EVALUATION OF COLOR FASTNESS AND SKIN IRRITATION OF HAIR DYE CREAM FROM HENNA LEAVES (*LAWSONIA INERMIS* L.)

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ABSTRACT

Objectives: This study aims to evaluate the color fastness and study the skin irritation of a natural hair dye cream from Henna leaves. **Materials and method:** Nineteen experimental (F1-F19) are conducted using the D-optimal model to evaluate the cause-effect relations and evaluate the color fastness of hair dye cream. Three independent variables were selected for the survey namely Henna extract concentration (X_1 includes 5%, 10%, 15%), pH of cream (X_2 includes 6, 7, 8), hydrogen peroxide concentration (X_3 includes 0%, 2%, 4%). Three dependent variables include color fastness after 1 washes (Y_1), 6 washes (Y_2) and 12 washes (Y_3). Test for skin irritation hair dye cream was applied for rabbits. **Results:** the optimal parameters of hair dye cream were identified, which included Henna extract concentration of 10%, pH of 6, hydrogen peroxide concentration of 4%. The fastness property of the dye was observed to be very good even after washing 12 times and the absence of sensitivity or reaction on the skin. **Conclusion:** The results showed that the product has the ability to dye hair and is safe for the skin.

Keywords: *hair dye cream, Henna leaves, BCPharSoft software, color fastness, skin irritation study.*

I. INTRODUCTION

Henna leaves (*Lawsonia inermis* L.) has been used to dye hair, nails, skin and wool for thousands of years. The leaves of this plant posess a red dye molecule called lawsone (2- hydroxy-1, 4-naphthaquinone), which has the ability to bond with the protein. Lawsone acts as a non oxidizing hair coloring agent at a maximum concentration of 1.5% in the hair dyeing product. Other constituents in Henna such as flavonoids and gallic acid act as organic mordants to the process of coloring. Carbohydrates give the Henna paste a suitable consistency for adherence to the hair [1], [2].

Herbal hair dyes consider to be effective in minimising the use of chemical agents which in turn overcome pollution problems. Herbal hair dyes are pleasant odors, easily available, less expensive, safe, and efficient and rarely have side effects [5], [6]. Due to these reasons, this study aimed to evaluate the color fastness and study the skin irritation of hair dye cream from Henna leaves. Currently, there is no publication on the formulation of hair dye from Henna leaves in Vietnam.

II. MATERIALS AND METHODS

2.1. Materials

Henna leaves provided by Dai Nam herbal store (Ho Chi Minh city, Vietnam) which were ground into raw powder (size 1.0 - 1.6 mm). This powder contained no more than 11 percent of water and meet the testing standards according to Vietnam's pharmacopoeia V [1]

All materials used in this study included distilled water, glycerin, cosmagel 305, ajidew NL-50, argan oil, geodard ultra were purchased

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from Thanh Đat Chemical in Can Tho city, Vietnam and meet the analytical standards.

Henna leaves were extracted by heat reflux method at a temperature of 60°C in 45 minutes in two times and filtrate. The extraction solvent was distilled water and the ratio of medicinal herbs/solvent was 1:12. All filtrates were combined and concentrated using Heidolph Hei-VAP Core XL vacuum rotary evaporator until giving concentrated extract.

Coffee extract was prepared by decoction method in 20 minutes and filtrate. The extraction solvent was distilled water and the ratio of medicinal herbs/solvent was 1:8. The filtrate were concentrated using Heidolph Hei-VAP Core XL vacuum rotary evaporator until giving concentrated extract.

2.2. Methods

The formula ingredients of hair dye cream from Henna leaves were presented in Table 1.

Table 1. The formula ingredients of hair dye cream

No	Ingredients	Quantity
1	Henna extract (X_1)	-
2	Coffee extract	1%
3	Buffer solution* (X_2)	-
4	H_2O_2 (X_3)	-
5	Glycerin	10%
6	Cosmagel 305	4%
7	Argan oil	5%
8	Ajidew NL-50	5%
9	Geogard ultra	1%
10	Distilled water	q.s. 100%

** The buffer solution of glycerin - triethanolamine - citric acid for pH 6, 7 and 8*

The parameters of the independent and dependent variables were presented in Table 2.

Table 2. Variables in experimental design

Independent variables	Level 1	Level 2	Level 3
X_1: concentration of Henna extract (%)	5	10	15
X_2: pH variations	6	7	8
X_3: concentration of hydrogen peroxide (%)	0	2	4
Dependent variables	Constraints		
Y_1: color fastness after 1 shampoo washes	Color fastness (maximum)		
Y_2: color fastness after 6 shampoo washes	Color fastness (maximum)		
Y_3: color fastness after 12 shampoo washes	Color fastness (maximum)		

Color fastness study

Nineteen experimental (F1-F19) were designed according to D-optimal model using Design Expert software (version 6.0.6, Stat-Ease Inc., Minneapolis, USA). The data were analyzed by BCPharSoft software to investigate the cause-effect relations and optimized formulation. The optimized formulation was experimentally repeated in triplicate for further validation.

The hair dye cream were studied for efficiency on human blonde hair strands (length 20 cm). Human hair including 19 test samples were dyed in turn into 19 formula contained in becher for 30 minutes, washed with herbal shampoo and dried with a hair dryer on cool setting. Then check the color lasting capacity after 1 shampoo washes, 6 shampoo washes, 12 shampoo washes.

Different grades (1-7) were assigned to colors depth ranging from jet black to blonde using an experimental color grade scale. Color fastness was determined in terms of number of shampoo washes that a color can withstand [7], [8].

Skin irritation study

Skin irritation study was conducted according to TCVN 7391 - 10:2007 [3]. The dorsal aspect of rabbits was cleared and the hair was removed by shaving. The formulation (500 mg/rabbit) was applied and the site of application was covered with cotton bandage. The patch was removed after 4 hours and the score of erythema was recorded as follows: 0-0,4: No reaction; 0.5-1.9: slight, patchy erythema; 2-4.9: moderate erythema; 5-8: Severe erythema with or without edema.

III. RESULTS

The formula of hair dye cream was designed by Design Expert software including 19 experiments. These results corresponding to the experiments were summarized in Table 3.

Table 3. The independent variables of 19 experiments (F1-F19) and their responses

Experiment	Independent variables			Dependent variables		
	X_1 (%)	X_2	X_3 (%)	Y_1	Y_2	Y_3
F1	10	6	4	2	2	3
F2	15	6	2	2	3	4
F3	15	8	2	3	4	6
F4	10	6	2	3	4	6
F5	15	7	4	3	3	4
F6	5	7	0	2	3	5
F7	15	6	0	2	6	7
F8	5	8	2	2	6	7
F9	15	8	0	2	6	7
F10	10	8	4	2	6	7
F11	5	6	4	2	4	5
F12	10	7	0	3	3	5
F13	5	8	4	3	3	6
F14	10	8	0	3	3	4
F15	10	7	2	3	3	7
F16	15	8	4	3	6	7
F17	5	7	2	2	3	6
F18	5	7	8	2	3	6
F19	5	6	0	3	5	6

Where X_1: concentration of Henna extract (%); X_2: pH variations; X_3: concentration of hydrogen peroxide, Y_1: color fastness after 1 shampoo washes, Y_2: color fastness after 6 shampoo washes, Y_3: color fastness after 12 shampoo washes.

Color grade after number of shampoo washes from 1 to 7 namely jet black: 1; very dark brown: 2; dark brown: 3; medium brown: 4; light brown: 5; dark blonde: 6; medium blonde: 7.

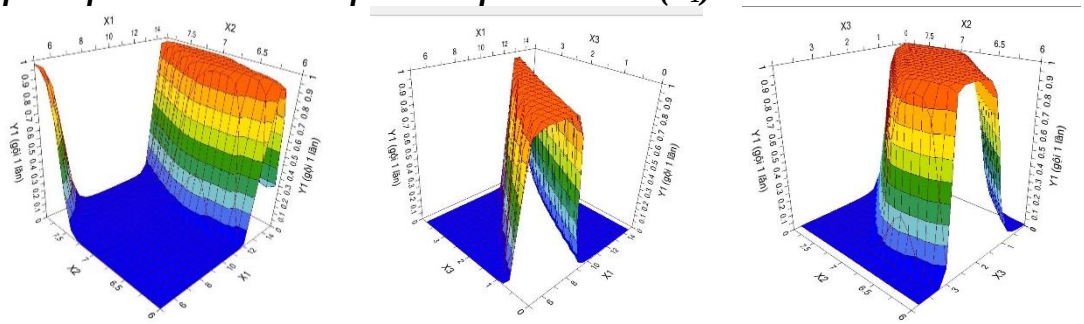
Analyzing the cause-effect between the conditions of the formulation and the color fastness of the hair dye cream. The data in Table 3 were used as inputs for BCPharSoft to investigate the cause-effect relations and optimize the formula. The results of the accuracy of model statistics from BCPharSoft outputs were presented in Table 4.

Table 4. Model statistics from BCPharSoft outputs

Dependent variables	**Y_1**	**Y_2**	**Y_3**
R^2 training	1.00	0.99	0.97
R^2 test	0.99	0.98	0.93

Table 4 demonstrates that all R2 training and R2 test values were more than 0.9, indicating that the models were extremely reliable. These models have the potential to be utilized for multivariate optimization.

Effects of variables on color fastness after 1 washes (Y_1)

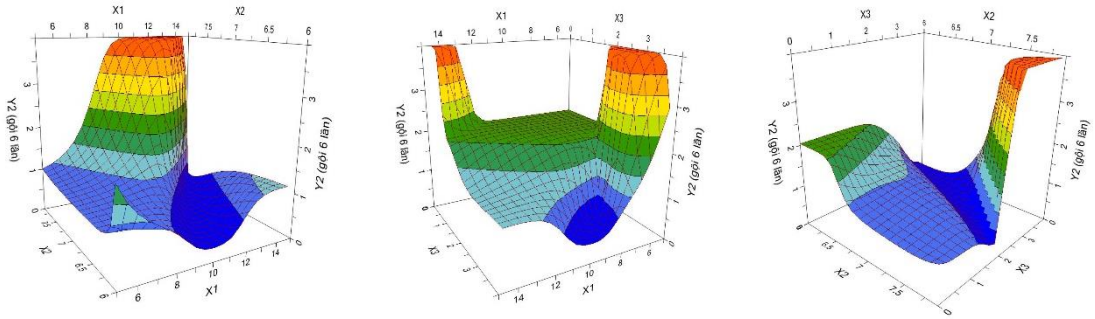


(a) $X_1X_2Y_1$ (b) $X_1X_3Y_1$ (c) $X_2X_3Y_1$

Figure 1. Response surface plots showing the effects of (a) concentration of Henna extract (X_1) and pH value (X_2); (b) concentration of Henna extract (X_1) and concentration of hydrogen peroxide (X_3); (c) pH value (X_2) and concentration of hydrogen peroxide (X_3) on color fastness after 1 washes (Y_1).

With the ideal circumstances as shown in Table 2, color fastness after 1 washes - Y_1 has to be as high as feasible. When all X factors are considered in the 3D diagram in Fig. 1, it can be seen that the concentration of Henna extract - X_1 is high (level 2 - 10% or level 3 - 15%), the value of pH - X_2 needs to be low (pH = 6), hydrogen peroxide (percent) - X_3 is high (level 3 - 4%).

Effects of variables on color fastness after 6 washes (Y_2)

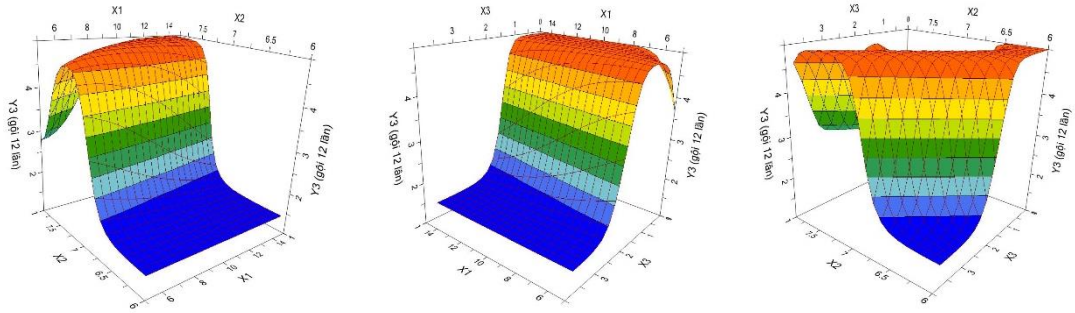


(a) $X_1X_2Y_2$ (b) $X_1X_3Y_2$ (c) $X_2X_3Y_2$

Figure 2. Response surface plots showing the effects of (a) concentration of Henna extract (X_1) and pH value (X_2); (b) concentration of Henna extract (X_1) and concentration of hydrogen peroxide (X_3); (c) pH value (X_2) and concentration of hydrogen peroxide (X_3) on color fastness after 6 washes (Y_2).

With the ideal circumstances as shown in Table 2, color fastness after 6 washes – Y_2 has to be as high as feasible. When all X factors are considered in the 3D diagram in Fig. 2, it can be seen that the concentration of Henna extract – X_1 is high (level 2 - 10% or level 3 - 15%), the value of pH – X_2 has little effect on color fastness after 6 washes however a low pH value needs to be low (pH = 6), hydrogen peroxide (percent) – X_3 is high (level 3 - 4%).

Effects of variables on color fastness after 12 washes (Y_3)



(a) $X_1X_2Y_3$ (b) $X_1X_3Y_3$ (c) $X_2X_3Y_3$

Figure 3. Response surface plots showing the effects of (a) concentration of Henna extract (X_1) and pH value (X_2); (b) concentration of Henna extract (X_1) and concentration of hydrogen peroxide (X_3); (c) pH value (X_2) and concentration of hydrogen peroxide (X_3) on color fastness after 12 washes (Y_3).

With the ideal circumstances as shown in Table 2, color fastness after 12 washes - Y_3 has to be as high as feasible. When all X factors are considered in the 3D diagram in Fig. 3, it can be seen that the concentration of Henna extract - X_1 is high (level 2 - 10% or level 3 - 15%), the value of pH – X_2 has little effect on color fastness after 12 washes however a low pH value (pH = 6) should be chosen to avoid skin irritation, hydrogen peroxide (percent) - X_3 is high (level 3 - 4%).

Color fastness study

BCPharSoft OPT program optimizes the formula by setting variables X_1, X_2 and X_3 to 10%, 6 and 4%, respectively. Three replicated batches of the improved method are created to

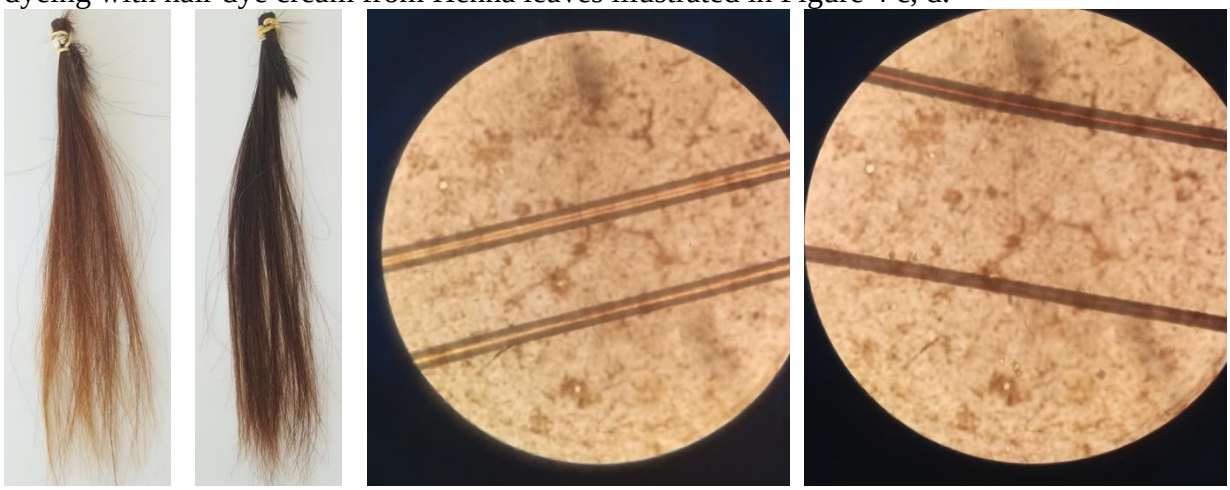
confirm the validity of the optimization approach. Table 5 displays the experimental outcomes.

Table 5. Comparison of the predicted and observed responses (n = 3)

Responses	Y_1	Y_2	Y_3
Predicted	2	2	3
Observed	2	2	3

Color grade after number of shampoo washes from 1 to 7 namely jet black: 1; very dark brown: 2; dark brown: 3; medium brown: 4; light brown: 5; dark blonde: 6; medium blonde: 7.

The photographs of hair samples before and after dyeing with hair dye cream from Henna leaves illustrated in Figure 4 a, b. The microscopic view of hair samples before and after dyeing with hair dye cream from Henna leaves illustrated in Figure 4 c, d.



(a) (b) (c) (d)

Figure 4. The photographs of hair samples before (a) and after dyeing with hair dye cream (b) and microscopic view of hair samples before (c) and after dyeing with hair dye cream from Henna leaves (d).

Skin irritation study

The total scores of the optimal hair dye cream for skin irritation in terms of erythema and oedema was calculated after 24, 48 and 72 hours according to TCVN 7391 - 10:2007 [3]. The results showed no skin irritation or erythema or edema on the skin treated with hair dye cream when observed for 24, 48 and 72 hours. Therefore, skin irritation studies showed absence of erythema and or edema indicating absence of sensitivity or reaction on the skin.

IV. DISCUSSION

Hair coloring, or hair dyeing, is the practice of changing the hair color. The main reasons for this are cosmetic: to cover gray or white hair, to change to a color regarded as more fashionable or desirable. Hair color is determined by the amount of a pigment called melanin in hair. An abundance of one type of melanin, called eumelanin, gives people black or brown hair. An abundance of

another pigment, called pheomelanin, gives people red hair [5].

The mechanism of hair color change occurs in an alkaline medium that promotes the opening of the cuticles that allows the penetration of the dyes' molecules into the cortex. The oxidizing agent such as hydrogen peroxide causes a chemical reaction in the hair cortex that leads to the new hair color. In hair dye, the coloring process goes through 2 stages. The first stage, hydrogen peroxide will dissolve or oxidize hair pigments so that the hair will fade in color. The second stage, the hair cuticle (the outermost part of the hair) must be opened before the hair dye enters the hair using alkaline agent [8].

In Asia and North Africa, the leaves of Henna are widely used for hair and skin coloration [5]. Henna when used alone as colorant, gave an unappealing orange-red color whereas a combination of Henna and coffee powders show appealing reddish black color with good dyeing effect.

As can be seen from the Figures 1 a, b, Figure 2 a, b and Figure 3 a, b, the color fastness of natural dyes can be improved by increasing the concentration of Henna extract. This is clearly shown in experiments F2, F5 in Table 3. In contrast, the experiments F7, F9, F16 showed complete color loss after 12 washes whereas Henna extrac concentrate was 15%. Maybe the large amount of color molecules does not penetrate into the cortex and are deposited on the surface of the hair, so they also diffuse out of the hair after washing. This means that the concentration of Henna extract was not a decisive factor in the color fastness of hair dye cream.

The natural pH of hair strands and scalp is on the acidic side, between 4.5 - 5.5, which is to secrete sebum for the scalp. At an acidic or pH-balanced, it will help tighten the cuticles and bring shine. On the contrary, the pH/alkaline medium promotes the opening the cuticle and it beneficial to the penetration of dye molecules into the cortex in a fast manner. However, the more acidic the pH value (pH < 3), the more irritating the skin, and the more alkaline the pH value (pH > 10), the more scaly or rough the skin. In addition, natural dyes include organic compounds with low molecular weight that require a low pH in order to pass through the hair cuticle and diffuse throughout the cortex [7]. Because of the above reason, we choose the pH values of the hair dye cream is selected 6, 7 and 8, respectively.

The range of pH value from 6 to 8 change the color of the hair and the above pH value does not affect on the color fastness of the product. The results of the test of the effectiveness of hair dye on washing can be seen in Figures 1 a, c, Figure 2 a, c and Figure 3 a, c. Hair dye cream in cosmetic should choose a low pH value (pH = 6) [7]. Moreover, the choice of pH too determines strength and health of the hair. When bleached, highlighted or balayed, hair has undergone techniques involving chemicals that will most likely damage it. Then, the closer the pH of the hair dye product is to the balanced pH level, the better it is for the hair

These types of dyeing such as semi-permanent, temporary can also be called direct dyeing, which take place without an oxidizing agent. Temporary dyes act through dye deposition on cuticles, but semipermanent may penetrate a little into the cortex. Demi-permanent and permanent dyes are based on color precursors, called oxidation dyes, and the final shade is developed by their interactions with an oxidizing agent, but they differ from the

alkalizing agent used. These dyes are long-lasting color and allows a wide range of hair color choices. In addition, these dyes alter the structure of the hair as it lightens, darkens, or changes natural tones [8].

In oxidation systems, there is an intense diffusion of the molecules into the cortex, what promotes a longer color resistance and the color fastness after washes (Y_1, Y_2, Y_3 maximum). This result can be seen in Figures 1 b, c, Figures 2 b, c and Figures 3 b, c. The high concentration of oxidizing agent, the easier it is to stain and color fast. This means that the independent variables simultaneously affect each other on the color fastness of the hair dye cream and the number of washes.

In addition to the above independent variables, the pH value of the shampoo is also an important factor for color fastness for hair dye cream. The pH of the shampoo (pH > 6) is high to open the hair cuticle, diffuse the pigment out and reducing the color dye on the hair [8].

BCPharSoft OPT program optimizes the following parameters: 10% extract of Henna percentage, pH value 6, 4% hydrogen peroxide percentage. The R^2 test and R^2 train values are used to examine the cause-effect relationship. In general, if the R^2 training value is more than 95% and the R^2 test value is greater than 70%, the model is acceptable. If the R^2 test value is greater than 100%, the model's predictability is improved. According to Table 4, the values Y_1, Y_2, and Y_3 exhibited high compatibility based on the R^2 test (R^2 training = 97% > 95%). The values Y_1, Y_2, and Y_3 have strong predictability (R^2 test = 93% > 85%) based on the value of R^2 test.

The dyeing effect of optimal formula is observed in Figure 4 a, b. The results of this study show that human hair is reddish - black color on blonde hair. This formula hair dye cream resistance up to 12 washes compared to the dyed color. The active ingredient of of Henna extract is naphthoquinone. In addition, there is flavonoids which is considered as color fixatives that help improve color fastness through shampoo washes [1].

Various factors may affect the color fastness of hair dyeing cream, such as the type and amount of melanin in the hair, the number of shampoo washes, the type of shampoo, etc. Nowadays, young people like to follow fashion trends. They like to change their hair color in a short period of time, but want it to be safe to use. Therefore, hair dye cream from herbal with a minimum of synthetic chemicals are a reasonable choice.

Skin irritation study was done by applying prepared formulation on the skin of rabbits. The irritation, edema and erythema were evaluated and it was found that no irritation and erythema appear on skin of rabbits.

V. CONCLUSION

The study revealed that hair dye cream from Henna leaves offers benefits such as promotion of hair color, enhancement of color fastness while being safe and eco-friendly.

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