



**NOVEL NANO-HERBAL HAIR DYE: FORMULATION, EVALUATION AND
QUANTITATIVE ESTIMATION****NAGULWAR VP*, DESHMUKH MV AND BANKAR AA**

Government College of Pharmacy, Kathora-Naka, Amravati-444604

Corresponding Author: Dr. V. P. Nagulwar: E Mail: vaishalinagulwar@yahoo.com;*Tel: 9890150775/ 9834032537**Received 18th March 2020; Revised 21st April 2020; Accepted 13th May 2020; Available online 1st Nov. 2020<https://doi.org/10.31032/IJBPAS/2020/9.11.5240>**ABSTRACT**

The present study demonstrates the formulation, evaluation and quantitative estimation of nano-herbal hair dye cream. Nano herbal hair dye cream was formulated using various natural constituents like Henna, Walnut, Chamomile, Rosemary, Cinnamon and Fenugreek. Three batches of the hair dye cream were formulated and evaluated for their organoleptic, physico-chemical, rheological properties along with skin irritation testing and stability testing. Colour dyeing activity was carried out on white human hair. The effective contact time for hair colouring was found to be 60 min as compared to 90 min of henna and walnut powder cream/paste. Quantitative estimation of constituents e.g. Lawsone and Apigenin was performed by TLC and HPTLC techniques. Content of Lawsone and Apigenin in nano herbal hair dye cream was found to be 12.6 mg and 19.68 mg per 30 g of nano herbal hair dye cream respectively.

Keywords: Nano herbal hair dye cream, Henna, Lawsone, Apigenin**INTRODUCTION**

Ageing is associated with progressive decreases in the maximal function and reserve capacity of all organs in the body including the skin. The most common phenomenon of ageing in hair is greying. Maintenance of hair pigmentation is

dependent on the presence and function of melanocytes, which are maintained by the stem cells of the bulge area of the hair follicle. Loss of melanocytes and melanocyte stem cells is associated with the loss of hair pigmentation seen with human

ageing [1]. From ancient days, various plants like Henna, Chamomile, Indigo, etc. are used to dye the grey hair to get natural black colour [2-6]. However, these materials tend to impart red to copper colour instead of the desired black colour. Hair dyes include various components such as dyes modifiers, antioxidants, alkalizers, soaps, ammonia, wetting agents etc. to impart desired properties. Some hair dyes marketed as natural dyes, contain 1-3% of phenylenediamine which is a synthetic hair dye and stain the skin and clothes during use [7]. Permanent dyes have the chief disadvantage of producing hypersensitive reactions in some individuals and continuous usage of these dyes on natural hair causes so numerous side effects such as skin irritation, erythema, loss or damage of hair and skin cancer. Owing to these disadvantages, research on development of herbal preparations such as herbal tablets, herbal tonics, herbal paste, herbal shampoo, herbal contraceptives and herbal dyes is gaining momentum in recent past [8-11].

Nanomaterials have been extensively researched and used as a vehicle for the delivery of bioactive agents to the skin within the cosmetic arena [12]. Because of their unique and intrinsic properties, nanomaterials are being increasingly explored for the development of advanced skincare products, specifically targeting the

hair follicle and shaft [13]. The hair formulation is prepared from part of plant e.g. leaves, roots, seed, flowers and flowering bud and used for prepared shampoo, conditioner, lotion, gel, cream, oil which is externally applying on hair [14]. Therefore, a need was felt to formulate a completely herbal nano-formulation, which will be safe for use and free of hypersensitive reactions. Nano herbal hair dye cream was formulated using various constituents like Henna, Walnut, Chamomile, Rosemary, Cinnamon and Fenugreek. Henna obtained *Lawsonia innermis* is most commonly used ingredient in hair dye. Its principle coloring ingredient of is lawsone, which acts as a non-oxidizing hair coloring agent. Henna balances the pH of the scalp and graying of hair, thus preventing premature hair fall [15]. Walnut (*Juglans regia*) contains the aromatic secondary metabolite Juglone (also called 5-hydroxy-1,4-naphthoquinone), a brown pigment having hair-dyeing property that occurs naturally in the nuts and plant parts [16]. Chamomile contains main chemical component apigenin(4',5,7-trihydroxyflavone) which is used as dye [17]. Rosemary (*Rosmarinus officinalis*) is known for its anti-inflammatory, antioxidant, antimicrobial activities [18]. Cinnamon (*Cinnamomum zeylanicum*) contains aromatic essential oils

and other constituents, such as cinnamaldehyde, cinnamic acid, and cinnamate [19]. Fenugreek (*Trigonella foenumgraceum*) is used as a conditioner for the hair. It contains significant amount of flavanoid, which has the antioxidant activity by scavenging free radicals and preventing hair fall [20]. The present study demonstrates the formulation, evaluation of nano herbal hair dye cream and quantitative estimation of active constituents i.e. lawsone and apigenin.

MATERIALS AND METHODS

Materials

Henna leaves (*Lawsonia inermis*), Fenugreek seeds (*Trigonella foenumgraceum*) and Rosemary leaves (*Rosmarinus officinalis*) were procured from Yucca Enterprises, Mumbai. Walnut husk (*Juglans regia*) and Cinnamon bark (*Cinnamomum zeylanicum*) was procured from Barsaiya herbs, Amravati and Chamomile flowers were obtained from R. R. Agro foods, Ahmedabad. Marker compounds Apigenin, Lawsone were obtained from Yucca Enterprises, Mumbai. Pre-coated TLC Plates and HPTLC Plates were obtained from IPER College, Wardha.

Methods

Size reduction and sieving

Plant materials were procured from authentic suppliers, dried in shade and powdered by using laboratory blender

(Remi sales & Eng. Ltd.) and subjected to sieving by sieve 44 (Jayant test sieves). The powdered material was finally passed through sieve 120 to get a fine powder.

Washing Fastness test for Henna leaves powder and walnut husk

Accurately weighed quantities of henna and walnut husk as 0.5, 1, 1.5, 2, 2.5, 3 gm were taken and dissolved in individual quantities in 10 ml water, 10 ml hexane and 10 ml ethanol. The prewashed white pieces of cotton cloth were taken in a vessel containing henna in the respective solvent and were kept aside for 30 min. Then the specimen was removed and rinsed in cold water. The colour fastness is rated by the presence of the colour in fabric cloth.

Formulation of Nano Herbal Hair Dye by Nanoprecipitation technique [21]

Stearic acid and potassium hydroxide were melted in a beaker at 70°C. Gelatin, henna, rosemary with alcohol was added (oil phase). Accurately weighed walnut, chamomile, cinnamon and fenugreek were dissolved in water and glycerin (aqueous phase) was added. The oil phase was added slowly into the aqueous phase (containing walnut, chamomile, cinnamon and fenugreek) and stirred continuously till a creamy emulsion was formed. The emulsion was allowed to cool and solution of methyl paraben and propyl paraben in water was added slowly. Finally, perfume

was added at room temperature and creamy emulsion was magnetically for 48 hours. Three batches of nano herbal hair dye cream were formulated as per formulae given in **Table 1**.

Hair dyeing activity

White human hairs were washed with water and dried. 1g cream of batch A, B and C was applied on white human hair and kept aside for 30 min. The cream was washed

and brown colour shade was observed as shown in **Figure 1**. Batch B was selected as an optimized batch for colour activity and stability study as it gave better brown colour as compared to batch A and C. 1 gm cream of batch B was applied on white human hair and allowed for different contact times such as 25 min, 35 min, 45min and 60 min. After washing, different brown color shade was observed.

Table 1: Formula for Nano Herbal Hair dye cream (o/w) 30 g

Ingredients (g)	Batch A	Batch B	Batch C
Part A (oil phase)			
Stearic acid	2.5	3.0	3.0
Gelatin	3.0	2.0	2.5
Henna	8.5	9.0	9.0
Rosemary	0.1	0.2	0.2
Alcohol	2.0	2.0	2.0
KOH	0.2	0.3	0.3
Part B (aqueous phase)			
Walnut	6.5	7.0	7.5
Chamomile	2.5	1.0	1.5
Cinnamon	0.04	0.06	0.08
Fenugreek	1.0	1.2	1.2
Glycerine	2.0	2.0	2.0
Methyl Paraben	0.06	0.06	0.06
Propyl Paraben	0.03	0.03	0.03
Flavours	Q.S	Q.S	Q.S
Water	Q.S	Q.S	Q.S
Total weight (g)	30	30	30

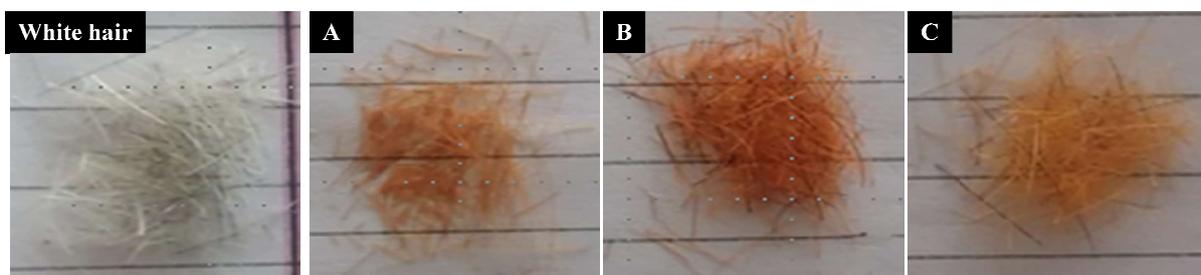


Figure 1: Hair dyeing activity (Batch A-C) on human white hair

Evaluation of nano herbal hair dye cream

The formulated nano herbal hair dye cream (o/w) was evaluated for parameters like physical appearance, pH, viscosity,

spreadability, extrudability, stability, skin irritations and particle size.

a) Physical Appearance

The cream was observed visually against light background. The average of three readings was recorded.

b) Phase Separation

The formulated cream was kept intact in a closed container at 25-30°C away from light. The cream was observed for phase separation carefully every 24 hrs for 30 days.

c) Viscosity

The viscosity determinations were carried out on Brook-field viscometer (LVF model, USA) using spindle number 4 and the average of three reading was recorded.

d) pH

The pH of cream was determined by pH meter (Analab, Model- pH cal) into a depth 0.5 cm in a beaker containing cream. Average of three reading was recorded.

e) Spreadability

Spreadability was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load. The spreadability (S) can be calculated using the formula given below, where, M = Weight tied to upper slide, L = Length of glass slide, T = Time taken to separate the slides.

$$S = \frac{M * L}{T}$$

f) Extrudability

The formulations were filled in standard capped collapsible tube and sealed. The tube was weighed and recorded. The tube was

placed between two glass slides and was clamped. A 500 gm weight was placed over the glass slide and cap was opened. The amount of cream extruded was collected and weighed. The percent of cream extruded was calculated and grades were allotted (++++ Excellent, +++ Good, ++ Fair, + Poor).

g) Accelerated Stability Study

The stability studies of formulated cream were carried out 40/75 (°C/%RH) and at room temperature for 30 days. The effects of temperature, humidity and time on the physical characteristics of the creams were assessed. The stability studies were carried out at temperature of 20-25°C.

h) Skin Irritation study

A small quantity of cream was applied on the forearm and any local reaction like irritation and erythema was checked within three hours of application.

i) Particle Size

Particle size was determined by Nano Zeta Sizer (Malvern's Zetasizer Nano ZS- 90).

Quantitative estimation

a) Thin Layer Chromatography

The glass plate of 10×10 cm size was cleaned and coated with silica gel and allowed to dry. After drying, it was activated at 110°C for 30 min. For selection of appropriate mobile phase, various solvent systems were tried on trial and error basis as mentioned in **Table 2**. The mobile

phase for HPTLC work selected was Toluene: Ethyl acetate: Formic acid in the ratio of 8:2:0.6 selected which gave better separation.

b) High performance thin layer chromatography [22]

Preparation of standard Lawsone stock solution [23]

Accurately weighed quantity of 10 mg Lawsone was dissolved in 10 ml of methanol in 10 ml of volumetric flask to prepare stock solution (1000 µg/ml). The stock solution was further diluted with methanol to give a working standard solution of lawsone (100 µg/ml). From it, for linearity studies 2, 4, 6, 8 and 10 µl per band (Lawsone 0.2, 0.4, 0.6, 0.8 and 1 µg per band respectively) were applied on TLC plates with the help of Hamilton syringe (100 µl), using Linomat 5 sample applicator. The chromatogram of standard drug (4µl/band) is shown in **Figure 4a**.

Preparation of standard drug stock solution (Apigenin) [24]

Accurately weighed quantity of 10 mg Apigenin was dissolved in 10 ml of methanol in 10 ml of volumetric flask to prepare stock solution (1000 µg/ml). The stock solution was further diluted with methanol to give a working standard solution of Apigenin (100 µg/ml). From it, for linearity studies 2, 4, 6, 8 and 10 µl per

band (Apigenin 0.2, 0.4, 0.6, 0.8 and 1 µg per band respectively) were applied on TLC plates with the help of Hamilton syringe (100 µl), using Linomat 5 sample applicator. The chromatogram of standard drugs (10 µl/band) is shown in **Figure 4c**.

Preparation of sample cream (o/w) stock solution [25, 26]

Methanol was used for solution preparation. The stationary phase was used aluminum HPTLC plate (20×10 cm) precoated with silica gel 60 F254. Mobile phase consisting of Toluene: Ethyl acetate: Formic acid (8 : 2 : 0.6) was used. The 5 gm of cream was dissolved in 40 ml of methanol and subjected to reflux for 20 min. The mixture was filtered and the filtrate was concentrated to 5 ml to get the working solution of sample cream. The sample was applied on TLC plates with the help of Hamilton Syringe (100µl), using Linomat 5 sample applicator. The chromatogram of cream is shown in **Figure 4b and 4d**. The HPTLC parameter for quantitative estimation is shown in **Table 3**. The quantitative estimation of chemical constituent Lawsone and Apigenin present in nano herbal hair dye cream was carried out by HPTLC technique and evaluated by comparison with standard Lawsone and Apigenin.

Table 2: Selection and optimization of mobile phase

Mobile Phase	Ratio	R _f	
		Lawsone	Apigenin
Toluene : Ethylacetate : Glacial acetic acid	8:1:1	0.71	0.68
Toluene : Ethylacetate : Formic acid	6:4:0.3	0.47	0.25
Toluene : Ethylacetate : Formic acid	8:2:0.3	0.6	0.28
Toluene : Ethylacetate : Formic acid	8:2:0.8	0.7	0.37
Toluene : Ethylacetate : Formic acid	8:2:1	0.67	0.42
Toluene : Ethylacetate : Formic acid	8:2:0.5	0.57	0.26
Toluene : Ethylacetate : Formic acid	8:2:0.6	0.58	0.28

Table 3: Optimized HPTLC parameters for quantitative estimation

Parameters	Stabilizing Condition
Stationary phase	Precoated silica gel 60 F ₂₅₄ (MERCK)
Mobile phase	Toluene :Ethylacetate :Formic acid (8:2:0.6)
Thickness	0.2 mm
Mode of application	Band
Band width	5 mm
Separation technique	Ascending
Temperature	Ambient
Saturation time	20 min.
Scanning mode	Single level
Measurement mode	Absorbance/Reflectance
Slit dimension	6 × 0.45
Detection TLC	UV-Densitometry Scanning
Wavelength	274 nm and 334 nm

RESULTS AND DISCUSSION

Three batches (A, B and C) of nano herbal hair dye cream were formulated and subjected to evaluation for parameters like physical appearance, pH, Viscosity, Spreadability, extrudability, stability study, skin irritations and particle size. Particle size was determined by Nano Zeta Sizer (Malvern's Zetasizer Nano ZS- 90) and found to be 98.56 nm i.e. less than 100 nm. Color dyeing activity of Batch A-C was carried out on white human hair. After 60 min washing time, applied dye gives better brown colour as compared to 25, 35, 45 minutes contact time (**Figure 2**). Thus, the effective contact time for hair colouring was found be 60 minutes as compared to 90 minutes of normal henna and walnut powder cream/paste.

Human hair dyed with batch B was subjected to shampoo washing for consecutive seven days. The brown colour of dyed white human hair was found to be lightly faded after the fifth day of washing with shampoo (**Figure 3**). Based on the colour dyeing activity and particle size, Batch B was selected for accelerated stability study and quantitative estimation. Formulated nano herbal hair dye (Batch A-C) was found to be smooth, homogenous and brown in colour with a characteristic smell. The formulated cream was observed for phase separation carefully every 24 hrs for 30 days. The cream was kept intact in a closed container at 25-30°C away from light. No phase separation was observed after 30 days in all three batches. The pH, viscosity, spreadability and extrudability of

nano herbal hair dye cream (batch A-C) are summarized in **Table 4**.

The accelerated stability studies of formulated cream batch B were carried out in 40/75 ($^{\circ}\text{C}/\% \text{RH}$) and at room temperature for 30 days (**Table 5**). After 30 days, the colour of the cream was found to be the same. pH value changed from 7.32 to 7.3. Viscosity decreased from 24533 cps to 23742 cps. Spreadability changed from 5.72 g.cm/sec to 7.43 g.cm/sec.

For quantitative estimation of constituents, the focus was on the content of active Lawsone and Apigenin in cream formulation. Trial and error basis TLC was carried out and finally the mobile phase selected was Toluene: Ethyl acetate:

Formic acid (8:2:0.6 % v/v) which showed better separation (Lawsone R_f 0.58; Apigenin R_f 0.28). The study was then extended for HPTLC. Marker compounds used were Lawsone and Apigenin for estimation the content of Lawsone and Apigenin in Henna and Chamomile respectively added in the nano cream. The quantitative estimation of chemical constituent by HPTLC technique has revealed that the nano cream contains Lawsone (12.6 mg) and Apigenin (19.68 mg) per 30 g respectively. For evaluation of skin irritation, open patch test was performed. No local reaction on forearm like irritation and erythema was observed within three hours of application.

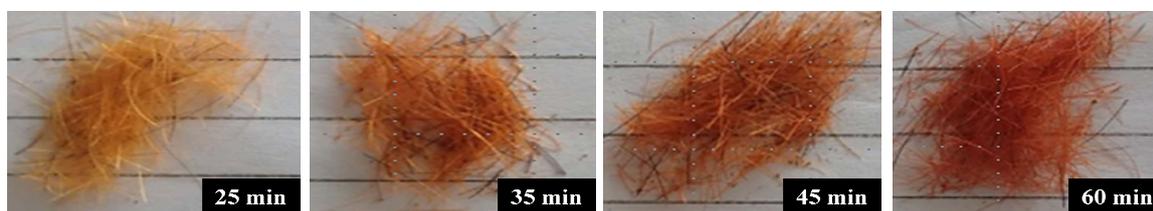


Figure 2: Hair dyeing activity of Batch B

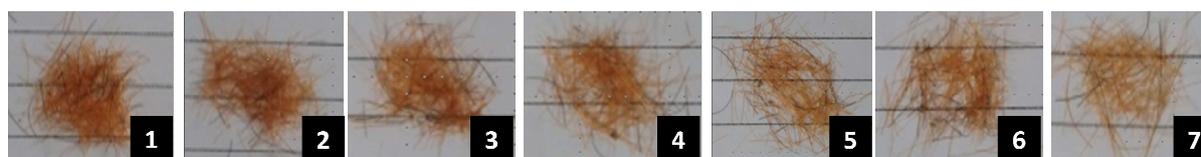


Figure 3: Effect of washing on hair dye applied human hair for consecutive seven days

Table 4: Evaluation of pH, viscosity, spreadability, extrudability of Batch A-C

Batch	pH	Viscosity (cps)	Spreadability (g.cm/sec)	% Extrudability
A	7.30	25241	8.54	91.74%
B	7.32	24533	5.72	93.01%
C	7.37	24853	7.61	88.12%

Table 5: Accelerated stability study of Batch B

Parameters	Initial (Day 0)	After 30 days 40/75($^{\circ}\text{C}/\% \text{RH}$)
Color	Brown	Brown
pH	7.32	7.3
Viscosity	24533 cps	23741 cps
Spreadability	5.72 gm.cm/sec	7.43 g.cm/sec

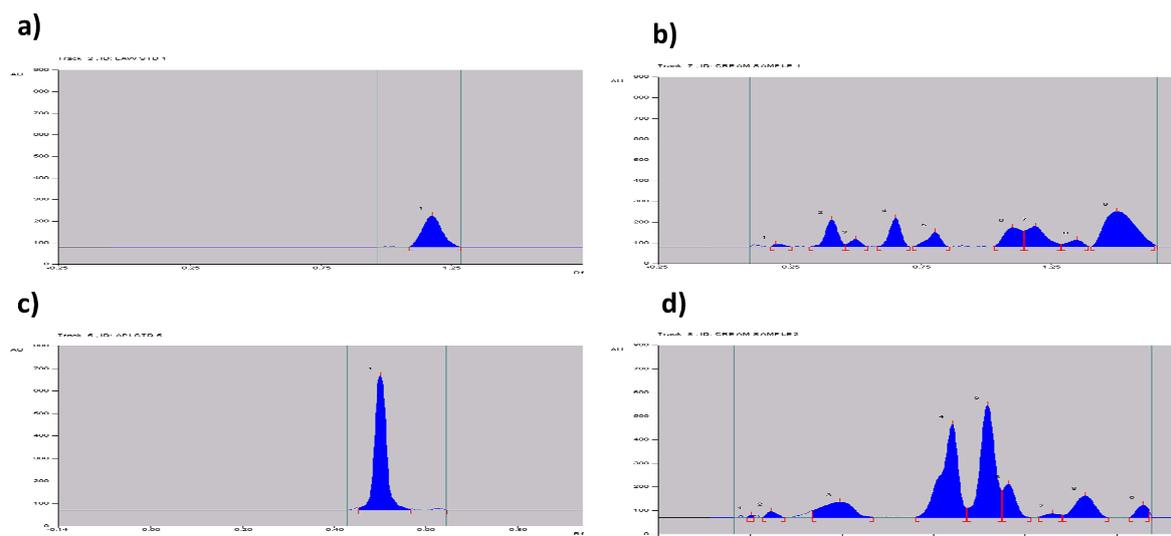


Figure 4. Chromatogram of a) standard Lawsone (0.4 µg/band), b) Sample cream- 0.4 µg/band (Lawsone), c) standard Apigenin (1 µg/band), d) Sample cream-1 µg/band (Apigenin)

CONCLUSION

Nano herbal hair dye cream (o/w) was formulated using various natural constituents like Henna, Walnut, Chamomile, Rosemary, Cinnamon and Fenugreek. Three batches of the hair dye cream were formulated and evaluated for parameters like physical appearance, pH, viscosity, spreadability, extrudability, stability, skin irritations and particle size. The developed nano herbal hair dye cream was found to be effective for colouring human hair within 45-60 minutes as compared to normal henna and walnut powder cream/paste (90-120 min). Quantitative estimation of constituents e.g. Lawsone and Apigenin was performed by TLC and HPTLC techniques. Content of Lawsone and Apigenin in nano herbal hair dye cream was found to be 12.6 mg and 19.68 mg per 30 g of nano herbal hair dye cream respectively. Thus, this completely

herbal nano hair dye cream can be an alternative to synthetic hair dyes as it is very safe to use and free of side effects attributed to synthetic/ semisynthetic hair dyes.

ACKNOWLEDGEMENTS

Authors are thankful to the Principal, Govt. College of Pharmacy for providing the facilities for the successful completion of the work.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

- [1] B. Buffoli *et al.* (2014). "The human hair: from anatomy to physiology," *Int. J. Dermatol.* 53 (3), 331–341.
- [2] M. Yusuf, M. Shahid, M. I. Khan, S. A. Khan, M. A. Khan, and F. Mohammad (2015). "Dyeing studies with henna and madder: A research on effect of tin (II) chloride

- mordant,” *J. Saudi Chem. Soc.* 19 (1), 64–72.
- [3] J. K. Srivastava, E. Shankar, and S. Gupta (2010). “Chamomile: A herbal medicine of the past with a bright future (review),” *Molecular Medicine Reports.* 3 (6), 895–901.
- [4] E. Vadivel and Y. T. Kandolkar (2014). “Formulation and evaluation of polyherbal formulation as hair colorant,” *Int. J. Pharm.* 4 (3), 226-233.
- [5] S. Cosmulescu, I. Trandafir, G. Achim, M. Botu, A. Baciú, and M. Gruia (2010). “Phenolics of green husk in mature walnut fruits,” *Not. Bot. Horti Agrobot. Cluj-Napoca*, 38 (1), 53–56.
- [6] A. Vallverdú-Queralt, J. Regueiro, M. Martínez-Huélamo, J. F. Rinaldi Alvarenga, L. N. Leal, and R. M. Lamuela-Raventos (2014). “A comprehensive study on the phenolic profile of widely used culinary herbs and spices: Rosemary, thyme, oregano, cinnamon, cumin and bay,” *Food Chem.* 154, 299–307.
- [7] C. F. Cruz, C. Costa, A. C. Gomes, T. Matamá, and A. Cavaco-Paulo (2016). “Human hair and the impact of cosmetic procedures: A review on cleansing and shape-modulating cosmetics,” *Cosmetics.* 3 (3), 26-48.
- [8] Y. Madhusudan Rao, Shayeda, and P. Sujatha (2011). “Formulation and evaluation of commonly used natural hair colorants,” *Indian J. Nat. Prod. Resour.* 7 (1), 45–48.
- [9] K. K. Kumar, K. Sasikanth, M. Sabareesh, and N. Dorababu (2011). “Formulation and evaluation of diacerein cream,” *Asian J. Pharm. Clin. Res.* 4 (2), 93–98.
- [10] P. Naishadham, P. R. Sushma, R. Dasika, S. Tangirala, and S. Tangirala (2013). “Evaluation of organic hair dye formulation by an environment friendly process,” *Int. J. Pharm. Sci. Rev. Res.* 21 (2). 152–157.
- [11] A. Aswal, M. Kalra, and A. Rout (2013). “Preparation and evaluation of polyherbal cosmetic cream,” *Der Pharm. Lett.* 5 (1), 83–88.
- [12] A. G. Sourav Ghosh (2014). “Herbonanocuticals: A New Step Towards Herbal Therapeutics,” *Med. Aromat. Plants.* 3 (3), 1-9.
- [13] J. Rosen, A. Landriscina, and A. J. Friedman (2015). “Nanotechnology-based cosmetics for hair care,” *Cosmetics.* 2 (3), 211–224.
- [14] A. S. Dhase, S. S. Khadbadi, and

- S. S. Saboo (2014). "Formulation and Evaluation of Vanishing Herbal Cream of Crude Drugs," *Am. J. Ethnomedicine. 1* (5), 313–318.
- [15] K. M. Patel and P. R. Patel (2017). "Review on Lawsonia inermis Linn.: An Update ," *Asian J. Pharm. Technol.* 7 (4), 237-250.
- [16] A. Thakur (2011). "Juglone: A therapeutic phytochemical from Juglans regia L.," *J. Med. Plants Res.* 5 (22), 5324–5330.
- [17] R. Gevrenova (2010). "Determination of natural colorants in plant extracts by high-performance liquid chromatography," *J. Serbian Chem. Soc.* 75 (7), 903–915.
- [18] J. R. De Oliveira, S. E. A. Camargo, and L. D. De Oliveira (2019). "Rosmarinus officinalis L. (rosemary) as therapeutic and prophylactic agent," *J. Biomed. Sci.* 26 (1), 5-27.
- [19] P. V. Rao and S. H. Gan (2014). "Cinnamon: A multifaceted medicinal plant," *Evid. Based Complement. Alternat. Med.* 2014.
- [20] Z. Shahi, M. Khajeh Mehrizi, and M. Hadizadeh (2017). "A review of the natural resources used to hair color and hair care products," *J. Pharma. Sci. Res.* 9 (7), 1026–1030.
- [21] S. Galindo-Rodriguez, E. Allémann, H. Fessi, and E. Doelker (2004). "Physicochemical parameters associated with nanoparticle formation in the salting-out, emulsification-diffusion, and nanoprecipitation methods," *Pharm. Res.* 21 (8), 1428–1439.
- [22] M. Srivastava, *High-performance thin-layer chromatography (HPTLC)*. 2011.
- [23] G. RK, T. R, B. N, and S. RK (2017). "Phytochemical High Performance Thin Layer Chromatography based Estimation of Lawsonia inermis (Henna) obtained from Two Natural Habitats and Dye Products Collected from Local Market," *Med. Aromat. Plants.* 6 (3), 1-7.
- [24] F. Jo et al. (2016). "Matricaria recutita and its Isolate-Apigenin : Economic Value , Ethnopharmacology and Chemico-Biological Profiles in Retrospect," *Res. Rev. J. Pharmacogn. Phytochem.* 4 (4), 17–31.
- [25] M. Patel, B. Solanki, N. Gurav, P. Patel, and S. Verma (2013). "Method development for

- Lawson estimation in Trichup herbal hair powder by high-performance thin layer chromatography,” *J. Adv. Pharm. Technol. Res.* 4 (3), 160–165.
- [26] N. G. Patel, K. G. Patel, K. V. Patel, and T. R. Gandhi (2015). “Validated HPTLC method for quantification of luteolin and apigenin in *prema mucronata* roxb., verbenaceae,” *Adv. Pharmacol. Sci.* 2015.