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Obtaining and evaluation of natural hair dye based on the green husk of *Juglans* sp.

Obtención y evaluación de tinte natural para cabello con base en ruezno de nogal *Juglans* sp.

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Resumen

El ruezno o percarpio de nogal *Juglans* sp., es un desecho agrícola con fuente de compuestos orgánicos que aportan colorantes de forma natural. Estos pigmentos representan una alternativa a los productos de belleza que en su mayoría contienen alérgenos y carcinógenos. El objetivo del presente estudio fue evaluar la efectividad y pérdida de color de un colorante utilizando los extractos del pericarpio de *Juglans* sp. en mechones de cabello decolorado. Se obtuvieron extractos de pericarpio secos y molidos (10% p/v) a base de agua, etanol y metanol y se evaluaron en mechones de cabello decolorados con peróxido de hidrógeno. El cambio de color se evaluó utilizando la escala de Fisher-Saller asignando un número en lugar de una letra a cada mechón de cabello tratado. Los resultados de la escala modificada se evaluaron mediante análisis de varianza y se registró la pérdida de color lavando cada mechón 30 veces. Como resultados, no se encontró una pérdida de color significativa después de 30 lavados con detergente; tanto el extracto etanólico como el metanólico desarrollaron colores oscuros (U-Y en la escala de Fisher-Saller, 21-25), mientras que los extractos acuosos desarrollaron colores cenizoa (M-O en la escala de Fisher-Saller, 13-15). No existe ningún tinte para el cabello

comercial a base de cáscara verde en el mercado. Esta investigación muestra una formulación semipermanente como una opción ecológica a los tintes sintéticos.

Palabras clave: *Aloe vera*, *Euphorbia antisyphilitica,* Fisher-saller, mordientes, colorante sintético.

Abstract

The green husk of *Juglans* sp., is an agricultural waste with a source of organic compounds that provide naturally coloring agents. Natural pigments represent an alternative to beauty products most of which contain allergens and carcinogens. The objective of this study was to evaluate the effectiveness and color loss of a dye using extracts from the pericarp of *Juglans* sp. in strands of bleached hair. Dried and ground pericarp extracts (10% w/v) based on water, ethanol, and methanol, were obtained and evaluated on bleached hair strands using hydrogen peroxide. Color change was assessed using the Fisher-Saller scale by assigning a number instead of a letter to each treated hair strand. The results of the modified scale were evaluated by analysis of variance and color loss was recorded by washing each strand 30 times. As results, no significant color loss was found after 30 washes with detergent; both ethanolic and methanolic extracts developed dark colors (U-Y on the Fisher-Saller scale, 21-25), while aqueous extracts developed ashy colors (M-O on the Fisher-Saller scale, 13-15). There is no commercial green peel-based hair dye on the market. This research shows a semi-permanent formulation as an ecological option to synthetic dyes.

Keywords: *Aloe vera*, *Euphorbia antisyphilitica*, Fisher-saller, mordants, synthetic dye.

1. Introduction

Some reports indicate that more than a third of women over the age of 18 and approximately 10% of men over the age of 40, use hair coloring products [1]. The use of hair dye is extremely common worldwide, this practice starts at very young age, and it has been reported that hair dyes are used excessively and incorrectly, most of the time people do not know about the risks associated with excessive hair dye use [2].

Synthetic hair dyes can generate adverse effects such as cancer, [3, 4] allergic reactions, [5] and skin diseases [6] in humans. Some of these products can cause toxicity [7] and mutagenicity, in the environment. Synthetic dyes contain high amounts of chemical compounds [8] compared to natural hair dyes. [9] The manufacturing process can be dangerous to the health of the employees [10] and could cause potential residual effects to the final consumer, as well as beauty salon personnel involved in the application of hair dyes. Through the years, epidemiological studies have reported an increased risk of developing bladder cancer in hairdressers and barbers. [11,12] In a study conducted by IARC [13] concluded that some of the chemicals to which these workers are occupationally exposed are probably carcinogenic to humans. Some aromatic amines used in most synthetic dyes bind with DNA and are genotoxic [14]. The risk of developing cancer has been associated with the use of synthetic dyes; this is probably due to the oxidative mechanism of the components that may be involved in the carcinogenic trigger [15].

Hair dyes of natural origin have advantages over synthetic ones, since they are obtained from agricultural products and are a

renewable, biodegradable and non-toxic source. [16] In recent years, the demand among consumers of natural hair products has grown considerably; [17] most of these products are obtained from biological sources, especially plants. [18] It has been reported by Jahanban- Esfahlan [19] that walnut pericarp is often discarded and produced in large quantities, with little known uses [20]. In Mexico more than 30,000 tons of pecan husks are disposed annually, of which 5% is used and the remaining is eliminated [21]. Cosmulescu et al. [22 report that these residues can cause pollution and especially can be toxic to surrounding plant species and water bodies. Few reports indicate that the industrial use of this waste is for preparation of polymeric compounds, [19] coloring of various textile products and raw material for the cosmetic and pharmaceutical industries. [23, 24] Green husk (pericarps) could be a valuable source of natural compounds for hair dyeing because of its content of juglone, a brown pigment, [5] that appears naturally in roots, bark, leaves and husks of plants of the Juglandaceae family [25].This work analyses the obtention of a semi-permanent hair dye product based on the green husk extract from aqueous, ethanolic or methanolic solvents that were combined with mordants and natural ingredients such as *Aloe vera* and candelilla wax.

2. Materials and methods

Biological Material

Dried and fresh pericarps (husks) were collected from the *Juglans* sp.walnuts orchard located in Ramos Arizpe Coahuila, Mexico. These were washed with running water for 10 minutes and dried afterwards in a laboratory oven at 40-50 °C for four days. After being dried, they were ground in a home blender, sieved, and stored in plastic zyploc ® plastic bags for extraction using different solvents.

Aqueous Extract

For the preparation of aqueous extracts, 20 g of ground pericarps were mixed with 200 mL of distilled water to obtain a final concentration of 10% (weight/volume, w/v). This mixture was placed in a baker and consequently boiled for 15 minutes while stirring continuously [26]. Then cooled at room temperature, covered with aluminium foil, and refrigerated for seven days. Once the standing time had passed, the mixture was filtered with a coffee maker filter.

Ethanolic Extract

The Methodology modified by Vieria et al. [27] was followed using 5 g of ground pericarps dissolved in 50 mL of a mixture of 50% ethanol and 50% distilled water (v/v), with a final concentration of 10% (w/v). The mixture was placed under constant stirring for 15 minutes, then transferred to a container covered with aluminium foil and left to a room temperature setting for 7 days. The mixture was filtered using a coffee filter and then heated at a 60 °C for 10 minutes.

Methanolic Extract

The methanolic extract was obtaining using 5 g of ground pericarps into 50 mL of 98% methanol (Fermont ®) to a final concentration of 10% (w/v), the mixture was stirred for 10 minutes at room temperature and remained covered with aluminium foil for 7 days at 25 °C. After the standing time, the mixture was filtered with a coffee machine paper filter.

Mordant Solutions

A mordant solution was prepared using 4% (w/v) citric acid [26] for a final volume of 50 mL as well as the following solutions: 10% (w/v) potash alum, 3% (w/v) ferrous sulphate and 12% (w/v) ascorbic acid [5] separately. Each mixture was stirred for 10 minutes.

Aloe vera Gel

The leaves (shoots) of *A.* vera were collected, washed with tap water and then the epidermis we removed to obtain the hydro-parenchymatous gel, processed in a

home blender until we obtain a semi-solid consistency.

Bleach of Hair

Hair samples 15 cm long and approximately 4 mm in diameter, were collected from voluntary donors with dark brown hair. The Samples were bleached using 40 g of bleaching powder (Vezia ® Care Collection) and 60 mL of hydrogen peroxide cream 9% (30 vol) purchased from local stores. The strands were left 24 hours after we applying the hydrogen peroxide cream and wrapped in aluminium foil to generate heat. The strands were identified according to Fischer-Saller scale [28] to an extra light blonde or category A (Fig. 1). The samples were then rinsed under running water until the cream was removed.

Figure 1. Fisher-Saller scale, Evayanti and Artaria [28].

Evaluated Treatments

Hair dye mixtures were obtained by mixing the type of extract (ground pericarps with water, ethanol, or 10% w/v methanol), mordant solution (ascorbic acid 12%, citric acid 4%, ferrous sulphate 3%, potassium alum 10%) and *Aloe vera* gel according to Beiki et al. [5] A total of 7 treatments were evaluated; Table 1 shows the percentages of the amounts used. Once mixtures were prepared, the hair strands were immersed in each treatment for 45 minutes, then rinsed with water for 30 seconds or until the residual water was crystallized. This procedure was repeated in triplicate for each treatment. The Dyed strands were left to dry at room temperature for one day. After that time, the samples were washed intensively with 28% sodium lauryl ether sulphate (SLES) and rinsed under running water; this procedure was repeated 30 times for each treatment. At the end, they were left to dry at room temperature.

Table 1. Dyeing mixtures (treatments)of pericarp extracts, according to the amount and type of solvent, mordants and color developers used in hair strands.

Treatment	Ethanol extract 10% (w/v)	Aqueous extract 10% (w/v)	Methanol extract 10% (w/v)	*Aloe vera* gel	Ferrous sulphate 3% (w/v)	Ascorbic acid 12% (w/v)	Potash alum 10% (w/v)	Citric acid 4% (w/v)
1	3 mL	0 mL	0 mL	2 mL	2 mL	4 mL	0 mL	0 mL
2	0 mL	0 mL	3 mL	2 mL	2 mL	4 mL	0 mL	0 mL
3	0 mL	3 mL	0 mL	2 mL	2 mL	4 mL	0 mL	0 mL
4	3 mL	0 mL	0 mL	2 mL	2 mL	0 mL	2 mL	4 mL
5	0 mL	0 mL	3 mL	2 mL	2 mL	0 mL	2 mL	4 mL
6	0 mL	3 mL	0 mL	2 mL	2 mL	0 mL	2 mL	4 mL
Control	0 mL	0 mL	0 mL	0 mL	0 mL	0 mL	0 mL	0 mL

Hair Color Evaluation

To verify the degree of coloration, comparisons were made by matching treatments against the Fischer-Saller scale proposed by Birngruber and Verhoff [28] and Evayanti and Artaria [29] (Fig. 1) and converting the letter designations to numbers. In physical anthropology, The Fischer-Saller scale is made up of thirty strands of human hair. In general, it is detailed as follows: A (1) = blond, B-E (2-5) =light blond (straw), F-L (6-12) = blond, M-O (13-15) = dark blond, P-T (16-20) =light brown to brown, U-Y (21-25) =dark brown to black, I-IV (26-29) = reddish, V-VI (29-30) = reddish blond. The control treatment was a bleached strain classified as A (1) as very light blond. Once the evaluation was concluded, an analysis of variance (ANOVA) was performed ($p< 0.05$) with number of colors assigned on the Fisher-Saller scale, then, a Tukey's HSD Test for multiple comparisons was done in R software 4.1.2 version [30].

Formulation

The treatment with the most intense coloration (U-Y or 21-25 numeration) was chosen for final formulation of 20 mL. The formula consisted of 6 mL of extract, 4 mL of *Aloe vera*, 4 mL of 3% ferrous sulphate and 6 mL of 12% ascorbic acid with 1.86% (w/v) xanthan gum, 5.21% (w/v) candelilla wax and 5.21% (w/v) coconut oil. The last two ingredients (coconut oil and candelilla wax) were melted at 50 °C on a stirring plate and xanthan gum was added; Finally, the dye was added to the mixture and stirred manually. The formulated treatment was applied using a brush to a bleached strand of hair, emulating salon dye application, then the sample was left to rest on aluminium foil for 45 minutes. After the time had elapsed, the sample was washed with running tap water. This procedure was performed by triplicate and the final sample was intensively washed with SLES 28% and rinsed under running water. The obtained formula was compared to the control (bleached strains) and with a synthetic Schwarzkopf ® dye (light brown nude-563) using the same methodology described above.

3. Results

All evaluated treatments were highly significant different compared to the control (natural blonde, 1±0.00). Treatment 1,

induced the darkest color results (22.66±0.57) classified as dark brown to black in the Fisher-Saller scale (W); it was observed that mixtures with ascorbic acid and ferrous sulphate in combination with *A. vera* gel mixed with the three different types of extracts, ethanolic, methanolic and aqueous solvents (22.6, 19.6 and 13.0 respectively) induced darkest colors than the use of citric acid, potassium alum, and ferrous sulphate in combination with *A. vera* gel in ethanol, methanol, and water solvents(16.3, 14.3 and 11.3 respectively) (Fig. 2- Fig. 4).

Figure 2. Control= bleached hair strains with hydrogen peroxide, Trat 1= treatment 1 with 10% ethanolic extract *Juglans* sp., ferrous sulphate 3%, ascorbic acid 12% and *A. vera*, Trat 4= treatment 4 with 10% ethanolic extract *Juglans* sp., ferrous sulphate 3%, potassium alum 10%, citric acid 4% and *A. vera.*

It has been proven that the treatments with 3% ferrous sulphate, 12% ascorbic acid and *A. vera* as mordants (treatments 1,2 and 3) presented more intensive coloration after 30 washes with 28% sodium lauryl ether sulphate and were highly significant different than those that were treated with 4% citric acid, 10% potassium alum, 3% ferrous sulphat, and *A. vera* (treatments 4,5 and 6) (Table 2).

Table 2. Hair dyeing results obtained according to Fisher-Saller scale and assigned numeration after 30 washes with 28% sodium lauryl ether sulphate.

Treatment	Assigned number	Tukey HSD	Fisher-Saller scale letter
Control	1±0.00	e	A= very light blond
1	22.66±0.57	a	W= dark brown to black
2	19.66±2.08	ab	T= light brown to brown
3	13.0±1.00	cd	M= dark blond
4	16.33±1.52	bc	P= light brown to brown
5	14.33±3.21	cd	N= dark blond
6	11.33±1.52	d	K= blond
Formulation	17±2.6	bc	Q= light brown
Synthetic dye	17±1.0	bc	Q= light brown

ANOVA, Tukey´s multiple comparison test (significance p< 0.05).

The best result was obtained with the use of ethanolic extract of *Juglans* sp. pericarps 10% (w/v) in a mixture of ferrous sulphate 3%, ascorbic acid 12% and *A. vera* gel (treatment 1). Dark brown to black colors were obtained in the dyed strains; however, there was no statistical difference with the use of treatment 1 and treatment 2 (methanolic extract of *Juglans* sp. Pericarps 10% w/v, ferrous sulphate 3%, ascorbic acid 12% and *Aloe vera* gel) where light brown to brown colors were obtained in hair strains. Slight coloration was observed when using the aqueous extracts of the *Juglans* sp. pericarps (10% w/v) with results of dark blond with ferrous sulphate, ascorbic acid, and *A. vera*, treatment 3) and blond when ferrous sulphate, citric acid,

potash alum and *A. vera* mixture was evaluated (treatment 5 and 6).

Figure 3. control= bleached hair strains with hydrogen peroxide, Trat 2= treatment 2 with 10%methanolic extracts of *Juglans* sp., ferrous sulphate 3%, ascorbic acid 12% and *A. vera*, Trat 5= treatment 5 with 10% methanolic extract of *Juglans* sp., ferrous sulphate 3%, potassium alum 10%, citric acid 4% and *A. vera.*

Figure 4. control= bleached hair strains with hydrogen peroxide, Trat 3= treatment 3 with 10% aqueous extract of *Juglans* sp., ferrous sulphate 3%, ascorbic acid 12% and *A. vera*, Trat 6= treatment 6 with 10% aqueous extract of *Juglans*, ferrous sulphate 3%, potassium alum 10%, citric acid 4% and *A. vera.*

Figure 5. Color comparison with a= synthetic dye (Schwarzkopf ®, light brown nude color); b= cream formulated with *Juglans* sp ethanol extract after 30 washes with 28% sodium lauryl ether sulphate.

There was no statistical difference between the use of aqueous extract of *Juglans* sp. (treatment 3, dark blond), when compared to the methanolic extract of *Juglans* sp. (treatment 5, dark blonde) (Table 2). The formula in treatment 1, was obtained using an emulsion of candelilla wax, xanthan gum and coconut oil (see methodology), however, the intensity of the color was diminished when combined with these ingredients (Fig. 5). Color results using the formula of ethanolic extract, varied from treatment 1 (22.6±0.57) to 17±2.6 on the modified Fisher-Saller scale. No statistical difference was found between the ethanolic extract formula (17±2.64) compared to synthetic dye schwarzkopf ® (17±1.0) developing light brown to brown colors according to Fisher-Saller scale.

The stability of the formula was considered by leaving it in a sealed glass bottle and placed in a cabinet at a temperature not higher than 30 °C, it was found that after 8 months of storage, this mixture did not lose effectiveness in tests on bleached hair strands.

4. Discussion

It has been reported that the main component of the plant responsible for the color is the juglone, that belongs to the

Juglandaceae family, contains numerous bioactive polyphenolic groups quercetin and juglone, which is found in various parts, such as leaves, husk and bark [31]. Jahanban-Esfahlan [19], recapitulated the uses and chemical constituents of the walnut nut, some records about its compounds are the content of phenols, flavonoids, hydroxybenzoic acid, hydroxycinnamic acid, ellagitannins and gallotannins.

There were few limitations throughout the general research of this work, starting with the collection of the biological material such as *A. vera* and pericarps. In this study, *A. vera* was assessed as a color enhancer, however, the mucilage obtained from this plant varies in concentration determined by the variety of the species and the climatic conditions when it is collected. Significative differences have been reported in the composition and concentration of the active compounds found in the genus *Aloe* sp., [32,33] in addition, the age of the plant can affect the concentration of active principles, as mature leaves have higher concentration than younger ones.

Some tests (not mentioned in the methodology part) were carried out to find out the concentration of mucilage extracted from *A. vera*. Some samples were taken in spring, summer, and winter, finding that specimen from summer had higher density than the winter sample; *Juglans* extract dyed *A. vera* mucilage collected in summer, instead of the hair strand. The plant used in this study was not identified taxonomically or molecularly. Another point to note is the collection of pericarps, since it was evaluated with freshly collected pericarps, and the waste is not generated all year round, so it is necessary to wait to obtain this material. In a study by Lee [34], the authors found significant variations in juglone content during the four-month period in an Ohio cultivar and in seedlings. The leaves of seedlings in July contained Juglone at their maximum levels and in September at their minimum level. Another fact to consider is the storage of the husks; it reveals the need for further research on pericarps to find if there is a variation in the dye obtention from different collected samples over the years.

Another limitation is the lack of knowledge on beauty techniques, which is why we lengthened the time for discoloring the samples, a situation that generally occurs in 45 minutes to an hour. It would be convenient to continue dyeing the hair sample with different degrees of discoloration to identify its effectiveness in people who currently use a synthetic dye or who stopped dyeing at a certain time.

In the present study, the ethanolic and methanolic treatments presented the darkest colors results, while the aqueous treatments presented lighter ashy colors. It was found that the addition of alum to the mixtures conferred lighter and greenish tones. There are reports of other mordants of natural origin that can be used [35,36] but, potassium alum, *A. vera* and ferrous sulphate has been utilized as mordants due to their eco friendliness compared to other commonly used metal salt mordants [35,37] however, the intensity of the color was lighter than with the application of the liquid extracts without the addition of emulsifiers. When comparing the obtained color from the formulation with a commercial dye (Schwarzkopf ®) purchased in the supermarket, the ash characteristic was similar in both products although the ingredients of the synthetic dye are hydrogen peroxide, ammonium hydroxide, ammonia, potassium hydroxide, diamino-toluenes, the product package warns about the possible cause of irritation or allergy in some people. In addition, over 5000 different types of chemicals are used in hair dyes, some have been described as carcinogenic in animals. [11, 38] Due to its extent use, scientists have tried to determine if the exposure to the chemicals used in hair dye products, is linked to increased cancer

risk in people. Most chemical dyes contain ammonia, acetic acid, hydrogen peroxide, ammonium hydroxide, 2-methyl resorcinol, p-aminophenol, cetearet, crotein Q and toluenes, substances that cause nasal disorders and irritant-type contact dermatitis [39]. Patel et al. [6], quotes that the frequent use of hair dyes, increases allergies due to their chemical content; itchy scalp, hair loss and breakage, redness on the scalp, conjunctivitis, pigmentation, and headaches. It may also increase the risk of cancer [3].

The ingredients of this study were chosen to support small-scale producers dedicated to the use of candelilla, a plant from the Chihuahuan desert. For many of these producers, the use of candelilla represents their only source of income. It has multiple industrial uses, including the production of paints, polishes, candles, leather products, cosmetics, disposable containers, wax paper and electronic items [40].

5. Conclusions

The effectiveness of extracts of green husks of *Juglans* sp. used as natural dyes on bleached hair strands was evaluated. It was found that ethanolic and methanolic extracts developed the darkest colors. No significant color loss was found in the treated hair after 30 washes with detergent. The proposed formula consists of natural ingredients such as: *A. vera*, *Juglans* sp. extract, vitamin C (ascorbic acid), and ferrous sulphate, materials that have been reported to be sustainable. Hair dye based on green walnut shells are not available in the market. There is scattered information about the use of pecan leaves, shell, and bark in infusions, however, few studies report the use of pecan in the cosmetic industry. The tests carried out for the evaluation of this product show that it is possible to dye hair with pecan extract using mordants of either natural origin or low ecological footprint.

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