such as non-ionic dyes at 0.95 nm, cationic dyes at 1.4 nm, and anionic dyes at 1.2–1.3 nm [23], can easily enter the hair cuticle. On the other hand, larger molecules have a tougher time penetrating. However, they can still be attracted to the hair fiber through various forces such as electrostatic, van der Waals, and hydrogen bonding [24]. Natural dyes from henna leaves and walnut husks are commonly used for direct dyeing. In henna, lawsone, reacts with amino groups in hair keratin fibers at a slightly acidic pH (4.5–6.0), resulting in hair coloration. Scanning electron microscopy (SEM) observations have shown that henna dye may help repair cuticle damage and provide a smooth, moisturized appearance to dyed hair cuticles [25].

Synthetic dyes used to be popular for long-lasting hair color, but now ammonia-free commercial dyes are available to deposit color without damaging hair structure [26]. Permanent hair dyes are synthetic oxidative hair widely used for their strong dyeing performance and predictable colors [27] that use intermediates such as p-aminophenol and p-phenylenediamine, and some couplers such as m-aminophenol, resorcinol and m-hydroxyphenol [28]. However, studies have reported potential health risks associated with synthetic ingredients, including allergenicity [29,30], carcinogenicity [31], mutagenicity [32,33], and environmental toxicity [34].

The interest in natural hair dyes, which replace the toxic compounds in synthetic permanent and semi-permanent dyes, is increasing [22,11]. Using natural and renewable raw materials to develop a hair color that can permanently dye hair without causing damage is a highly sought-after goal in the industry [11]. Natural dyes are considered eco-friendly, biodegradable, and have a low probability of causing allergies or toxic reactions. Additionally, using "green formulations" in hair dyes ensures that the compounds used do not harm the environment [35].

Previously, iridoids like genipin were reported as dye protein fibers and recommended as "natural reactive dyes" for materials containing amino groups, including leather, wool, and silk [1]. Brazil's Indigenous tribe members used this fruit's contents to dye their textiles, create pottery, and paint their bodies during ritual ceremonies and battles [36]. Many countries, such as Brazil, have a long tradition of folk medicine in their various geographical regions and cultural communities [37]. Ethnomedical knowledge encompasses intricate practices, particularly those related to using herbs from Indigenous plant life [38]. There are experiences in the development of pharmaceuticals and cosmetics based on traditional knowledge about the use of plants and their components, which is an opportunity that should be prioritized through research in academic and research institutions [39]. Brazil has developed some drugs; for example, Lapachol, originally from the *Handroanthus impetiginosus* tree, was used to treat malaria and served as the model for atovaquone, a drug used to treat acute uncomplicated malaria caused by *Plasmodium falciparum* [40]. The latest research in cosmetics focuses on using nontraditional oils and butter from Brazilian tropical plants, promoting sustainability and benefiting small farms. These oils have the potential to replace traditional skincare ingredients and are increasingly being incorporated into new commercial products [41].

A tree of the Rubiaceae family, *Genipa americana* L., is found in Asia and Latin America, and it was established that it contains the iridoids geniposide and genipin, which are monoterpenes that can react with proteins and amino acids to create colored compounds [42]. These iridoids in fruit are initially colorless. However, upon contact with the amino groups, they turn blue in green fruit and crystalline yellow in ripe fruit [1].

Hair dye is one of the most popular cosmetics, although some components are dangerous. People continue to use it even if they have suffered discomfort or developed an allergy. Therefore, it is necessary to investigate new sources to develop safer dyes. Otherwise, these products will continue to be dangerous [43]. New technologies have been developed to expand the range of available colors, simplify processes, and make them more efficient and faster while minimizing damage to hair and human health [44]. Various cosmetic technologies have been employed in hair dyes, leading to the accessibility of pigment agents and a wider range of products, including permanent, semi-permanent, and temporary dyes. For this reason, the popularity of products containing semi-permanent dyes has increased even though they last only for about four to

six washes and interact moderately with the hair shaft [45] but are gentler on the hair compared to permanent options [46].

The present investigation proposes using *G. americana* fruit extract as a hair dye shampoo formulation component.

MATERIAL AND METHODS

Samples preparation

Green fruits of G. *americana* L., (Fig. 1A) also known as "Huito," were collected during February in Sicllabamba, Echarati, La Convención, Cusco, Perú. The botanical identification was done at the Vargas CUZ Herbarium of the Universidad Nacional de San Antonio Abad del Cusco using dichotomous keys, consulting specialized bibliography, and comparing with herbarium samples while agreeing with the classification of the angiosperm phylogenetic system group IV-2016. The fruits were sliced (Fig. 1B), dried in a stove at 30°C–40°C for a week, and ground in a coffee mill, obtaining a particle size of 3–4 mm.

Preparation of *G. americana* green fruit extract

Genipin is a colorless molecule that reacts with primary amines in the presence of oxygen to form water-soluble blue Pigments (Fig. 1C) [47].Choosing a water-soluble solvent, such as a 70% ethanolic solution, was necessary for the extraction procedure [48] and to prevent sample contamination. The grounded sample (100 g) was macerated with 250 ml of a 70% ethanolic solution for 20 days at room temperature and shaken vigorously daily. It was then filtered with a Buchner funnel connected to a vacuum bomb and evaporated at 40°C using a rota-evaporator.

Formulation of hair dye shampoo

To formulate the hair dye shampoo, sodium lauryl sulfate (25%), coconut fatty acid diethanolamine (1.5%), methylparaben (0.2%), Ethylenediaminetetraacetic acid (EDTA) (0.1%), and Butylated hydroxytoluene (BHT) (0.05%) were mixed in hot water (at 55°C) until complete dissolution and foam formation were avoided. After the solution was cooled at room temperature (17°C–20°C), *G. americana* extract (7.5%, 10%, and 12.5%) was added, and the pH was adjusted to 6 using triethanolamine. Distilled water was added to a final volume of 100 ml, and a 20% solution of NaCl (1.5%) was added to adjust the viscosity.

Physicochemical and microbiological evaluation of formulations

The prepared shampoo underwent a physical and chemical evaluation to determine its quality. The color, odor, appearance, consistency, and precipitation formation after 7 days were visually inspected [49]. Foam stability and foam expansion were also measured. The modified cylinder shake method involved adding 50 ml of 1% shampoo solution to a 250 ml graduated cylinder, covering it with parafilm, and shaking it upside down ten times. The foam volume was measured immediately and then after 1, 2, 3, 4, and 5 minutes to account for foam stability [50,51].

The pH was measured at a constant temperature using a Jenway 3510 pH meter after being calibrated with a standard solution of pH 7. Distilled water with a pH range of 6.24–6.88 was necessary [52]. The viscosity was measured at 25°C with a Brookfield viscometer (Viscoqc 100-L model, Anton Paar, Graz, Austria) [53]. In addition, after production, the microbiological quality of the shampoo was analyzed. The total viable bacterial count was determined using standard laboratory procedures. The sample was dispersed in a phosphate buffer, and 10-fold serial dilutions were made under aseptic conditions. The pour plate technique was used to count bacteria and yeast. A 1 ml sample was taken from the proper dilution. Bacterial counts were conducted using soya bean casein digest (SBCD) agar, while yeast and mold counts were determined using Sabouraud dextrose agar containing 40 ppm chlortetracycline. A positive result was considered if bacterial colonies were observed on the solidified agar plates after 48 hours of incubation at 37°C and if fungal growth was observed on the plates after 2 weeks of incubation at 25°C. To identify isolates, 1 g of the sample was transferred into a flask containing 50 ml of SBCD broth and 0.5% Polysorbate 80. After 48 hours of incubation at 37°C, a loopful of the flask contents was streaked onto SBCD agar (supplemented with 0.5% Polysorbate 80), and the plates were then incubated for an additional 48 hours at 37°C. To identify the isolates, 1 g of the sample was placed into a flask containing 50 ml of SBCD broth and 0.5% Polysorbate 80. The flask was then incubated for 48 hours at 37°C. After incubation, a sample was spread on SBCD agar (supplemented with 0.5% Polysorbate 80), and the plates were further incubated for 48 hours at 37°C.

Colonies that formed were obtained in pure culture. The colonies were incubated on Mannitol Salt Agar to confirm the presence of *Staphylococcus aureus*. Cetrimide Agar was used for the isolation and enumeration of *Pseudomonas aeruginosa*. MacConkey Agar was used to isolate Gram-negative bacteria and determine *Escherichia coli* [54,55].

Evaluation of the ability of the shampoo dye hair to color bleached hair

Bleaching application

Black human hair untreated with chemicals was obtained from a local salon in Cusco, Perú, for dye testing. It was then bleached to observe the color change during the use of natural dyes [22].

The hair tresses were bleached with commercial bleaching products. The bleaching powder (BlondMe Premium Lightener 9+), whose main components are potassium persulfate, ammonium persulfate, and magnesium carbonate hydroxide,

Figure 1. *Genipa americana* plant (A); Green fruits (B); Dried and sliced fruits (C).

and developer (Schwarzkopf Professional BlondMe Premium Developer), with hydrogen peroxide, paraffinum liquidum, sodium cetearyl sulfate, peg-35 castor oil, and potassium hydroxide as main components, and were mixed in a non-metallic bowl at a ratio of 1:2.5. The bleaching product was applied to each sample using a brush, using 9 g for each sample, which equates to 1.4 mg per 1 mg of hair [56]. The hair was left for 30 minutes and then rinsed with demineralized water. After rinsing, the hair tresses were combed and detangled before being dried with a commercial dryer.

Application of hair dye shampoo

Four tresses of bleached hair were selected and then dyed with a shampoo containing *G. americana* extract for 1 month. The hair tresses were massaged, and the shampoo was left over the hair for 45 minutes at 20°C and 45%–50% humidity and then rinsed with demineralized water. Following the previous step, a commercial hair dryer was used to dry all the tresses. The shampoo dye was reapplied as follows:

- The first group had 30 applications and 30 washes.
- The second group had 15 applications and 15 washes.
- The third group had 10 applications and 10 washes.
- The fourth group did not receive any treatment and served as a control.

Color measurement

The measure of color changes was made in triplicate using a Chromameter CR-400 (Minolta Camera Co., Osaka, Japan). The L*, a*, and b* color coordinates indicated the hair color. L* represents the lightness scale, with 0 being black and 100 being white. a* indicated green-red (-a = green, +a = red), while b* indicated blue-yellow (-b = blue, +b = yellow). To obtain precise measurements, calibration was carried out using the white calibration plate under the same temperature conditions as the measurements. The following data should be displayed on the chromameter screen: $y = 93.5$; $y = 0.3190$ and $x = 0.3114$. The test results were presented as ΔL, Δa, and Δb values, indicating the variation between the bleached hair and the samples in terms of L*, a*, and b* values. Positive ΔL values indicate more luminosity samples than the standard, while negative values indicate darker ones. Positive Δa values indicate redder samples, while negative values show greener ones. Positive Δb values indicate more yellow samples, while negative values indicate bluer ones. The absolute color differences can be measured by using the following formula: [57].

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

$$\Delta E = \sqrt{(L_i - L_0)^2 + (a_i - a_0)^2 + (b_i - b_0)^2} \quad (2)$$

where:

L_i, a_i, b_i = bleached hair tresses

L_0, a_0, b_0 = samples of hair tresses with *G. americana.*

ΔE values compare color samples, with smaller values indicating closer colors. Ranges of differences are: 0–0.2 = imperceptible; 0.2–0.5 = very small; 0.5–1.5 = small; 1.5–3.0 = visible; 3.0–6.0 = much; 6.0–12.0 = big; > 12 = very great [58,59,60].

STATISTICAL ANALYSIS

Significance differences between treatments were evaluated using one-way ANOVA and Scheffé test ($p < 0.05$) with statistical software SPSS (version 22).

RESULTS AND DISCUSSION

Hair dye shampoo formulations

Table 1 presents the composition of *G. americana* hair dye shampoo formulations at 7.5%, 10%, and 12.5%.

Natural ingredients in cosmetics are increasingly popular. Shampoo cleans hair using a surface-active agent. It comes in cream, gel, or liquid form and contains properties that emulsify, wet, detangle, and foam. This leaves hair soft, clean, and manageable [61].

Table 1 shows a formula that contains 60% water, 25% anionic surfactant, and 1.5% secondary surfactant. Other ingredients, including EDTA and BHT, provide stability. Cocamide DEA has been the most used secondary surfactant with alkyl sulfates and alkyl ether sulfates for the last 30 years. It enhances foam heights and stability, solubilizes effectively, and helps to increase viscosity [62,63,64]. Cocamide DEA is a versatile ingredient, solubilizing agent, thickening aid, and foam stabilizer [65]. The formula also contains *G. americana* extract for added coloring properties.

Most shampoo formulations need to contain both primary and secondary surfactants. The primary surfactants provide most of the fundamental properties. In contrast, secondary surfactants enhance the effects of the primary surfactants in specific ways, such as boosting foam or making the shampoo milder on the scalp and eyes. Surfactants are

Table 1. Formulation of hair dye shampoos containing *G. americana* extracts at 7.5, 10, and 12.5%.

Constituent	Inci Name	Quantity (%)	Function
Sodium lauryl sulfate	Sodium Lauryl Sulfate	25	Anionic surfactant
Coconut fatty acid diethanolamine	Cocamide DEA	1.5	Secondary surfactant
Ethylene diamine tetra-acetic acid (EDTA)	Disodium EDTA	0.1	Chelator
Butylated Hydroxytoluene (BHT)	BHT	0.05	Antioxidant
Sodium chloride	Sodium Chloride	1.5	Thickener
Methylparaben	Sodium Methylparaben	0.2	Preservative
G. americana extract	----------	7.5, 10, 12.5	Natural dye
Triethanolamine	Triethanolamine	q.s pH= 6	pH corrector
Water	Aqua	q.s 100	Vehicle

essential for cationic polymer-based deposition systems, which deliver actives and benefit agents to the hair and scalp [66].

EDTA is frequently utilized as a chelating agent, forming chemical complexes with metals. Shampoos are most effective in water with low levels of magnesium and calcium, as these elements can react with shampoos and create insoluble foam. When magnesium, calcium, and other metal ions bind to EDTA, shampoos' effectiveness is unaffected.

Antioxidants like BHT help prevent and slow down the oxidation process of other chemicals by binding to radical molecules and reducing their decomposition power [67]. Some studies have shown that samples incorporating BHT exhibit minor absolute color difference (ΔE) fluctuations and less color degradation [68].

Sodium chloride is an effective thickening and viscosity agent. This salt increases the surfactant micelles' swelling and resistance to movement [69].

Physicochemical and microbiological evaluation of formulationsTable 2 displays the results of physicochemical tests conducted on the shampoo dye formulation at three different extract concentrations.

All three formulations possess similar physicochemical characteristics, with slight color variations (Fig. 2). The 7.5% formulation had less intense coloration than the other formulations. Additionally, the odor varies with the concentration of the extract, with higher concentrations leading to a more intense odor. A precipitate formation after 7 days was present in the 12.5% formulation, so this formulation was discarded for the coloration evaluation. However, there was no appearance, consistency, foam formation, foam stability, pH, or viscosity variation. The precipitation observed in the shampoo formulation using 12.5% of *G. americana* extract may be due to several factors; among them, we can mention the higher number of solid residues from the higher amount of extract [70]. It is also known that anionic surfactants such as sodium lauryl sulfate can interact with some positively charged groups, giving rise to precipitates [71]. This precipitation negatively impacts the quality and efficacy of the formulated shampoo, so it was not used to color bleached hair tresses.

Table 2. Physicochemical parameters of hair dye shampoos containing *G. americana* extracts.

Physicochemical parameters	*G. americana* extract (%)		
	7.5	10	12.5
Color	Light bluish-black	Moderate bluish-black	Intense bluish-black
Odor	Light odor of Genipa	Moderate odor of Genipa	Moderate odor of Genipa
Appearance	Black shiny	Black shiny	Black shiny
Consistency	Slightly thick	Slightly thick	Slightly thick
Foam expansion (cm^3)	105	108	109
Foam stability	Good	Good	Good
Precipitate formation after 7 days	No	No	Yes
pH	6	6	6
Viscosity (mPa*s)	4849	4849	4849

The pH level of shampoo is crucial for improving hair quality, minimizing eye irritation, and maintaining the scalp's ecological balance. Mild acidity helps prevent swelling, promotes scale setting, enhances hair shine, and minimizes hair damage [72]. A stable pH level of 5–7 in shampoo prevents scalp damage and is close to the skin's pH. In other words, shampoos should have a neutral or slightly acidic pH. Citric acid is commonly used in formulations to achieve the right pH for the shampoo [73,74].

Shampoos containing *G. americana* extracts have a pH level of 6, in compliance with skin health and safety regulations [75]. In a study by Al Badi and Khan [76], the pH of samples of herbal shampoo was reported to be 7.2. Another study developed by Seema *et al.* [77] found that the pH of a polyherbal shampoo was 5.5, which is considered slightly acidic. Most commercial shampoos have a pH between 6.0 and 7.0 at 25°C [76], ideal for conditioning hair and creating a proper electric charge [78]. The pH level of the environment affects the absorption of proteins in hair, with absorption being higher when the pH level is between 6 and 9. Using conditioners with a pH of 6 makes hair softer, so our product has the proper conditioning status [79].

Consumers often base their choice of shampoo on its ability to produce foam, a measure of its effectiveness in cleaning hair. The amount of foam produced by shampoo has a psychological impact and is seen as an indicator of how well it can clean hair [80]. However, when evaluating its efficacy, shampoo users should consider factors such as the duration, consistency, and stability of foam production [81]. Foaming and consistency are important properties in shampoo, as people often link more foam with better cleaning, although the two aren't always connected. Foam is created when foaming agents in the shampoo interact with water and air. The greater the shampoo's foaming potential, the higher the foam. Additionally, the more stable the foam, the longer it will last without breaking or disappearing [76]. Although foam generation has little to do with the cleaning ability of shampoos, it is extremely important to consumers and, therefore, a significant criterion in evaluating shampoos [81]. The volume and stability of foams are important factors in assessing shampoos' quality and consumer acceptance [82]. Shampoos that produce large volumes and stable foams, which maintain their volume for an extended period, are generally preferred by consumers. High-quality shampoos can produce a large volume of stable foams after shaking. In our study, the *G. americana* shampoo showed a foam expansion of 105, 108, and 109 cm^3 for 7,5%, 10%, and 12,5%, respectively. Previous studies established that shampoo

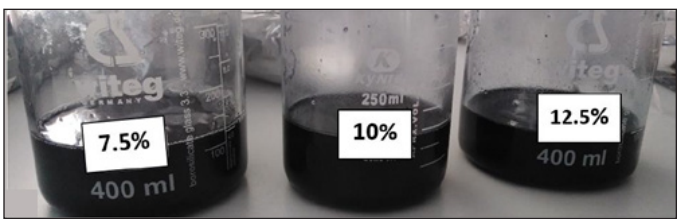


Figure 2. *G. americana* shampoo at different concentrations.

should have a foam volume of 100 cm^3 or more for 5 minutes to demonstrate good stability. In the same study, Pantene, Sunsilk, and Herbal Essence commercial shampoos showed 107, 106, and 104 cm^3 foam volumes, respectively [83].

Shampoo viscosity is influenced by the amount of solids present, which is vital in defining and controlling attributes like shelf-life stability [72]. Viscosity and rheological properties affect the quality of liquid shampoos. These factors can impact various aspects of the product, such as appearance, transparency, ease of dispensing, consistency, and foam formation [84]. The rheological properties of a shampoo formulation are essential to ensure the desired consistency and ease of dispensing from the container. The viscosity of our formulations at different percentages of *G. americana* extracts was the same (4849 mPa*s), which seems an adequate value. Sunsilk, Pantene, Ultra Doux, Herbal essences, L'Oreal Elvive, and Syoss at 25°C showed 6213.67, 10131.67, 5297.33, 9226.67, 9593.67, and 3457 mPa*s, respectively [72].

Table 3 shows the results of microbiological analysis for hair dye shampoos containing *G. americana* extracts. The concentrations used were 7.5%, 10%, and 12.5%. The formulations were free from *pathogenic microorganisms P. aeruginosa, S. aureus, and E. coli*. This indicates that the preparations were appropriately made and safe from a microbiological quality control standpoint. Additionally, yeast and mold counts were within the acceptable criteria for this product type, and the total aerobic mesophilic bacteria also met the criteria.

Shampoos do not necessarily require sterility. However, they must be devoid of harmful microorganisms and should not contain excessive microbial contaminants [86]. Research studies have demonstrated that *Pseudomonas* species in shampoos containing sodium lauryl sulfate as a surfactant can lead to product separation and color change. Cosmetics industries are particularly concerned about the presence of *P. aeruginosa* in their products. This bacterium is an opportunistic pathogen that can cause spoilage and is the most common microorganism associated with recalls of cosmetic formulations in the United States and Europe [87]. Shampoos, like any product containing water and organic or inorganic compounds, must be preserved to prevent microbial contamination. This is crucial for consumer safety and to prolong the product's shelf life. The primary goal of microbiological safety is to shield consumers from potentially harmful microorganisms and to maintain the product's quality by preventing biological and physicochemical deterioration [88].

Table 3. Microbiological analysis results of hair dye shampoos containing *G. americana* extracts.

Analysis	*G. americana* extract (%)			Acceptance criteria for microbiological quality [85]
	7.5	10	12.5	
Staphylococcus aureus	Absence	Absence	Absence	Absence per 1 g or ml
Pseudomonas aeruginosa	Absence	Absence	Absence	Absence per 1 g or ml
Total coliforms and *Escherichia coli*	Absence	Absence	Absence	Absence per 1 g or ml
Total aerobic mesophilic	$< 5 \times 10^2$ CFU/ml	$< 5 \times 10^2$ CFU/ml	$< 5 \times 10^2$ CFU/ml	$\leq 1 \times 10^3$ CFU/g or ml
Molds and yeast	<10 CFU/ml	<10 CFU/g ml	<10 CFU/g ml	$\leq 1 \times 10^3$ CFU/g or ml

CFU: Colony Forming Units.

Evaluation of color: Table 4 shows the results of the shampoo dye coloration, including different concentrations and application numbers, showing that L*, a*, and b* vary considerably in the data obtained from bleached hair tresses; it is also observed that when the exposure of the hair to the shampoo dye is more constant (30 applications), the coloration change is more significant. The concentration of *G. americana* extract directly affects hair color, with higher concentrations (10%) resulting in more significant differences compared to bleached hair, as shown in Figure 4.

Using natural plant colorants as a substitute for toxic synthetic colors in hair dyeing has garnered a growing interest. However, natural hair dyeing has yet to overcome the challenge of achieving consistent color and long-lasting results on human hair [89]. Extracted plant dyes can be directly applied or combined with metal ion-based mordants to address this issue [90]. Direct dyes are commonly applied to the surface of hair fibers, but their use results in a limited chroma range and poor color fastness [11,91].

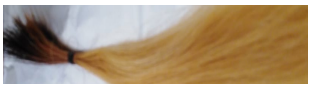
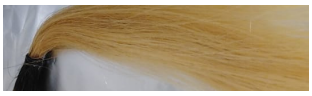
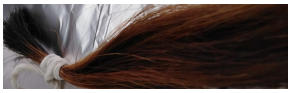
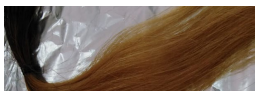
In our investigation, we applied hair dye formulations directly onto hair tresses. We discovered that the number of applications and the concentration of the extract affected the hair's coloration. We found the most effective conditions for better coloration to be 30 applications and 10% extract concentration, resulting in a scale of ΔE from 6.0 to 12.0, which means a great difference from bleached hair tresses.

Historically, plant extracts such as indigo and henna were used in Europe and Asia to dye hair from light brown to black [92]. However, synthetic dyes can cause skin discoloration, allergies, skin irritation, unexpected hair colors, and hair breakage [93]. Prolonged use of chemical dyes can lead to erythema, dry scalp, hair loss, and even skin cancer [94].

Synthetic hair dyes are classified as permanent or temporary, depending on their ability to penetrate the hair and color longevity. Temporary dyes are less damaging because they do not penetrate the cortex [95] and last for 5–8 washes, while permanent dyes provide long-lasting results. Natural dyes are similar to temporary hair dyes but face certain challenges. For instance, they do not penetrate deeply into the hair or prevent the dyed hair from fading or washing out due to weak color and long-term deposition [96]. To improve hair dyeability, developers can be added to open the hair cuticle and break down chemical bonds. This process allows dye molecules to interact with the proteins of the hair. Additionally, mordants can be added to fix the dye in hair fibers and enhance hair color fastness [22].

As shown in Figure 3, there were differences between the treatments using *G. americana* shampoo: T1: *G. americana* 7.5% + 30% applications; T2: *G. americana*

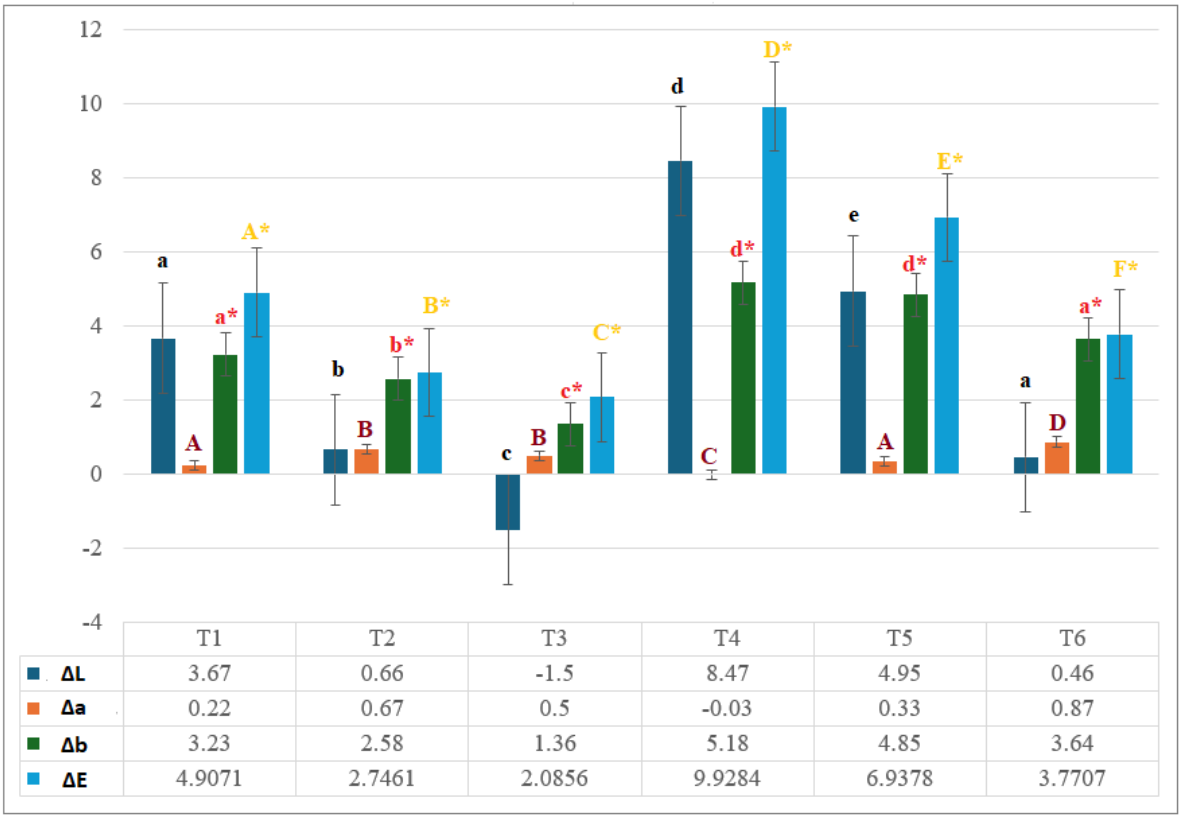
Table 4. Absolute color differences (ΔE) of hair dye shampoos containing *G. americana* extracts.

Hair tresses	Applications number	Photographic image	L	A	b	ΔE*
Bleached	--		55.4 ± 0.21	7.32 ± 0.35	22.01 ± 0.11	0.0
G. americana 7.5%	30		51.73 ± 0.12	7.1 ± 0.23	18.78 ± 0.28	**4.9071**[a]
	15		54.74 ± 0.06	6.65 ± 0.19	19.43 ± 0.32	**2.7461**[b]
	10		56.9 ± 0.41	6.82 ± 0.36	20.65 ± 0.08	**2.0856**[b]
G. americana 10%	30		46.93 ± 0.17	7.35 ± 0.34	16.83 ± 0.29	**9.9284**[c]
	15		50.45 ± 0.06	6.99 ± 0.27	17.16 ± 0.13	**6.9378**[d]
	10		54.94 ± 0.19	6.45 ± 0.11	18.37 ± 0.22	**3.7707**[e]

*Different letters indicate significant differences, as Scheffé's test indicates, at $p < 0.05$.

7.5% + 15% applications; T3: *G. americana* 7.5% + 10% applications; T4: *G. americana* 10% + 30% applications; T5: *G. americana* 10% + 15% applications; T6: *G. americana* 10% + 10% applications. Positive ΔL values indicate more luminosity samples than the control, while negative values indicate darker ones. Positive Δa values indicate redder samples, while negative values show greener ones. Positive Δb values indicate more yellow samples, while negative values indicate bluer ones. According to our results, the T4 (T4: *G. americana* 10% + 30% applications) showed better results regarding the absolute color differences (ΔE).

Temporary hair colors in shampoos, lotions, or foams only last until the next wash. They add color highlights, neutralize yellow tones in white hair, or cover up a few gray hairs. These colors contain high molecular weight ingredients that deposit on the hair's surface and are easily removed. The dye is combined with a cationic polymer to make it less soluble, adhere better to the hair, and then dispersed in a base using surfactants to create the final product [97]. One example is henna, derived from the *Lawsonia alba* plant, also used on nails and skin [95]. Semi-permanent hair colors do not contain ammonia or ethanolamine but may contain hydrogen peroxide or resorcinol. These colors cannot lighten hair and are typically used to cover white hair or enhance natural hair color. Their low molecular weight enables them to penetrate the middle layers of the hair cuticle without tightly binding to the hair protein itself [98]. As a result, applying semi-permanent colors does not require prior hair modification, as is necessary with permanent hair color. Many organic compounds, such as quinones, carotenoids, tannins, curcuminoids, and flavonoids, are used as coloring agents in hair dye plants and have been studied for their effectiveness in dyeing hair [91]. Previous studies have discussed using plant pigment extracts in natural hair dye formulations. For instance, *Indigofera tinctoria* (Indigo), *Lawsonia inermis* (Henna), *Emblica officialis* (Amla), *Juglans nigra* (walnut hull), and *Hibiscus rosasinensis* (Shoe flower) there have also been documented in the literature of their use as natural sources of hair dye [99,100]. The blackcurrant's discarded fruit skins are used to create hair dye through an extraction and purification process. Research shows that this blackcurrant hair dye is fade-resistant [101]. Thai plants, including False daisy, Thao yanang, Sappan tree, Kae lae, and turmeric extracts, are greenish, brownish, and yellowish in color. When combined with a natural developer and metal as a mordant agent, these extracts were utilized for hair dye, resulting in a color range from dark reddish-brown to orangish-brown [22]. Rich anthocyanins black beans can be used as a hair dye when extracted with hydrochloric ethanol, and the color lasts through four washings [96]. Research has shown that anthocyanin extracted from black beans is a potential natural hair dye. However, the durability of this dye is not as good as other natural dyes [96]. For example, studies have demonstrated that natural pigments derived from Thai plants and ferrous sulfate as a mordant enhance color retention in up to fifteen shampoo washes [22]. This is because ferrous sulfate



	T1	T2	T3	T4	T5	T6
ΔL	3.67	0.66	-1.5	8.47	4.95	0.46
Δa	0.22	0.67	0.5	-0.03	0.33	0.87
Δb	3.23	2.58	1.36	5.18	4.85	3.64
ΔE	4.9071	2.7461	2.0856	9.9284	6.9378	3.7707

Figure 3. The difference in color coordinate values compared with the bleached hair (control); lightness values (ΔL), redness (Δa), yellowness (Δb), and total difference (ΔE) of hair dyed with T1: *G. americana* 7.5% + 30% applications; T2: *G. americana* 7.5% + 15% applications; T3: *G. americana* 7.5% + 10 applications; T4: *G. americana* 10% + 30 applications; T5: *G. americana* 10% + 15% applications; T6: *G. americana* 10% + 10% applications.

a,b,c,d,e**;** A, B, C, D**;** a*,b*,c*,d***;** A*, B*, C*, D*, E*, F* means followed by a different letter are significantly different to Scheffe's test ($p < 0.05$) of lightness values (ΔL), of redness values (Δa), of yellowness values (Δb), and total difference (ΔE).

creates a complex with the hair fiber, improving the natural dye's adherence to the hair [102].

The black bean dye takes longer to dye hair compared to commercially marketed hair dyes. Typically, most hair dyes require 45–60 minutes of dyeing, whereas the black bean dye needs 1.30 hours for a better effect. However, because all ingredients in this formulation are natural, they have a minimal impact on the dyeing process. Previous studies have also shown that hair dyed with plant extracts is more durable, even though it may take longer to dye the hair (around 2 hours). Natural dyes adhere better to the hair, allowing the dye molecules to penetrate the cuticle and diffuse into the cortex [103]. Phycocyanin from *Arthrospira* (Spirulina) *platensis* can be used as hair dye. It retains 50% of its color after five shampoo washes and has good physical stability in hair dye powder formulation with no color degradation issues [104].

According to Machado *et al.* [1], geniposide is the primary extracted substance from *G. americana* ripe fruit. It could react with hair keratin, resulting in changes in color in response to pH and temperature. The study found that mild treatment conditions such as 5% *G. americana*, pH 5.5, and 25°C caused visible differences in white hair. Increasing the *G. americana* concentration or pH level achieved a significant color change. This change was classified as a big difference

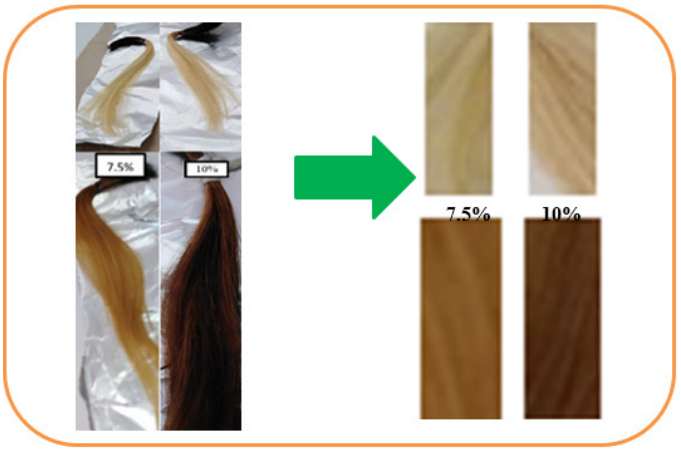


Figure 4. Color changes of hair tresses with *G. americana* extract at different concentrations after 30 applications. The incorporation of *G. americana* extracts into a shampoo with a pH of 6 results in the development of brown tones, which are distinctly visible.

with an ΔE of 6.7. Furthermore, exposing the sample to high temperatures led to a red color, which differed considerably from untreated white hair samples with an ΔE greater than 12.0. The concentration of *G. americana* influenced the

color change. 5% and 20% resulted in ΔE of 37.6 and 54.0, respectively. Our findings agree with these results because we obtained an ΔE of 9.9284, which means a big difference with the bleached tresses.

Yang *et al.* [105] found that the color of hair changes due to the reaction between proteins and genipin, a byproduct of geniposide. The study produced pigments with different colors based on the pH level used. The color varied from magenta (pH 9) to dark red (pH 12) at higher pH levels and from brown (pH 5) to blue (pH 7) at neutral or slightly acidic pH levels. Hair color depends on geniposide concentration and the availability of deprotonated amino groups of keratin [105].

Based on our research, we conclude that *G. americana* contains a natural pigment suitable for dyeing hair. When this pigment is applied to bleached hair strands using a 10% ethanolic extract for 30 applications, it produces an interesting brown color. This natural alternative could be safe and effective for covering gray hair.

When using natural colorants in hair dye cosmetics, it is important to consider stability issues such as thermal, light, and acidity/alkalinity. Over the years, various encapsulation systems have been developed to protect plant extracts from environmental stresses such as extreme pHs, UV, and heat [106]. Encapsulation shields dye molecules by enclosing them in a shell, ensuring the stability and dyeability of natural colorants in hair dye products. Techniques include emulsion polymerization, suspension polymerization, and spray drying [107]. For example, microcapsules of colorants from Chinese gallnut and henna were produced using spray drying techniques, improving stability and formulation compatibility [108,109]. Encapsulation has solved compatibility and stability issues in natural dyeing with plant colorants. Inorganic nano-carriers, such as nanoparticles, nanofibers, and nanotubes, stabilize plant-based hair dyes and improve the dyeing process. For instance, with their small size and increased surface-to-volume ratio, carbon nanotubes can be easily absorbed into the hair cuticles, enhancing affinity and leading to long-lasting coloring effects [110].

Mordant dyeing is a process that helps fix dyes on hair fibers to improve colorfastness [111]. Commonly used mordants include metal salts such as iron (II) sulfate, copper (II) sulfate, and alum [22], which form a bond with the dye through dative covalent bonding. This bonding affects the colors' hue and ability to remain colorfast [112]. Bio-mordants, like *Aloe vera* extract and tannin-rich plant extracts, are being explored as effective alternatives to metallic mordants [113,114].

CONCLUSION

Genipa americana green fruits, commonly known as Huito, produce a dye that can be used effectively as a hair dye. After 30 applications, hair treated with shampoo containing 7.5% and 10% *G. americana* extract showed satisfactory development of visible brown tones. The organoleptic, physicochemical, and microbiological characteristics remained stable throughout the analysis period. When hair samples were bleached and washed with the developed formulations, the most successful outcomes were achieved with a 10% shampoo concentration applied 30 times per month, each application lasting 45 minutes, followed by a water rinse. The shampoo used had a pH of 6, and the resulting color was brown. The absolute color difference (ΔE) of the hair dye shampoo containing 10% *G. americana* extract was 9.9284, indicating a significant difference from the bleached hair.

Recently, a growing demand for safer, more environmentally friendly hair coloring products from plants has been growing. This is due to a global awareness of the harmful effects of synthetic hair dyes, leading to a trend in the hair dye market. The scientific community is exploring natural colorants as substitutes for synthetic dyes due to a focus on sustainability and health benefits. Natural colorants offer advantages such as being eco-friendly, less toxic, and biodegradable. Many plant colorants also have health-promoting properties, such as antioxidants, anti-inflammatory, and antimicrobial effects. Further research is needed to understand how plant-derived colorants interact with human hair. This will help develop effective plant-based hair dyes and broaden the variety of plants or engineered microorganisms used for large-scale production. Additionally, more research is needed to develop better encapsulation systems, nanocarriers, and bio-mordants to enhance the effectiveness of natural colorants in hair dyes. Natural dyes act as temporary hair dyes but develop weak colors and have long-term deposition problems because they do not penetrate the hair sufficiently to prevent fading. To improve hair dyeability, ingredients such as a developer can be added. A developer opens the hair cuticle, allowing dye molecules to enter the hair shaft and interact with hair proteins. A substance called mordant fixes the dye in hair fibers and improves color fastness.

AUTHOR CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

FINANCIAL SUPPORT

There is no funding to report.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the

publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Machado AV, Menezes LRO, Santana AAM, Marques MN, de Lima AK, Cano A, *et al. Genipa americana* L.: a new phytochemical for white hair coloring. Chem Biodivers. 2023;20(10):e202300083. Doi https://doi.org/10.1002/cbdv.2023000832.
2. Zviak C. Hair bleaching. ln: Zviak C, editor. The science of hair care. New York, NY: Marcel Delcer; 1986. pp. 213–33.
3. Khalili F, Mahvi AH, Nasseri S, Yunesian M, Yaseri M, Djahed B. Health risk assessment of dermal exposure to heavy metals content of chemical hair dyes. Iran J Public Health. 2019;48(5):902–11.
4. Goebel C, Diepgen TL, Krasteva M, Schlatter H, Nicolas JF, Blömeke B, *et al.* Quantitative risk assessment for skin sensitisation: consideration of a simplified approach for hair dye ingredients. Regulatory Toxicol Pharmacol. 2012;64(3):459–65. doi: https://doi.org/10.1016/j.yrtph.2012.10.004
5. Han JH, Lee HJ, Bang CH, Lee JH, Park YM, Lee JY. P-phenylenediamine hair dye allergy and its clinical characteristics. Ann Dermatol. 2018;30(3):316–21. doi: https://doi.org/10.5021/ad.2018.30.3.316
6. Søsted H, Agner T, Andersen KE, Menné T. 55 cases of allergic reactions to hair dye: a descriptive, consumer complaint-based study. Contact Dermatitis. 2002;47(5):299–303. doi: https://doi.org/10.1034/j.1600-0536.2002.470508.x7.
7. AlGhamdi KM, Moussa NA. Local side effects caused by hair dye use in females: cross-sectional survey. J Cutan Med Surg. 2012;16(1):39–44. doi: https://doi.org/10.2310/7750.2011.10144
8. Sharma VK, Gupta V, Pahadiya P, Vedi KK, Arava S, Ramam M. Dermoscopy and patch testing in patients with lichen planus pigmentosus on face: a cross-sectional observational study in fifty Indian patients. Indian J Dermatol Venereol Leprol. 2017;83(6):656–62. doi: https://doi.org/10.4103/ijdvl.IJDVL_469_16
9. Shenoi SD, Rao R. Pigmented contact dermatitis. Indian J Dermatol Venereol Leprol. 2007;73:285.
10. Fujita F, Azuma T, Tajiri M, Okamoto H, Sano M, Tominaga M. Significance of hair-dye base-induced sensory irritation. Int J Cosmet Sci. 2010;32(3):217–24.
11. Boga C, Delpivo C, Ballarin B, Morigi M, Galli S, Micheletti G, *et al.* Investigation on the dyeing power of some organic natural compounds for a green approach to hair dyeing. Dyes Pigment. 2013;97(1):9–18. doi: https://doi.org/10.1016/j.dyepig.2012.11.020
12. Beiki T, Najafpour GD, Hosseini M. Evaluation of antimicrobial and dyeing properties of walnut (*Juglans regia* L.) green husk extract for cosmetics. Color Technol. 2017;134(1):71–81. doi: https://doi.org/10.1111/cote.12322
13. Dweck AC. Natural ingredients for colouring and styling. Int J Cosmet Sci. 2002;24(5):287–302. doi: https://doi.org/10.1046/j.1467-2494.2002.00148.x
14. Da França SA, Dario MF, Esteves VB, Baby AR, Velasco MVR. Types of hair dye and their mechanisms of action. Cosmetics. 2015;2(2):110–26. doi: https://doi.org/10.3390/cosmetics2020110
15. Oumeish OY. The cultural and philosophical concepts of cosmetics in beauty and art through the medical history of mankind. Clin Dermatol. 2001;19(4):375–86. doi: https://doi.org/10.1016/s0738-081x(01)00194-8
16. Ali A, Moinuddin, Allarakha S, Fatima S, Ali SA, Habib S. Risk of carcinogenicity associated with synthetic hair dyeing formulations: a biochemical view on action mechanisms, genetic variation and prevention. Indian J Clin Biochem. 2022;37(4):399–409. doi: https://doi.org/10.1007/s12291-022-01051-x
17. Schäfer N, Balwierz R, Krzeszewska-Zaręba A, Skotnicki Z, Skotnicka-Graca U, Kalarus K. The use of botanical raw materials in hair dyeing. Aesthetic Cosmetol Med. 2021;6(10):263–9. doi: https://doi.org/10.52336/acm.2021.10.6.01
18. Wei G, Bhushan B, Torgerson PM. Nanomechanical characterization of human hair using nanoindentation and SEM. Ultramicroscopy. 2005;105(1-4):248–66. doi: https://doi.org/10.1016/j.ultramic.2005.06.033
19. Swift JA, Chahal SP, Challoner NI, Parfrey JE. Investigations on the penetration of hydrolyzed wheat proteins into human hair by confocal laser-scanning fluorescence microscopy. J Cosmet Sci. 2000;51(3):193–203.
20. Kelch A, Wessel S, Will T, Hintze U, Wepf R, Wiesendanger R. Penetration pathways of fluorescent dyes in human hair fibres investigated by scanning near-field optical microscopy. J Microsc. 2000;200(3):179–86. doi: https://doi.org/10.1046/j.1365-2818.2000.00756.x
21. Malinauskyte E, Shrestha R, Cornwell PA, Gourion-Arsiquaud S, Hindley M. Penetration of differentmolecularweight hydrolysed keratins into hair fibres and their effects on the physical properties of textured hair. Int J Cosmet Sci. 2020;43(1):26–37. doi: https://doi.org/10.1111/ics.12663
22. Boonsong P, Laohakunjit N, Kerdchoechuen O. Natural pigments from six species of Thai plants extracted by water for hair dyeing product application. J Clean Prod. 2012;37:93–106. doi: https://doi.org/10.1016/j.jclepro.2012.06.013
23. Morel O, Christie RM, Greaves A, Morgan KM. Enhanced model for the diffusivity of a dye molecule into human hair fibre based on molecular modelling techniques. Color Technol. 2008;124(5):301–9. doi: https://doi.org/10.1111/j.1478-4408.2008.00157.x
24. Robbins CR, Reich C, Patel A. Adsorption to keratin surfaces: a continuum between a charge-driven and a hydrophobically driven process. J Soc Cosmet Chem. 1994;45(2):85–94.
25. Bianchi S, Bernardi S, Continenza MA, Vincenti E, Antonouli S, Torge D, *et al.* Scanning electron microscopy approach for evaluation of hair dyed with *Lawsonia inermis* Powder: *In vitro* study. Int J Morphol. 2020;38(1):96–100.
26. He L, Michailidou F, Gahlon HL, Zeng W. Hair dye ingredients and potential health risks from exposure to hair dyeing. Chem Res Toxicol. 2022;35(6):901–15. doi: https://doi.org/10.1021/acs.chemrestox.1c00427
27. Morel OJX, Christie RM. Current trends in the chemistry of permanent hair dyeing. Chem Rev. 2011;111(4):2537–61. doi: https://doi.org/10.1021/cr1000145
28. Ros MM, Gago-Dominguez M, Aben KKH, Bueno-De-Mesquita HB, Kampman E, Vermeulen SH, *et al.* Personal hair dye use and the risk of bladder cancer: a case–control study from The Netherlands. Cancer Causes Control. 2012;23:1139–48. doi: https://doi.org/10.1007/s10552-012-9982-1
29. Hamann D, Yazar K, Hamann CR, Thyssen JP, Lidén C. p-Phenylenediamine and other allergens in hair dye products in the United States: a consumer exposure study. Contact Dermat. 2014;70(4):213–8. doi: https://doi.org/10.1111/cod.12164
30. Schuttelaar MLA, Vogel TA. Contact allergy to hair dyes. Cosmetics. 2016;3(3):21. doi: https://doi.org/10.3390/cosmetics3030021
31. Chong HP, Reena K, Ng KY, Koh RY, Ng CH, Chye SM. Para-phenylenediamine containing hair dye: an overview of mutagenicity, carcinogenicity and toxicity. J Environ Anal Toxicol. 2016;6(5):1000403. doi: https://doi.org/10.4172/2161-0525.1000403
32. Nohynek GJ, Fautz R, Benech-Kieffer F, Toutain H. Toxicity and human health risk of hair dyes. Food Chem Toxicol. 2004;42(4):517–43. doi: https://doi.org/10.1016/j.fct.2003.11.003

33. Burnett CM, Loehr RF, Corbett JF. Dominant lethal mutagenicity study on hair dyes. J Toxicol Environ Health Part A. 1977;2(6):657–62. doi: https://doi.org/10.1080/15287397709529467
34. Tkaczyk A, Mitrowska K, Posyniak A. Synthetic organic dyes as contaminants of the aquatic environment and their implications for ecosystems: a review. Sci Total Environ. 2020;717:137222. doi: https://doi.org/10.1016/j.scitotenv.2020.137222
35. Kirkland D, Marzin D. An assessment of the genotoxicity of 2-hydroxy-1, 4-naphthoquinone, the natural dye ingredient of Henna. Mutat Res Genet Toxicol Environ Mutagen. 2003;537(2):183–99. doi: https://doi.org/10.1016/S1383-5718(03)00077-9
36. Bentes ADS, Mercadante AZ. Influence of the stage of ripeness on the composition of iridoids and phenolic compounds in genipap (*Genipa americana* L.). J Agri Food Chem. 2014;62(44):10800–8. doi: https://doi.org/10.1021/jf503378k
37. De Mello JF. Plants in traditional medicine in Brazil. J Ethnopharmacol. 1980;2(1):49–55. doi: https://doi.org/10.1016/0378-8741(80)90030-6
38. Calaça CE. Medicinas e plantas medicinais nos tropicos: aspectos da constituiçao da ciencia farmaceutica ocidental. Historia, Ciencias, Saúde-Manguinhos. 2002;9(1):221–6. doi: https://doi.org/10.1590/S0104-59702002000100012
39. Castro Braga F. Brazilian traditional medicine: historical basis, features and potentialities for pharmaceutical development. J Tradit Chinese Med Sci. 2021;8(1):S44–S50. doi: https://doi.org/10.1016/j.jtcms.2020.06.005
40. Newman DJ. The influence of Brazilian biodiversity on searching for human use pharmaceuticals. J Braz Chem Soc. 2017;28(3):402–14. doi: https://doi.org/10.21577/0103-5053.20160225
41. de Paula Pereira N. Sustainability of cosmetic products in Brazil. J Cosmet Dermatol. 2009;8(3):160–1. doi: https://doi.org/10.1111/j.1473-2165.2009.00452.x
42. Touyama R, Takeda Y, Inoue K, Kawamura I, Yatsuzuka M, Ikumoto T, *et al.* Studies on the blue pigments produced from genipin and methylamine. I. Structures of the brownish-red pigments, intermediates leading to the blue pigments. Chem Pharm Bull. 1994;42(3):668–73. doi: https://doi.org/10.1248/cpb.42.668
43. Palaniappan V, Karthikeyan K, Anusuya S. Dermatological adverse effects of hair dye use: a narrative review. Indian J Dermatol Venereol Leprol. 2024;90(4):458–70. doi: https://doi.org/10.25259/IJDVL_745_2022
44. Couto EP. O impacto da biodiversidade na cadeia produtiva de higiene pessoal, perfumaria e cosméticos. Gestão Contemp. 2014;4(1):144–6.
45. Harrison S, Sinclair R. Hair colouring, permanent styling and hair structure. J Cosmet Dermatol. 2003;2(3-4):180–5. doi: https://doi.org/10.1111/j.1473-2130.2004.00064.x
46. Andrisano V, Gotti R, Roveri P, Cavrini V. Analysis of semi-permanent hair dyes by HPLC with on-line post-column photochemical derivatisation. Chromatographia. 1997;44(7-8):431–7. doi: https://doi.org/10.1007/BF02466323
47. Neri-Numa IA, Pessoa MG, Paulino BN, Pastore GM. Genipin: a natural blue pigment of food and health purposes. Trends Food Sci Technol. 2017;S0924–2244(17):30180–2. doi: http://dx.doi.org/10.1016/j.tifs.2017. 06.018
48. Gao Y, Xu J, Liu G, Nie R, Duan J, Xu D, *et al.* Preparation and characterization of water-insoluble gardenia blue pigment. Materials. 2021;14(21):6594. doi: https://doi.org/10.3390/ma14216594
49. Aghel N, Moghimipour B, Dana RA. Formulation of a herbal shampoo using total saponins of Acanthophyllum squarrosum. Hamdard Medicus. 2008;51(4):111–7.
50. Malpani T, Jeithliya M, Pal N, Puri P. Formulation and evaluation of pomegranate based herbal shampoo. J Pharmacogn Phytochem. 2020;9(4):1439–44.
51. Revansiddappa M, Sharadha R, Abbulu K. Formulation and evaluation of herbal Antidandruff shampoo. J Pharmacogn Phytochem. 2018;7(4):764–7.
52. Tarun J, Susan J, Susan VJ, Criton S. Evaluation of pH of bathing soaps and shampoos for skin and hair care. Indian J Dermatol. 2014;59(5):442–4. doi: https://doi.org/10.4103/0019-5154.139861
53. Mainkar AR, Jolly CI. Evaluation of commercial herbal shampoos. Int J Cosmet Sci. 2000;22(5):385–91. doi: https://doi.org/10.1046/j.1467-2494.2000.00047.x
54. Geis PA. Cosmetic microbiology: a practical approach (3rd ed.). Boca Raton, FL: CRC Press; 2020. doi: https://doi.org/10.1201/9780429113697
55. Abu Shaqra QM, Al-Groom RM. Microbiological quality of hair and skin care cosmetics manufactured in Jordan. Int Biodeter Biodegr. 2012;69:69–72. doi: https://doi.org/10.1016/j.ibiod.2011.12.009
56. 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(4):320–7. doi: https://doi.org/10.1111/ics.12624
57. Kidawa N, Simon C. Perception of noise depending on spatial frequency and contrast with the aid of the CIE-lab color space. [Dissertation], Imaging Science and Media Technology University of Applied Sciences, Cologne, Germany, 2003.
58. Silva FM, Silva CLM. Colour changes in thermally processed cupuaçu (Theobroma grandiflorum) puree: critical times and kinetics modelling. Int J Food Sci Technol. 1999;34(1):87−94. doi: https://doi.org/10.1046/j.1365-2621.1999.00246.x
59. Young KW, Whittle K. Colour measurement of fish minces using Hunter L, a, and b values. J SciFood Agri. 1985;36(5):383−92. doi: https://doi.org/10.1002/jsfa.2740360511
60. 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(4):829. doi: https://doi.org/10.3390/molecules26040829
61. Barata E. Cosméticos—Arte e Ciência. Lisboa, Portugal: Lidel; 2002.
62. Trüeb RM. Shampoos: ingredients, efficacy and adverse effects. JDDG. 2007;5(5):356–65. doi: https://doi.org/10.1111/j.1610-0387.2007.06304.x
63. Mainkar AR, Jolly CI. Formulation of natural shampoos. Int J Cosmet Sci. 2001;23(1):59–62. doi: https://doi.org/10.1046/j.1467-2494.2001.00057.x
64. Christov NC, Denkov ND, Kralchevsky PA, Ananthapadmanabhan KP, Lips A. Synergistic sphere-to-rod micelle transition in mixed solutions of sodium dodecyl sulfate and cocoamidopropyl betaine. Langmuir. 2004;20(3):565–71. doi: https://doi.org/10.1021/la035717p
65. Duarte AT, Silva LT, Salústio P, Ribeiro HM. Abordagem do comportamento reológico de champôs contendo diferentes espessantes. Revista Portuguesa de Farmácia. 2003;52(1):124.
66. Cornwell PA. A review of shampoo surfactant technology: consumer benefits, raw materials and recent developments. Int J Cosmet Sci. 2018;40(1):16–30. doi: https://doi.org/10.1111/ics.12439
67. Kusumawati I, Indrayanto G. Natural antioxidants in cosmetics. Stud Nat Prod Chem. 2013;40:485–505. doi: https://doi.org/10.1016/b978-0-444-59603-1.00015-1
68. Tran TH, Tran TKN, Ngo TCQ, Pham TN, Bach LG, Phan NQA, *et al.* Color and composition of beauty products formulated with lemongrass essential oil: cosmetics formulation with lemongrass essential oil. Open Chem. 2021;19(1):820–9. doi: https://doi.org/10.1515/chem-2021-0066
69. Im SH. Shampoo compositions. In: Preedy VR, editor. Handbook of hair in health and disease. Human Health Handbooks. Wageningen Academic Publishers; vol. 1 no. 1, 2012. doi: https://doi.org/10.3920/978-90-8686-728-8_22
70. Panontin JF, Rambo MKD, Isaac V, Seibert CS, Scapin E. New antioxidant lauryl-free herbal shampoo formulation with a Brazilian plant extract. Brazil J Biol. 2022;82:e264677. doi: https://doi.org/10.1590/1519-6984.264677

71. Khan N, Brettmann B. Intermolecular interactions in polyelectrolyte and surfactant complexes in solution. Polymers. 2019;11(1):51. doi: https://doi.org/10.3390/polym11010051
72. Bushra T, Eram K, Rana A, Lama A. Pharmaceutical evaluation of different shampoo brands in local Saudi market. Saudi Pharm J. 2018;26(1):98–106. doi: https://doi.org/10.1016/j.jsps.2017.10.006
73. Abu-Jdayil B, Mohameed H. Rheology of dead sea shampoo containing the antidandruff climbazole. Intern J Cosm Sci. 2004;26(6):281–9. doi: https://doi.org/10.1111/j.1467-2494.2004.00240.x
74. Krunali T, Dhara P, Meshram D, Mitesh B. Evaluation of standards of some selected shampoo preparation. World J Pharm Pharmacol Sci. 2013;2(5):3622–30.
75. Sbhatu DB, Berhe GG, Hndeya AG, Abraha HB, Abdu A, Gebru HA, *et al.* Formulation and physicochemical evaluation of lab-based aloe adigratana reynolds shampoos. Int J Anal Chem. 2020;2020(1):6290617. doi: https://doi.org/10.1155/2020/6290617
76. Al Badi K, Khan SA. Formulation, evaluation and comparison of the herbal shampoo with the commercial shampoos. Beni-Suef Univ J Basic Appl Sci. 2014;3(4):301–5. doi: https://doi.org/10.1016/j.bjbas.2014.11.005
77. Seema Y, Arti S, Pratiksha B, Jadhay S, Gaikward D. Formulation and evaluation of polyherbal shampoo and compare with marketed shampoos. World J Pharm Pharmacol Sci. 2017;6(12):1388–97. doi: https://doi.org/10.20959/wjpps201712-10644
78. Noudeh GD, Sharififar F, Khazaeli P, Mohajeri E, Jahanbakhsh J. Formulation of herbal conditioner shampoo by using extract of fenugreek seeds and evaluation of its physicochemical parameters. Afr J Pharm Pharmacol. 2011;5(22):2420–7. doi: https://doi.org/10.5897/AJPP11.121
79. Berthiaume MD. Formulation of conditioning shampoos. Drug Cosmet Indus. 1997;160(5):54–64.
80. Arzhavitina A, Steckel H. Foams for pharmaceutical and cosmetic application. Int J Pharm. 2010;394(1-2):1–17. doi: https://doi.org/10.1016/j.ijpharm.2010.04.028
81. Bezerra KGO, Meira HM, Veras BO, Stamford TCM, Fernandes EL, Converti A, *et al.* Application of plant surfactants as cleaning agents in shampoo formulations. Processes. 2023;11(3):879. doi: https://doi.org/10.3390/pr11030879
82. Deeksha P. Malviya P, Kumar S. Evaluation of marketed shampoo (synthetic and natural) for their hair cleansing, dirt dispersion, wetting time, soild content and foaming capacity properties. Glob J Pharmacol. 2014;8(4):490–3.
83. AlQuadeib BT, Eltahir EKD, Banafa RA, Al-Hadhairi LA. Pharmaceutical evaluation of different shampoo brands in local Saudi market. Saudi Pharm J. 2018;26(1):98–106. doi: https://doi.org/10.1016/j.jsps.2017.10.006
84. Al-Achi A, Baghat T, Chukwubeze O, Dembla I. Rheological profile, specific gravity, surface tension, and pH of fifteen overthe-counter preparations. Int J Pharm Comp. 2007;11(3):252–8.
85. ISO 17516:2014. Cosmetics—Microbiology—Microbiological limits. Geneva, Switzerland: ISO; 2014.
86. Ravita TD, Tanner RS, Ahearn DG, Arms EL, Crockett PW. Post-consumer use efficacies of preservatives in personal care and topical drug products: relationship to preservative category. J Indus Microbiol Biotechnol. 2009;36(1):35–8. doi: https://doi.org/10.1007/s10295-008-0468-9
87. Lundov MD, Zachariae C. Recalls of microbiologically contaminated cosmetics in EU from 2005 to May 2008. Int J Cosmet Sci. 2008;30(6):471–4. doi: https://doi.org/10.1111/j.1468-2494.2008.00475.x
88. Halla N, Fernandes IP, Heleno SA, Costa P, Boucherit-Otmani Z, Boucherit K, *et al.* Cosmetics preservation: a review on present strategies. Molecules. 2018;23(7):1571. doi: https://doi.org/10.3390/molecules23071571
89. 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. doi: https://doi.org/10.1016/j.scp.2023.101285
90. Bechtold T. Hair coloration with natural dyes and pigments. In: Stevens C, Bechtold T, Manian A, Pham T, editors. Handbook of natural colorants. New Jersey, NJ: John Wiley & Sons, Ltd.; 2023. Vol. 25, pp 543–55; doi: https://doi.org/10.1002/9781119811749.ch25
91. Cui H, Xie W, Hua Z, Cao L, Xiong Z, Tang Y, *et al.* Recent advancements in natural plant colorants used for hair dye applications: a review. Molecules. 2022;27:8062. doi: https://doi.org/10.3390/molecules27228062
92. PK J, Dass DJ. Evaluating hair growth potential of some traditional herbs. Asian J Pharm Clin Res. 2015;8(6):150–2.
93. Rao YM, Sujatha P. Formulation and evaluation of commonly used natural hair colorants. Indian J Nat Prod Resour. 2008;7(1):45–8.
94. Mielke HW, Taylor MD, Gonzales CR, Smith MK, Daniels PV, Buckner AV. Lead-based hair coloring products: too hazardous for household use: some hair coloring products sold in pharmacies contain more lead than paint, making them a public health hazard. J Am Pharm Assoc (1996). 1997;37(1):85–9.
95. Dias MFRG. Hair cosmetics: an overview. Int J Trichol. 2015;7(1):2–15. doi: https://doi.org/10.4103/0974-7753.153450
96. Inman C, Lourith N, Kanlayavattanakul M. Alternative application approach on black bean: hair coloring product. Chem Biol Technol Agric. 2020;7(1):1–7. doi: https://doi.org/10.1186/s40538-019-0163-2
97. Guerra-Tapia A, Gonzalez-Guerra E. Hair cosmetics: dyes. Actas Dermosifiliográficas. 2014;105(9):833–9. doi: https://doi.org/10.1016/j.adengl.2014.02.003
98. Neuser F, Schlatter H. Hair dyes. In: Draelos ZD, editors. Cosmetic dermatology: products and procedures. Oxford, UK: Wiley-Blackwell; 2010. pp. 256–310.
99. Shin YS, Lee SH. Natural dyeing of hair using juglone. J Korean Soc Clothing Textiles. 2006;30(12):1708–13.
100. Komboonchoo S, Bechtold T. Natural dyeing of wool and hair with indigo carmine (CI natural blue 2), a renewable resource based blue dye. J Clean Prod. 2009;17(16):1487–93. doi: https://doi.org/10.1016/j.jclepro.2009.05.007
101. Rose PM, Cantrill V, Benohoud M, Tidder A, Rayner CM, Blackburn RS. Application of anthocyanins from blackcurrant (*Ribes nigrum* l.) fruit waste as renewable hair dyes. J Agric Food Chem. 2018;66(26):6790–8. doi: https://doi.org/10.1021/acs.jafc.8b01044
102. Burkinshaw S, Kumar N. The mordant dyeing of wool using tannic acid and FeSO4, part 1: Initial findings. Dyes Pigments. 2009;80(1):53–60. doi: https://doi.org/10.1016/j.dyepig.2008.05.008
103. Gargote M. Formulation of hair colors from nature. Int J Sci Eng Res. 2012;3(8):1–3.
104. Kraseasintra O, Tragoolpua Y, Pandith H, Khonkarn R, Pathomaree W, Pekkoh J, *et al.* Application of phycocyanin from *Arthrospira* (Spirulina) *platensis* as a hair dye. Front Mar Sci. 2022;9:1024988. doi: https://doi.org/10.3389/fmars.2022.1024988
105. Yang D, Zhou M, Wei W, Zhu H, Fan X. Preparation of a genipin blue from egg protein and genipin. Nat Prod Res. 2012;26(8):765–9. doi: https://doi.org/10.1080/14786419.2010.547859
106. Yang S, Liu L, Han J, Tang YA. Encapsulating plant ingredients for dermocosmetic application: an updated review of delivery systems and characterization techniques. Int J Cosmet Sci. 2019;42(1):16–28. doi: https://doi.org/10.1111/ics.12592
107. Casanova F, Santos L. Encapsulation of cosmetic active ingredients for topical application–a review. J Microencapsul. 2015;33(1):1–17. doi: https://doi.org/10.3109/02652048.2015.1115900
108. Tang Y, He W, Yang S, Liu L. Stabilisation and detoxification of henna (*Lawsonia inermis* L.) extract for hair dye cosmetics by spray-drying encapsulation. Color Technol. 2019;135(6):439–50. doi: https://doi.org/10.1111/cote.12433

109. Tang Y, Yang S, He W, Liu L, Zhang Z. Stabilization of Chinese Gallnut (*Galla Chinensis*) Tannins by spray-drying microencapsulation for natural hair coloring. Fibers Polym. 2020;21:1283–92. doi: https://doi.org/10.1007/s12221-020-9634-0
110. Rosen J, Landriscina A, Friedman AJ. Nanotechnology-based cosmetics for hair care. Cosmetics. 2015;2(3):211–24. doi: https://doi.org/10.3390/cosmetics2030211
111. Mathur JP, Gupta NP. Use of natural mordant in dyeing of wool. Indian J Fibre Text Res. 2003;28:90–3.
112. İşmal ÖE, Yıldırım L. Metal mordants and biomordants. Green Chem Text Technol. 2019;3:57–82. doi: https://doi.org/10.1016/b978-0-08-102491-1.00003-4
113. Packianathan N, Karumbayaram S. Formulation and evaluation of herbal hair dye: an ecofriendly process. J Pharm Sci Res. 2010;2(10):648–56.
114. Rani NU, Jajpura L, Butola BS. Ecological dyeing of protein fabrics with *Carica papaya* L. leaf natural extract in the presence of bio-mordants as an alternative copartner to metal mordants. J Inst Eng India Ser E. 2020;101:19–31. doi: https://doi.org/10.1007/s40034-020-00158-1

How to cite this article:
Gutierrez-Ninachoque D, Ortiz de Orue-Meza JO, Del Carpio-Jiménez C. Formulation and evaluation of hair dye shampoo using *Genipa americana* L. (Huito) fruit extract. J Appl Pharm Sci. 2025;15(01):243-255.