

Formulation and Evaluation of Natural Herbal Hair Dye Gel Using *Lawsonia inermis* (Henna Leaves) and Skin Irritation Studies in Albino Rats

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Abstract: **Introduction:** Hairs are protective appendages on the body that remain on one or other part of body from the time of birth to death. People who carry luxuriant and lustrous hair are regarded as young and beautiful. Since the conventional methods of hair coloring by the use of natural or synthetic colorants has limitations, an attempt has been made in this study to formulate a hair dye using crude drugs having good coloring property that is safe and ready to use. **Objectives:** To formulate and evaluate natural polyherbal hair dye gel using henna, tea, hibiscus leaves powder and amla fruit extract. **Materials and Methods:** For this purpose, powders of Henna leaves, Hibiscus leaves, Amla fruits and Tea leaves were procured from the local market in Sikkim and Kathmandu. The extract was prepared by cold maceration using water as solvent. Preliminary identification of phytochemical constituents was performed. Various physical parameters were used for standardization. Formulation of hair dye gel were done using carbopol-934, extract of henna, tea, hibiscus leaves powder, amla fruit extract, glycerin, sodium hydroxide solution (1%), methyl paraben and distilled water. Dyeing studies of formulated gel and marketed gel on human hair was performed. Skin Irritation study of formulated gel was performed in albino rats (n = 6) as per experimental protocol approved by Institutional animal ethical committee IAEC No. HPI/2018/IAEC/PP-0165. **Observation and Results:** The study revealed that all the selected plants contain alkaloids, flavonoids, tannins & phenolic compounds, glycosides, carbohydrate, saponin, fat & volatile oils, gum & mucilage, sterols and protein which imparts benefits such as promotion of hair growth and prevention of hair greying while being safe and eco-friendly. Comparison of physicochemical properties of formulated and marketed formulation both has similar color, appearance and consistency. Skin irritation studies showed absence of erythema and or edema indicating absence of sensitivity or reaction on the skin. **Conclusions:** The above findings suggest that the prepared formulation was good and can be used for hair dyeing purposes without irritation and erythema.

Key words: Hair dye gel, henna, skin irritation study.

1. Introduction

The Food Drug and Cosmetic Act defines cosmetics by their intended use, as 'articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance [1]. Cosmeceuticals are topical cosmetic-pharmaceutical hybrids intended to enhance the beauty through ingredients that provide additional health-related function or benefit. These

cosmeceuticals, serving as a bridge between personal care products and pharmaceuticals, have been developed specifically for their medicinal and cosmetic benefits [2]. With the emerging interest in the world to adopt and study the traditional system and to exploit their potentials based on different health care systems, the evaluation of the rich heritage of the traditional medicine is essential [3]. Nowadays, herbal preparations are used to enhance beauty and increase the attractiveness of the person as sunburn, complexion brighter and hair growing. Herbal cosmetics can be classified on the basis of dosage form like- cream, powder, soaps and solutions and

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according to part or organ of the body to be applied like- cosmetics for skin, hair, nail, teeth and mouth [4]. Plant products have been part of phytomedicines and herbo-cosmeceutical products since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits and seeds i.e., any part of the plant may contain active components [5].

Hair, one of the vital parts of the body derived from ectoderm of skin, is protective appendages on the body. It grows in three cyclic phases i.e. anagen (growth), catagen (involution) and telogen (rest). The anagen phase can be as short as 2-6 years. In the catagen phase, the growth activity increases and hair moves to the next phase, catagen phase is between 2-3 weeks. The telogen phase is a state at which the hairs move into resting state. This phase lasts for 2-3 months. In general, 50-100 hairs at random are shed every day. Genetic predisposition and hormonal factors predominantly contribute to the hair fall. The use of chemotherapeutic agents also causes hair fall [6]. Management of hair fall is extremely complex. Hormone therapy, α -reductase inhibitors, vasodilators like minoxidil are widely used to reduce the hair fall/loss. The use of some of the herbal oils is also reported to reduce the hair fall/loss.

Even though varieties of synthetic hair care products are available in the market, the consumer requirement is eco friendly, natural and environmentally safe cosmeceuticals without producing any side effects. Synthetic hair colorants involve the use of chemicals like 1-3% phenylenediamine, ammonia, peroxide and coal tar dyes that are capable of removing and replacing or covering the natural hair color. Inorganic salts like aluminum sulphate, copper sulphate, lead acetate and potassium dichromate which act as mordants are also added to improve and protect the color produced by the dye. Use of these chemicals can result in unpleasant side effects, including temporary skin irritation and allergy, hair breakage, skin discoloration, unexpected hair color and cancer [7].

Table 1 Classification of Henna (*Lawsonia inermis*) [10].

Kingdom	Plantae
Subkingdom	Viridaeplantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Order:	Myrtales
Family	Lythraceae
Genus	Lawsonia
Species	inermis
Local name	Mendhi(Nepali)

Herbal plants are the richest sources of antioxidants like vitamin A, vitamin C, vitamin E and other components like gallic acid, saponins, amino acids, elemental sulphur, enzymes, mucilages, flavanoids, tannins, essential oils and polysaccharides. A number of herbal products have been acclaimed with hair growth-promoting activity [8]. Compared with commercial cosmeceuticals the herbo-cosmeceutical preparation is more potent, safe, enhanced activity and without any side effects [9].

Natural dyes are the colors derived from plant, animal or insect matter without any chemical processing. Henna (*Lawsonia inermis*) known as Mendhi in Nepali is selected principle herb to prepare hair dye (Table 1). It has been used traditionally used for colouring palms and hairs. Since the conventional methods of hair coloring by the use of natural or synthetic colorants has limitations, an attempt has been made in this study to formulate a hair dye using crude drugs having good coloring property that is safe and ready to use.

The aim of the study is to formulate and evaluate natural and safe hair color gel and to optimize the hair dyes gels from *Lawsonia inermis* leaves, hibiscus leaves, amla fruit and tea leaves extracts which contain a mixture of saponin, alkaloids, flavonoids and tannins by using carbopol as gelling agent.

2. Materials and Methods

2.1 Collection of Plant Materials

Powders of *Lawsonia inermis* (Henna leaves),

Hibiscus leaves, *Embelica officinalis* (Amla fruit), tea leaves were procured from the local market. The powdered material was stored in a well closed container.

2.2 Physico-chemical Analysis

Physicochemical values such as the percentage of ash values and extractive values were performed according to official methods prescribed in Indian Pharmacopeia 1996 and WHO guidelines on quality control methods for medicinal plant material [11-14].

2.3 Preparation of Extract

The powdered leaves of henna, tea and Hibiscus, fruits of Amla were extracted with water with three times each by cold maceration at room temperature. In cold maceration, whole powdered plant drug is kept in contact with the solvent in a stopper container for 48 hours with frequent agitation until soluble matter is dissolved. The extracts thus obtained were concentrated by distilling off the solvent under reduced pressure by using Rota Evaporator [15, 16].

2.4 Organoleptic Study of Powdered Plant Materials

The powder drugs were evaluated by its appearance, colour, odour and taste.

2.5 Physicochemical Evaluation of Powdered Plant Materials

The individual aqueous extracts were subjected to qualitative chemical investigations for the identification of the phytoconstituents such as sterols, flavonoids, triterpenes, alkaloids, glycosides, tannins, proteins, carbohydrates. The preliminary phytochemical tests were performed for each extract.

2.6 Qualitative Analysis of Phytochemical Constituents

The individual aqueous extracts were subjected to qualitative chemical investigations for the identification of the phytoconstituents such as sterols, flavonoids, triterpenes, alkaloids, glycosides, tannins, proteins, carbohydrates. The preliminary phytochemical tests

Table 2 Composition of the herbal gel formulation.

Ingredients	Quantity
Carbopol-934	1 g
Extract of leaves of <i>Lawsonia inermis</i>	5%
Extract of leaves of <i>Camellia sinensis</i>	5%
Extract of leaves <i>Hibiscus rosa-sinensis</i>	5%
Extracts of fruits of <i>Embelica officinalis</i>	5%
Water	20 mL
Glycerin	10 mL
Sodium hydroxide solution (1%)	q.s
Methyl Paraben	0.5 g
Distilled water	q.s upto 100 g

were performed for each extract by using standard protocols (Table 2) [11].

2.7 Preparation of Herbal Hair Dye Gel

The gel was prepared with defined quantity of carbopol-934 polymer. The specified quantity (1 g) of carbopol-934 was added in to distilled water with vigorous stirring and left for overnight for dissolving the polymer. 5% aqueous extracts of henna leaves powder, tea leaves powder, hibiscus leaves powder and amla fruit extract were dissolved in 20 ml of water with constant stirring. This aqueous extract solution was added into the polymer solution and mixed well by magnetic stirrer. Methylparaben was added as a preservative into this mixture and mixed well by magnetic stirrer. After complete dispersion of the extracts and preservatives, the pH of the gel was adjusted to neutral pH 7 by using sodium hydroxide. Glycerin 10 ml was added and mixed well in a magnetic stirrer. Distilled water was added and made up to 100 g [17, 18].

2.8 Physicochemical Evaluation of Formulations [19, 20]

2.8.1 Physical evaluation

Physical parameters such as color, appearance and consistency were checked visually for the prepared gel.

2.8.2 pH

Aqueous solution (1%) of the formulation was measured by using a calibrated digital pH meter at constant temperature.

2.8.3 Viscosity

Viscosity of the prepared herbal gel formulation was measured by using Brookfield Viscometer with spindle # C 50-1 having a speed of 50 rpm at room temperature and the determination of viscosity was done in triplicate.

2.8.4 Spreadability

Two sets of glass slide with standard dimension were taken. Polyherbal formulation gel was placed in between the two slides and sandwiched about the length of 60 mm. Removed the adhered excess gel on the surface of the glass slides and fixed to a stand without any disturbance. In the upper slide 20 g weight was tied and noted the time taken for movement of upper slide to the distance of 60mm under the influence of weight. Mean time was calculated by repeating the experiment three times and the spreadability was calculated using the following formula.

$$\text{Spreadability} = (\text{Weight} \times \text{Length}) / \text{Time}.$$

2.9 Evaluation of Herbal Hair Dye Gel

2.9.1 Studies on dyeing effect

(1) Colored hair was pasted on a white paper sheet covered with transparent cellophane tape and kept for 30 days under the following conditions:

- Room temperature $25 \pm 30^\circ\text{C}$
- Sunlight 2 hours/day

(2) Effect of natural detergent on colored hair:

10% w/v aqueous dispersion of Reetha (*Sapindus mukorossi*) was prepared and the dyed hair was washed with the same on alternate days for a period of 30 days.

2.9.2 Selection of Animal Species

Albino rats of either sex ($n = 6$) weighing between 200-250 g were selected and animals were maintained

under uniform laboratory conditions (12 h light and 12 h dark cycle) with room temperature (37°C) and Relative Humidity (RH) 40-60% [22]. The experimental protocol was approved by Institutional animal ethical committee IAEC No. HPI/2018/IAEC/PP-0165.

2.9.3 Skin irritation study

Animals were acclimatized for at least 7 days before experimentation. The dorsal abdominal skin of the rats was shaved 24 h before study. The formulation (200 mg/rat) was applied and the site of application was covered with cotton bandage. The patch was removed after 24 h and the score of erythema was recorded as follows [22]:

- point-No reaction;
- points-Slight, patchy erythema;
- points-Slight but confluent or moderate but patchy erythema;
- points-Moderate erythema;
- points-Severe erythema with or without edema).

3. Results and Discussion

3.1 Organoleptic Study of Powdered Plant Materials

The powder drugs were evaluated by its appearance, color, odor and taste (Table 3). Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash, acid insoluble ash and water soluble ash, Aqueous Extractive Value, Loss on drying etc (Table 4) [14].

3.2 Preparation of Extract

The extraction of various plants such as henna leaves, hibiscus leaves, tea leaves, amla fruits were done by cold extraction method and evaluated for its color, consistency and percentage yields. Results are presented in Table 5.

Table 3 Organoleptic characteristics of powdered plant materials.

Powder Sample	Appearance	Color	Taste	Odor
Henna	Powder	Light Green	Aromatic Bitter	Characteristic
Hibiscus Leaves	Powder	Dark Green	Slight Bitter	Pungent flavored
Tea	Powder	Bitter Astringent	Bitter Astringent	Characteristic
Amla	Powder	Brown	Sour	Aromatic

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Table 4 Physicochemical evaluation of powdered plant materials.

S. No.	DRUGS	Total Ash (%)	Acid Insoluble Ash (%)	Water Soluble Ash (%)	Alcohol Soluble Extractive Value	Aqueous Extractive Value	Loss On Drying
1	Henna leaves	7.70	4.5	3.0	3.8	5.0%	4.5
2	Hibiscus leaves	8.1	4.9	3.8	2.4	4.2	3.8
3	Tea leaves	3.29	1.25	2.83	3.1	3.4	3.7
4	Amla fruits	9.5	7.9	1.5	2.9	2.8	2.5

Table 5 The percentage yield, color and consistency of aqueous extract of plant materials.

Sample	Solvent	Color	Consistency	% w/w
Henna leaves	Water	Orange	Semisolid	15.5
Hibiscus leaves	Water	Dark brown	Semisolid	10.2
Tea leaves	Water	Brown	Semisolid	8.2
Amla fruit	Water	Brown	Semisolid	7.2

Table 6 Phytochemical screening of aqueous extracts of plant materials.

S. No	Phytoconstituents	Henna Leaves	Hibiscus Leaves	Tea leaves	Amla Fruits
1	Alkaloids	-	+	+	-
2	Glycosides	-	-	-	+
3	Carbohydrates	+	-	-	+
4	Saponin	+	+	-	+
5	Fats & oils	+	+	+	+
6	Volatile oils	+	-	+	+
7	Tannis& phenolic Compounds	+	+	+	+
8	Protein	+	-	-	+
9	Gums & mucilage	+	-	-	+
10	Sterols	-	+	-	+
11	Flavonoids	+	+	+	+

Note: (+) Present, (-) Absent

Table 7 Physicochemical evaluation of formulated and marketed preparation.

S. No	Parameters	Formulated preparation	Marketed formulation
1	Color	Reddish Brown	Reddish Brown
2	Appearance	Semisolid	Semisolid
3	Consistency	Homogenous, smooth	Homogenous, smooth
4	pH	6.8	7
5	Viscosity	4173 cps	4711 cps
6	Spreadability	11.08 gm.cm/sec	8 gm.cm/sec

The extracts were subjected to phytochemical analysis and the results represented in the Table 6.

Comparison of physicochemical properties of formulated and marketed formulation was done and results are shown in Table 7.

3.3 Dyeing Studies of Formulated Gel and Marketed Gel

Dyeing studies of formulated gel and marketed gel on human hair presenting a brownish color. It has seen

that exposure to 2 hours of sunlight daily caused hair color to fade after 16 days whereas it remained unchanged for 30 days when kept at room temperature protected from sunlight. There was no change in color after washing the hair every alternate day in natural soap solution for 10 days after which the intensity of the color started reducing. The effect of the color lasted for around 20 days even after frequent washing and exposure to sunlight.

3.4 Skin Irritation Study of Formulated Gel and Marketed Gel

The total scores of both the formulations for skin irritation in terms of erythema and oedema was calculated after 24 hours according to OECD scoring system. Results revealed that the both the gel formulation did not cause any erythema or oedema on the rat skin when observed for 24 hours. Therefore, according to OECD guidelines the formulation can be classified as non-irritant to the rat skin. Skin irritation studies showed absence of erythema and or edema indicating absence of sensitivity or reaction on the skin.

4. Discussion and Conclusions

In this study, the raw materials for the preparation of gel were procured from the local market in Sikkim. It is concluded from the pharmacognostical and physicochemical studies that the samples are genuine and no adulteration is reported. The powdered plant parts and their extracts were subjected to preliminary physicochemical and phytochemical analysis which was found to be very promising. Various physical parameters were used for standardization viz. Total ash value, acid insoluble ash value, sulphated ash value, water soluble ash value, water soluble extractive value, alcohol soluble extractive value, were carried according to the methods given in IP 1985. LOD measures the volatile substances present in the formulation and it is the index for volatile oils and moistures.

The aqueous extracts showed the presence of alkaloids, carbohydrates, phenolic compounds, protein, sterols, fixed & volatile oils, saponins, flavonoid and tannin.

Formulation of hair dye gel were done using carbopol-934, extract of *Senna auriculata*, extract of *Camellia sinensis*, extract of *Ocimum gratissimum*, amla fruit extract, glycerin, sodium hydroxide solution (1%), methylparaben and distilled water. The consistency and organoleptic properties were good.

The formulation was compared with marketed formulation.

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

The study revealed that all the selected plants contain alkaloids, flavonoids, tannins & phenolic compounds, glycosides, carbohydrate, saponin, fat & volatile oil, gum & mucilage, sterols and protein which imparts benefits such as promotion of hair growth and prevention of hair greying while being safe and eco-friendly. The overall results show that the prepared formulation was good and can be used for hair dyeing purposes without irritation and erythema.

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